Chapter 15

Mosquitoes (Culicidae)

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Since ancient times, mosquito bites or habitats have been associated with human disease, and, in 1878, mosquitoes were the first arthropods formally incriminated as intermediate hosts of vertebrate parasites. During the past century it has become established that mosquitoes are the most important arthropods affecting human health. They attain their greatest impact as vectors for the organisms causing such well-known human diseases as malaria, filariasis, encephalitis, yellow fever, and dengue. These afflictions are especially severe in developing regions of the tropics. They cause early death and chronic debilitation that can strain the resources of health services and reduce human productivity, thereby perpetuating economic hardship.

Mosquito-borne diseases also persist in industrialized temperate countries. Yet, human discomfort from bites is often the chief concern. In the United States, hundreds of millions of dollars are spent annually to control them for this reason alone. Additionally, large populations of mosquitoes can cause intense irritation and extensive blood loss to livestock and wildlife, resulting in reduced productivity and even death.

Mosquitoes occur in practically every region of every continent in the world except Antarctica. They develop in an extremely broad range of biotic communities: arctic tundra, boreal forests, high mountains, plains, deserts, tropical forests, salt marshes, and ocean tidal zones. The greatest species diversity occurs in tropical forests, but extremely high densities of mosquitoes are common even in the species-poor biomes, such as the tundra. Many species have benefited from human alteration of the environment, and a few have become domesticated. Because of their immense importance, mosquitoes have been the subject of many major books. Among the more important books that deal exclusively with mosquito biology are texts by Christophers (1960), Clements (1992, 1999), Forattini (1962, 1965), Gillett (1971), Bock and Cardew (1996), Horsfall (1955), Lounibos et al. (1985), Mattingly (1969), and Service (1990, 1993a). *Journal of the American Mosquito Control Association* is devoted mainly to studies of mosquitoes. A substantial proportion of the scientific articles in the *Journal of Medical Entomology* and *Medical and Veterinary Entomology* also report mosquito research, as other scientific journals increasingly do. *Wing Beats* is a trade magazine dedicated to mosquitoes.

TAXONOMY

The family **Culicidae**, derived from *culex*, the Latin name for "gnat," is a member of one of the main stocks of Nematocera, the infraorder Culicomorpha. It consists of two superfamilies that include all of the piercing/sucking nematocerans, both predators and blood-feeding biters. The superfamily Chironomoidea comprises the families Chironomidae and Thaumaleidae, which have nonpiercing mouthparts, and Simuliidae and Ceratopogonidae, which pierce either vertebrates or invertebrates. The superfamily Culicoidea comprises the Dixidae, Corethrellidae, Chaoboridae, and Culicidae, the second and fourth of which feed on vertebrate blood. Several of these families are superficially similar. However, among all the culicomorphs, the long proboscis of mosquitoes is distinctive. It is considered the most specialized of biting mouthparts among Nematocera and indicates a long and close association of mosquitoes with vertebrate animals. Wood and Borkent (1989) provide an overview of nematoceran phylogeny and classification.

Culicidae consists of about 3,500 recognized species. The largest number remaining to be discovered probably inhabits tropical rainforests, where faunas are more diverse but less well surveyed than temperate regions. Species that have been studied intensively often reveal that they consist of complexes of closely related species, indicating that many reproductively isolated and niche-specific forms remain to be identified or are undergoing speciation.

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TABLE 15.1 Classification of Culicidae

Subfamily	Tribe	Genera
Anophelinae		*Anopheles* (*An.*), *Bironella* (*Bi.*), *Chagasia* (*Ch.*)
Culicinae	Aedeomyiini	*Aedeomyia* (*Ad.*)
	Aedini	*Aedes* (*Ae.*), *Armigeres* (*Ar.*), *Eretmapodites* (*Er.*),
		Haemagogus (*Hg.*), *Heizmannia* (*Hz.*), *Opifex* (*Op.*), *Psorophora* (*Ps.*),
		Udaya (*Ud.*), *Verrallina* (*Ve.*), *Zeugnomyia* (*Ze.*)
	Culicini	*Culex* (*Cx.*), *Deinocerites* (*De.*), *Galindomyia* (*Ga.*), *Lutzia* (*Lu.*)
	Culisetini	*Culiseta* (*Cs.*)
	Ficalbiini	*Ficalbia* (*Fi.*), *Mimomyia* (*Mi.*)
	Hodgesiini	*Hodgesia* (*Ho.*)
	Mansoniini	*Coquillettidia* (*Cq.*), *Mansonia* (*Ma.*)
	Orthopodomyiini	*Orthopodomyia* (*Or.*)
	Sabethini	*Isostomyia* (*Is.*), *Johnbelkinia* (*Jb.*), *Kimia* (*Km.*), *Limatus* (*Li.*), *Malaya* (*Ml.*), *Maorigoeldia* (*Mg.*), *Onirion* (*On.*), *Runchomyia* (*Ru.*), *Sabethes* (*Sa.*), *Shannoniana* (*Sh.*), *Topomyia* (*To.*), *Trichoprosopon* (*Tr.*), *Tripteroides* (*Tp.*), *Wyeomyia* (*Wy.*).
	Toxorhynchitini	*Toxorhynchites* (*Tx.*)
	Uranotaeniini	*Uranotaenia* (*Ur.*)

The classification of all mosquitoes into two subfamilies, 11 tribes of Culicinae, and 41 genera is based on Knight and Stone (1977) and modified according to updates to the online Systematic Catalog of the Culicidae, Walter Reed Biosystematics Unit website. In parentheses are the two-letter generic abbreviations recognized by the American Mosquito Control Association and used in several journals and books.

Current culicid classification (Table 15.1) recognizes two subfamilies: **Anophelinae** and **Culicinae**. Anophelinae is considered to be a primitive group. The former subfamily Toxorhynchitinae has been reduced to tribe status on the basis of cladistic analysis of morphological and nucleotide-sequence data (Harbach and Kitching, 1998). Anopheline eggs bear characteristic floats, their larvae lack air tubes, and adults have elongate palps in both sexes. Typical culicine larvae have air tubes, and adult females have short palps.

There are 41 genera of mosquitoes, 38 of which are in the subfamily Culicinae. Culicines are organized into 11 tribes, the most diverse of which are Aedini and Sabethini in terms of numbers of genera and species worldwide. The 13 genera in North America north of Mexico, and the number of species in each, are *Anopheles* (23), *Aedes* (83), *Psorophora* (17), *Haemagogus* (1), *Culex* (37), *Deinocerites* (3), *Culiseta* (8), *Coquillettidia* (1), *Mansonia* (2), *Orthopodomyia* (3), *Wyeomyia* (5), *Uranotaenia* (3), and *Toxorhynchites* (2) (Darsie and Ward, 1981, 2004; online-updated classification of Knight and Stone, 1977, in Systematic Catalog of Culicidae, Walter Reed Biosystematics Unit). A proposed elevation of many subgenera to the rank of genus led to some confusion in the mosquito nomenclature used by researchers during 2000–2015. A classification of the tribe Aedini, one that takes into account both the need for stability of names and the currently understood evolutionary relationships of taxa, has been presented by Wilkerson et al. (2015) and is used in this chapter.

Three important species groups of mosquitoes worldwide are the *Anopheles gambiae* and *Culex pipiens* complexes and the *Aedes* subgenus *Stegomyia*. The ***Anopheles gambiae* complex** of Africa consists of eight recognized species. Two of these, *An. gambiae* (Fig. 15.33) and *An. arabiensis*, are widespread and important vectors of malaria and lymphatic filariasis. *Anopheles arabiensis* tends to occur in somewhat drier regions than does *An. gambiae*. Both prefer to bite humans, but *An. gambiae* is more anthropophilic, endophilic, and endophagic, and therefore it is the more important vector. Another species in that complex, *Anopheles coluzzii*, is a regionally important vector. Other important anopheline complexes, such as the Asian *An. dirus* complex and the African *An. funestus* group, also are recognized.

The *Culex pipiens* species assemblage is a ubiquitous group of two closely related domestic and peridomestic species distributed worldwide: 1) the temperate species *Cx. pipiens*, the northern house mosquito (including the facultatively autogenous *Culex pipiens*, form molestus, often breeding in subterranean habitats); and 2) the tropical and subtropical *Cx. quinquefasciatus* (formerly called *Culex*

fatigans), the southern house mosquito (Fig. 15.29). Their ranges overlap in the central latitudes of the United States, where they commonly hybridize. They are vectors of several human pathogens, such as St. Louis encephalitis virus, West Nile virus, and lymphatic filariasis, as well as bird malaria. Two possible members of the species assemblage, *Cx. australicus* and *Cx. globocoxitus*, inhabit Australia. A form occurring in temperate China and Japan, *Cx. pallens*, has no formal status as a species. A full examination of this problematic assemblage is presented in a series of papers introduced and summarized by Linthicum (2012).

Several brightly marked *Aedes* spp. in the large **subgenus *Stegomyia*** are medically important, including *Ae. aegypti* and *Ae. albopictus*. *Aedes aegypti*, the yellow fever mosquito (Fig. 15.23), has a worldwide distribution in the tropics and subtropics. It is the primary vector of dengue, urban yellow fever, Chikungunya, and Zika viruses. It exists in at least two forms, *aegypti* and *formosus*, considered to be either subspecies or separate species. *Aedes aegypti formosus* is the original feral form and is found in large parts of interior Africa. It has a black body, develops in tree holes, feeds on a wide variety of animals, and rarely enters houses. It has adapted to some domestic situations in Africa, where it develops in rain-filled containers. *Aedes aegypti aegypti* is a paler, brownish-black domestic form. It occurs mainly in coastal regions of Africa and is distributed throughout much of southern Asia and most warmer parts of the New World, including the southern United States. In Africa it has become independent of rain, developing in hand-filled water jars without regard to season. On other continents, where it does not compete with *Ae. a. formosus*, it uses both rain-filled and hand-filled containers. Some authorities recognize a still paler and more domestic type of *Ae. a. aegypti* as the subspecies *Ae. a. queenslandensis*, but this is probably only a localized variant.

Aedes albopictus, the Asian tiger mosquito (Fig. 15.26), similarly occupies water-filled containers and also transmits dengue, Chikungunya, and Zika viruses. It was largely confined to Asia, where it occurs in tropical and subtropical rural settings. It readily oviposits in tree holes. A cold-hardy, egg-diapausing strain of this mosquito has been carried from northern Japan to other parts of the world via the trade in used automobile and truck tires. The first established population was detected in Texas (USA) in 1985. It has since spread through much of the southern, central, and eastern United States, including foci in the upper Midwest, much farther north than the nondiapausing *Ae. aegypti*. It also has gained a foothold in several other parts of the world. In most of its range in the southern United States, *Ae. albopictus* has replaced *Ae. aegypti* as the predominant mosquito in artificial containers in suburban and rural environments.

Other important members of the subgenus *Stegomyia* include *Ae. africanus*, *Ae. bromeliae*, and *Ae. luteocephalus*, which transmit yellow fever and dengue viruses in parts of Africa; and *Ae. polynesiensis* and *Ae. pseudoscutellaris*, which transmit lymphatic filariasis in South Pacific islands.

Keys to the mosquito genera worldwide were provided by Mattingly (1971). Keys for the identification of species of restricted geographical regions are available for most states in the United States, for Canadian provinces, and for many countries throughout the world. These include handbooks that also present biological and medical information on individual species. Good examples of statewide handbooks are written for New Jersey (Headlee, 1945), California (Bohart and Washino, 1978), Indiana (Siverly, 1972), Minnesota (Barr, 1958), New York (Means, 1979), Florida (Darsie and Morris, 1998), and Alaska (Gjullin et al., 1961). The US regional handbooks include the southeastern United States (King et al., 1960; Burkett-Cadena, 2013) and the northwestern states (Stage et al., 1952). Some handbooks include keys to pupae, and the handbook for Illinois (Ross and Horsfall, 1965) is noteworthy in particular for its egg keys.

The most recent comprehensive treatments of North American species are Wood et al. (1979), which contains keys to larvae and adults of Canada, plates of taxonomic structures for each species, distribution maps, and biological information; and Darsie and Ward (1981), updated in 2004, which covers all of North America north of Mexico and has illustrated keys and distribution maps. These works were preceded by Carpenter and LaCasse (1955), which contains formal descriptions, biology, and meticulously crafted full-page plates of adults of each species. A thorough treatment of North American genera was presented by Stone (1981).

Other parts of the world covered by notable works include the South Pacific (Belkin, 1962), United Kingdom (Marshall, 1938), the Neotropical Region (Lane, 1953), and Japan and Korea (LaCasse and Yamaguti, 1950).

For details on morphological terminology and anatomical features of mosquitoes, the work of Harbach and Knight (1980) is recommended. Members of species complexes often are indistinguishable morphologically. Specialists have overcome some of these problems by using chromosome banding patterns, isozyme profiles, DNA probes and DNA restriction-fragment patterns, or nucleotide sequences of various genes to distinguish such species from one another. These molecular methods for identification are widely available for routine field work.

All known mosquito species in the world are listed in *A Catalog of the Mosquitoes of the World* (Knight and Stone, 1977), plus its four supplements (Knight, 1978; Ward, 1984; Gaffigan and Ward, 1985; Ward, 1992). Updates to the catalog are presented online by the Walter Reed Biosystematics Unit. This work provides the taxonomic history

and current standing of all recognized species, their distributions by country, and references to the general literature and to taxonomic works for all regions of the world. The most recent large treatment of a major geographic area is the 12-volume catalog on the Australasian Region, edited by Debenham, Hicks, Lee, and others (1980−1989).

