Chapter 14

Black Flies (Simuliidae)

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As small, powerful fliers adapted for blood-feeding, black flies can be formidable pests of humans, domestic animals, and wildlife, affecting virtually all facets of outdoor life. They are distributed worldwide, with the exception of Antarctica and some oceanic islands (e.g., Hawaii), and inhabit elevations from below sea level to at least 5,000 m above sea level. Areas with large altitudinal variation have more species. Their distribution is largely influenced by the availability of flowing water, which is required for development of the immature stages, probably driven by respiratory needs and the filter-feeding mode of life. Many of the worst pest species breed in large rivers, some of which can produce nearly a billion flies per kilometer of riverbed per day (Adler et al., 2016). Other pest species inhabit the myriad small streams of heavily wooded terrain, making management efforts difficult.

Often ranked third globally among arthropods in importance as vectors of disease agents, black flies also are among the few arthropods that have killed animals via exsanguination during massive attacks. Even when not biting, their persistent swarming behavior can create an intolerable nuisance as the blood-seeking females dart into facial orifices and crawl on the skin. As often is the case, the behavior of a minority defines the reputation of the group. So it is with black flies, for only about 10%−20% of the world's species are actually pests of humans and their animals. Among these species, however, are the vectors of the agents of human onchocerciasis and mansonellosis, bovine onchocerciasis, and avian leucocytozoonosis. The majority of species go unnoticed, because either they do not feed as adults or their hosts are of little economic concern.

TAXONOMY

More than 2,200 species of black flies have been described worldwide (Adler and Crosskey, 2018). The Palearctic Region contains the most described species, about 700, followed by the Oriental and Neotropical Regions with well over 500 and 300 species, respectively (Currie and Adler, 2008; Takaoka, 2017). The Nearctic Region has about 256 known species (Adler et al., 2004).

The Simuliidae consist of two subfamilies. The most primitive subfamily, **Parasimuliinae,** includes one genus and four described and one undescribed species endemic to the Pacific Northwest. The females of these species do not have biting mouthparts. The subfamily **Simuliinae** contains all remaining species and is divided into two tribes, the **Prosimuliini** and the **Simuliini**, the latter including the majority of pest species. The most universally accepted classification system below the tribal level is summarized by Adler and Crosskey (2018), who recognize 25 extant genera in the subfamily Simuliinae. The largest genus of black flies is *Simulium*, which contains 37 subgenera and about 81% of all species, including more than 90% of the major pest and vector species.

The morphological uniformity of black flies creates difficulty for species identification. For this reason, a holistic approach to identification is typically used, relying on characters from larvae, pupae, males, females, the polytene chromosomes, and DNA barcodes, as well as distributional and ecological information. The need for accurate identifications, particularly in programs for pest and vector management, has driven the taxonomy of black flies. As a result, black flies are taxonomically one of the best known groups of arthropods at the species level; for example, about 98% of North American species are known as larvae and pupae.

More than 150 identification keys exist for black flies in various parts of the globe. Crosskey and Howard (1997) provide a comprehensive list of identification keys by zoogeographic region. Keys to the genera and species of adults, pupae, and larvae of the Nearctic Region are provided by Adler et al. (2004). The most comprehensive

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English-language treatment of the Palearctic fauna is by Rubtsov (1956), and of the Neotropical fauna, by Coscarón and Coscarón Arias (2007) and Shelley et al. (2010). Keys to the supraspecific taxa of the Australasian and Afrotropical Regions are given by Crosskey (1967, 1969, respectively). Comprehensive keys for the Oriental Region are lacking, but the keys by Takaoka and Davies (1995), Takaoka (2003), and Chen (2016) provide a helpful starting point.

The giant polytene chromosomes (usually $n = 3$), which are best developed in the larval silk glands, provide a highly useful tool for discovering and identifying species. Studies of these giant chromosomes in the Simuliidae have generated the largest literature on the genetics of natural populations for any group of organisms (Adler et al., 2010). Giant chromosomes, particularly their banding patterns, reveal that many black flies regarded as single species are actually complexes of two or more species known as cryptic species or sibling species, each of which is biologically unique. The existence of cryptic species has far-reaching implications for biological studies and population management of pests and vectors. For example, *Simulium damnosum*, the black fly known for much of the 20th century as a vector of the agent of human onchocerciasis or river blindness, is the largest species complex among all hematophagous arthropods worldwide, consisting of more than 55 cytologically distinct entities (Post et al., 2007). Many of these entities are distinct species, but not all are vectors of the parasite that causes human onchocerciasis.

Cytotaxonomy of black flies has been reviewed for the world fauna (Adler and Crosskey, 2015). Molecular analyses are becoming an increasingly routine addition to species discovery and identification (Colorado-Garzón et al., 2016).

FIGURE 14.2 Larvae of black flies. (A) North American species, *Simulium venustum*, attached to aquatic vegetation, filter feeding. (B) Head of the larva of the Australian black fly *Paracnephia strenua*, showing details of the labral fans used in filter feeding. *(A) Photograph by Stephen A. Marshall. (B) © Douglas A. Craig.*

FIGURE 14.1 Embryonated eggs of the North American black fly *Simulium vittatum*, which deposits eggs in masses on in-stream substrates. *© Jena Johnson.*

MORPHOLOGY

The immature stages of black flies are adapted for aquatic life, although the nonmobile pupa also has terrestrial adaptations that are useful if the water recedes. The egg is roughly oval or triangular with rounded angles (Fig. 14.1). It has a glutinous outer layer and a smooth, pigmented inner shell. A micropyle, consisting of a simple hole in the egg for the entry of sperm, is present in some species but not others; if a micropyle is absent, sperm entry is facilitated enzymatically.

The larva (Fig. 14.2A) hatches with the aid of an egg burster, a small tubercle on the dorsum of the head capsule. The basic larval design consists of a well-sclerotized head capsule bearing an anterior pair of labral fans (Fig. 14.2B) and an elongate body with one thoracic proleg and a terminal abdominal proleg. Rows of tiny hooks on the prolegs enmesh with silk pads spun from a pair of larval silk glands and applied to a substrate. These silk glands extend from the anterior of the head into the posterior portion of the abdomen, where they enlarge and double back onto themselves. The adhesiveness of the silk is correlated with the velocity of the flowing water to which each species is adapted.

While clinging to a pad by its posterior proleg, the larva extends its body to filter feed. The prominent labral fans, each with about 20–80 individual rays bearing microtrichia (minute hairs) on their inner surface, are used to filter particulate matter from the water current. Larvae of some species (e.g., *Gymnopais* spp.) that live in habitats, such as glacial meltwaters, with little suspended food have lost the labral fans over evolutionary time. These species rely on their mandibles, specialized labrum, and hypostoma to scrape food from the substrate.

Additional features of the head and body are conspicuous and taxonomically important. The antennae, which consist of three articles and a terminal cone sensillum, are elongate, slender, and variously pigmented. A pair of dark eyespots is prominent on each side of the head capsule. Pigmentation patterns of the head capsule and body and the shape of the **postgenal cleft**, an area of weakly sclerotized cuticle on the ventral side of the head capsule, are important for interpreting the taxonomy of the family. The anteroventral portion of the head capsule bears the **hypostoma**, an anteriorly toothed plate used in conjunction with the mandibles to cut strands of silk and to scrape food from the substrate. Mature larvae are recognized by the presence of a prominent, dark gill histoblast on each side of the thorax.

The pupa (Fig. 14.3), which resembles an adult with its appendages held close to the body, is housed in a silk cocoon. Cocoons are shapeless sacs in the evolutionarily older species but are well-formed, slipper- or boot-shaped coverings sometimes bearing anterior processes and lateral windows in the more derived species. The pupa is held firmly in its cocoon by numerous anteriorly directed sets of hooklets. A pair of conspicuous gills arises from the thorax. The gills are among the most taxonomically useful and fascinating structures in any life stage. They vary in arrangement from thick, clublike structures to clusters of two to more than 100 slender filaments.

Adult black flies (Figs. 14.4–14.6) are characterized by a small but robust body; conical or beadlike antennae with seven to nine flagellomeres, in addition to the scape and pedicel; and an arched thorax bearing a pair of wings that typically span 6–10 mm and have thickened veins near the leading margin. Most species are blackish, but orange, yellow, and variously patterned species also exist. Males (Fig. 14.6) of nearly all species are holoptic, with eyes that occupy most of the head and meet at the midline. Male eyes consist of enlarged dorsal facets, in addition to the

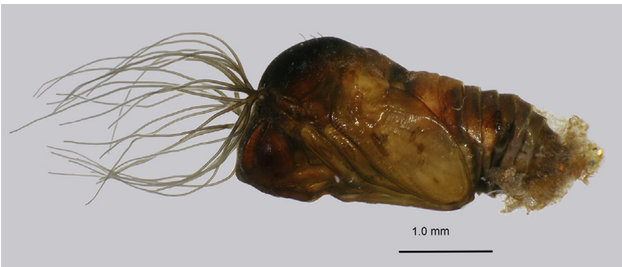

FIGURE 14.3 Pupa of the Australian black fly *Nothogreniera fergusoni*; the cocoon of this species is reduced to a small bit of silk at the posterior end. © *Douglas A. Craig.*

FIGURE 14.4 Female of the North American black fly *Prosimulium mixtum* feeding on a human. *Photograph by Stephen A. Marshall.*

FIGURE 14.5 Female of *Simulium vittatum*, one of the most common and widely distributed black flies in North America. © *Jena Johnson.*

FIGURE 14.6 Male of the North American black fly *Simulium vittatum*, showing the enlarged upper facets of the compound eye. © *Jena Johnson.*

typical-sized ventral facets, an arrangement that enhances the ability of males to locate females entering a mating swarm from overhead. Females are dichoptic, with smaller eyes separated by the frons.

The mouthparts arise ventrally from the head. A conspicuous pair of long maxillary palps attaches near the base of the proboscis. The third palpal segment accommodates the sensory vesicle (Lutz's organ), which has many chemosensilla that detect odors such as carbon dioxide. The labium forms the back of the proboscis and envelops the other mouthparts, including the minutely serrated mandibles and the toothed laciniae, with a pair of large, fleshy lobes called the labella. The mouthparts of the male are similar to those of the female, except the mandibles and laciniae are not adapted for blood-feeding and, therefore, do not bear teeth.

The stout thorax bears a pair of wings, either smoky or hyaline but never patterned. The venation, including the setation, is taxonomically important at the generic level. The color patterns of the legs and thoracic scutum are useful for species identification. The tarsal claws exist in one of three conditions. Species that feed on mammals have either a simple, unarmed claw or a minute tooth at the base of each claw. Bird feeders are endowed with a large thumblike lobe at the base of each claw. The abdomen is weakly sclerotized except the genitalia, which are of the utmost importance in the identification of species. To interpret the taxonomically important characters of the genitalia of both males and females, the abdomens must be treated with a clearing agent such as potassium hydroxide or hot lactic acid and examined in a depression slide with glycerin.

LIFE HISTORY

Immature black flies are found in virtually any water that flows, even if only imperceptibly and temporarily, from the smallest trickles to the largest rivers. Most species occupy specific habitats, and some higher taxa are characteristic of particular environments. For example, members of the genus *Gymnopais* occupy small, icy streams of the Far North, species of *Simulium* (subgenus *Trichodagmia*) live on the lips of waterfalls and in swift rocky flows, members of the *S. noelleri* sp. group are found below impounded waters, and species of *Simulium* (subgenus *Psilozia*) are found in warm, highly productive streams and rivers with open canopies.

Each species of black fly has a specific pattern of seasonal occurrence. Nearly all species in the tribe Prosimuliini are univoltine, completing a single generation annually. The tribe Simuliini contains both univoltine and multivoltine species. Some multivoltine species can complete seven or more generations per year in areas of North America with mild climates. In certain tropical areas of the world, some species (e.g., members of the *S. damnosum* complex) might cycle through more than 20 generations each year.

Eggs typically cannot resist desiccation, although those of some species (e.g., *Austrosimulium pestilens*) can survive in moist soil of dry streambeds for several years, hatching when streams are inundated. During the summer, eggs of multivoltine species (e.g., *S. vittatum*, *S. damnosum*) can hatch in fewer than 4 days. In northern temperate regions, univoltine species (e.g., *Prosimulium* spp.) often spend the warm months as eggs, whereas multivoltine species spend the cold months as eggs. Accordingly, the potential for long-term survival of eggs must be considered in management programs. Eggs of some species (e.g., *S. rostratum*) remain viable in the laboratory just above freezing for up to 2 years.

The larval stage lasts from about a week, or even less, to nearly half a year, depending on species, stream temperature, and food availability. At one extreme, the larvae of some species in the West African *S. damnosum* complex complete development in 4 days. At the other extreme, larvae of many univoltine, temperate species hatch in the fall, develop during the winter, and pupate in the spring. The number of larval instars varies from six to 11, depending on species and environmental conditions, such as food supply.