Original systematic studies of mosquitoes are published in several scientific journals, but the one devoted solely to this subject, *Mosquito Systematics*, was subsumed under the *Journal of the American Mosquito Control Association* in 1995. Two long series of valuable papers of international scope on mosquito taxonomy and distribution have been published in the journal *Contributions of the American Entomological Institute: Contributions to the Mosquito Fauna of Southeast Asia* and *Mosquito Studies*. Preferred common names of mosquito species are listed by the Entomological Society of America (Bosik, 1997) and by Pittaway (1992). An internationally accepted set of two-letter abbreviations for all mosquito genera is shown in Table 15.1. These abbreviations appear in most mosquito publications and are used in this chapter.

MORPHOLOGY

The **eggs** of most mosquitoes are elongate, ovoid, or spindle-shaped; others are spherical or rhomboid. The outermost layer of the egg shell, or **chorion** (Fig. 15.1), often has intricate surface structures and patterns diagnostic of the particular species. The chorions of *Anopheles* species have unique, transparent air-filled compartments flanking the egg that serve as **floats** (Fig. 15.1A). Eggs of *Anopheles*, *Toxorhynchites*, *Wyeomyia*, *Aedes*, *Psorophora*, and *Haemagogus* spp. are laid individually, whereas in *Culex*, *Culiseta*, *Coquillettidia*, and *Mansonia* spp., they are attached together in a single clump, forming a floating egg raft (Fig. 15.2A) or submerged cluster (Fig. 15.2B). *Culex* eggs have a cup-shaped corolla at one end (Fig. 15.1B), allowing them to sit vertically on the water surface in a raft (Fig. 15.2A); the upper ends have apical droplets with a chemical thought to maintain the raft upright.

Mosquito larvae, commonly known as **wigglers** or **wrigglers**, pass through four instars, which closely resemble one another except for their size. Larvae are rich in taxonomic characters that are easy to see on slide-mounted specimens (Fig. 15.3). The head is defined by a distinct capsule bearing a pair of "eyes" composed of clusters of lateral ocelli, a pair of antennae of varying shape and length, and chewing mouthparts bearing a variety of brushes, combs, and sweepers used in feeding (Fig. 15.4). The lateral **palatal brushes** on the labrum create water currents that draw floating or suspended particles toward the mouth. Sweepers and brushes on the mandibles, and brushes on the maxillae, are thought to collect and pack particles to create a bolus of food in the pharynx. In

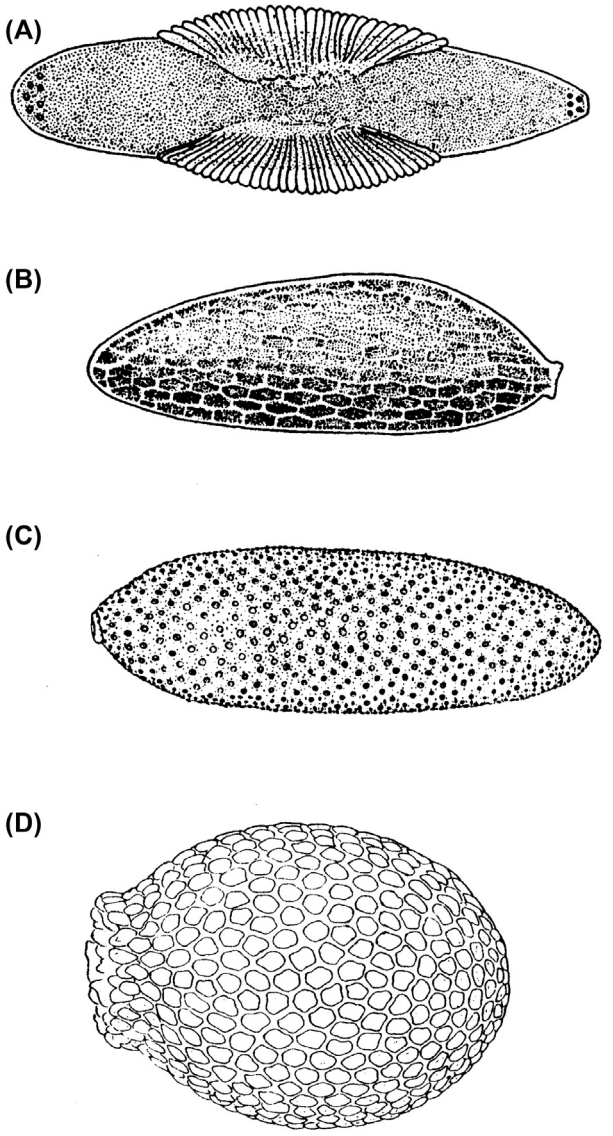

FIGURE 15.1 Eggs of mosquitoes, showing variations in shape and chorionic sculpturing. (A) *Anopheles*. (B) *Culex*. (C) *Aedes aegypti*. (D) *Toxorhynchites brevipalpis*. *A and B from Ross, 1947; C and D from Harbach and Knight, 1980.*

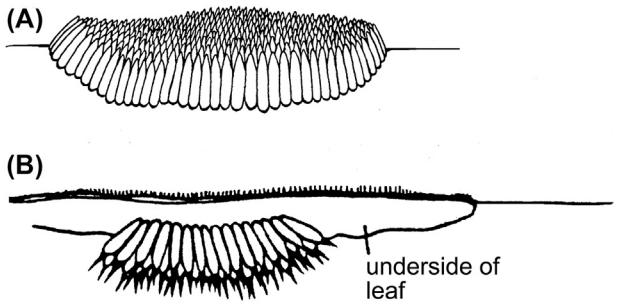

FIGURE 15.2 Mosquito egg rafts and clusters. (A) Floating egg raft of *Culex restuans* (Ross, 1947). (B) submerged egg cluster of *Mansonia*, attached to underside of floating leaf. (Gordon and Lavoipierre, 1962).

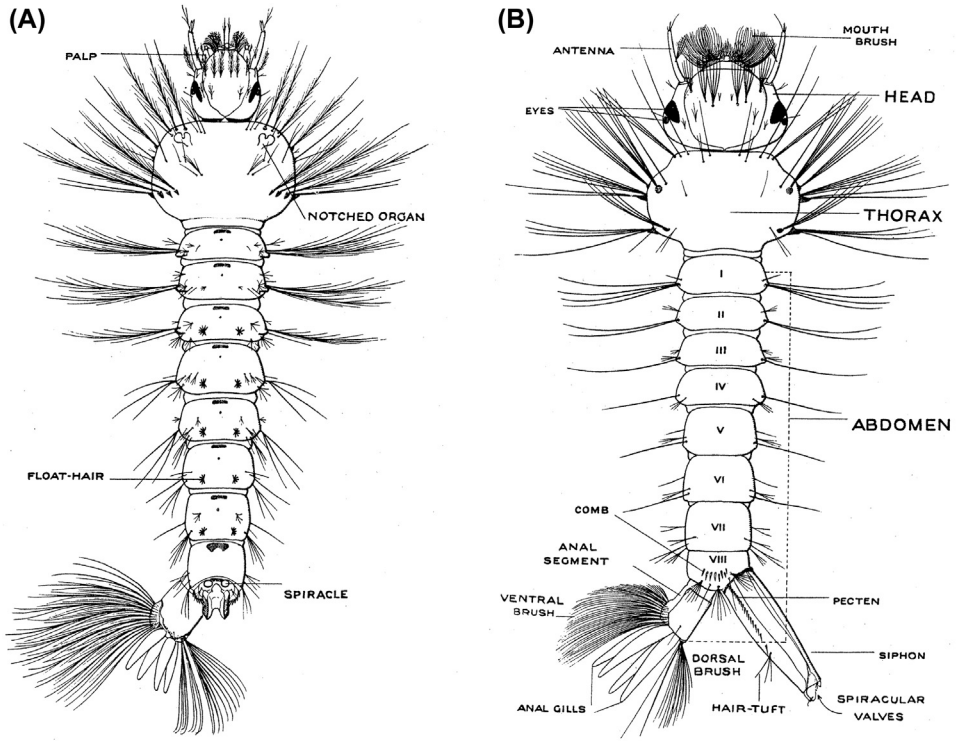

FIGURE 15.3 External anatomy of mosquito larvae, dorsal view, with anal segment and siphon at posterior end rotated to provide better view. (A) Anopheline form (*Anopheles maculipennis*). (B) Culicine form (*Aedes cinereus*). (Marshall, 1938).

predatory larvae, the mandibles and/or maxillae are heavy and sharply toothed for seizing or holding prey. The thorax is wide, with three indistinct, legless segments.

The larval abdomen is narrower than the thorax, cylindrical, and composed of eight apparent segments, the second-to-last being a composite of segments 8 and 9. A pair of spiracles opens on the dorsal side of this segment. In culicines the spiracles open at the end of the **respiratory siphon**, an elongate air tube extending dorsally. The siphon of *Coquillettidia* and *Mansonia* is short, ending in a heavily sclerotized point with a dorsal sawlike edge used to pierce and remain lodged in plant tissue. In anophelines the siphon

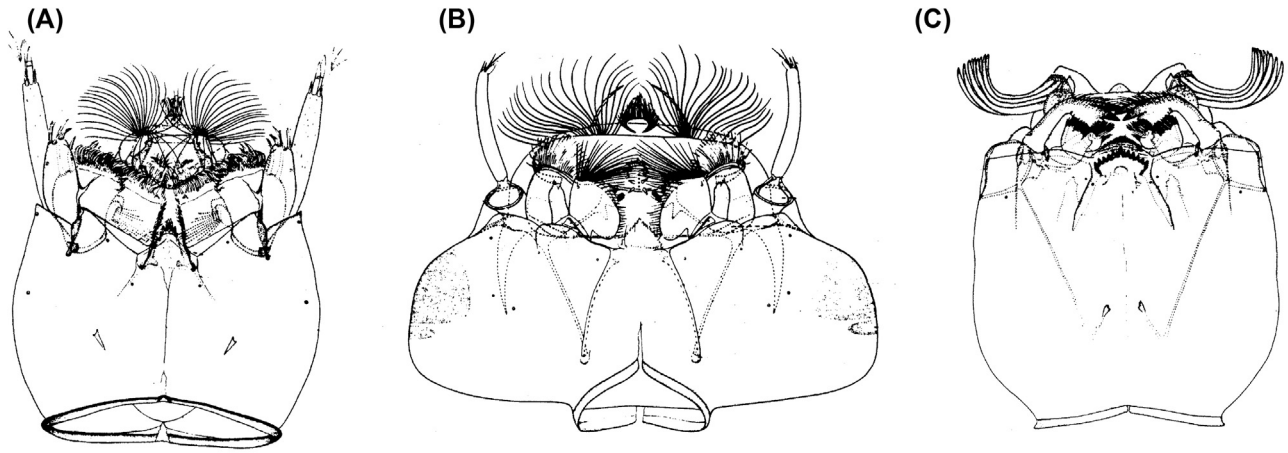

FIGURE 15.4 Heads of mosquito larvae, postero-ventral view. (A) Anopheline form (*Anopheles quadrimaculatus*). (B) typical culicine form (*Aedes fulvus pallens*). (C) toxorhynchitine form (*Toxorhynchites brevipalpus*). The lateral palatal brushes of most larvae are used to generate water currents for filter feeding; in *Toxorhynchites* they are modified for seizing prey. (Harbach and Knight, 1980).

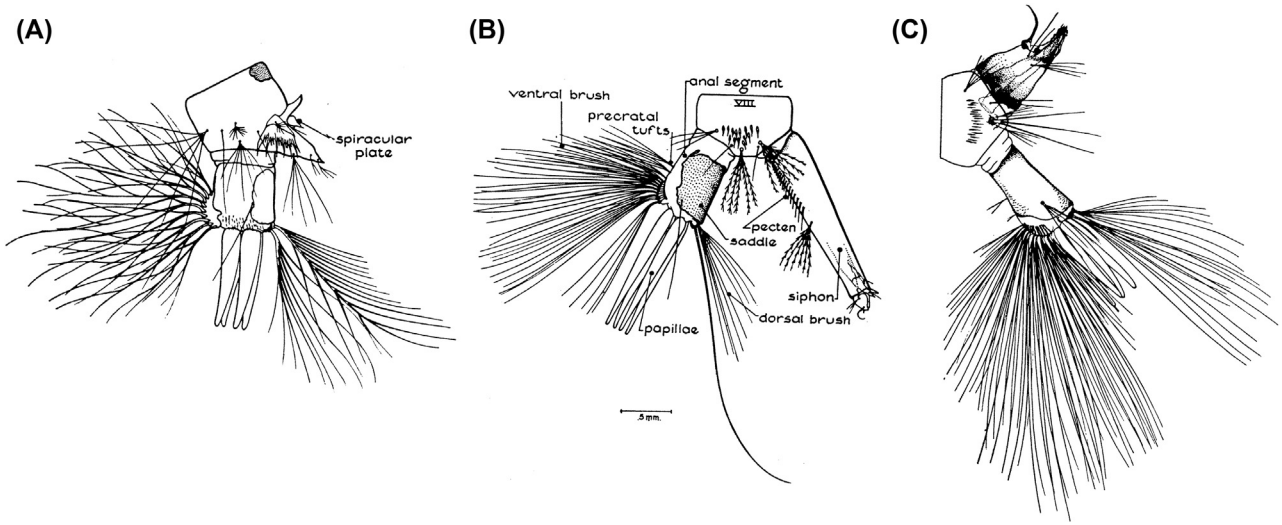

FIGURE 15.5 Terminal segments of mosquito larvae. (A) Anopheline form (*Anopheles earlei*), lacking a siphon. (B) typical culicine form (*Aedes fitchii*), showing elongate siphon. (C) specialized culicine form (*Coquillettidia perturbans*), showing short, stout siphon suited for piercing and clinging to plants. (Barr, 1958).

is lacking, and the spiracles are borne on a short spiracular plate. Segment 10, the anal segment, extends ventrally at an angle from the rest of the abdomen. It typically bears four anal papillae used primarily in osmoregulation. The terminal region of the larva bears several structures useful in identification (Fig. 15.5). They include comb scales on segment 8, pecten spines on the siphon, a saddle sclerite encircling the anal segment, and various tufts and brushes of setae. Some of these terminal structures apparently are used to groom the mouthparts when the larva bends its body around to form a loop.

Larval internal anatomy conforms to the general insect plan. The alimentary tract is almost straight, the only notable features being eight large gastric caeca at the junction of the foregut and midgut in the thorax and five Malpighian tubules at the midgut–hindgut junction. Because most of the cuticle is semitransparent, two large tracheal trunks are obvious, extending forward from the spiracles to the thorax.

Mosquito pupae, commonly known as **tumblers**, are comma-shaped, with the head and thorax fused to form a cephalothorax and the abdomen curled beneath it (Fig. 15.6). Projecting from the dorsal mesothorax is a pair of respiratory tubes, or **air trumpets**, through which the pupa obtains oxygen at the water surface. Within the cephalothorax, the developing appendages of the adult head and thorax usually can be seen coiled ventrally; they envelop an air pocket, the ventral air space that provides buoyancy to help maintain the pupa at the water surface when resting. At the end of the abdomen two broad paddles are attached to the eighth segment. The pupa can flex its abdominal segments, causing the paddles to flap downward, propelling it through the water when it is disturbed.

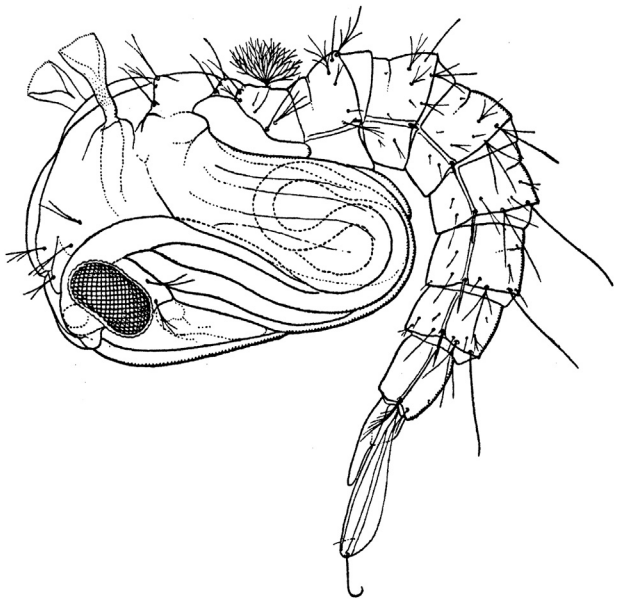

FIGURE 15.6 Mosquito pupa. Lateral view of *Anopheles gambiae* in resting position at water surface; presence of adult structures visible within pupal cuticle. (Smart, 1948).

Adult mosquitoes are slender, with thin legs and narrow, elongate wings (Fig. 15.7). The body surface is covered with scales, setae, and fine pile, creating the characteristic markings and colors of each species. The two compound eyes, each represented by 350–900 ommatidial lenses, wrap around the front and sides of the head. The antennae arise between the eyes, are long and filamentous, and are usually sexually dimorphic. In species in which sound is used to locate females in flight, the flagellum of the male antenna has whorls of much longer fibrillae,

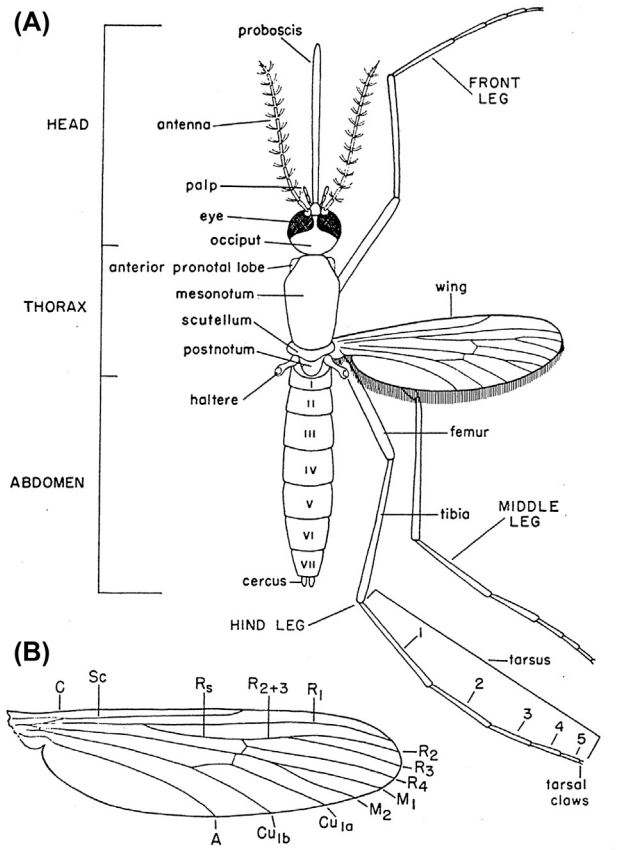

FIGURE 15.7 External anatomy of mosquito adult. (A) Generalized adult, dorsal view. (B) wing, showing typical venation and vein nomenclature. (Ross and Horsfall, 1965).

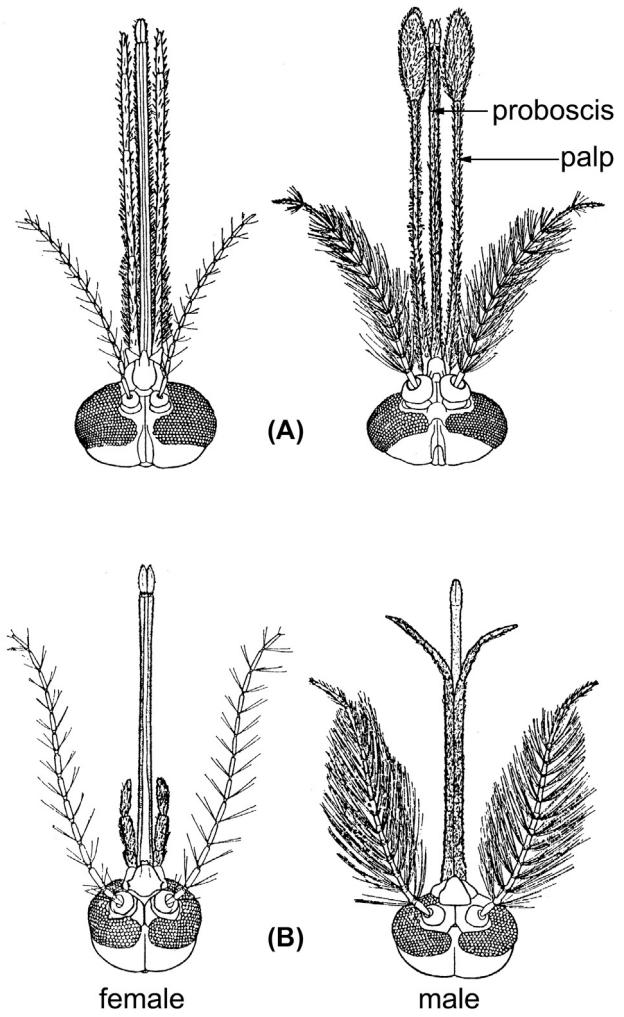

FIGURE 15.8 Heads of anopheline and culicine mosquitoes, females (left), males (right); males typically with plumose antennae. (A) *Anopheles* (anopheline), both males and females with palps about as long as the proboscis; male with plumose antennae and tips of palps broadened. (B) *Culex* (culicine), females typically with short palps and males with long, curved or brushlike palps. (Gordon and Lavoipierre, 1962).

giving it a plumose appearance (Fig. 15.8). The pedicel at the base of the antenna is a large globular structure that contains **Johnston's organ**, a mass of radially arranged mechanoreceptors that respond to vibrations of the flagellum induced by sound. In addition, to the long fibrillae, the antenna has a variety of sensory structures, including those for detecting host odors.

The mosquito proboscis is prominent, projecting anteriorly at least two-thirds the length of the abdomen. It consists of the basic complement of insect mouthparts: the labrum, paired mandibles, hypopharynx, paired maxillae, and labium. The first four structures have evolved into fine stylets, forming a tightly fitting fascicle that in females is used to penetrate host skin (Fig. 15.9). The fascicle is cradled within the groove of the large and conspicuous labium (Fig. 15.10), which comprises the bulk of the proboscis. The tip of the labium bears a pair of small taste-sensitive labella and a short, pointed ligula between them. The latter allows water and sugar solutions to make contact with the inner surfaces of the labella. Of the fascicle of stylets, the hypopharynx and mandibles are narrowly pointed at their tips, whereas the maxillae end in serrated blades. Both mandibles and maxillae puncture the skin and advance the fascicle into the host's tissue. A salivary channel runs the length of the hypopharynx, delivering saliva to the tissue during probing. The labrum is curled laterally to form a food canal for drawing the host's blood or a sugar solution up the proboscis. In males, and in females of non–blood-feeding species, the mandibles and maxillae have atrophied, so they cannot pierce skin. In both sexes of *Toxorhynchites*, the nonpiercing proboscis is curved downward (Fig. 15.11). Maxillary palps arise at the base of the proboscis and bear several kinds of sensilla. Though there are many exceptions, palps usually are short in female culicines but longer than the proboscis in most male culicines and in both sexes of anophelines (Fig. 15.8).

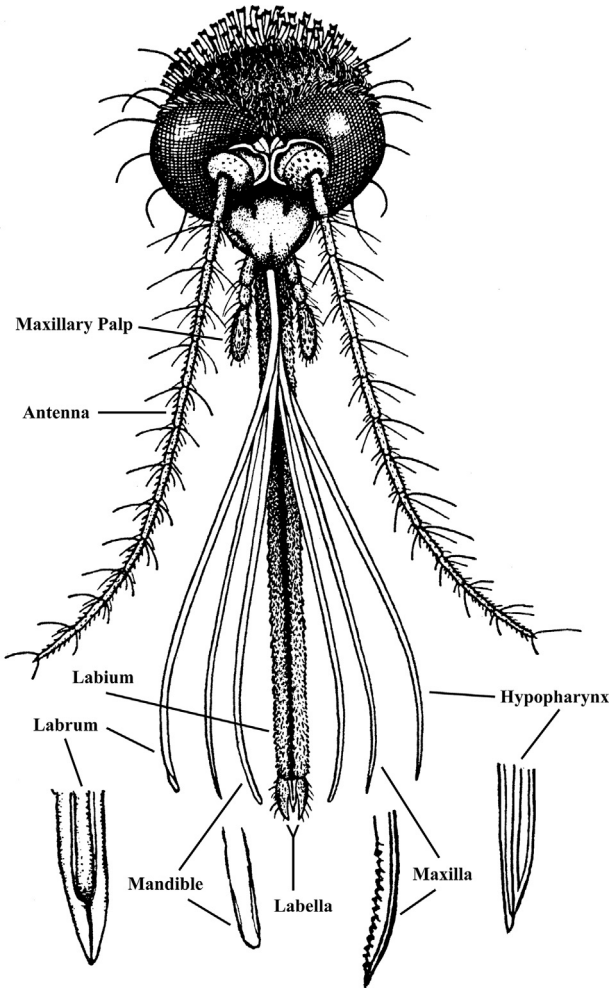

FIGURE 15.9 Mouthparts of adult female mosquito, showing labium, splayed stylets, and variations in structure of their tips. (Matheson, 1944).

The mosquito thorax forms a single, relatively rigid muscle-filled locomotor unit with obscured segmentation. The mesothorax and metathorax each has a pair of lateral spiracles. The slender legs are attached close together on the underside of the thorax by elongate, downward-projecting coxae; the tarsi are tipped with two claws and a central pad, the empodium. The wings are narrow, have a distinctive pattern of veins, and bear scales along the veins and the hind margin, the latter forming a fringe. The **halteres**, tiny modified hindwings used in flight control, are located right behind the insertions of the wings.

The abdomen is clearly segmented and capable of extensive expansion and some movement, owing to the membranous areas between each set of tergites and sternites. This allows for expansion of the abdominal wall to accommodate large blood and sugar meals and developing clutches of eggs. Abdominal segments 5–8 are progressively smaller so that the abdomen tapers toward the posterior end. Segment 9 is quite small and bears the cerci, the postgenital lobe of the female, and the claspers and other genitalic structures, or **terminalia**, of the male (Fig. 15.12). At emergence, the male genitalia are inverted. During the first hours of adulthood, segments 8 and 9 of males together rotate 180 degrees to reach the mature position. The complex and varied male genitalia provide a useful source of characters for species identification.