Final-instar larvae typically move to slower water before pupating in a silk cocoon that is spun on a substrate. Some species (e.g., *Prosimulium magnum*) pupate in masses, but most pupate individually. The duration of the pupal stage depends largely on temperature and species, lasting from several days to a few weeks. When the adult is ready to emerge, it expels air from its respiratory system, thus splitting the pupal cuticle along the dorsal eclosion line.

The newly emerged adult, partially covered in air, rises to the surface of the water with enough force to break the water-air interface. It then seeks a resting site, often streamside, to tan and harden. Adults generally live less than a month, during which time mating, sugar-feeding, host location, blood-feeding, and oviposition must be accomplished. Crosskey (1990) and Adler et al. (2004) provide detailed treatments of simuliid life history and bionomics.

BEHAVIOR AND ECOLOGY

After hatching, early instars often disperse short distances to more suitable sites for development. Larvae lead a largely sessile life attached to silk pads on substrates such as stones, trailing vegetation, sticks, aquatic plants, and leaf packs. The larvae of about 30 species, mostly in tropical Africa and Central Asia, are obligatorily phoretic, anchoring themselves to the bodies of larval mayflies and freshwater crabs and prawns. When disturbed, a larva repositions itself by looping over the substrate in inchworm-like fashion or by releasing itself from the silk pad and drifting downstream, often on a lifeline of silk. Downstream drift is usually greatest around dusk and during the night; its extent and timing should be considered in management programs.

The majority of larval life is spent feeding, usually by passively filtering suspended matter from the current (Fig. 14.2A) or actively grazing adherent material from the substrate. The larvae of some species are also predaceous, consuming small invertebrates such as chironomid midges. Larvae that filter their food lean with the current and twist their bodies longitudinally 90−180 degrees. In this position, one labral fan receives particulate matter that is resuspended by vortices arising from the substrate, while the other fan receives material from the main flow. Larvae filter particles that are about 0.09−350 μm in diameter, with the majority of ingested particles less than 100 μm in diameter. Larval diet consists of detritus, bacteria, small invertebrates, larval fecal pellets, and algae, with gut contents largely reflecting particle size and composition of available material in the water. Feeding efficiency (i.e., the ability to remove particles from the water column) is low, typically less than 2%. Retention of material in the gut typically varies from 20 min to longer than 2 h, depending mainly on larval age, species, and water temperature. Where larvae achieve extraordinarily high densities, as in the boreal region, their fecal pellets provide significant nutrition for freshwater organisms and might even fertilize river margins, leading ecologists to refer to the larvae as "ecosystem engineers" (Malmqvist et al., 2004a).

Species composition in streams and the distribution patterns of larvae and pupae are associated with a variety of environmental factors, such as oviposition behavior and competition, and abiotic conditions such as water chemistry, substrate availability, and stream size (McCreadie and Adler, 2012). Distributions in a small section of stream or on a specific substrate are customarily referred to as microdistributions. Factors influencing microdistributions are those that vary over a few centimeters or meters, including substrate texture, water depth, hydrodynamics, and interactions with other organisms. Microdistributions can be species specific. For example, last instars of *Simulium truncatum* and *S. rostratum*, two morphologically similar, boreal species often found in the same section of stream, select different microhabitats on the basis of water velocity. The patterns of larval dispersion on a substrate are either spaced (e.g., *S. vittatum*), with a well-defined area surrounding each larva, or clumped (e.g., *S. noelleri*), with each larva occupying only enough space to attach its silk pad. Larvae with spaced patterns vigorously defend their space from other larval black flies.

Macrodistributions encompass a scale of many meters to hundreds of kilometers. The most important factors influencing macrodistributions are stream size, water velocity, temperature, water chemistry, food quality and quantity, and the presence of lake outlets. Within a stretch of stream, species distributions can be predicted by gradients of physical and chemical factors such as temperature and conductivity. Larval densities are usually greatest within a short distance downstream of impoundment outflows. For example, densities as high as 1.2 million larvae/m^2 have been recorded for *S. noelleri* at lake outlets in Europe. Some species rarely are found far from lake outlets and, therefore, species assemblages at these outflows are often distinct from those farther downstream (Adler and McCreadie, 1997). Distributions of species among streams can be predicted by factors such as stream width and elevation. For example, species in genera such as *Twinnia*, *Gymnopais*, *Greniera*, and *Stegopterna*, as well as many species of *Prosimulium* and *Simulium* occur in trickles and small streams. Species such as *Metacnephia lyra*, *Simulium jenningsi*, *S. reptans*, *S. vampirum*, and members of the genus *Ectemina* occupy large streams and rivers. The influence of stream size and impoundment outflows on the distribution of black flies is important throughout most of the world. We know little of the oviposition choices of female black flies, which are a primary determinant of larval distributions. At larger scales, biogeographic factors are useful in predicting species distributions. Streams in one ecoregion (e.g., mountains) tend to be more similar to one another than to streams in a different ecoregion (e.g., coastal plain), with respect to physical, chemical, and riparian characteristics. Simuliid faunas also show significant differences among ecoregions.

Species richness, the number of species in a specified location, varies from one to 13 for any given stream site, but is typically less than eight. At a larger scale, the mean number of species per stream reach in a given region is remarkably consistent, ranging from three to four species (McCreadie et al., 2005). Thus, even though the total number of species in regions varies across North and South America, the mean number of species per stream reach remains relatively invariant (McCreadie et al., 2017).

After emergence, adults of most species of black flies undertake short dispersal flights, usually less than 5 km. Males disperse to find mates and a source of sugar, whereas females of most species have the additional need to find hosts for blood and sites for oviposition. Although exceptions have been reported, black flies are diurnal fliers, generally taking to the wing when temperatures exceed 10ºC. Local meteorological conditions can modify or even halt flight, but the primary factors that control daily flight patterns are wind, light, and temperature. Most species show a propensity to be on the wing at particular times of the day, these times varying with species, sex, physiological state, season, and nature of the activity.

Some species of black flies (e.g., *S. vampirum*) undertake far longer flights in search of hosts or breeding sites. These long-range movements are wind assisted, with the direction of movement being controlled by prevailing winds. Movements of hundreds of kilometers by some species (e.g., members of the *S. damnosum* complex) have implications for control strategies, requiring that breeding grounds remote from problem areas be treated. Long-distance flights typically occur in species that feed on mammals, especially those that inhabit open areas such as savannas or prairies.

The universal energy source used by males and females for flight is sugar. Adults are opportunistic in their choice of carbohydrate sources, using floral or extrafloral nectar, plant sap, and honeydew from aphids and related insects (Burgin and Hunter, 1997). Water markedly increases longevity, and a 10% sugar solution further increases longevity. The sugar meal is stored in the crop and passed to the gut as needed for digestion.

Mating is necessary for all but about 10 parthenogenetic species (e.g., *Prosimulium ursinum*). These parthenogenetic species lack males and are triploid and northern in distribution. In the sexual species, mating occurs shortly after emergence. Males use a variety of strategies to encounter females. The most commonly reported method is the formation of precopulatory swarms. These aerial swarms usually form 2−3 m above ground, either beside or above a marker. Swarm markers tend to be visually apparent aspects of the environment such as a tree branch, rock, waterfall, or host. Females enter the swarm, sometimes immediately after emergence, and are seized by males. Coupled pairs fly out of the swarm or fall to the ground or lower vegetation. Some species (e.g., *S. decorum*) do not form swarms but instead couple on the ground during large, synchronous emergences. Males of some species also perch on vegetation and seize passing females. Visual cues mediate mating, but contact pheromones might also play an important role. Black flies generally are refractive to mating under laboratory conditions, which has impeded attempts to colonize most species and to elucidate details of mating behavior. The long-term successful colonization of *S. vittatum* is a notable exception (Gray and Noblet, 2014).

Copulation lasts from a few seconds (e.g., *S. vittatum*) to 2 h (e.g., *Gymnopais* spp.). During copulation, the male passes a spermathophore (i.e., a package of spermatozoa) to the female. The tip of the spermatophore is opened enzymatically by the female and sperm move into the female's storage structure, the spermatheca. Stored sperm are released to fertilize eggs as they are being deposited.

A bloodmeal is required for the females of more than 90% of the world's simuliid species to mature the eggs. Males do not feed on blood. Females of those species that never take blood have feeble, untoothed mouthparts unable to cut host skin. These females are obligatorily autogenous; that is, they are able to produce eggs without taking blood, relying instead on energy acquired during the larval stage for all of their egg production. Females of species with biting mouthparts are anautogenous (i.e., they mature their eggs with the aid of a bloodmeal). Nonetheless, the females of some species with biting mouthparts can mature the first batch of eggs without a bloodmeal (facultative autogeny) if conditions for larval growth have been optimal. In these facultatively autogenous species, however, each subsequent batch of eggs requires a bloodmeal. Each ovarian or gonotrophic cycle (i.e., maturation of an egg batch) varies from about 2 days to 2 weeks, depending on the species and ambient temperature. Most females probably do not survive long enough to complete more than two or three ovarian cycles. Because the transmission of pathogens is usually horizontal, passing from host to host via the simuliid vector, anautogenous females have a greater potential than facultatively autogenous females to acquire and transmit disease agents.

The majority of simuliid species in the world probably feed on mammals (mammalophily), although those that feed on birds (ornithophily) also are common; no other groups of organisms serve as hosts for black flies. About two-thirds of the blood-feeding species in North America are principally mammalophilic, and the other one-third are mainly ornithophilic (Adler et al., 2004). A number of these species (e.g., *S. johannseni*, *S. venustum*), however, feed on both mammals and birds. Molecular analyses of bloodmeals in wild-caught black flies have proved valuable in determining the hosts of different species of black flies (Malmqvist et al., 2004b). Host specificity varies from highly specific in species such as *Simulium annulus*, which feeds chiefly on loons and cranes, to those such as *S. rugglesi,* which have been recorded feeding on nearly 30 different host species. Most simuliid species attack thinly haired or sparsely feathered regions of the host body and areas that are difficult for the host to groom. Thus, mammals often are attacked along the ventral region of the body and inside the ears. Birds are attacked especially on the neck, bases of the legs, and around the eyes. Humans are bitten wherever flesh is exposed, although specific areas are often attacked, such as along the hairline (e.g., *S. venustum*), the arms and hands (e.g., *S. parnassum*), the upper torso (e.g., members of the *S. ochraceum* and *S. oyapockense* complexes), and the ankles and feet (e.g., members of the *S. damnosum* and *S. metallicum* complexes).

A number of host attractants have been identified. Carbon dioxide released from the host and color, shape, and size of the host provide some of the major cues and attractants that females use to locate an appropriate blood source (Sutcliffe, 1986). Traps used to monitor populations often exploit these cues. For example, sticky silhouettes and carbon dioxide in gaseous or dry-ice form often are used to monitor females.

Biting (Fig. 14.4) and engorging require a series of appropriate cues, especially temperature and various phagostimulants, such as adenosine phosphates, in the host's blood. When the fly begins to bite, the labella are withdrawn, and small teeth and spines at the apex of the labrum and hypopharynx pull the host skin taut (Sutcliffe and McIver, 1984). The serrated mandibles cut the host flesh, allowing the labrum and hypopharynx to enter the wound, along with the laciniae, which are armed with backwardly directed teeth that anchor the mouthparts. Blood from the wound forms a small pool that is drawn up the food channel formed when the mandibles overlap the labral food canal. Because of their method of feeding from pooled blood, black flies are termed pool feeders or telmophages. Saliva is applied to the host flesh via a salivary groove along the anterior surface of the hypopharynx. Various salivary components promote local anesthesia, enhance vasodilation, inhibit platelet aggregation, and prevent clotting (Cupp and Cupp, 1997). Chemosensilla on the mouthparts help determine that blood will be directed to the midgut.

Female black flies are determined feeders. Once the host skin is penetrated, females typically do not leave until they are satiated. Because most black flies are not nervous, easily interrupted feeders, they make poor mechanical vectors of pathogens. Most species feed for about 3−6 min, taking approximately their own weight in host blood.

Most blood-feeding activity is restricted to outdoor settings (exophily), with females infrequently entering shelters to feed. This behavioral trend has implications for vector control. For example, residual house treatments effective for the control of mosquitoes are of no use for controlling black flies. Nonetheless, some cavity-nesting wild birds can experience severe assaults by ornithophilic black flies that enter through the cavity opening.

Biting activity occurs within certain optimal ranges of temperature, light intensity, wind speed, and humidity, with optima differing for each species. Given the appropriate range of meteorological conditions, many species bite throughout the day. Other species show a particular pattern of biting activity, such as a single peak in the morning (e.g., members of the *S. exiguum* complex) or a bimodal pattern with peaks in the morning and early evening (e.g., those in the *S. damnosum* complex). For all black flies, feeding typically is restricted to the hours of daylight and dusk. A rapid decrease in air pressure, combined with increased cloud cover, produces a sudden flush of biting activity.