Located in the thorax are a pair of three-lobed salivary glands, whose ducts join anteriorly to form a common salivary duct that enters the hypopharynx (Fig. 15.13). In males, these glands produce saliva used only in sugar-feeding; in females, some portions are devoted to sugar-feeding and others to blood-feeding. The foregut, which begins in the head with the muscular cibarium and pharynx, pumps food up the labral food canal. The tubular esophagus extends

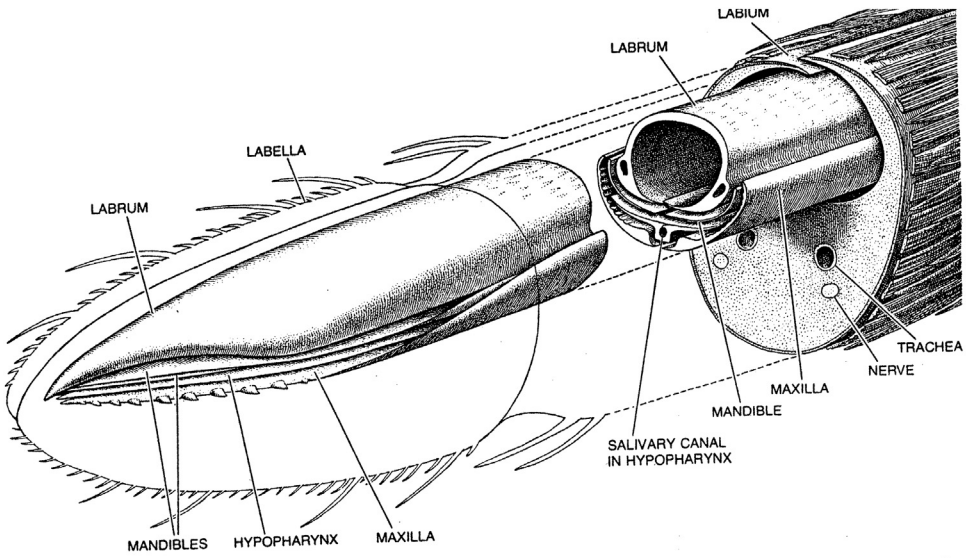

FIGURE 15.10 Fascicle of stylets of adult female mosquito. Mouthparts near tip of proboscis, showing natural arrangement of stylets in a single bundle, or fascicle, within a groove in the labium, which forms a sheath. *Jones, 1978; illustration by Tom Prentiss.*

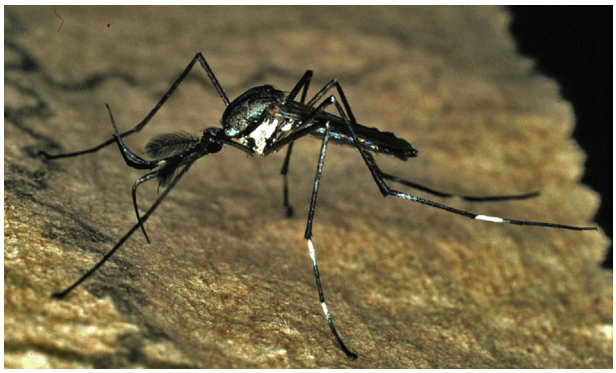

FIGURE 15.11 *Toxorhynchites amboinensis*, adult male. The form of the palps and antennae in females and males is similar to that of other culicines; however, the proboscis of both sexes is bent downward at an angle of 90° or more. *Photograph by Woodbridge A. Foster.*

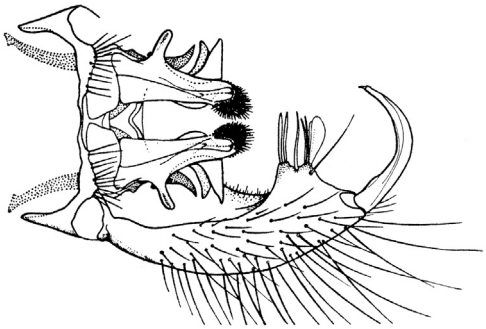

FIGURE 15.12 Male genitalia of *Culex quinquefasciatus*, showing principal copulatory structures used in male taxonomic identification. Gonocoxite and gonostylus on left (upper) side have been omitted. (Ross and Roberts, 1943).

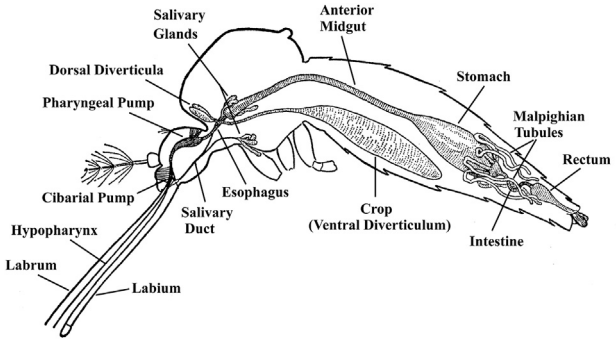

FIGURE 15.13 Digestive system of the adult mosquito. Semi-diagrammatic view of major structures, including the salivary glands, foregut-midgut junction, and rectum. (Snodgrass, 1959).

through the cervix, or neck, into the thorax. There it is modified to form three diverticula, including two small dorsal outpocketings and a large ventral crop; the crop extends through the thorax and expands to form a large sac in the abdomen. Imbibed sugar solutions are stored in these diverticula and pass, a little at a time, through the proventricular valve into the midgut. A bloodmeal, on the other hand, passes directly into the widened posterior midgut, or stomach (Fig. 15.13). There it becomes surrounded by a semipermeable, saclike **peritrophic membrane** secreted by the midgut epithelium. The resulting blood bolus then is digested and absorbed. The pyloric valve separates the midgut from hindgut; five Malpighian tubules empty into the hindgut just beyond the valve in the pyloric chamber. The anterior portion of the hindgut is tubular and loosely coiled; the posterior part is enlarged to form a bulbous rectum with large papillae projecting into it. The papillae probably are involved in resorption of salt ions.

Paired gonads are located in the posterior third of the abdomen. The testes of males contain packets of sperm in various stages of maturation. A duct extending posteriorly from each testis widens to form a seminal vesicle, which stores mature sperm. The two seminal vesicles lie together and unite posteriorly to form the ejaculatory duct. Two large accessory glands open into this duct, which leads to the aedeagus. In females, the reproductive system consists of a pair of ovaries and accessory structures (Fig. 15.14). Each ovary includes a few dozen to over 200 polytrophic ovarioles, the egg-forming units. A duct from each ovary extends posteriorly, and the two unite to form a common oviduct, which connects to the gonopore via a genital chamber. Opening into the genital chamber by tiny ducts are one (in anophelines) to three (in most culicines) sperm-storing spermathecae, a small accessory gland, and the seminal bursa (lacking in most *Anopheles*), which receives semen from the male during mating.

LIFE HISTORY

The holometabolous life cycle of mosquitoes is completed in two different environments, one aquatic, the other terrestrial. The larvae and pupae develop in a wide range of aquatic habitats. These include temporary surface water (e.g., tidal pools in salt marshes, rain pools, and flood water), permanent surface water (e.g., pools, streams, swamps, and lakes), and diverse natural and artificial water-holding containers (e.g., tree holes, leaf axils, fruit husks, mollusk shells, drinking water pots, and discarded tires). An extensive analysis and classification of larval habitats have been presented by Bates (1949), Mattingly (1969), Laird (1988), and Service (1993). The only absolute requisite of all development sites is that they maintain at least a film of water for the duration of the larval and pupal periods. However, individual species tend to oviposit, and therefore develop, in sites with specific structural and chemical properties. Adults are much more mobile than the immatures, but they also tend to occupy characteristic resting, foraging, and overwintering habitats.

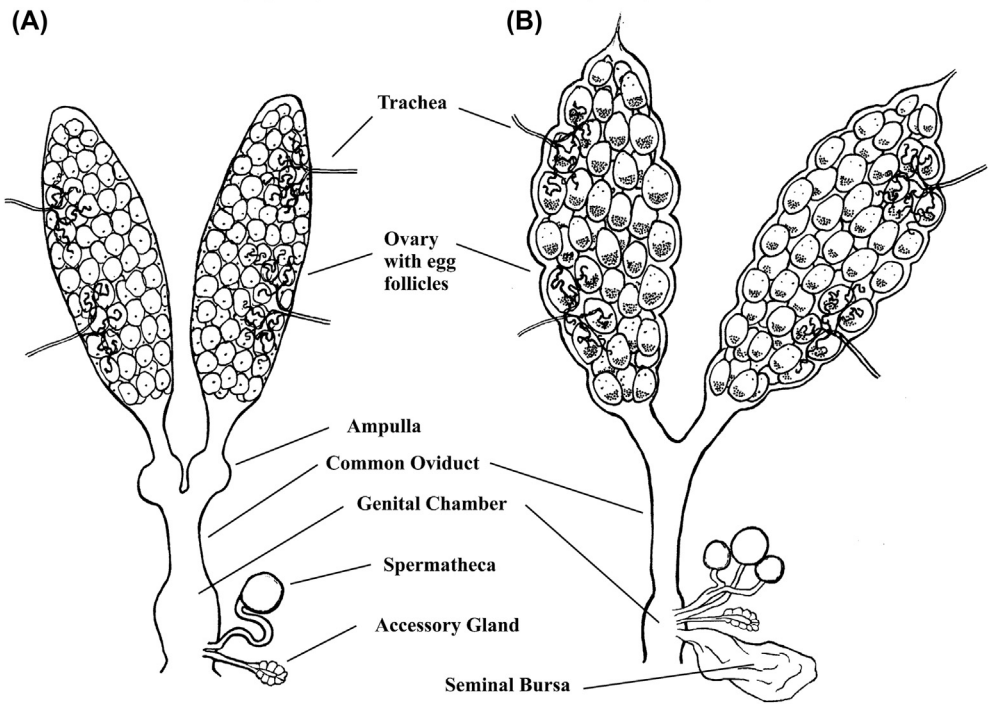

FIGURE 15.14 Female reproductive system of the mosquito. (A) Anopheline form, based on *Anopheles gambiae*. (B) Culicine form, based on *Aedes aegypti*. *Original by Woodbridge A. Foster.*

Mosquito eggs are laid either on or in water, or on solid substrates that are likely to become indundated. Females in the subfamilies Anophelinae and most of the Culicinae in the tribe Sabethini scatter their eggs individually on the water surface, whereas those in the tribe Aedini (e.g., *Aedes*, *Psorophora*, and *Haemagogus*) attach their eggs individually on a substrate that later will become inundated with water. Clumped eggs are laid in boat-like rafts on the water surface by several genera of Culicinae (e.g., *Culex*, *Culiseta*, *Coquillettidia*, *Uranotaenia*, *Armigeres*, and *Trichoprosopon*) or in a radial cluster attached underwater to vegetation in the case of *Mansonia*.

At first the eggs are white, but most turn dark within hours as the chorion tans. In lowland tropics, subtropics, and summers of some temperate regions, eggs usually complete embryonic development within 2−3 days after being laid, but may take up to a week or longer in cool climates. Larvae hatch soon after embryonation in species that lay their eggs directly in water, including all Anophelinae and most tribes of Culicinae. The best known exceptions are members of the genera *Aedes*, and other Aedini that typically develop in temporary water. Their eggs are laid on solid substrates out of water, and the larvae within them remain quiescent until inundated. The eggs can tolerate periods of cold and desiccation and may remain viable for years. Hatching usually occurs at warm temperatures after the eggs have been submerged and microbial activity has caused the oxygen level in the water to drop.

Depending on the species and particular conditions of the water, most mosquito **larvae** spend most of their time either at the water surface or at the bottom of the water column, coming to the surface for air only occasionally, or not at all. At ideal conditions of food and temperature (26−28°C), the entire larval phase of *Aedes aegypti*, a tropical and subtropical mosquito, may last as few as 5−6 days. The first three instars are completed in about 1 day each and the fourth lasts about 3 days. In males these periods are slightly shorter, so the males pupate about 1 day earlier than females. Larvae of many species grow even faster, as when the water is heated by direct sunlight, whereas others develop slowly. *Toxorhynchites* and *Wyeomyia* spp. usually take 2−3 weeks even under ideal conditions. At cooler temperatures, or when food is scarce, growth becomes slower and can practically cease, with larvae remaining alive for months. Larvae of some species that inhabit high latitudes or high altitudes, or that develop in the early spring in temperate regions, have growth thresholds close to freezing and can tolerate even temporary entrapment in solid ice. This is typical of the snow pool *Aedes* spp. and of mosquitoes that overwinter as larvae, such as *Wyeomyia smithii* in pitcher plants and *Orthopodomyia alba* in tree holes.

The **pupa** spends nearly all of its time at the water surface. By the time it has molted to form a pharate adult within the pupal cuticle, it is very dark. In warm water the entire pupal stage typically lasts about 2 days in both sexes.

In some mosquitoes, such as *Toxorhynchites* and *Wyeomyia* spp., the shortest pupal periods may be 5−6 days. In all species the pupal period lasts longer at lower temperatures.

Adult males tend to emerge earlier than females, because of their shorter larval growth periods. As **adult emergence** approaches, the pupa remains stationary at the water surface, and the abdomen gradually straightens over 10−15 min. The adult emerges from the pupal cuticle by ingesting air, causing the cephalothorax to split and the adult to rise up out of the cuticle and stand on the water surface. The entire process takes only a few minutes. The newly emerged adult is capable of short flights a few minutes later but cannot sustain long flights for many hours, until after the cuticle becomes fully sclerotized. Lipids and glycogen, carried over from larval reserves, provide sufficient energy for a few days of flight and survival.

It is typically during the first 3−5 days of adult life that both sexes obtain **sugar** from plant **nectar** or **honeydew**, become sexually mature, and then mate. In some species (e.g., *Culiseta inornata*, *Wyeomyia smithii*, and *Deinocerites cancer*) sexual maturation is complete at the time of emergence or only a few hours later, and mating occurs almost immediately. Mosquitoes typically first feed on sugar to obtain enough energy for sexual maturation and for the flight necessary for mating, dispersal, and finding vertebrate blood. Natural sugar is taken repeatedly throughout adult life by both sexes of most species. Females typically mate only once. Males can inseminate several females before their supplies of mature sperm and accessory gland secretion become depleted. The semen supply is replenished in a few days.

Amorphous masses of fat body line the inner walls of the abdomen. The fat body synthesizes and stores both glycogen for flight and lipids for maintenance, using the digestive products of sugar and bloodmeals. Glycogen is stored also in the fibrillar flight muscles of the thorax, serving as a source of energy for immediate flight if the sugars in the crop and hemolymph have been exhausted.

Only females feed on vertebrate blood. In most mosquitoes, ingestion and digestion of a bloodmeal initiate **egg development** by stimulating a cascade of hormones from the brain and ovaries. The large amount of protein contained in hemoglobin and the blood serum provides the amino acids for synthesizing **vitellogenin**, the proteinaceous precursor of egg yolk. The protein also serves as the substrate for building lipid and glycogen, which contribute both to egg yolk and to the maternal energy reserves used for survival and flight. A bloodmeal will stimulate egg development only if it is sufficiently large and if the female's ovarian follicles have reached the resting stage, at which point they are considered to be **gonoactive**. If a female has had poor larval nutrition, the follicles may not have reached the resting stage, and she will be unable to develop any eggs until having ingested sugar or a preliminary bloodmeal. Such a **gonoinactive** female, needing food to bring the ovarian follicles to the resting stage, is sometimes said to be "pre-gravid." Details of the hormonal control of these processes are discussed by Brown and Lea (1990), Hagedorn (1994, 1996), and Klowden (1996).

In most species, females are **anautogenous**, the egg follicles remaining in the resting stage until a bloodmeal is taken. Following each bloodmeal, the female develops one mature clutch of eggs, exhibiting what is known as gonotrophic concordance. However, females of **autogenous** species or populations can develop eggs without a bloodmeal; among these there are obligate and facultative types. A facultatively autogenous female typically develops only the first clutch of eggs without blood; she does so only if she emerges with sufficient reserves and cannot readily find blood. Thereafter, a bloodmeal is required for each gonotrophic cycle. Species that are obligately autogenous have atrophied feeding stylets, never take blood, and subsist entirely on their larval reserves and plant sugar. Autogeny has been reviewed by O'Meara (1985). At the other extreme, there are some anautogenous species that take blood not only at the beginning of each gonotrophic cycle but also once or twice during egg development. These supplementary bloodmeals can provide extra energy, acting as a substitute for sugar (e.g., domestic *Aedes aegypti*, *Anopheles gambiae*).

Ordinarily, all eggs develop synchronously and become mature in 2−5 days after blood-feeding at favorable temperatures. During this time, the most advanced follicle within each ovariole passes through a series of five easily observed **physiological stages** (Fig. 15.15), originally described by Christophers (1911) and concisely summarized by Clements (1992). These stages comprise four physiological phases. The follicles develop synchronously, beginning with the **previtellogenic phase** (stages G through II). The follicle typically stops growing at stage IIa or IIb, the **resting stage**, until the female takes a bloodmeal. Within hours of blood-feeding, the follicle enters the **initiation phase** (stage IIIa), when yolk (vitellogenin) synthesis by the fat body begins, followed by the **trophic phase** (stages IIIb and IV), the main period of yolk incorporation and follicle growth. In the **post-trophic phase**, the egg has reached its mature shape, and the **chorion**, or egg shell, is formed. The eggs are then ready to be oviposited.

By this time, the next follicle in each ovariole either has progressed to stage I or already has reached the resting stage (stage II). In the first case, it awaits oviposition before developing to the resting stage. Once oviposition has occurred and these new follicles are in the resting stage, the next bloodmeal is necessary for subsequent follicle development. The entire cycle of egg production, from bloodmeal through oviposition, is the **gonotrophic cycle**. A female typically undergoes many such cycles, each starting

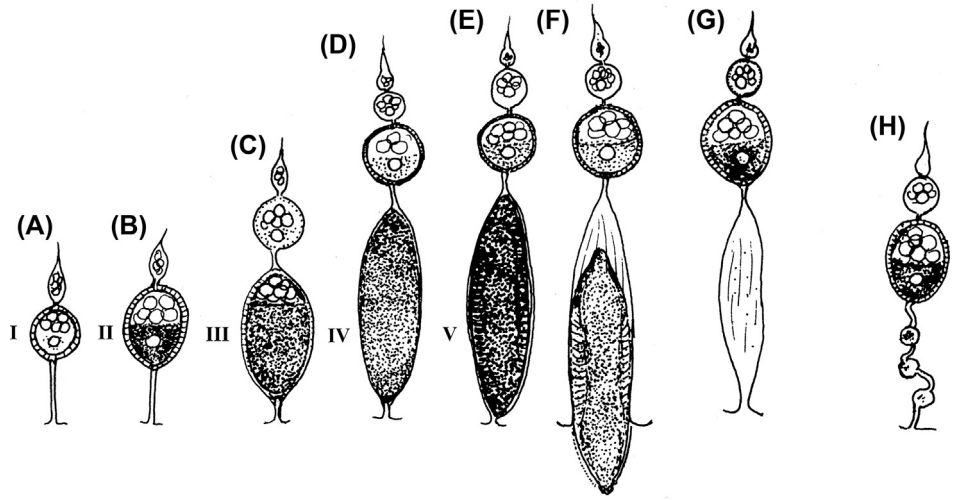

FIGURE 15.15 Egg follicle development in mosquitoes, showing stages in the development of an egg follicle within a single ovariole of the ovary. (A)–(E), Stages I–V of Christophers in an *Anopheles* female. After oviposition (F), the ovariole stalk remains swollen for awhile, called the "sac stage" (G). Note: formerly, the sac was thought to shrink to a dilatation. Now it is believed that the dilatations (three of them shown in H) generally form from follicles that fail to develop into eggs after a bloodmeal and are then resorbed. (World Health Organization, 1975).

with a bloodmeal, an important fact when considering how most mosquito-borne pathogens are transmitted.

The number of ovarioles that produce mature eggs depends on the sizes of the female body, energy reserves, and bloodmeal. The follicles that do not pass beyond stage II degenerate. When most or all the ovarioles contain mature eggs, the ovaries may occupy nearly the entire volume of the distended abdomen. The gravid female fertilizes one egg at a time, as each passes down the oviduct to be oviposited on the water or a damp substrate.

When all eggs have been fertilized and expelled during oviposition, the ovaries return to their pretrophic size, but the tracheae on the ovaries, which had been tightly coiled as **tracheal skeins** before the eggs developed, become stretched and straightened. In *Anopheles* spp., a swelling at the base of each lateral oviduct, the **ampulla**, becomes permanently stretched during the first oviposition. These signs serve to distinguish **parous** females, those that have completed at least one gonotrophic cycle, from **nulliparous** females, those that have not.

The number of completed **gonotrophic cycles** also can be determined. According to current interpretations, each ovariole ovulating a mature egg is left with an egg sac, which becomes reduced to a zone of granules in the calyx, the ovariole's connection to the lateral oviduct. Furthermore, a dilatation is formed in the stalk of each ovariole where a follicle has degenerated after a bloodmeal, instead of developing into an egg (Fig. 15.15). Thus, a count of the maximum numbers, per ovariole, of dilatations in the stalk and zones of granules in the calyx yields an estimate of the number of gonotrophic cycles completed. This **physiological age grading** can provide the medical entomologist with valuable information on the age of individuals and the age structure of a mosquito population. Details of these processes and their interpretation and application are given by Detinova (1962), Sokolova (1994), Fox and Brust (1994), and Hoc (1996).

Univoltine mosquito species complete only one generation per year. This occurs either if the developmental time is slow in relation to the season favorable for development or if the life cycle includes an obligate form of diapause, a compulsory phase of arrested development. **Bivoltine** and **multivoltine** species can complete two or more generations, respectively, during each breeding season, but the number actually completed may depend on temperature, available larval habitats, or available hosts. Mosquitoes pass through the winter or dry season as eggs, larvae, or adults, depending on the species and the climate. In cold climates, overwintering takes place in a state of diapause.

BEHAVIOR AND ECOLOGY

Eggs that are laid on or in water generally are not resistant to desiccation and hatch shortly after embryonation, provided that they are wet and not too cold. This is typical of *Anopheles*, *Culex*, *Culiseta*, and *Toxorhynchites* spp. *Aedes*, *Psorophora*, and *Haemagogus* eggs, on the other hand, typically are laid on damp substrates, display great resistance to desiccation, and remain quiescent for months or years after embyrogenesis until they receive a hatching stimulus. Sometimes moisture by itself is sufficient to induce hatching. Usually, however, the requisite stimulus is a reduction of dissolved oxygen in the water caused by microbial activity and decomposition of organic matter.

Among quiescent eggs that are eventually submerged, only a portion of a single egg clutch may hatch during any one inundation, resulting in **installment hatching**. This apparently is the combined result of intrinsic variations among eggs in their hatching-stimulus thresholds and of local variations in microbial activity, causing differences in oxygen tension around the eggs. Even during a single inundation, hatching may not occur all at once but over a period of many days. In both quiescent and non-quiescent types of eggs, physical agitation of the water sometimes serves as a hatching stimulus.

When an egg is ready to hatch, the first-instar larva uses a dorsal hatching spine on its head, the egg breaker or egg burster, to apply pressure to a preformed weakness in the chorion. This causes the chorion to pop open at one end, and the larva wriggles free. Because the eggs of *Culex*, *Culiseta*, and *Coquillettidia* usually stand vertically on the water surface in rafts, the larvae develop inside them with their anterior end oriented downward and hatch directly into the water.

Mosquito larvae are not buoyant and must, at rest, be suspended at the surface by special hairs and spiracular structures that cling to the surface tension while obtaining oxygen directly from the air. Culicinae typically migrate up and down in the water column, so they occur both at the surface and at the bottom of a body of water, depending on the availability of food. At the surface the tip of the siphon opens above the surface film, as the larvae hang diagonally downward most of the time (Fig. 15.16B). *Mansonia*, *Coquillettidia*, and some *Mimomyia* spp. are unusual in remaining submerged throughout larval and pupal development, with their siphons embedded in the tissues of aquatic plants from which they derive some oxygen (Fig. 15.16C). Mosquitoes that live in water-filled leaf axils (e.g., *Wyeomyia* spp.) are adept at flattening themselves against vertical surfaces and maneuvering in narrow spaces. Anopheline larvae spend most of their time at the water surface, often close to vegetation or floating material. They are able to remain suspended horizontally at the surface (Fig. 15.16A) due to pairs of dorsal palmate setae (float hairs) on several abdominal segments (Fig. 15.3A).

Larvae propel themselves by a back-and-forth lashing movement of the abdomen. Anopheline larvae usually swim horizontally at the surface film. When larvae of typical culicine mosquitoes are feeding below the surface, they periodically swim actively back to the surface to obtain oxygen. However, in many microenvironments dissolved oxygen also is absorbed from the water through the cuticle, requiring only infrequent trips to the surface by some species.

Mosquito larvae feed on a variety of organic detritus, suspended material, and small organisms in their aquatic habitats. The organisms include bacteria, protists, fungi, algae, microinvertebrates, and small macroinvertebrates in the case of predatory species. The organic detritus usually consists of dead plant material and dead macroinvertebrates.