Most female black flies can produce a batch of about 100−600 eggs, although the number varies from 25 (some *Gymnopais* spp.) to about 800 (some *Simulium* spp.). Females of some species can produce several of these egg batches in a lifetime, depending on the number of bloodmeals and how long the female lives. Oviposition usually occurs in the late afternoon and early evening. Eggs are deposited freely into the water during flight (e.g., *S. venustum* complex) or attached in strings or masses (Fig. 14.1) to substrates such as rocks and trailing vegetation at the water line (e.g., *S. vittatum*). Some species, however, oviposit in moist fissures in riverbanks (e.g., *S. posticatum*) or in streamside mosses (e.g., some *Prosimulium* spp.). Females of some species participate in communal oviposition, mediated by oviposition pheromones, resulting in masses of thousands of eggs (McGaha et al., 2015).

PUBLIC HEALTH IMPORTANCE

The importance of black flies to humans centers largely around the pestiferous habits of the blood-seeking females and the disease agents they transmit. The human disease agents transmitted by black flies are those that cause onchocerciasis in the tropics of Africa and Central and South America and mansonellosis in northwestern Argentina, southern Panama, and the western Amazon Region. No other human pathogens or parasites are known definitively to be transmitted by black flies, and no endemic simuliid-borne disease of humans has been reported from North America.

The biting and nuisance problems inflicted by black flies have had severe consequences for most outdoor activities including agriculture, forestry, industrial development, military exercises, mining, and tourism. Industrial and recreational development in some regions of Canada and Russia has been impeded or halted by overwhelming attacks from black flies. Yet, these negative effects gain balance through the protection that the attacking black flies afford to sensitive environmental areas that otherwise might suffer from development. Actual monetary losses due to biting and nuisance problems in different sectors of the economy, although significant and sometimes crippling (Sariözkan et al., 2014), are typically poorly documented.

Biting and Nuisance Problems

The black flies that bite humans (i.e., anthropophilic species) constitute 10% or less of the total simuliid fauna in any zoogeographic region (Table 14.1), with some areas of the world being nearly free of biting problems. No black fly is known that feeds exclusively on humans. In North America, where the name "black fly" originated, fewer than 60 species have been recorded to bite humans. Less than one-third of these hold any real status as biting pests, but those that bite regularly can be unrelenting in their attacks. Individual reactions to bites vary from a small red spot at the puncture site, often with initial streaks of oozing blood (Fig. 14.7) to an enlarged swelling the size of a golf ball (Stokes, 1914). Swelling from bites around the eyes can impede vision, and bites on the legs can impair walking.

A general syndrome, sometimes called **black fly fever**, is common in areas such as northeastern North America

TABLE 14.1 Species of Black Flies Regarded as Significant Biting and Nuisance Pests of Humans, Livestock, and Poultry

Species	Geographic Region
Humans	
Austrosimulium australense	New Zealand
Austrosimulium ungulatum	New Zealand
Prosimulium mixtum group	Eastern North America
Simulium amazonicum complex	South America (Amazon Basin)
Simulium arakawae	Japan
Simulium buissoni	Marquesas Islands
Simulium cholodkovskii	Russia
Simulium decimatum	Russia
Simulium jenningsi	Eastern North America
Simulium johannseni	Midwestern North America
Simulium jujuyense	Argentina
Simulium meridionale	Western North America
Simulium nigrogilvum	Thailand
Simulium ochraceum complex	Galapagos Islands
Simulium oyapokense complex	South America (Amazonian Region)
Simulium parnassum	Eastern North America
Simulium penobscotense	Northeastern North America
Simulium pertinax	Brazil
Simulium posticatum	England
Simulium quadrivittatum	Central America
Simulium sanguineum	Northwestern South America
Simulium tescorum	Southwestern United States
Simulium turgaicum	Western Asia
Simulium venustum complex	North America
Simulium vittatum complex	North America
Livestock	
Austrosimulium pestilens	Australia (Queensland)
Cnephia pecuarum	United States (Mississippi River Valley)
Simulium cholodkovskii	Russia
Simulium chutteri	South Africa
Simulium colombaschense	Europe (historical)
Simulium decimatum	Russia
Simulium equinum	Europe, Russia
Simulium erythrocephalum	Europe
Simulium incrustatum	Paraguay
Simulium jenningsi group	Eastern North America
Simulium kurense	Western Asia
Simulium lineatum	Europe
Simulium luggeri	Western Canada
Simulium maculatum	Russia
Simulium ochraceum complex	Galapagos Islands
Simulium ornatum complex	Europe, Russia
Simulium reptans	Europe, Russia
Simulium turgaicum	Russia, western Asia
Simulium vampirum	Western Canada
Simulium vittatum complex	North America
Poultry	
Cnephia ornithophilia	Eastern North America
Simulium meridionale	North America
Simulium rugglesi	North America
Simulium slossonae	Southeastern United States

where biting problems can be intense. It is presumably a response to salivary components from the fly, and is characterized by headache, nausea, fever, and swollen lymph nodes in the neck. Many people experience some itching from the bites, intensified by scratching the wounds. Severe allergic reactions, including asthmatic responses, are infrequent; however, medical treatment, including hospitalization, is sometimes necessary (Gudgel and Grauer, 1954). No human deaths from simuliid bites have been recorded since the beginning of the 20th century, although anecdotal accounts suggest that an unclothed human can be exsanguinated in about 2 h in some areas of Russia. Exposure to fierce attacks of biting and swarming black

FIGURE 14.7 Bite wounds on legs of a river guide, caused by a North American black fly of the *Simulium venustum* complex. *Photograph by Peter. H. Adler.*

FIGURE 14.8 Overwhelming swarm of blood-seeking flies along Nunavut's Dubawnt River in the Canadian Arctic. *Photograph by Nicolas Perrault; Creative Commons CC0 1.0 Universal Public Domain Dedication.*

flies (Fig. 14.8) can affect a person's emotional state and produce short-term psychological effects that reduce individual efficiency.

Many species of black flies are attracted to humans but do not bite, or they bite infrequently in proportion to the number of flies actually attracted. These species can create enormous nuisance problems. One such species is *Simulium jenningsi*, a major pest in North America. Females of this species sometimes bite humans and occasionally cause allergic reactions, but they are more of a nuisance because of their habit of swarming about the head and entering the eyes, ears, nose, and mouth. Outdoor activities in afflicted areas, such as Pennsylvania (USA), can become unbearable as the females ceaselessly swarm around the head. More than $5 million is spent annually in the management of *S. jenningsi*.

Occasional nuisance problems have been caused by large numbers of flies attracted to incandescent lights and by mating swarms that form over bicycle and foot paths at about the same height as a person walking or riding a bicycle. These kinds of problems usually are caused by members of the North American *S. vittatum* species complex, which breed abundantly in human-altered habitats, such as lake outlets and polluted waters.

Human Onchocerciasis

The greatest public health problem associated with black flies is onchocerciasis, or **river blindness**, a tropical disease caused by the filarial nematode *Onchocerca volvulus*, which is transmitted solely by black flies during blood-feeding. River blindness is the second leading infectious cause of blindness in the world. In the Old World, river blindness is found in 27 countries in the central belt of Africa, with small foci in southern Yemen (Fig. 14.9). In the New World, where the disease possibly was introduced during the slave trade, its current and historical distributions are patchy, with foci in northern Brazil, Colombia, Ecuador, Guatemala, Mexico, and Venezuela (Fig. 14.10). The World Health Organization (1995) conservatively estimated that about 17.7 million people are infected (17.5

FIGURE 14.9 Geographic distribution of human onchocerciasis in Africa and Yemen. *Compiled from various sources.*

FIGURE 14.10 Geographic distribution of human onchocerciasis in the New World, showing foci where the disease has been eliminated and where it is ongoing. *Modification of map; The Carter Center, Atlanta, Georgia, USA.*

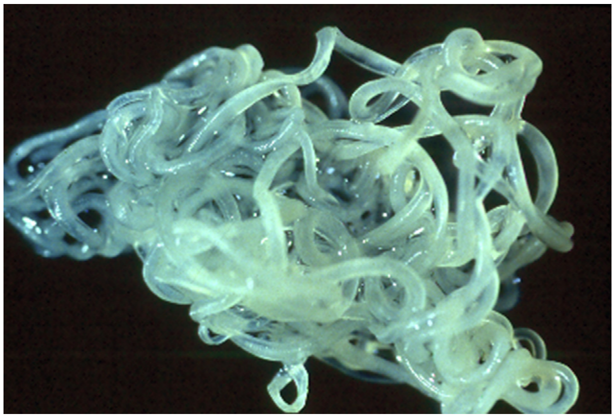

FIGURE 14.11 *Onchocerca volvulus*, after digesting away the nodular human tissue. *Courtesy of Armed Forces Institute of Pathology, USA.*

FIGURE 14.12 Children at a clinic in Guatemala for surgical removal of adults of *Onchocerca volvulus* from subcutaneous nodules on their heads, either waiting (scalps shaved) or returning to be checked following previous surgery (heads bandaged). *Photograph by E. W. Cupp.*

million in Africa and Yemen; 140,500 in tropical America), with approximately 270,000 cases of blindness and another half million individuals with severe visual impairment. Subsequent evidence suggested that the 1995 figures were underestimates and should have been 120 million people at risk, with 37 million heavily infected (Remme et al., 2006). Onchocerciasis is occasionally diagnosed in patients in North America who have traveled from endemic areas. Research on the disease and its vectors has generated a massive body of literature (Muller and Horsburgh, 1987) and numerous reviews (Shelley, 1988b; Crosskey, 1990; World Health Organization, 1995).

Onchocerca volvulus typically is found only in humans (definitive host) and adult flies of the genus *Simulium* (intermediate host). Various strains of *O. volvulus* are recognized, such as forest and savanna strains in West Africa, and these form highly compatible parasite—vector complexes with distinct clinical features. When the female black fly ingests a bloodmeal from an infected human, the **microfilariae** (220—360 μm long) penetrate the gut of the fly and make their way to the thoracic flight muscles. Once in the thoracic muscles, the microfilariae lose their motility and transform to first-stage larvae (L1), which then molt to become second-stage larvae (L2). The final molt in the fly produces the infective third-stage larvae (L3), which migrate to the fly's head and mouthparts. Vector incrimination is based on the presence of L3 worms in the head capsules of female black flies. In West Africa, DNA tests allow animal parasites and the human parasites of savanna and forest to be distinguished. Development in the black fly, which is influenced by ambient temperature, typically requires 6—12 days, but the time between successive bloodmeals taken by the fly is usually 3—5 days. Consequently, the infective larvae will be passed to a human host no earlier than the third bloodmeal when the fly is about 8—10 days old.

In humans, the infective larvae molt to the L4 stage within about a week. One more molt yields juvenile adults, which grow to mature adult worms over the next 12—18 months and begin reproducing. Adult worms (Fig. 14.11) typically become encapsulated in fibrous nodules (onchocercomas) that vary in size from about 0.5 to 10.0 cm and can be subcutaneous over bony prominences (Fig. 14.12) or deep in muscular and connective tissues; they cause no inflammatory response and no great discomfort. Mating between the small male worms (3—5 cm long) and the large females (30—80 cm) occurs in the nodules. Adult female worms can produce microfilariae, potentially millions of them, for up to 14 years. These microfilariae migrate from the nodules to the skin, where they can be acquired by a vector, as well as to the eyes and various other organs (e.g., liver) of the human host. A diagnostic clinical feature of onchocerciasis is the presence of hundreds of microfilariae in skin snips.

River blindness is essentially a rural disease, afflicting those people most vulnerable to both the medical consequences and social stigmas of infection. Symptoms of the disease depend on factors such as geographical location, microfilarial transmission rates, and frequency of reinfection. Where transmission rates are low, the disease can be asymptomatic. With heavy infections, however, the classical manifestations of the disease appear (i.e., dermal changes, lymphatic reactions, nodules [Fig. 14.12], and ocular disturbances). Other than the nodules in which the adults are enveloped, all symptoms are caused by the microfilariae.

Large numbers of microfilariae migrating throughout the dermis cause horrific itching that can lead to bleeding, secondary bacterial infections, inability to sleep, fever, headache, and even suicide. In addition, to itching, chronic infections in Africa and Yemen can cause dermal lesions, patches of depigmentation ("leopard skin"), fibrosis, loss of elasticity (e.g., "elephant knees"), and dry wrinkling ("lizard skin"). In Yemen, the itching symptoms of the disease, which can result in dark, swollen skin, are known as *aswad* or *sowda*. In Central America, two unique, chronic skin conditions occur: a painful, reddish rash on the face (*erisipela de la costa*) and lesions associated with reddish skin on the trunk and arms (*mal morado*). The lymphatic nodes also can be affected, especially in the groin and thighs; combined with loss of skin elasticity, the result is a condition known as hanging groin. The various skin conditions associated with onchocerciasis can create a social stigma that hinders treatment and exacerbates patient suffering (Tchounkeu et al., 2012).