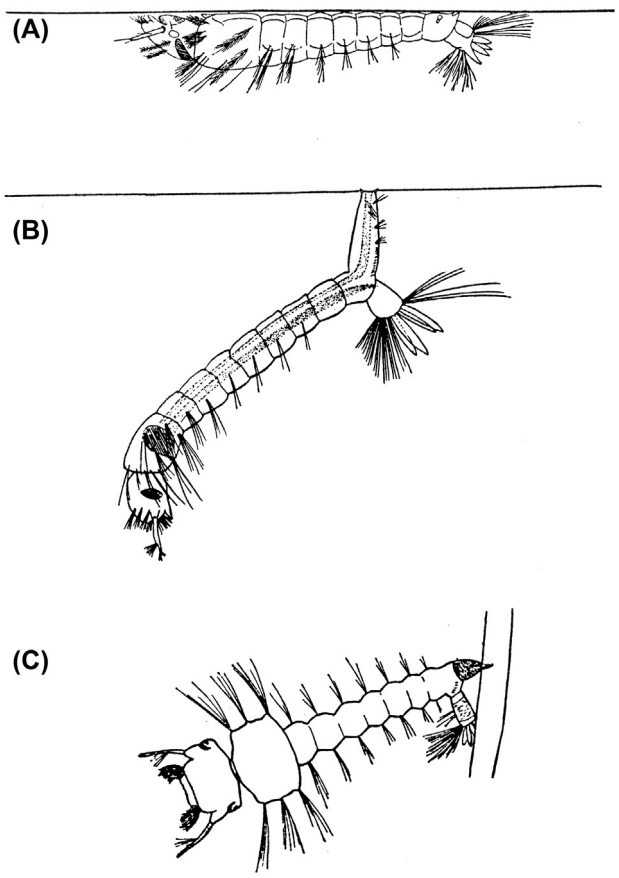

FIGURE 15.16 Resting and feeding positions of mosquito larvae. (A) *Anopheles*, showing horizontal position at the water surface, dorsal-side up; larva has rotated its head 180° so that the ventral side of the head is uppermost and the mouthparts are applied to the water's surface for filter feeding on floating detritus. (B) *Culex*, showing typical diagonal position while suspended from water surface. (C) *Mansonia*, attached to submerged part of aquatic plant (stem or root) by its siphon. *A and B from Ross, 1947; C from Gordon and Lavoipierre, 1962.*

They collect these food items in five basic ways: filtering, gathering, scraping, shredding, and preying. **Filterers** generate water currents with their lateral palatal brushes on the labrum, drawing suspended particles though fine combs where they are collected and directed to the mouth. **Gatherers** use their mouthparts in a similar manner, but only after stirring up the particles from solid surfaces. **Scrapers** obtain food by scraping it off solid surfaces, whereas **shredders** gnaw, chew, and bite off pieces of organic matter. **Predators** grasp insects and other small, mobile prey in their large and sharp mandibles or maxillae (e.g., some *Psorophora* spp.) or with long, curved palatal brushes (e.g., *Toxorhynchites*) (Figs. 15.4C and 15.17).

Most species use more than one of the above techniques. *Anopheles* primarily filter-feed at the water surface

FIGURE 15.17 *Toxorhynchites amboinensis* larva feeding on larva of *Culex pipiens*. This predaceous species has been used in biological control trials and naturally exerts a damping effect on populations of pest and vector mosquitoes. *Photograph by Woodbridge A. Foster.*

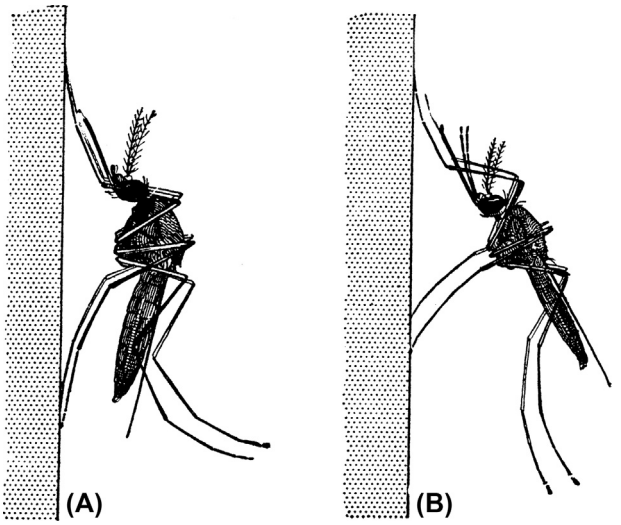

FIGURE 15.18 Comparison of typical positions of mosquito adults resting on a vertical surface. (A) Culicine. (B) anopheline. Culicines typically hold the abdomen parallel to the substrate or pointed toward it, and the proboscis and abdomen form an angle. Anophelines characteristically tilt the body at a sharp angle to the substrate, with the abdomen pointing away from it, and with the proboscis and abdomen in line. (Marshall, 1938).

by rotating their heads 180 degrees so that the oral opening becomes dorsal. Many *Aedes* and *Culex*, on the other hand, filter-feed near the surface but also gather, scrape, or shred organic matter at the bottom, depending on food availability. *Coquillettidia* and *Mansonia*, which are anchored to submerged vegetation, use a combination of filter-feeding, gathering, and scraping techniques within their immediate surroundings. Larval feeding has been reviewed by Merritt et al. (1992).

Mosquito pupae normally remain motionless at the water surface with the tips of their thoracic air trumpets in contact with the air. Like larvae, they dive when disturbed, propelling themselves with their caudal paddles by extending the abdomen, then snapping it back inward toward the cephalothorax. Pupae of most species are buoyant, due to the ventral air space beneath the cephalothorax, and rise to the surface without swimming. They remain submerged by repeatedly swimming downward or by wedging or lodging themselves under debris. After sufficient submergence time, they lose their buoyancy as their air supply dwindles, and they must swim actively to the surface. Pupae of a few mosquitoes (e.g., *Limatus* spp.) are never buoyant and can keep from sinking only by clinging to the surface film, much as most mosquito larvae do. The plant-piercing pupae of *Mansonia* and *Coquillettidia* spp. do not rise to the water surface until they release their attachment to plants when ready for adult emergence.

On **adult emergence** from the pupal stage, adults typically seek shelter in vegetation, cavities, and other resting sites, where they remain except during periods of activity. When resting, they typically position themselves head-up on vertical surfaces, with forelegs and midlegs on the substrate and hindlegs raised (Fig. 15.18). Culicines hold the abdomen in various positions, but the proboscis is always at an angle to it (Fig. 15.18A). Anophelines, on the other hand, hold the proboscis and abdomen in line, oblique to the substrate (Fig. 15.18B). This distinctive position also is apparent during feeding. While at rest, adults perform various stereotyped grooming movements and frequently wave their hindlegs.

Dispersal and foraging may occur only a few dozen meters from their larval habitats. Most species fly less than 2 km in a lifetime. Such ranges are typical of domestic species and result from random elements in their repeated foraging flights for mates, sugar sources, hosts, resting sites, and oviposition sites. Other species enter a specific dispersal mode that is wind-assisted or light-directed and carries them dozens or even hundreds of kilometers from their origins. These flights are most obvious following massive adult emergences of species with quiescent eggs, such as the salt-marsh mosquito *Aedes taeniorhynchus* after high tides, and floodwater mosquitoes such as *Aedes vexans* in bottomlands after heavy rain. The average dispersal distance of such broods is difficult to determine accurately,

because efforts to recapture marked specimens must be made over vast areas, and relatively few are caught. Some salt-marsh species and African anophelines must make extended, round-trip flights to complete their gonotrophic cycles when development sites and blood-feeding sites are many kilometers apart.

Average flight speed also is difficult to determine under natural conditions. *Aedes aegypti* can fly upwind at air speeds up to 5.4 km/hr, while other species in a dispersing mode are estimated to fly much faster. However, their ground speed drops nearly to zero as headwind velocities approach their maximum air speed. Thus mosquitoes tend to avoid flight under windy conditions, except when assisted by a tailwind. Mosquito dispersal in its various forms has been reviewed by Service (1997).

Each mosquito species has a characteristic pattern of adult **diel activity**, under the control of an endogenous circadian rhythm that is entrained by the daily light-dark cycle. Generally one or two **flight periods** occur each 24 hours, characterized as being diurnal, nocturnal, or crepuscular (dawn and dusk). During these periods, both sexes will take flight without external cues other than ambient light. Mosquitoes likely engage in a generalized search pattern during **foraging flights**, then respond to specific stimuli associated with either mating sites, sugar sources, hosts, or oviposition sites as they encounter them, depending on their needs.

Mosquito species vary in the habitats where they forage for mates and food. Some fly over varied terrain; others tend to be active in either wooded or open areas; still others perform all activities close to larval and resting sites. There is some evidence that adult females become familiar with local habitats that have provided either food or oviposition sites in the past and tend to return there, based on experience.

Field studies and experiments indicate that some species are guided in their foraging flights by specific visual features along the horizon, or they fly along the edges of tree stands bordering open terrain. Others apparently simply fly crosswind or downwind, depending on wind speed. When they can see likely sites that supply needed resources, they alter their flight paths to move directly toward the object. In the absence of visual information, if they detect salient odors at a distance, they fly upwind within a downwind-drifting odor plume and then use those and other cues at close range until they arrive at their source. Drinking water, imbibed from the surface of moist substrates, presumably is located similarly, with elevated humidity as an indicator of its proximity. Nectar meals and blood meals also provide water.

Mating usually takes place a few days after adult emergence. The males typically form flight swarms at particular times at swarm markers (prominent objects or other contrasting features of the environment). Each male follows a looping flight path over the marker. In species such as *Aedes aegypti* and *Ae. albopictus*, the females' preferred host serves as the swarm marker. Females probably use the same visual or host-chemical swarm markers that males do, to locate their specific meeting place, but there is some evidence that swarming males also may release a volatile pheromone that attracts females from a distance. When a female enters a swarm, males detect the characteristic frequency of her wing beat and her position with their plumose antennae and Johnston's organs and fly toward her. Her fundamental flight tone varies from about 150 to 600 Hz, depending on the temperature and her size and species. It is about 100−250 Hz lower than the males' flight sound. Each sex can detect the other's audible flight tone or one of the ultrasonic harmonics of it, and at close range adjust these frequencies so that they converge. These duets and their mutual sonic convergence appear to be necessary for successful mating. Swarms usually are species-specific, but mixed swarms do occur; hybrid matings are rare. Interspecific pairings may be avoided either by the lack of frequency-matching or by the recognition of species-specific contact pheromones.

Successful copulation typically occurs only after the duetting and close-range maneuvering, after which the male orients his body with respect to her, venter-to-venter, clasping her genitalia with his. Even then, the female may reject the male by using her genitalia to make full copulation and intromission impossible. If there is mutual acceptance, the couple drifts or flies from the swarm, often shifting to an end-to-end position, with the female flying forward and the male facing backward, clinging to her only by his genitalia. Mating may be completed in the air or on vegetation. Copulation lasts from 12 seconds to several minutes.

There are many exceptions to this standard method of mating. Males of *Deinocerites* and *Opifex* guard pupae at the water surface and mate with the females as they emerge. Males of *Culiseta inornata* remain at the emergence site for long periods and locate newly emerged females at random while crawling about, recognizing them by a specific contact pheromone on their legs. A male *Eretmapodites chrysogaster* will follow a female in tandem flight to a host, wait beside her while she takes a bloodmeal, and then will copulate when she is finished. Males of the sabethine genera *Sabethes*, *Limatus*, *Wyeomyia*, *Runchomyia*, *and Topomyia* locate females at rest on vines, sticks, and tree trunks, and most perform a variety of leg-waving and genitalic courtship rituals on the substrate before insemination. Several aspects of mosquito mating behavior have been reviewed by Downes (1969). Lees et al. (2014), a lead review followed by a series of papers in a special issue of Acta Tropica, together provide a comprehensive summary of male biology and behavior, with emphasis on their relevance to genetic control.

During **copulation** the male deposits a mixture of sperm and accessory gland secretion in the female's seminal bursa or genital chamber. The semen often produces a distinct seminal mass in the bursa or genital chamber, sometimes called a **mating plug**. It disappears in 1−2 days and therefore does not, by itself, prevent subsequent insemination. Within an hour the sperm move into the spermathecae where they are stored. At least in culicine mosquitoes, the swollen bursa itself and, later, substances in the male accessory gland collectively called **matrone**, or MAGS, cause the female to become unreceptive to males. Substances in the accessory fluid also can affect feeding behavior and promote egg development and oviposition. A single insemination usually is sufficient for the life of a female. Most evidence indicates that females only rarely receive sperm from more than one male.

Adult mosquitoes of both sexes of most species regularly feed on plant sugar throughout life, but only females feed on vertebrate blood. Water presumably is taken from the surface of moist substrates as well as in sugar meals and bloodmeals. Field studies and experiments indicate that some species are guided in their foraging flights by specific visual features along the horizon, or they fly along the edges of tree stands bordering open terrain. Others apparently simply fly crosswind or downwind, depending on wind speed. When they can see likely sources of sugar or blood, they alter their flight paths to move directly toward the object. If they detect odors of flowers or hosts in the absence of visual information about a food source, they turn to fly upwind within the downwind drifting odor plume, eventually arriving at the odor source.

Sugar-feeding starts soon after emergence, usually before females begin responding to host stimuli. Sugar is taken frequently by both sexes throughout adult life, both between and during gonotrophic cycles. Females of some domestic species may take sugar infrequently or never (e.g., *Aedes aegypti* and *Anopheles gambiae* in some localities); they typically live in close association with their hosts and utilize blood for both energy and reproduction. **Sugar sources** include floral and extrafloral nectaries, homopteran honeydew, spoiled or damaged fruit, tree sap, and damaged or even undamaged plant leaves and stems. *Malaya* spp. solicit regurgitated nectar and honeydew from ants.

Nectar and honeydew are the most important sugar sources. They contain not only sugar but also amino acids; these are insufficient to initiate egg development but probably promote longevity. Most mosquitoes obtain sugar from a variety of plant species that provide accessible nectar; others seem to be fairly specific in their choices, and a few are important pollinators of particular plant species whose flowers they visit. Mosquitoes generally feed from fragrant, light-colored, clustered flowers with short corollas that allow easy access to the nectar.

Details of sugar-feeding biology have been reviewed by Foster (1995). The fragrances serving as mosquito attractants commonly include blends of several forms of terpenoids and benzenoids, among other compounds (Nyasembe and Torto, 2013).

Blood-feeding by female mosquitoes rarely begins until at least 1−3 days after adult emergence, and often not until after mating and sugar-feeding. Their hosts include all classes of vertebrates: mammals, birds, reptiles, amphibians, and even amphibious fishes, earthworms, and leeches. They have been reported to take hemolymph from other insects, but perhaps this occurs only when the insects have been contaminated with vertebrate odors.

Host specificity and **host preference** vary widely. Some species feed almost entirely on members of one genus of animal, others opportunistically attack members of two or three vertebrate classes. Bloodmeal identification methods, used to determine a mosquito's host, have been reviewed by Washino and Tempelis (1983) and Clements (1999). The immunological methods have been replaced largely by DNA profiling techniques, reviewed by Kent (2009). Host specificity is a function of both the mosquito's innate host preference and the hosts available to the mosquito when and where it is active. Some species forage over a broad range of habitats. Others are active principally in either wooded or open areas or remain close to sites of larval development or adult resting. Still other species attack their hosts in rather narrowly defined zones within a habitat. For example, within tropical forests, different species feed in different vertical strata: ground level, intermediate levels, or just below the leaf canopy.

Host-finding behavior in mosquitoes usually involves the use of volatile chemicals to locate vertebrate hosts. Carbon dioxide, lactic acid, and octenol are among the most widely documented host attractants. Other skin emanations also are known to be important, because odors from live hosts are always more attractive than any combination of those three chemicals in a warm, humid airstream. Several fatty acids produced by the normal bacterial flora of the skin are particularly effective in attracting *Anopheles gambiae* to human feet. Mixtures of these fatty acids and other compounds probably play a major role in attracting most mosquitoes (see Smallegange and Takken, 2010). Subtle differences in these odors of different host species and even different individuals undoubtedly play a role in host preference. These odors commonly have a combined effective range of 7−30 m, but up to 60 m for some species.

Vision also is important in orienting to hosts, particularly for diurnal species and especially in an open environment and at intermediate or close ranges. Dark, contrasting, and moving objects are particularly attractive. As the female approaches to within one to 2 m of a potential host, chemical and visual cues are still important, but convective heat and humidity surrounding the body also

come into play. Odors, carbon dioxide, heat, and humidity all are detected by sensilla on the antennae and palps. In the special case of some frog-feeding species, the calls of frogs serve as auditory attractants.

Host-finding behavior in mosquitoes was reviewed by Bowen (1991) and Cardé and Gibson (2010). Specific behavioral and physiological aspects of attraction have been reviewed by several authors (Anon., 1994). Takken and Verhulst (2013) have reviewed the entire subject of mosquito host preferences.

If the suite of **host stimuli** is acceptable, the female attempts to land on the host animal, often preferring certain body parts, such as the head or legs. On landing, she proceeds through four phases of feeding behavior: exploration, penetration and vessel-seeking, imbibing, and withdrawal. She typically remains motionless for a few seconds, then begins exploratory movements, including contacting the skin surface with her proboscis in probing motions. If the host is not suitable, she may wander for a considerable time and leave without feeding. Even on a suitable animal she usually explores at least briefly before selecting a spot that is likely to be well vascularized. Probing activity is stimulated by heat and moisture, and probably also by chemicals on the surface of the skin. As in the case of airborne attraction, these stimuli are detected by antennal and palpal receptors, but receptors on the proboscis, tarsi, and elsewhere on the legs apparently also are important.

FIGURE 15.19 Female mosquito (*Haemagogus lucifer*, a vector of jungle yellow fever), showing position of mouthparts during blood-feeding. The fascicle is exsheathed from the labium along part of its length while the labium buckles backward, allowing the fascicle to enter the skin in search of a blood vessel. *Photograph by Woodbridge A. Foster.*

Adult blood-feeding has been studied extensively. Mosquitoes can feed from a variety of skin surfaces, including the moist skin of frogs and the scaly legs of reptiles and birds. They can penetrate mucus, matted hair, light layers of feathers, and heavy cloth such as denim, provided it is not thicker than the length of the proboscis. Once a feeding site is selected, the fascicle of stylets pierces the skin while the labium serves as its guide and is bent backwards without penetrating (Fig. 15.19). The maxillae and mandibles on each side of the fascicle alternately slide by each other in quick stabbing/puncturing movements. While doing so the tissue is gripped with the backward-directed maxillary teeth as the stylets penetrate epidermal and subepidermal tissue.

Saliva flows from the tip of the hypopharynx as the flexible end of the fascicle bends at sharp angles, probing in various directions within the subepidermal tissue in search of a small arteriole or venule. The saliva contains an antihemostatic enzyme, apyrase, which inhibits platelet aggregation and causes randomly punctured vessels to bleed freely into the surrounding tissue spaces. This makes it easier for the mosquito to locate a vessel and shortens the total time on the host. The saliva also contains anticoagulants, which facilitate vessel location and blood ingestion by preventing the blood from clotting. Sensilla on the labrum and in the cibarium apparently detect plasma and cellular factors, including adenyl nucleotides such as ATP, which help the mosquito to locate a blood vessel and stimulate ingestion. Upon finding a vessel, the female slips the tip of her fascicle into the lumen and draws blood up through the food canal by pumps in the cibarium and pharynx. The blood accumulates in the midgut, allowing the mosquito to engorge fully in 1–4 min.

During this time, the female begins to extract water from the bloodmeal and may deposit small droplets of urine on the host's skin. In some *Anopheles* spp., this fluid is copious, to a degree coming directly from the midgut, rather than being processed through the malpighian tubules. It includes some blood cells from the accumulating meal and appears red. When abdominal stretch receptors signal the presence of sufficient blood in the midgut, the female pushes with her forelegs to withdraw her stylets and flies away. Usually she is too heavy to fly far until a substantial amount of water and salt in the bloodmeal has been excreted in the urine, after 1–2 h.

While digesting the meal and developing eggs, females locate species-characteristic **resting sites** and may remain there until the eggs are mature. However, females of many species are known to leave their resting sites during each daily activity period throughout the gonotrophic cycle. These flights allow them to obtain sugar meals or supplementary bloodmeals, to relocate closer to an oviposition site, or perhaps simply to find a more suitable resting site. In at least some species, a hormone from the ovaries in the trophic phase inhibits the mosquito's responsiveness to host attractants, by blocking host-odor receptors on the antennae, provided she has substantial energy reserves (see Klowden, 1996, for a concise review).

The interval of time between bloodmeals, an important component of **pathogen transmission**, is determined by two things: the occurrence of supplementary bloodmeals within the gonotrophic cycle and the duration of the cycle. The latter depends not only on how fast the blood is digested and eggs matured but also on how soon the female lays her entire batch of eggs. In addition, feeding success and frequency are modified by pathogens that appear to manipulate the mosquito to enhance its likelihood of transmission between vertebrate hosts. For example, the malaria parasite *Plasmodium* interferes with salivation, causing the mosquito to pierce one or more hosts and inject the parasite multiple times within a single night. Furthermore, infected mosquitoes are more strongly attracted to hosts, and infected hosts are more attractive to mosquitoes than are uninfected hosts. Some of these interactions have been reviewed by Cator et al. (2012).

Oviposition generally occurs during the same part of the day as mating and feeding. Gravid females locate and evaluate suitable sites by using chemical and visual cues, including organic chemicals, salts, high humidity, dark cavities, and reflective surfaces. The organic chemical cues are derived from decaying organic matter, microorganisms, the chemical byproducts of larvae or pupae that have previously developed there, and the presence of mosquito eggs that have been deposited by other females. The apical droplets on the eggs of *Culex quinquefasciatus* contain an oviposition-attractant pheromone.

Within each genus of mosquito, there is considerable variation among species in their **oviposition site preferencs**, and therefore their larval habitats. In general, *Anopheles* spp. occur in permanent or semipermanent water, such as the edges of lakes, ponds, streams, and pools; others develop in temporary rain puddles, leaf axils, and tree holes. *Culex* typically lay eggs in permanent or semipermanent pools, ponds, and water containers. Several medically important species of both *Anopheles* and *Culex* develop in water with large surface areas, taking advantage of irrigated fields and reservoirs created by dams. *Culiseta* are found in several kinds of permanent surface pools; some species have very narrow requirements. *Coquillettidia* and *Mansonia* oviposit in permanent bodies of water that contain floating or emergent aquatic plants to which the submerged immatures can attach.

Aedes, *Psorophora*, and *Haemagogus* spp. lay their eggs on damp surfaces where they will be inundated by temporary water or a rising water level. *Aedes* spp. and ecologically similar mosquitoes form two general categories, according to typical habitat: (1) **floodwater mosquitoes**, which include floodplain species, saltmarsh species, and snow pool and spring species—some floodplain species have become prolific in rice fields and other forms of irrigation; (2) **container mosquitoes**, including leaf-axil species, tree-hole species, and artificial-container species. Several medically important species use both tree holes and artificial containers. Among genera of minor importance, *Toxorhynchites* lay their eggs only in natural and artificial containers in wooded areas; *Wyeomyia* oviposits primarily in leaf axils, liana crevices, flower bracts, or pitcher plants, depending on the species, and *Deinocerites* oviposits exclusively in crab holes in tidal mudflats and mangrove swamps.

The distribution of mosquito eggs reflects the availability, size, and stability of the larval habitats used by a species. Though mosquito life histories are highly variable, the oviposition behavior of mosquitoes tends to follow along taxonomic lines. Most mosquitoes fall into one of three behavioral categories:

1. **Eggs laid out of water**. Species in this group may distribute eggs of a single clutch individually among several widely scattered potential development sites, particularly if those sites are common but small. Container species such as *Aedes aegypti*, *Ae. albopictus*, and *Haemagogus* species deposit the eggs at varying distances above the water line, and at least *Ae. aegypti* lays only a portion of the clutch in each water container, sometimes called skip oviposition. Floodwater species such as *Ae. vexans*, *Ae. dorsalis*, and the saltmarsh-inhabiting *Ae. taeniorhychus* generally scatter their eggs widely over areas where water will accumulate, inserting them into crevices of drying mud or plant debris in low ground.
2. **Eggs placed on or in water**. Mosquitoes in this category lay the entire clutch in a clump at one site while standing on the water surface or on floating vegetation. The egg rafts of *Culex*, *Culiseta*, *Coquillettidia*, and *Uranotaenia* spp. are formed between the female's hindlegs as she deposits each egg on end in the water, one against the next. Some *Armigeres* spp. suspend the egg raft above the water with their hindlegs while forming it and then carry it with them before placing it on the water. *Trichoprosopon digitatum* females stand guard over the raft until the eggs hatch. *Mansonia* spp. prepare their egg clusters underwater, attached to a

plant, while standing on the floating leaves of aquatic plants. Exceptional species in various genera deposit egg rafts on top of floating vegetation, lay their eggs singly underwater on the sides of rock pools, or enter beetle holes in bamboo and extrude the eggs in ribbons.

3. **Eggs dropped onto water**. Species in this group oviposit aerially while hovering. *Anopheles* drop all of them at one site or distribute them among several smaller sites. *Toxorhynchites* and most culicine species in the tribe Sabethini (e.g., *Wyeomyia* and *Sabethes*) propel a few eggs into each of many container habitats with a flick of the abdomen, often through small openings in tree limbs or bamboo. If a mosquito cannot find suitable oviposition sites when the eggs are mature, it may lay them in suboptimal situations or retain them until a suitable site is found. Retained eggs gradually lose their viability over several weeks or months. An extensive review of oviposition behavior is given by Bentley and Day (1989).

Dormancy occurs in all species of mosquitoes except those living in tropical and subtropical habitats that provide conditions for year-round larval development. The life stage that becomes dormant depends on the severity of a region's winter or dry season and on the species. Species of *Aedes*, *Psorophora*, and *Haemagogus*, which all have quiescent eggs requiring inundation regardless of season, typically overwinter (hibernate) or oversummer (aestivate) as embryonated eggs. Larvae serve as the dormant stage in mosquitoes whose adult activity is precluded seasonally but whose breeding sites are protected from severe cold or complete drying. When adults overwinter as the dormant stage, typically in *Anopheles* and *Culex*, they seclude themselves in well-protected harborages, or **hibernacula**.

Prior to dormancy, mosquitoes often enter **diapause**, a physiological state of arrested development that is induced or broken only by specific environmental cues. Mosquito diapause is reviewed by (Denlinger and Armbruster, 2014). **Facultative egg diapause**, a feature of multivoltine species, is induced by exposure of the pupae or adult females to lowered temperatures and short photoperiods. They lay diapausing eggs, which will not hatch until the day length is appropriate, even during unseasonably warm periods in autumn, winter, or early spring, when the resulting larvae and adults might not survive. **Obligate egg diapause** occurs in univoltine species, regardless of preceding conditions, and is maintained despite warm, long-day conditions. This is typical of snow pool and spring *Aedes* spp. in cold and temperate climates. Diapause is broken after the eggs have been subjected to winter conditions (in the case of obligate diapause) and when favorable temperatures and long days resume. **Larval diapause** is similar to facultative egg diapause in its induction and termination. Diapausing larvae feed and grow little or not at all, and they do not molt.