Migrating microfilariae also enter the eye, resulting in a severe ocular pathology that can involve all tissues of the eye. The discovery of the co-involvement of the symbiotic bacterium *Wolbachia* and its filarial host, *O. volvulus*, in ocular pathology (Pearlman, 2003) opened the possibility of reducing ocular onchocerciasis through antibiotic treatments (Taylor et al., 2014). Ocular problems manifest in many forms, including cataracts, retinal hemorrhages, corneal opacities, secondary glaucoma, sclerosing keratitis, and optic neuritis. Various forms of visual impairment occur, such as night blindness and reduction in peripheral vision, but the most severe consequence is irreversible blindness with complete loss of light perception (Fig. 14.13). Blindness usually takes years to occur; at age 20, for example, it is rare in infected people, but at 50 years of age, half of the infected victims can be blind (Fig. 14.14). The incidence of blindness is highest in the savannas of West Africa, with 15% of some villages experiencing blindness. At these high levels of disease, the village is often abandoned. Outside West Africa, ocular pathology is rare.

At least 26 species of *Simulium* are known vectors of *O. volvulus* (Table 14.2). Most of these vectors are members of species complexes, and considerable taxonomic work is still needed to resolve all of the vector species in areas such as East Africa and the Americas. In West Africa and Yemen, all vectors are members of the *S. damnosum* species complex, and include *S. damnosum sensu stricto* and *S. sirbanum*, the principal vectors associated with the savanna form of the disease and ocular pathology. The vectors in East Africa are members of the *S. damnosum* complex, the *S. neavei* group, and *S. albivirgulatum*. In the

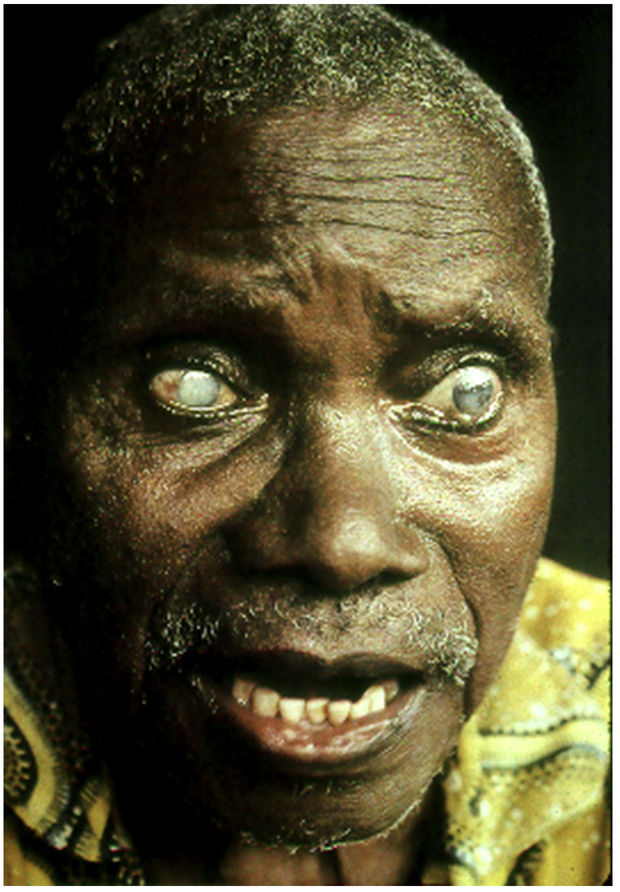

FIGURE 14.13 Human blindness caused by the filarial nematode *Onchocerca volvulus* transmitted by black flies of the *Simulium damnosum* complex in West Africa; note opacity of corneas. © *Eric Poggenpohl.*

FIGURE 14.14 Young boy leading a man blinded by onchocerciasis in Burkina Faso. *Photograph by E. W. Cupp.*

TABLE 14.2 Disease Agents Transmitted by Black Flies

Disease Agent	Vectors[1]	Hosts	Geographic Areas	Select References
Protozoa[2]				
Leucocytozoon cambournaci	*Helodon decemarticulatus, Cnephia ornithophilia, Simulium aureum* complex, *S. vernum* group	Sparrows	North America	Adler et al., 2004
Leucocytozoon dubreuili	*H. decemarticulatus, C. ornithophilia, S. aureum* complex, *S. vernum* group	Thrushes	North America	Adler et al., 2004
Leucocytozoon icteris	*H. decemarticulatus, C. ornithophilia, S. anatinum, S. annulus, S. aureum* complex	Blackbirds	North America	Adler et al., 2004
Leucocytozoon lovati	*S. aureum* complex, *S. vernum* group	Grouse	North America	Adler et al., 2004
Leucocytozoon neavei	*Simulium* spp., especially *S. adersi*	Guinea fowl	Eastern Africa	Fallis et al., 1974
Leucocytozoon sakharoffi	*H. decemarticulatus, S. aureum* complex, *S. angustitarse*	Corvids	North America, England	Adler et al., 2004 Fallis et al., 1974
Leucocytozoon schoutedeni	*Simulium* spp., especially *S. adersi*	Chickens	Eastern Africa	Fallis et al., 1973
Leucocytozoon simondi	*Cnephia ornithophilia, S. anatinum, S. fallisi, S. rendalense, S. rugglesi, S. usovae, S. venustum* complex	Ducks, geese	North America, Norway	Adler et al., 2004
Leucocytozoon smithi	*S. aureum* complex, *S. congareenarum, S. jenningsi* group, *S. meridionale, S. slossonae*, possibly *S. ruficorne* group	Turkeys	North America, introduced to Africa	Adler et al., 2004
Leucocytozoon tawaki	*Austrosimulium ungulatum*	Penguins	New Zealand	Allison et al., 1978
Leucocytozoon toddi	*H. decemarticulatus, S. aureum* complex, *S. vernum* group	Hawks	North America	Adler et al., 2004
Leucocytozoon ziemanni	*H. decemarticulatus, S. aureum* complex, *S. vernum* group	Owls	North America	Adler et al., 2004
Trypanosoma avium	*Metacnephia lyra, S. aureum* complex, *S. latipes, S. vernum*	Grouse, raptors	Europe	Votýpka et al., 2002; Reeves et al., 2007
Trypanosoma confusum	*H. decemarticulatus, Simulium* spp.	Birds	North America	Bennett, 1961
Trypanosoma corvi	*S. latipes*	Kestrels	England	Dirie et al., 1990
Trypanosoma numidae	*Simulium* spp., especially *S. adersi*	Chickens, guinea fowl	Eastern Africa	Fallis et al., 1973
Zelonia australiensis	*S. dycei*	Macropods	Australia	Barratt et al., 2017
Filarial Nematodes				
Dirofilaria ursi	*S. venustum* complex	Bears	North America	Addison, 1980
Mansonella ozzardi	*S. amazonicum* complex, *S. argentiscutum*, possibly *S. exiguum* complex, *S. oyapockense* complex, *S. sanguineum*	Humans	Northern South America, north-western Argentina, Panama	Shelley, 1988a; Shelley and Coscarón, 2001

Continued

TABLE 14.2 Disease Agents Transmitted by Black Flies—cont'd

Disease Agent	Vectors[1]	Hosts	Geographic Areas	Select References
Onchocerca cervipedis	*Prosimulium impostor*, *S. decorum*, *S. venustum* complex	Deer, moose	North America	Pledger et al., 1980
Onchocerca dewittei	*S. bidentatum*	Wild boar	Japan	Uni et al., 2015
Onchocerca dukei	*S. bovis*	Cattle	Africa	Wahl and Renz, 1991
Onchocerca gutturosa	*S. erythrocephalum*, *S. bidentatum*	Cattle	Japan, Ukraine	Crosskey, 1990; Takaoka, 1999
Onchocerca lienalis	*S. erythrocephalum*, *S. jenningsi*, *S. ornatum* complex, *S. reptans*, *S. arakawae*, *S. daisense*, *S. kyushuense*	Cattle	North America, Russia, western Europe, Japan	Lok et al., 1983; Crosskey, 1990; Takaoka, 1999
Onchocerca lupi	*S. vittatum*	Dogs	Southwestern North America	Hassan et al., 2015
Onchocerca ochengi	*S. damnosum* complex	Cattle	West Africa	Wahl et al., 1998
Onchocerca ramachandrini	*S. damnosum* complex	Wart hogs	West Africa	Wahl, 1996
Onchocerca skrjabini	*S. arakawae*, *S. bidentatum*, *S. daisense*, *S. oitanum*	Japanese deer, serows	Japan	Takaoka, 1999
Onchocerca takaokai	*S. bidentatum*	Wild boar	Japan	Uni et al., 2015
Onchocerca tarsicola	*P. tomosvaryi*, *S. ornatum* complex	Deer, reindeer	Western Europe	Crosskey, 1990
Onchocerca volvulus	Africa: *S. albivirgulatum*, *S. damnosum*, *S. dieguerense*, *S. ethiopiense*, *S. kilibanum*, *S. konkourense*, *S. leonense*, *S. mengense*, *S. neavei*, *S. sanctipauli*, *S. soubrense*, *S. squamosum*, *S. thyolense*, *S. woodi*, *S. yahense*, Americas: *S. callidum*, *S. exiguum* complex, *S. guianense* complex, *S. incrustatum*, *S. limbatum*, *S. metallicum* complex, *S. ochraceum* complex, *S. oyapockense* complex, *S. quadrivittatum*, Yemen: *S. rasyani*	Humans	Africa, Central America, South America	Shelley, 1988b; Crosskey, 1990; World Health Organization, 1995; Post et al., 2008
Splendidofilaria fallisensis	*S. anatinum*, *S. rugglesi*	Ducks	North America	Anderson, 1968
Viruses				
Vesicular stomatitis virus	*S. notatum*, *S. vittatum*	Cattle, horses, pigs; occasionally goats, llamas, sheep	Americas	

[1]*Vector species also have the potential to be pests, depending on host specificity and population size.*
[2]*Many* Leucocytozoon *lineages have been found in female black flies, but transmission has not been demonstrated (Murdock et al., 2015; Lotta et al., 2016).*

Americas, at least nine species have been incriminated as vectors, the most important of which are members of the *S. exiguum*, *S. guianense*, *S. metallicum*, *S. ochraceum*, and *S. oyapockense* complexes; their importance varies with location and the eradication status of the disease.

An understanding of the unique life history and behavior of each vector species is key to the control of onchocerciasis. Breeding sites of the vectors represent the ideal targets for control. The immature stages of the *S. damnosum* complex primarily inhabit swift sections of medium to large rivers, from dry savannas to forest highlands, depending on the species. Larvae and pupae of species in the *S. neavei* group live primarily in perennial, shaded forest streams where they have an obligatory phoretic relationship with river crabs. In Latin America, members of the *S. metallicum* and *S. ochraceum* complexes breed in large and small streams, respectively, that drain forested mountain slopes, whereas members of the *S. exiguum* and *S. oyapockense* complexes breed in large rivers of the rain forest, and members of the *S. guianense* complex inhabit the rocky shoals of large rivers.

Although each species breeds in a specific habitat, the adults of some species can travel great distances beyond their natal waterways. The adults of *S. sirbanum* and *S. damnosum sensu stricto*, for example, can travel more than 500 km, assisted by seasonally changing winds. In the wet season, moist monsoon winds from the southwest move flies in a northeastwardly direction. In the dry season, winds from the northeast assist flies in their reverse, southwestwardly flights. Continual reinvasions by vectors, therefore, occur with each season in both the northern and southern parts of West Africa, and must be considered in control efforts.

Mansonellosis

The filarial nematode *Mansonella ozzardi* is the causal agent of mansonellosis, a questionably pathogenic disease of humans. It is transmitted by at least five species of black flies in the Neotropical rain forests of Brazil, Colombia, Guyana, Venezuela, and southern Panama, as well as in northwestern Argentina (Table 14.2). The disease is now spreading into previously uninfected areas (Adami et al., 2014). Black flies first were incriminated as vectors of *M. ozzardi* in 1959 and subsequently confirmed experimentally as vectors in 1980. Mansonellosis also is found in the Caribbean Islands, where only ceratopogonid midges (*Culicoides* spp.) are known to transmit the causal agent (Shelley, 1988a).

Adult nematodes of *M. ozzardi* occur in the subcutaneous tissues of humans, and the microfilariae are found principally in the peripheral blood where they are acquired by blood-feeding flies. The life cycle of the nematode in black flies is similar to that of *O. volvulus*. A number of mammals and some birds and amphibians can be infected, but humans are the only significant reservoirs. In some highly endemic areas (e.g., Colombia Amazon), up to 85% of the human population can be infected. Mansonellosis generally is viewed as causing little or no pathology, but some reports have indicated that joint pains, headaches, hives, and pulmonary symptoms are associated with infections (Adami et al., 2014). Treatment with ivermectin can reduce microfilaremia. The discovery of *Wolbachia* bacteria in *M. ozzardi* (Casiraghi et al., 2001) opens the possibility for antibiotic treatment.

Other Diseases Related to Black Flies

Because black flies that feed on humans also feed on other hosts, the potential exists for certain disease-causing agents of domestic and wild animals to be transferred to humans. As examples, about 16 cases of zoonotic onchocerciasis are known in humans (Fukuda et al., 2011). Black flies also have been implicated as mechanical vectors of the bacterial agent of **tularemia** in the United States and Russia, suggesting that occasional cases of transmission of this pathogen to humans might occur. Similarly, **Eastern equine encephalitis** virus in the United States and **Venezuelan equine encephalitis** virus in Colombia have been isolated from several species of black flies, suggesting at least the potential for transmission of these pathogens to humans. Black flies in the Marquesas Islands have been implicated in the indirect transmission of **Hepatitis B** virus by causing numerous, itching lesions on the skin (Chanteau et al., 1993). Direct transmission of the virus by black flies also is theoretically possible.

Several additional diseases might be related to biting black flies. One such disease is **endemic pemphigus foliaceus** or **fogo selvagem**, a potentially lethal, autoimmune, blistering skin affliction. The disease is centered among poor, outdoor laborers in certain regions of Brazil (Eaton et al., 1998). Further work is needed to determine if black flies are the causal agents of the disease. Another affliction possibly associated with black flies in the New World is **thrombocytopenic purpura**, a disorder in which the platelet count is reduced. Again, more data are needed before black flies can be linked to the cause. A mysterious disease with a possible link to black flies is **nodding syndrome**, characterized by epileptic seizures in children of East Africa. Nodding syndrome has been known since the 1960s, but its incidence has increased in the past 10 years and is strongly associated with infection by the simuliid-borne filarial nematode *O. volvulus*, suggesting an autoimmune response (Johnson et al., 2017).

VETERINARY IMPORTANCE

The veterinary importance of black flies is manifested through pathogen transmission, biting, and nuisance swarming. Filarial nematodes, protozoans, and possibly several viruses are transmitted to animals. The most insidious parasites are those that cause leucocytozoonosis in domestic ducks, geese, and turkeys.

Deaths of birds and livestock have resulted from attacks by large numbers of black flies. Livestock under persistent attack sometimes stampede, trampling young animals, crashing into structures, and tumbling from precipices. Suffocation has been blamed for some deaths, with so many flies clogging the respiratory passages that breathing can become severely impaired. Deaths also have been attributed to respiratory tract infections caused by inhalation of flies. If enough blood is withdrawn, it may become too thick to transport oxygen efficiently, thereby killing the animal via **exsanguination**. Perhaps the most common cause of mortality can be attributed to the actual bites of the flies or, more specifically, to toxemia and acute shock caused by the various salivary components that are injected during blood-feeding.

More difficult to assess in economic terms, but equally harmful to the livestock and poultry industries, are the effects of harassment through biting and swarming (Fig. 14.15, Table 14.1). Biting is often aimed at weakly protected areas of the body, such as the ears, neck, and ventral midline (Fig. 14.16). Persistent attacks by black flies can cause unruly host behavior, weight loss, reduced egg and milk production, malnutrition in young animals, dermatitis and epidermal necrosis, impotence in bulls, delayed pregnancies, abortions, and possibly stress-related diseases such as pneumonia.

Actual monetary losses from black flies are not well documented but can be significant. The beef and dairy industries of Saskatchewan, for example, lost more than $3 million in 1978 from attacks by *Simulium luggeri* (Fredeen, 1985). In spring 1993, the ostrich and emu industry lost about $1.5 million along the Trinity River in eastern Texas as a result of attacks by black flies (Sanford et al., 1993). Effects of black flies on pets have rarely been documented, although *Cnephia pecuarum* has caused hospitalizations and deaths as recently as the end of the 20th century (Atwood, 1996). The death of even a single exotic bird, such as a parrot, from blood-feeding by black flies can reflect a loss of thousands of dollars (Mock and Adler, 2002).

Wildlife also succumb to withering attacks from black flies (Fig. 14.17), especially when the animals are stressed, as in years of lean food supply. Nestling birds are particularly vulnerable, including raptors and songbirds. Nestling bluebirds and tree swallows, for example, have been killed by *S. meridionale*, a species that routinely enters nest boxes

FIGURE 14.15 Cattle under attack by *Simulium vampirum* on the prairie of Alberta, Canada. *Photograph by Joseph A. Shemanchuk, Department of Agriculture and Agri-Food, Government of Canada.*

FIGURE 14.16 Damage to cow udder caused by black flies on the Canadian prairie. *Photograph by Joseph A. Shemanchuk, Department of Agriculture and Agri-Food, Government of Canada.*

FIGURE 14.17 Endangered whooping crane attacked by *Simulium annulus* in Wisconsin. *Photograph by Richard P. Urbanek, U.S. Fish and Wildlife Service.*

FIGURE 14.18 *Simulium annulus* and *Simulium johannseni* attracted to eggs of the whooping crane by odors from the bird's uropygial gland. *Photograph by Richard P. Urbanek, U.S. Fish and Wildlife Service.*

(Adler et al., 2004). Attacks on endangered species are of particular concern. The endangered Attwater's prairie chicken suffers attacks from the black fly *Cnephia ornithophilia*, which also carries a *Leucocytozoon* blood parasite (Adler et al., 2007). Endangered whooping cranes consistently abandon their nests during severe attacks from *Simulium annulus* and *Simulium johannseni* (Urbanek et al., 2010) (Figs. 14.17 and 14.18).

An odd, but indirect, nuisance problem mediated occasionally by black flies involves the Neotropical human bot fly *Dermatobia hominis*. Female bot flies capture hematophagous arthropods, including black flies, to which they glue their eggs. Once the carrier has landed on a host, the larvae of the bot fly hatch and bore into the host skin, causing myiasis. At least one species of black fly (*Simulium nigrimanum*) that feeds on domestic animals is known to be used as a carrier.

Bovine Onchocerciasis

At least 11 species of filarial nematodes in the genus *Onchocerca* are transmitted by simuliids to domesticated and wild animals (Table 14.2). Black flies transmit at least four species of filarial nematodes (genus *Onchocerca*) to cattle in the Afrotropical, Nearctic, and Palearctic Regions (Table 14.2). *Onchocerca lienalis* is the most widespread of these filarial parasites. *Simulium jenningsi* is its primary vector in the United States, whereas the *S. ornatum* complex is a principal vector in the Old World. The microfilariae of *O. lienalis* are concentrated in the umbilical region of the host. They are ingested during blood-feeding and transmitted to a new host after they have developed to the infective third stage in the simuliid vector. The percentage of infected cattle is often quite high, but symptoms and general effect on the host are usually not overt. Infected animals sometimes show dermatitis and inflammation of the skin and connective ligament.

A Palearctic species often confused with *O. lienalis* is *Ochocerca gutturosa*. Its microfilariae occur in the skin of the neck and back of the host. It has been confirmed from Japan and perhaps the Ukraine, where *S. bidentatum* and *S. erythrocephalum*, respectively, have been implicated in its transmission. Elsewhere, ceratopogonid midges (*Culicoides* spp.) are vectors. In West Africa, *O. ochengi* is transmitted to cattle by members of the *S. damnosum* complex, and *O. dukei* is transmitted by *S. bovis*. Both of these *Onchocerca* spp. can create nodules, either dermal (*O. ochengi*) or subcutaneous (*O. dukei*), in the inguinal region of the host. Economic losses resulting from bovine onchocerciasis rarely have been assessed, although a few reports have indicated that the quality of hides can be reduced.

At least four to seven additional species of *Onchocerca* are transmitted by black flies to nonbovine hosts (Table 14.2). *Onchocerca cervipedis* in North America and *O. tarsicola* in Europe infect the subcutaneous connective tissues, mainly in the legs, of deer, moose, and reindeer; consequently, they are sometimes called **legworms**. More than 60% of a host population can be infected. *Onchocerca ramachandrini* is a parasite of warthogs and is transmitted by members of the *S. damnosum* complex in West Africa. *Onchocerca lupi* parasitizes dogs in the southwestern United States. *Onchocerca skrjabini* parasitizes Japanese deer, and *Onchocerca takaoka* parasitizes wild boars in Japan.

Leucocytozoonosis

Species of protozoans in the genus *Leucocytozoon* are transmitted to birds by black flies, causing a malaria-like disease termed leucocytozoonosis (Table 14.2). More than 100 species of *Leucocytozoon* have been described, but the vectors—presumed to be primarily simuliids—have been demonstrated for only a fraction of these species. Molecular work has uncovered additional diversity of *Leucocytozoon* parasites in black flies (Murdock et al., 2015; Lotta et al., 2016). Most bird species and ornithophilic simuliid species are probably hosts of *Leucocytozoon* spp. The disease is known colloquially as **turkey malaria**, **duck malaria**, or **gnat fever**. The taxonomy of the genus *Leucocytozoon* is being revised using molecular techniques, and the parasite–vector–bird associations are expected to be reworked significantly. Two species of the parasite are of major economic concern, and both occur in North America. *Leucocytozoon simondi* is specific to ducks and geese, and its primary vectors are *S. anatinum* and *S. rugglesi*. *Leucocytozoon smithi* is specific to turkeys and is transmitted primarily by *S. meridionale* and *S. slossonae*.

Leucocytozoon species undergo a complex malaria-like life cycle. Gametocytes in the blood of an avian host are acquired by a female black fly. The parasite then undergoes

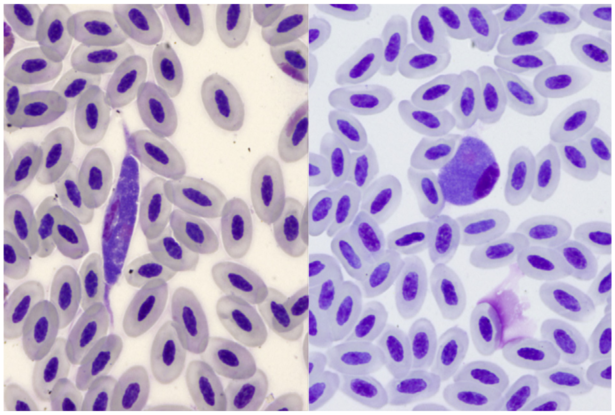

FIGURE 14.19 Mature macrogametocytes of *Leucocytozoon* cf. *toddi* from a buzzard (*Buteo buteo*), showing remarkable polymorphism. *Photograph by Jan Votýpka.*

both asexual and sexual development over a period of 3–4 days in the fly. During a subsequent bloodmeal, the fly transmits the parasites, as sporozoites, to another bird, which serves as a host for asexual development and gametocyte production (Fig. 14.19).

Leucocytozoonosis can be fatal in poultry, but its effects on wild hosts, with the exception of some populations of Canada geese (Herman et al., 1975), generally are less apparent or difficult to separate from the effects of bloodfeeding (Rohner et al., 2000). Birds with chronic infections have weakened immune systems and reduced reproduction. Severe infections produce emaciation, dehydration, and convulsions that lead to death. Internally, the liver and spleen of moribund hosts are enlarged, the heart muscle is pale, and the lungs are congested.

The disease had devastating effects on the poultry industry throughout much of North America when birds were held in outdoor arenas (Noblet et al., 1975). Entire flocks were killed and production facilities shut down in areas such as Nebraska, South Carolina, and Manitoba. The U.S. Agricultural Research Service estimated an annual average loss of nearly $750,000 in the United States from 1942 to 1951 as a result of leucocytozoonosis in domestic turkeys. The last major outbreaks of the disease in domestic turkeys were in the 1970s. Turkeys now are raised primarily in poultry houses, reducing the incidence of disease because the vectors generally do not venture inside shelters.

Other Parasites and Pathogens of Veterinary Importance

Black flies transmit additional parasites to wild animals (Table 14.2). The protozoan *Trypanosoma confusum* is specific to birds in North America and is transmitted when infected fecal droplets from the black fly contaminate the bite. Birds of numerous families serve as hosts. Other species of bird trypanosomes (e.g., *Trypanosoma corvi*) are believed to cause infections when the birds consume infected black flies or eat other birds that have been infected (Votýpka and Svobodová, 2004). The filarial nematodes *Splendidofilaria fallisensis* and *Dirofilaria ursi* are transmitted to ducks and black bears, respectively. The effects of these protozoan and filarial parasites on their wild hosts are poorly known.

Several North American species of simuliids, such as *S. notatum* and *S. vittatum*, serve as biological and mechanical vectors of vesicular stomatitis virus to livestock, primarily cattle, horses, and pigs (Smith et al., 2009). The virus causes lesions in various epithelial tissues, especially in the mouth. Millions of dollars can be lost during epizootics. Laboratory experiments have shown that a viremic host is not necessary for a female black fly to become infected; flies can become infected by feeding on the same host with an infected black fly (Mead et al., 2000).

Additional parasites of wildlife have been associated with black flies. Minute nematodes of the family Robertdollfusidae in the guts of African black flies might be transmitted to wildlife (Bain and Renz, 1993). **Bunyaviruses**, **Eastern equine encephalitis virus**, and **snowshoe hare virus** have been isolated from several North American black flies. Minimal mechanical transmission has been demonstrated for **Whataroa virus** in laboratory mice in New Zealand (Austin, 1967) and for **myxomatosis** in rabbits in Australia (Mykytowycz, 1957). These examples suggest that much is yet to be learned about the vector potential of black flies among wildlife. *Chlamydia* infections in sheep and **Rift Valley fever virus** are suspected of being simuliid-borne in South Africa (Palmer, 1995).

Simuliotoxicosis

Attacks by black flies have, at times, been so massive and virulent that livestock have been killed. Many of the deaths probably result from acute toxemia and anaphylactic shock caused by toxins introduced with the saliva as black flies are feeding. The diseased condition, either temporary or terminal, that results from the bites of black flies is known as simuliotoxicosis, a term first used to describe the toxic effects of simuliid bites on reindeer (Wilhelm et al., 1982). Cattle, especially calves, are vulnerable to simuliotoxicosis, but goats, horses, mules, pigs, and sheep also have been affected. Susceptible animals succumb in less than 2 h. Some immunity is apparent in animals living in afflicted areas. The biochemical nature of simuliotoxicosis requires more investigation.

Most of the species responsible for simuliotoxicosis breed in large rivers from which the adults emerge in astronomical numbers. They include *Austrosimulium pestilens* in Queensland (Australia), *Cnephia pecuarum* in the Mississippi River Valley (USA), *S. colombaschense* along the Danube River in central Europe, *S. vampirum* on the Canadian prairies, and *S. erythrocephalum*, the *S. ornatum* complex, and *S. reptans* in central Europe.

One of the worst attacks in recorded history killed about 22,000 animals in 1923 along Europe's Danube River in the southern Carpathian Mountains (Ciurea and Dinulescu, 1924). Prodigious attacks in this region during the 1700s prompted Empress Maria Theresa of the old Austro-Hungarian Empire to order one of the first biological studies of black flies, which eventually was published in 1795. On the Canadian prairies, thousands of livestock were killed from about 1886 into the 1970s by *S. vampirum*, a member of the *S. arcticum* complex (Fredeen, 1977). Massive mortality due to attacks by *Cnephia pecuarum* occurred in the United States during and immediately after the Civil War when the levees of the Mississippi River deteriorated, allowing the river to overflow and create extensive breeding areas for this species (Riley, 1887).

Simuliotoxicosis on a large scale is now rare, mainly because the former breeding sites of most of the responsible species have been altered by pollution, impoundment, and land development. Some of these species, however, still create nuisance problems for livestock, and occasional outbreaks cause deaths in localized areas of their ranges (Werner and Adler, 2005).

PREVENTION AND CONTROL

Management of black flies typically is aimed at the larval stage, in large part because in this life stage the pest species are concentrated in easily identifiable, specific habitats. Although adulticiding has sometimes offered temporary relief, it is typically more costly and has been used less frequently than larviciding. It usually has involved both aerial and ground fogging with DDT or permethrin products. Current efforts to manage black flies in the adult stage are restricted primarily to the application of repellents and pour-on insecticides.

The use of **chemical insecticides** in managing black flies dates to the dawn of the 20th century, reaching a peak from the mid-1940s into the 1970s when DDT was the principal means of control against both larvae and adults. The development of resistance and the undesirable effects on nontarget organisms led to the abandonment of DDT and the search for surrogate compounds, the most prominent of which were methoxychlor (chlorinated hydrocarbon) and temephos (organophosphate). These compounds, as well as insect growth regulators, were not selective and, therefore, had negative effects on nontarget organisms. The use of chemical insecticides to manage black flies became infrequent toward the end of the 20th century, although compounds such as methoxychlor and temephos continued to be used in a few areas of the world.

Black flies worldwide are managed primarily through the use of the entomopathogenic bacterium *Bacillus thuringiensis* var. *israelensis* (*Bti*, serotype H14), which is aimed at the larval stage. The actual killing agent is an endotoxin in the parasporal inclusions that disrupts the cells of the highly alkaline larval midgut. The efficacy and environmental safety of *Bti* are so superb that most other means of population suppression and management have disappeared since the commercial *Bti* product entered the scene in the early 1980s (Molloy, 1990; Gray et al., 1999). *Bti* can be applied by hand or aircraft. North America's largest suppression program for black flies is operated by the state of Pennsylvania (USA), which treats waterways for *S. jenningsi* in about half of its counties. Because of the intensive use of *Bti* for more than 30 years, target populations should be monitored for resistance.

The potential for exploiting **natural enemies** for simuliid control is enormous. Nearly 200 species of symbiotic organisms, in addition, to many species of bacteria, have been documented from the larval stage, and many have a parasitic relationship with their black fly hosts (McCreadie et al., 2011). Adult simuliids have many of the same symbiotes as the larvae, plus additional symbiotic species. Although natural enemies exert some control in most populations of black flies, attempts to mass produce them have not been made since the 1970s (Laird, 1981). Commonly encountered parasites include mermithid nematodes, microsporidia, the chytrid fungus *Coelomycidium simulii*, and several viruses. The prevalence of infection with these parasites and pathogens is usually less than 10% of a population. Infections typically slow development, however, so that parasitized larvae become relatively more frequent in a population over time as healthy individuals pupate first.

Mermithid nematodes probably hold the greatest promise for biological control of black flies. However, until more can be learned about their taxonomy and host specificity and how to cultivate them economically for mass release, they are unlikely to be useful in integrated pest management programs. Preparasitic mermithid nematodes crawl on stream substrates and use a protrusible stylet to penetrate the host body. As the mermithids mature, they can be seen through the host integument, coiled within the abdomen. Mermithids either exit and kill the host larva or pass into the adult, exiting shortly thereafter. Postparasitic worms molt to adults, mate, and deposit eggs in the streambed.

Patent infections with microsporidia are recognized by the large, irregular cysts that distort the larval host abdomen. Life cycles of microsporidia that attack black flies are poorly known, although transovarial transmission has been documented. Larvae with patent infections of the fungus *Coelomycidium simulii* are packed with minute, spherical thalli throughout their bodies. Thalli produce spores that are released into the water column after death of the host. Two common viruses that infect larvae are iridescent virus, which imparts an overall blue or violet cast, and cytoplasmic polyhedrosis virus, which creates white bands around the midgut. A significant number of bacterial species living in and on black flies, from parasites to mutualists, offer potential opportunities for control (Tang et al., 2012). Many predators consume black flies; most are typically opportunistic.

Physical control of the breeding habitat is occasionally effective in reducing pest populations, usually when the pest species is concentrated in a restricted area, such as directly downstream of an impoundment. In these situations, attachment sites (e.g., trailing vegetation) can be removed, or water levels can be altered to strand larvae above the water line.

Personal protection for humans involves primarily the use of **repellents**, both natural and synthetic, that are applied directly to the skin or impregnated in clothing. Among the more effective repellents are those with *N*,*N*-diethyl-*meta*-toluamide (DEET) as the active ingredient. Wearing light-colored clothing and minimizing openings in the clothing, such as button holes, through which black flies can gain access to skin, is standard practice when entering areas where black flies are a problem. Fine-mesh head nets are effective in areas where pest populations are intolerable. Fishermen often smoke cigars to ward off the flies, perhaps aided by the nicotine. Many additional means of protection can be found in the annals of folklore, but the utility of most remains suspect.

Various techniques have been devised to protect livestock and other animals, ranging from the use of smudges (i.e., smoldering fires that produce dense smoke) to the application of repellent substances and the use of shelters. Repellent products for livestock historically involved oils and greases, often laced with turpentine or other plant-derived products. Among the more commonly used repellents in recent times are permethrin solutions and ***eartags*** containing ivermectin. Various pour-on and spray formulations of insecticides and repellents are available commercially. White petroleum jelly can be applied inside the ears of horses to reduce biting problems. Providing shelters is an effective means of protecting livestock and poultry because many of the pest species of black flies infrequently enter enclosures. Providing the entries of shelters with self-application devices for repellents provides an added dose of protection. Black flies that enter houses of song birds can be managed by eliminating the vents or surrounding the holes with adhesives.

Onchocerciasis Control

The largest management program in the world for black flies was the World Health Organization's **Onchocerciasis Control Programme** (OCP) in West Africa from 1975 to 2002. Its history, as briefly summarized below, has been written by numerous authors (e.g., Davies, 1994; World Health Organization, 1995; Bump, 2004). The initial foundations for the program were laid in 1968, and in 1975 the program launched its first aerial treatments for the control of onchocerciasis. The goal of the OCP was to eliminate onchocerciasis as a major public health threat in seven West African countries: Benin, Burkina Faso, Ghana, Ivory Coast, Mali, Niger, and Togo. The program later was expanded to include the countries of Guinea, Guinea-Bissau, Senegal, and Sierra Leone, thus covering a total of 11 countries and 50,000 km of rivers. It was directed at the vectors of onchocerciasis, namely members of the *S. damnosum* species complex.

The primary strategy of the OCP was a massive aerial larviciding program aimed at reducing adult vector populations, thus interrupting transmission. Maintaining vectors at a sufficiently low number for a sufficiently long time prevents new cases of transmission while worms in the human reservoir die out, breaking the disease cycle. Given the longevity of adult worms, control programs in endemic areas must be maintained for approximately 15 years to eliminate the worm from the human reservoir (Remme et al., 1990; Plaisier et al., 1991). Prior to the OCP, aerial application of DDT was the main means of control, but by 1970 resistance had begun to develop. From 1975 into the 1980s, the OCP applied primarily temephos to the rivers. The first appearance (1980) of resistance to this compound by the vectors in the OCP area (Guillet et al., 1980) eventually led to the rotation of six insecticides, including *Bti*.

Vector control was integrated with an ivermectin chemotherapy program for the human reservoir in 1988. Ivermectin, originally developed for veterinary purposes, reduces the number of microfilariae in the skin, so that ingestion of sufficient microfilariae by the vectors becomes difficult. This microfilarialcidal drug, however, does not kill the adult worms. A single oral dose of Mectizan (the formulation of ivermectin for humans) every 6−12 months is not only nontoxic at levels higher than prescribed dosages but also sufficient to kill microfilariae in the skin and eyes and reverse progression of the disease. Dying microfilariae, however, can cause temporary adverse reactions in patients. Mass distribution of ivermectin has been possible through the humanitarian efforts of numerous organizations, including Merck and Co., which decided in 1987 to donate ivermectin tablets for the worldwide treatment of onchocerciasis for as long as necessary. In 1995, the **African Programme for Onchocerciasis Control** (APOC), which includes 19 African countries, was formed, with the goal of eliminating onchocerciasis from the continent. Its focus has been the mass distribution of ivermectin. Ten years later, more than 117,000 communities and 350,000 volunteers in 15 African countries were participating in the ivermectin distribution program (World Health Organization, 2007).

By 1995, vector control had interrupted transmission in about 90% of the original OCP area, protecting more than 30 million people from infection and sparing 100,000 from blindness at a cost of about $360 million. The combined use of ivermectin and weekly insecticide treatments of larval breeding sites was predicted to free the OCP area of onchocerciasis by 2002. Accordingly, the OCP terminated on December 31, 2002, but with approximately 46,000 new cases of onchocerciasis-related blindness in Africa each year, APOC was slated to continue until 2010. From 1995 to 2010, APOC was estimated to have prevented the loss of about 8.2 million years of life at a cost of $257 million (Coffeng et al., 2013). With new cases of onchocerciasis-related blindness in Africa, APOC was extended to 2015. The specter of resistance to ivermectin by *O. volvulus* (Osei-Atweneboana et al., 2011), however, also has threatened to compromise one of the pillars of onchocerciasis control. Civil wars, weakened infrastructure, poor surveillance, irregular financing, and other social disruptions also present formidable obstacles to achieving an onchocerciasis-free continent.

The Carter Center's **Onchocerciasis Elimination Program for the Americas** (OEPA), which was initiated in 1993, includes Brazil, Colombia, Ecuador, Guatemala, Mexico, and Venezuela (Blanks et al., 1998). It, too, relies on mass treatment with ivermectin donated by Merck, and tremendous progress has been made in fighting the disease. Transmission has been interrupted or eliminated in 11 of the 13 New World foci. Colombia was declared onchocerciasis-free in 2013, Ecuador in 2014, Mexico in 2015, and Guatemala in 2016 (World Health Organization, 2016). In Latin America, about 29,500 Yanomami people require continual treatment in the last-remaining foci, which are in the Amazonian region on the border of Brazil and Venezuela (World Health Organization, 2016; Botto et al., 2016). The *S. guianense* complex is the vector in these foci.

REFERENCES AND FURTHER READING

Adami, Y. L., Rodrigues, G., Alves, M. C., Moraes, M. A. P., Banic, D. M., & Maia-Herzog, M. (2014). New records of *Mansonella ozzardi*: A parasite that is spreading from the state of Amazonas to previously uninfected areas of the state of Acre in the Purus river region. *Memórias do Instituto Oswaldo Cruz, 109*, 87–92.

Addison, E. M. (1980). Transmission of *Dirofilaria ursi* Yamaguti, 1941 (Nematoda: Onchocercidae) of black bears (*Ursus americanus*) by blackflies (Simuliidae). *Canadian Journal of Zoology, 58*, 1913–1922.

Adler, P. H., & Crosskey, R. W. (2015). Cytotaxonomy of the Simuliidae (Diptera): A systematic and bibliographic conspectus. *Zootaxa, 3975*, 1–139.

Adler, P. H., & Crosskey, R. W. (2018). *World blackflies (Diptera: Simuliidae): A comprehensive revision of the taxonomic and geographical inventory [2018].* http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf.

Adler, P. H., & McCreadie, J. W. (1997). The hidden ecology of black flies: Sibling species and ecological scale. *American Entomologist, 43*, 153–161.

Adler, P. H., Currie, D. C., & Wood, D. M. (2004). *The black flies (Simuliidae) of North America*. Ithaca, New York: Cornell University Press. xv + 941 pp. + 24 color plates.

Adler, P. H., Roach, D., Reeves, W. K., Flanagan, J. P., Morrow, M. E., & Toepfer, J. E. (2007). Attacks on the endangered Attwater's prairie-chicken (*Tympanuchus cupido attwateri*) by black flies (Diptera: Simuliidae) infected with an avian blood parasite. *Journal of Vector Ecology, 32*, 309–312.

Adler, P. H., Cheke, R. A., & Post, R. J. (2010). Evolution, epidemiology, and population genetics of black flies (Diptera: Simuliidae). *Infection, Genetics and Evolution, 10*, 846–865.

Adler, P. H., Kúdelová, T., Kúdela, M., Seitz, G., & Ignjatović-Ćupina, A. (2016). Cryptic biodiversity and the origins of pest status revealed in the macrogenome of *Simulium colombaschense* (Diptera: Simuliidae), history's most destructive black fly. *PLoS One, 11*(1), e0147673.

Allison, F. R., Desser, S. S., & Whitten, L. K. (1978). Further observations on the life cycle and vectors of the haemosporidian *Leucocytozoon tawaki* and its transmission to the Fjordland crested penguin. *New Zealand Journal of Zoology, 5*, 663–665.

Anderson, R. C. (1968). The simuliid vectors of *Splendidofilaria fallisensis* of ducks. *Canadian Journal of Zoology, 46*, 610–611.

Atwood, D. W. (1996). *Distribution, abundance, control and field observations of the southern buffalo gnat, Cnephia pecuarum (Diptera: Simuliidae), in Arkansas and Texas* (Ph.D. thesis). Fayetteville, Arkansas: University of Arkansas, 127 pp.

Austin, F. J. (1967). The arbovirus vector potential of a simuliid. *Annals of Tropical Medicine and Parasitology, 61*, 189–199.

Bain, O., & Renz, A. (1993). Infective larvae of a new species of Robertdollfusidae (Adenophorea, Nematoda) in the gut of *Simulium damnosum* in Cameroon. *Annales de Parasitologie Humaine et Comparee, 68*, 182–184.

Barratt, J., Kaufer, A., Peters, B., Craig, D., Lawrence, A., Roberts, T., et al. (2017). Isolation of novel trypanosomatid, *Zelonia australiensis* sp. nov. (Kinetoplastida: Trypanosomatidae) provides support for a Gondwanan origin of dixenous parasitism in the Leishmaniinae. *PLoS Neglected Tropical Diseases, 11*(1), e0005215.

Blanks, J., Richards, F., Beltrán, F., Collins, R., Álvarez, E., Zea Flores, G., et al. (1998). The onchocerciasis elimination program for the Americas: A history of partnership. *Revista Panamericana de Salud Pública, 3*, 367–374.

Bennett, G. F. (1961). On the specificity and transmission of some avian trypanosomes. *Canadian Journal of Zoology, 39*, 17–33.

Botto, C., Basáñez, M. G., Escalona, M., Vivas-Martínez, S., Villamizar, N., Noya-Alarcón, O., et al. (2016). Evidence of suppression of onchocerciasis transmission in the Venezuelan Amazonian focus. *Parasites & Vectors, 9*(1), 1.

Bump, J. B. (2004). *The lion's gaze: African river blindness from tropical curiosity to international development* (Ph.D. thesis). Baltimore, Maryland: Johns Hopkins University, 421 pp.

Burgin, S. G., & Hunter, F. F. (1997). Nectar versus honeydew as sources of sugar for male and female black flies (Diptera: Simuliidae). *Journal of Medical Entomology, 34*, 605–608.

Casiraghi, M., Favia, G., Cancrini, G., Bartoloni, A., & Bandi, C. (2001). Molecular identification of *Wolbachia* from the filarial nematode *Mansonella ozzardi*. *Parasitology Research, 87*, 417–420.

Chanteau, S., Sechan, Y., Moulia-Pelat, J.-P., Luquiaud, P., Spiegel, A., Boutin, J.-P., et al. (1993). The blackfly *Simulium buissoni* and infection by hepatitis B virus on a holoendemic island of the Marquesas Archipelago in French Polynesia. *The American Journal of Tropical Medicine and Hygiene, 48*, 763–770.

Chen, H.-B. (2016). *Chinese black flies*. Guiyang, China: Guizhou Science and Technology Publishing House Co., Ltd., 673 pp.

Ciurea, I., & Dinulescu, G. (1924). Ravages causés par la mouche de Goloubatz en Roumanie; des attaques contre les animaux et contre l'homme. *Annals of Tropical Medicine and Parasitology, 18*, 323–342.

Coffeng, L. E., Stolk, W. A., Zouré, H. G. M., Veerman, J. L., Agblewonu, K. B., Murdoch, M. E., et al. (2013). African programme for onchocerciasis control 1995–2015: Model-estimated health impact and cost. *PLoS Neglected Tropical Disease, 7*(1), e2032.

Colorado-Garzón, F., Adler, P. H., García, L. F., Muñoz de Hoyos, P., Bueno, M., & Matta, N. E. (2016). Estimating diversity in the *Simulium ignescens* and *Simulium tunja* complexes in Colombia: Chromosomal rearrangements as the core of integrative taxonomy. *Journal of Heredity, 108*, 12–24.

Coscarón, S., & Coscarón Arias, C. L. (2007). Neotropical Simuliidae (Diptera: Insecta). In J. Adis, J. R. Arias, G. Rueda-Delgado, & K. M. Wantzen (Eds.), *Vol. 3. Aquatic biodiversity in Latin America*. Bulgaria: Pensoft, Sofia, 685 pp.

Crosskey, R. W. (1967). The classification of *Simulium* Latreille (Diptera: Simuliidae) from Australia, New Guinea and the western Pacific. *Journal of Natural History, 1*, 23–51.

Crosskey, R. W. (1969). A re-classification of the Simuliidae (Diptera) of Africa and its islands. In *Bulletin of the British Museurm (Natural History). Entomology Supplement 14* (pp. 1–195).

Crosskey, R. W. (1990). *The natural history of blackflies*. Chichester, England: John Wiley & Sons Ltd., 711 pp.

Crosskey, R. W., & Howard, T. M. (1997). *A new taxonomic and geographical inventory of world blackflies (Diptera: Simuliidae)*. London: The Natural History Museum, 144 pp.

Cupp, E. W., & Cupp, M. S. (1997). Black fly (Diptera: Simuliidae) salivary secretions: Importance in vector competence and disease. *Journal of Medical Entomology, 34*, 87–94.

Currie, D. C., & Adler, P. H. (2008). Global diversity of black flies (Diptera: Simuliidae) in freshwater. *Hydrobiologia, 595*, 469–475.

Davies, J. B. (1994). Sixty years of onchocerciasis vector control: A chronological summary with comments on eradication, reinvasion, and insecticide resistance. *Annual Review of Entomology, 39*, 23–45.

Dirie, M. F., Ashford, R. W., Mungomba, L. M., Molyneux, D. H., & Green, E. E. (1990). Avian trypanosomes in *Simulium* and sparrowhawks (*Accipiter nisus*). *Parasitology, 101*, 243–247.

Eaton, D. P., Diaz, L. A., Hans-Filho, G., dos Santos, V., Aoki, V., Friedman, H., et al., the Cooperative Group on Fogo Selvagem Research. (1998). Comparison of black fly species (Diptera: Simuliidae) on an Amerindian reservation with a high prevalence of fogo selvagem to neighboring disease-free sites in the state of Mato Grosso do Sul, Brazil. *Journal of Medical Entomology, 35*, 120–131.

Fallis, A. M., Jacobson, R. L., & Raybould, J. N. (1973). Experimental transmission of *Trypanosoma numidae* Wenyon to Guinea fowl and chickens in Tanzania. *Journal of Protozoology, 20*, 436–437.

Fallis, A. M., Desser, S. S., & Khan, R. A. (1974). On species of *Leucocytozoon*. *Advances in Parasitology, 12*, 1–67.

Fredeen, F. J. H. (1977). A review of the economic importance of black flies (Simuliidae) in Canada. *Quaestiones Entomologicae, 13*, 219–229.

Fredeen, F. J. H. (1985). Some economic effects of outbreaks of black flies (*Simulium luggeri* Nicholson & Mickel) in Saskatchewan. *Quaestiones Entomologicae, 21*, 175–208.

Fukuda, M., Otsuka, Y., Uni, S., Boda, T., Daisaku, H., Hasegawa, H., et al. (2011). Zoonotic onchocerciasis in Hiroshima, Japan, and molecular analysis of a paraffin section of the agent for a reliable identification. *Parasite, 18*, 185–188.

Gray, E. W., & Noblet, R. (2014). Black fly rearing and use in laboratory bioassays. In K. Maramorosch, & F. Mahmood (Eds.), *Rearing animal and plant pathogen vectors* (pp. 42–72). Boca Raton, Florida: Science Publishers, CRC Press.

Gray, E. W., Adler, P. H., Coscarón-Arias, C., Coscarón, S., & Noblet, R. (1999). Development of the first black fly (Diptera: Simuliidae) management program in Argentina and comparison with other programs. *Journal of the American Mosquito Control Association, 15*, 400–406.

Gudgel, E. F., & Grauer, F. H. (1954). Acute and chronic reactions to black fly bites (*Simulium* fly). *Archives of Dermatology and Syphilology, 70*, 609–615.

Guillet, P., Escaffre, H., Ouédraogo, M., & Quillévéré, D. (1980). Mise en évidence d'une résistance au téméphos dans le complexe *S. damnosum* (*S. sanctipauli* et *S. soubrense*) en Côte d'Ivoire (zone du programme de lutte contre l'onchocercose dans la région du bassin de la Volta). *Cahiers ORSTOM Séries Entomologie Médicale et Parasitologie, 23*, 291–299.

Hassan, H. K., Bolcen, S., Kubofcik, J., Nutman, T. B., Eberhard, M. L., Middleton, K., et al. (2015). Isolation of *Onchocerca lupi* in dogs and black flies, California, USA. *Journal of Emerging Infectious Diseases, 21*, 789–796.

Herman, C. M., Barrow, J. H., & Tarshis, I. B. (1975). Leucocytozoonosis in Canada geese at the Seney National Wildlife Refuge. *Journal of Wildlife Diseases, 11*, 404–411.

Johnson, T. P., Tyag, R., Lee, P. R., Lee, M.-H., Johnson, K. R., Kowalak, J., et al. (2017). Nodding syndrome may be an autoimmune reaction to the parasitic worm *Onchocerca volvulus*. *Science Translational Medicine, 9*. eaaf6953.

Laird, M. (1981). *Blackflies: The future for biological methods in integrated control*. New York: Academic Press.

Lok, J. B., Cupp, E. W., & Bernardo, M. J. (1983). *Simulium jenningsi* Malloch (Diptera: Simuliidae): A vector of *Onchocerca lienalis* Stiles (Nematoda: Filarioidea) in New York. *American Journal of Veterinary Research, 44*, 2355–2358.

Lotta, I. A., Pacheco, M. A., Escalante, A. A., González, A. D., Mantilla, J. S., Moncada, L. I., et al. (2016). *Leucocytozoon* diversity and possible vectors in the Neotropical highlands of Colombia. *Protist, 167*, 185–204.

Malmqvist, B., Adler, P. H., Kuusela, K., Merritt, R. W., & Wotton, R. S. (2004a). Black flies in the boreal biome, key organisms in both terrestrial and aquatic environments: A review. *Écoscience, 11*, 187–200.

Malmqvist, B., Strasevicius, D., Hellberg, O., Adler, P. H., & Bensch, S. (2004b). Vertebrate host specificity of wild-caught blackflies revealed by mitochondrial DNA in blood. *Proceedings of the Royal Society of London B (Supplement), Biology Letters, 271*, S152–S155.

McCreadie, J. W., Adler, P. H., & Hamada, N. (2005). Patterns of species richness for blackflies (Diptera: Simuliidae) in the Nearctic and Neotropical regions. *Ecological Entomology, 30*, 201–209.

McCreadie, J. W., Adler, P. H., & Beard, C. E. (2011). Ecology of symbiotes of larval black flies (Diptera: Simuliidae): Distribution, diversity, and scale. *Environmental Entomology, 40*, 289–302.

McCreadie, J. W., & Adler, P. H. (2012). The roles of abiotic factors, dispersal, and species interactions in structuring stream assemblages of black flies (Diptera: Simuliidae). *Aquatic Biosystems, 8*(14), 1–11.

McCreadie, J. W., Hamada, N., Grillet, M. E., & Adler, P. H. (2017). Alpha richness and niche breadth of a widespread group of aquatic insects in Nearctic and Neotropical streams. *Freshwater Biology, 62*, 329–339.

McGaha, T. W., Jr., Young, R. M., Burkett-Cadena, N. D., Iburg, J. P., Beau, J. M., Hassan, S., et al. (2015). Identification of communal oviposition pheromones from the black fly *Simulium vittatum*. *PLoS One, 10*, e0118904.

Mead, D. G., Ramberg, F. B., Besslesen, D. G., & Máre, C. J. (2000). Transmission of vesicular stomatitis virus from infected to noninfected black flies co-feeding on nonviremic deer mice. *Science, 287*, 485–487.

Mock, D. E., & Adler, P. H. (2002). Black flies (Diptera: Simuliidae) of Kansas: Review, new records, and pest status. *Journal of the Kansas Entomological Society, 75*, 203–213.

Molloy, D. (1990). Progress in the biological control of black flies with *Bacillus thuringiensis israelensis,* with emphasis on temperate climates. In H. de Barjac, & D. J. Sutherland (Eds.), *Bacterial control of mosquitoes and black flies: Biochemistry, genetics, and applications of Bacillus thuringiensis israelensis and Bacillus sphaericus* (pp. 161–186). New Brunswick, New Jersey: Rutgers University Press.

Muller, R., & Horsburgh, R. C. R. (1987). *Bibliography of onchocerciasis (1841–1985).* C. A. B. International Institute of Parasitology.

Murdock, C. C., Adler, P. H., Frank, J., & Perkins, S. L. (2015). Molecular analyses on host-seeking black flies (Diptera: Simuliidae) reveal a diverse assemblage of *Leucocytozoon* (Apicomplexa: Haemospororida) parasites in an alpine ecosystem. *Parasites & Vectors, 8*(343), 1–7.

Mykytowycz, R. (1957). The transmission of myxomatosis by *Simulium melatum* Wharton (Diptera: Simuliidae). *CSIRO Wildlife Research, 2*, 1–4.

Noblet, R., Kissam, J. B., & Adkins, T. R., Jr. (1975). *Leucocytozoon smithi:* Incidence of transmission by black flies in South Carolina (Diptera: Simuliidae). *Journal of Medical Entomology, 12*, 111–114.

O'Roke, E. C. (1934). A malaria-like disease of ducks caused by *Leucocytozoon anatis* Wickware. *University of Michigan School of Forestry Conservation Bulletin, 4*, 1–44.

Osei-Atweneboana, M. Y., Awadzi, K., Attah, S. K., Boakye, D. A., Gyapong, J. O., & Prichard, R. K. (2011). Phenotypic evidence of emerging ivermectin resistance in *Onchocerca volvulus*. *PLoS Neglected Tropical Diseases, 5*, e998.

Palmer, R. W. (1995). *Biological and chemical control of blackflies (Diptera: Simuliidae) in the Orange River*. Report to the Water Research Commission by the Onderstepoort Veterinary Institute. WRC Report 343/1/95 (pp. 1–10).

Pearlman, E. (2003). Immunopathogenesis of *Onchocerca volvulus* keratitis (river blindness): A novel role for endosymbiotic *Wolbachia* bacteria. *Medical Microbiology and Immunology, 192*, 57–60.

Plaisier, A. P., Van Oortmarssen, G. J., Remme, J. H. F., & Habbema, J. D. F. (1991). The reproduction lifespan of *Onchocerca volvulus* in West Africa savanna. *Acta Tropica, 48*, 271–284.

Pledger, D. J., Samuel, W. M., & Craig, D. A. (1980). *Black flies (Diptera: Simuliidae) as possible vectors of legworm (Onchocerca cervipedis) in moose of central Alberta*. Proceedings of the North American Moose Conference Workshop. 16 (pp. 171–202).

Post, R. J., Mustapha, M., & Krueger, A. (2007). Taxonomy and inventory of the cytospecies and cytotypes of the *Simulium damnosum* complex (Diptera: Simuliidae) in relation to onchocerciasis. *Tropical Medicine and International Health, 12*, 1342–1353.

Reeves, W. K., Adler, P. H., Rätti, O., Malmqvist, B., & Strasevicius, D. (2007). Molecular detection of *Trypanosoma* (Kinetoplastida: Trypanosomatidae) in black flies (Diptera: Simuliidae). *Comparative Parasitology, 74*, 171–175.

Remme, J. H. F., De Sole, G., & Van Oortmarssen, G. J. (1990). The predicted and observed decline in the prevalence and intensity of onchocerciasis infection during 14 years of successful vector control. *Bulletin of the World Health Organization, 68*, 331–339.

Remme, J. H. F., Feenstra, P., Lever, P. R., Medici, A. C., Morel, C. M., Noma, M., et al. (2006). Tropical diseases targeted for elimination: Chagas disease, lymphatic filariasis, onchocerciasis, and leprosy. In D. T. Jamison, J. G. Breman, A. R. Measham, G. Alleyne, M. Claeson, D. B. Evans, et al. (Eds.), *Disease control priorities in developing countries* (2nd ed., pp. 433–449). Washington, DC: World Bank.

Riley, C. V. (1887). *Report of the entomologist*. United States Department of Agriculture Report 1886 (pp. 459–492), 11 plates.

Rohner, C., Krebs, C. J., Hunter, D. B., & Currie, D. C. (2000). Roost site selection of great horned owls in relation to black fly activity: An anti-parasite behavior? *The Condor: Ornithological Applications, 102*, 950–955.

Rubtsov, I. A. (1956). Blackflies (fam. Simuliidae). In *"Fauna of the USSR". New series No. 64, insects, Diptera, 6 (6)*. Moscow: Akademii Nauk SSSR [In Russian; English translation: 1990. Blackflies (Simuliidae). Second Edition. "Fauna of the USSR". Diptera, 6 (6). E. J. Brill, Leiden.].

Sanford, D., Eikenhorst, B., Lamb, T., Cates, J. E., Robinson, J., Olsen, J., et al. (1993). Black flies cause costly losses in East Texas ostriches and emus. *Texas Agricultural Extension Service Veterinary Quarterly Review, 9*(2), 1–2.

Sariözkan, S., Inci, A., Yildirim, A., Düzlü, O., Gray, E. W., & Adler, P. H. (2014). Economic losses during an outbreak of *Simulium* (*Wilhelmia*) species (Diptera: Simuliidae) in the Cappadocia region of Turkey. *Turkiye Parazitolojii Dergisi, 38*, 116–119.

Shelley, A. J. (1988a). Biosystematics and medical importance of the *Simulium amazonicum* group and the *S. exiguum* complex in Latin America. In M. W. Service (Ed.), *Biosystematics of haematophagus insects* (pp. 203–220). Oxford: Clarendon Press.

Shelley, A. J. (1988b). Vector aspects of the epidemiology of onchocerciasis in Latin America. *Annual Review of Entomology, 30*, 337–366.

Shelley, A. J., & Coscarón, S. (2001). Simuliid blackflies (Diptera: Simuliidae) and ceratopogonid midges (Diptera: Ceratopogonidae) as vectors of *Mansonella ozzardi* (Nematoda: Onchocercidae) in northern Argentina. *Memórias do Instituto Oswaldo Cruz, 96*, 451–458.

Shelley, A. J., Hernández, L. M., Maia-Herzog, M., Luna Dias, A. P. A., & Garritano, P. R. (2010). The blackflies (Diptera: Simuliidae) of Brazil. In J. R. Arias, S. Golovach, K. M. Wantzen, & E. Domínguez (Eds.), *Vol. 6. Aquatic biodiversity in Latin America*. Bulgaria: Pensoft, Sofia, 821 pp.

Smith, P. F., Howerth, E. W., Carter, D., Gray, E. W., Noblet, R., & Mead, D. G. (2009). Mechanical transmission of vesicular stomatitis New

Jersey virus by *Simulium vittatum* (Diptera: Simuliidae) to domestic swine (*Sus scrofa*). *Journal of Medical Entomology, 46*, 1537–1540.

Stokes, J. H. (1914). A clinical, pathological and experimental study of the lesions produced by the bite of the “black fly” (*Simulium venustum*). *Journal of Cutaneous Diseases, 32*(751–769), 830–856.

Sutcliffe, J. F. (1986). Black fly host location: A review. *Canadian Journal of Zoology, 64*, 1041–1053.

Sutcliffe, J. F., & McIver, S. B. (1984). Mechanics of blood-feeding in black flies (Diptera, Simuliidae). *Journal of Morphology, 180*, 125–144.

Takaoka, H. (1999). Review on zoonotic *Onchocerca* species and their insect vectors in Japan. *Medical Entomology & Zoology, 50*, 1–8 (In Japanese, with English summary).

Takaoka, H. (2003). *The black flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya*. Fukuoka, Japan: Kyushu University Press.

Takaoka, H. (2017). Speciation, faunal affinities and geographical dispersal of black flies (Diptera: Simuliidae) in the Oriental Region. *Acta Tropica, 166*, 234–240.

Takaoka, H., & Davies, D. M. (1995). *The black flies (Diptera: Simuliidae) of West Malaysia*. Japan: Kyushu University Press.

Tang, X., Adler, P. H., Vogel, H., & Ping, L. (2012). Gender-specific bacterial composition of black flies (Diptera: Simuliidae). *FEMS Microbiology Ecology, 80*, 659–670.

Taylor, M. J., Hoerauf, A., Townson, S., Slatko, B. E., & Ward, S. A. (2014). Anti-*Wolbachia* drug discovery and development: Safe macrofilaricides for onchocerciasis and lymphatic filariasis. *Parasitology, 141*, 119–127.

Tchounkeu, Y. F. L., Onyeneho, N. G., Wanji, S., Kabali, A. T., Manianga, C., Amazigo, U. V., et al. (2012). Changes in stigma and discrimination of onchocerciasis in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 106*, 340–347.

Uni, S., Fukuda, M., Agatsuma, T., Bain, O., Otsuka, Y., Nakatani, J., et al. (2015). *Onchocerca takaokai* n. sp. (Nematoda: Filarioidea) in Japanese wild boars (*Sus scrofa leucomystax*): Description and molecular identification of intradermal females. *Parasitology International, 64*, 493–502.

Urbanek, R. P., Zimorski, S. E., Fasoli, A. M., & Szyszkoski, E. K. (2010). Nest desertion in a reintroduced population of migratory whooping cranes. *Proceedings of the North American Crane Workshop, 11*, 133–141.

Votýpka, J., & Svobodová, M. (2004). *Trypanosoma avium:* Experimental transmission from black flies to canaries. *Parasitology Research, 92*, 147–151.

Votýpka, J., Oborník, M., Volf, P., Svobodová, M., & Lukeš, J. (2002). *Trypanosoma avium* of raptors (Falconiformes): Phylogeny and identification of vectors. *Parasitology, 125*, 253–263.

Wahl, G. (1996). Identification of a common filarial larva in *Simulium damnosum* s. l. (Type D, Duke, 1967) as *Onchocerca ramachandrini* from the wart hog. *The Journal of Parasitology, 82*, 520–524.

Wahl, G., & Renz, A. (1991). Transmission of *Onchocerca dukei* by *Simulium bovis* in north-Cameroon. *Tropical Medicine & Parasitology, 42*, 368–370.

Wahl, G., Ekale, D., & Schmitz, A. (1998). *Onchocerca ochengi:* Assessment of the *Simulium* vectors in north Cameroon. *Parasitology, 116*, 327–336.

Werner, D., & Adler, P. H. (2005). A faunistic review of the black flies (Simuliidae, Diptera) of the federal state of Sachsen-Anhalt, Germany. *Abhandlungen und Berichte für Naturkunde, 27*, 205–245.

Wilhelm, A., Betke, P., & Jacob, K. (1982). Simuliotoxikose beim ren (*Rangifer tarandus*). In R. Ippen, & H. D. Schräder (Eds.), *Verhandlungbericht des XXIV Internationalen Symposium über die Erkrankungen der Zootiere Erkrankungen der Zootiere* (pp. 357–360). Berlin: Akademie-Verlag.

World Health Organization. (1995). Onchocerciasis and its control: Report of a WHO expert committee on onchocerciasis control. *WHO Technical Report Series, 852*, 1–10.

World Health Organization. (2007). *Onchocerciasis control in the WHO African region: Current situation and way forward. AFR/RC57/5*, 7 pp.

World Health Organization. (2016). Progress toward eliminating onchocerciasis in the WHO region of the Americas: Verification of elimination of transmission in Guatemala. *Weekly Epidemiological Record, 91*(43), 501–516.

World Health Organization. (2017). *Onchocerciasis. Fact Sheet 374*. http://www.who.int/mediacentre/factsheets/fs374/en/.