Temperate species destined to overwinter as adult females emerge in a state of **reproductive diapause** induced by larval and pupal exposure to shortening photoperiod and cool temperatures. Although these females mate, their egg follicles do not reach the resting stage, despite frequent sugar-feeding and accumulation of extensive fat reserves. Fattened female *Culex* spp. that hibernate through hard winters forego all further feeding until the onset of spring, whereas in milder climates they periodically leave their overwintering sites to take sugar meals.

Although diapausing *Culex* adults rarely feed on blood, some *Anopheles* species may take bloodmeals fairly regularly from hosts near these sites. They develop no eggs, however, exhibiting **gonotrophic dissociation**. Other overwintering *Anopheles* continue to feed and develop eggs, but these are not laid. Similarly, some tropical *Anopheles* take blood repeatedly during the dry season and remain continually gravid because there is nowhere to oviposit. These phenomena are sometimes referred to as **gonotrophic discordance**, a term that also applies to the taking of nonvitellogenic or otherwise supplementary bloodmeals, mentioned previously.

GENETICS

The formal and molecular genetics of moquitoes have been investigated in detail and remain the subject of intensive research, owing to two objectives: (1) controlling vector populations (Rai, 1996; Alphey, 2014; Burt, 2014) and (2) understanding genotype/phenotype relationships, such as the genetic basis for vector competence (Beerntsen et al., 2000) and insecticide resistance (Liu, 2015).

Mosquitoes typically have three pairs of **chromosomes**, with a single species (*Chagasia baffana*, of the Anophelinae) having four. Two pairs are autosomes and the third pair are sex chromosomes, which may be heteromorphic (anophelines) or homomorphic (culicines). Sex is determined by chromosomal heteromorphy in the former and by a sex-determining locus in the latter. In anophelines, males are the heterogametic sex and females are homogametic. When the sex-determining gene *Nix*, operating at the sex locus M, was knocked out in male *Aedes aegypti*, they were feminized; and when it was expressed in females, they developed male genitalia (Hall et al., 2015). This study thus isolated the genetic factor determining male sex in *Aedes aegypti* and illuminated the role of the *doublesex* gene in determining female sex.

Phenotypic expression of sex traits is also modulated in some groups of mosquitoes by temperature, such as when larval mosquitoes normally developing in cold water are forced to develop in warm water.

Formal genetics of mosquitoes has included isolation of morphological (e.g., eye color) and biochemical (e.g., enzymatic resistance to DDT) mutants. Crosses

between traits have revealed their Mendelian relationships, such as their segregation or linkage tendencies with other genes. Chromosomal morphology, karyotyping, description and quantification of translocations and crossovers, and genetic mapping are all well-developed endeavors in the field of mosquito research, using the banding patterns of polytene chromosomes. This research has yielded a wealth of information on genome structure; on population genetic structure; on species complexes in *Culex*, *Aedes*, and *Anopheles*; and on the potential to use genetic constructs such as translocation homozygotes or sterile-hybrid mating as tools for vector control. In their review of mosquito genomics, Severson and Behura (2012) note that genome sequence data for *Anopheles gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus* "have revealed detailed information on genome architecture as well as phenotype-specific transcriptomics and proteomics. These resources allow for detailed comparative analyses within and across populations as well as species."

The **genomes** of 16 *Anopheles* spp. have been sequenced (Neafsey et al., 2015). Although incomplete annotation and mapping of these genomes present challenges, there has been substantial progress in genome manipulation and genome engineering, using such tools as zinc-finger nucleases, TAL-effector nucleases, homing endonucleases, and the bacterial type II CRISPR-cas9 system (Kistler et al., 2015). Applications of these tools include dominant lethal constructs (Alphey, 2014) and mechanisms to drive beneficial genes into populations (Burt, 2014).

PUBLIC HEALTH IMPORTANCE

Mosquitoes are of public health significance because they feed on human blood. Blood-feeding compromises skin, presenting the possibility of secondary infection with bacteria. It introduces foreign proteins with saliva that stimulate histamine reactions, causing localized irritation, and that may be antigenic, leading to hypersensitivity; and it allows for acquisition and transmission of microorganisms that cause infection and disease in humans, domestic animals, and wild animals. Mosquito-borne diseases are caused by three groups of pathogens: viruses, malaria protozoans, and filarial nematodes. Mosquitoes are not known to transmit pathogenic bacteria to humans, with the exception of mechanical transmission of the causative agents of tularemia (*Francisella tularensis*) and anthrax (*Bacillus anthracis*).

Mosquito Bites

In addition to the tremendous impact of mosquitoes on human health as vectors of disease pathogens, the bites themselves are important. Aside from the mosquito's annoying flight and buzzing sound, a single bite can be irritating and a distracting nuisance. In Rangoon, Myanmar, *Culex quinquefasciatus* has been estimated to have densities of 15 million per square kilometer, and residents in poor districts receive 80,000 bites by this species per year. In Burkina Faso, in West Africa, residents of cities are estimated to experience 25,000 bites by this species per year. In northern Canada, the spring melt of snow brings with it hordes of snow pool *Aedes*; counts on an exposed human forearm can be as high as 280 to 300 bites per minute. It has been estimated that this rate of biting, without protection, can reduce the total blood volume in a human body by half in 90 min.

As with the other blood-feeding arthropods, the wound created at the bite site may allow secondary infection by bacteria, which can be exacerbated by scratching. In the absence of prior exposure to mosquitoes, a bite rarely produces more than a temporary tingling or burning sensation and sometimes a tiny spot of blood on or just beneath the surface of the skin. After one or more bites within a brief time, the proteins in mosquito saliva, which are injected both before and during feeding, normally stimulate the immune system so that subsequent bites give rise to one or both of two general kinds of cutaneous allergic response: **immediate reactions** and **delayed reactions**. The immediate reaction, called type I hypersensitivity, is an antibody-based inflammation of the skin known as **wheal-and-flare** caused by the release of histamine and arachidonic acid from antibody-sensitized mast cells. It usually starts within minutes of the bite and lasts a few hours at most. The typical delayed reaction, designated type IV hypersensitivity, involves a cellular immune response caused by lymphokines that are secreted by antigen-sensitized T cells. Both delayed and immediate reactions result in itching, redness, and swelling. The typical delayed reaction takes about 1 day to develop, may last for up to a week, and tends to result in a larger wheal with a deeper discoloration. The appearance of these two reactions, starting with the initial bites, changes after repeated bites by the same genus or species of mosquito over a period of days, weeks, and months. This timing, known as the Jones-Mote hypersensitivity series, typically proceeds as follows: (1) no reaction, (2) delayed only, (3) immediate and delayed, (4) delayed only, and, in some cases, (5) absence of any skin reaction (i.e., desensitization).

Mosquito-Borne Viruses

Among the more than 620 viruses associated with arthropods and registered in the US Centers for Disease Control and Prevention's Arbovirus Catalog. Somewhat less than half have biologic relationships with mosquitoes, and about 100 infect humans. The term **arbovirus** is a contraction of "arthropod-borne virus" and has no formal taxonomic meaning. The most significant mosquito-borne viruses causing human illness belong to four genera in three families (Table 15.2): the **Togaviridae**, genus

TABLE 15.2 Geographic Distribution of Selected Mosquito-Borne Viruses of Importance to Humans or Domestic Animals

Family (Genus)	Virus Species and Serotypes	Distribution
Togaviridae (*Alphavirus*)	Eastern equine encephalomyelitis	Americas
	Venezuelan equine encephalomyelitis	South and Central America, Mexico, United States (Florida)
	Western equine encephalomyelitis	North America, Mexico, South America (eastern)
	Chikungunya	Africa, Asia, incl. Philippines, South, Central, and North America
	O'nyong nyong	Africa
	Ross River	Australia, New Guinea, Fiji, American Samoa
	Semliki Forest	Africa, Asia, incl. Philippines
	Mayaro	South America (northern), Trinidad
Flaviviridae (*Flavivirus*)	Dengue (4 serotypes)	Tropics, especially southern Asia and Caribbean
	Yellow fever	Africa, Central and South America
	St. Louis encephalitis	Americas
	Murray Valley encephalitis	Australia, New Guinea
	Japanese encephalitis	Asia (eastern), incl. Philippines
	West Nile	Africa, Europe, Israel, Asia; South, Central, and North America
	Ilheus	Central and South America
	Rocio	Brazil
	Wesselsbron	Africa, Asia (southern)
	Zika	Africa, Asia, South Pacific, South and Central America, Caribbean, southern North America
Bunyaviridae (*Orthobunyavirus*)	Bunyamwera	Africa
	Germiston	Africa
	Ilesha	Africa
	Wyeomyia	Central America
	Itaqui	South America
	Marituba	South America
	Murutucu	South America
	Oriboca	South America
	Madrid	Central America
	Nepuyo	Central and South America
	California encephalitis	United States (western)
	Jamestown Canyon	North America
	La Crosse encephalitis	United States (eastern)
	Inkoo	Finland
	Tahyna	Europe
	Guaroa	South America
Bunyaviridae (*Phlebovirus*)	Rift Valley fever	Africa (northern, eastern)

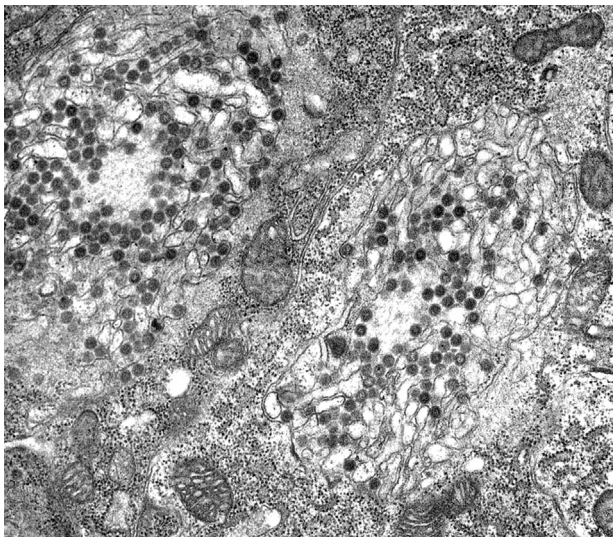

FIGURE 15.20 Rift Valley Fever virus virions in salivary gland tissues of *Culex pipiens* female, 42,000 X. *Courtesy of K. Lerthudsnee and W. S. Romoser.*

Alphavirus; the **Flaviviridae**, genus *Flavivirus*; and the **Bunyaviridae**, genera *Orthobunyavirus* and *Phlebovirus*. Some of the arboviruses infect both humans and animals and may cause disease in both. A representative of these anthropozoonotic arboviruses, Rift Valley fever virus, is shown in Fig. 15.20.

Aside from their genomic organization and morphology, mosquito-borne viruses may be viewed in terms of disease symptoms they cause. In humans, generally speaking, the mosquito-borne viruses cause infection with either no apparent symptoms, or acute disease of either systemic febrile illness (fever), encephalomyelitis (inflammation of the brain and spinal cord), hemorrhagic fever (bleeding and fever), or febrile myalgia and arthralgia (fever with muscle and joint pain, or arthritis). The case-fatality rate tends to be low for fevers, although morbidity (illness) may be high. For the encephalitis and hemorrhagic fevers, morbidity and mortality may range from low to high, depending on the virus and factors such as age. After the acute phase, humans either recover fully or show various sequelae, such as neurological problems after acute encephalitis. Long-term, chronic infection with the mosquito-borne viruses does not occur in humans, although the consequences of infection may be long lasting.

Table 15.2 is a list of the more important mosquito-borne arboviruses and their geographic distribution. The viruses may be classified hierarchically as follows: family, genus, serogroup, complex, species, serotype, and strain. The word **strain** is used to refer to different viruses of the same serotype that were isolated from different locations or different biologic sources or that show minor differences in antigenicity or genotype but not enough to justify elevating them to the varietal level. For example, Altamont virus is an eastern New York State strain of La Crosse virus, which along with Snowshoe hare virus is a serotype of the species California encephalitis virus in the California encephalitis virus complex of the California serogroup in the genus *Orthobunyavirus*, family Bunyaviridae. The relationships among the viruses are determined on the basis of similarities and differences in antigenic reactions to antibodies in immunological tests, and on genetic relationships determined by molecular analyses (i.e., nucleotide sequences or oligonucleotide fingerprint patterns). Arbovirus classification is presented by Fauquet et al. (2005).

Mosquito-borne viruses multiply in both invertebrate and vertebrate cells. Many arboviruses cause cytopathic effects and cell destruction in vertebrate cells; in invertebrate cells the same viruses typically cause a chronic cellular infection without cytopathology. Competent mosquitoes become infected when they feed on blood of a viremic vertebrate host in which there is sufficient circulating virus in its blood to provide an infectious dose to the mosquito. After blood has entered the midgut, the virions bind to and then pass through the microvillar membrane and into midgut epithelial cells. Within these cells, viruses replicate and virions bud off from the cells, pass through the basal lamina, and enter the hemolymph. Virions disseminate throughout the body of the mosquito and may infect and replicate in a variety of tissues, including salivary glands, fat body, ovaries, and nerves. A mosquito with a salivary-gland infection (Fig. 15.20) can transmit infectious virions during salivation as it probes the tissues of another vertebrate host. In some mosquitoes and for some viruses, **transovarial transmission** of virions occurs from the female mosquito to her progeny, and females of the next generation can transmit the virus orally without having become infected by a prior bloodmeal. Also, **venereal transmission** of some arboviruses from male to female mosquito has been documented experimentally. The rate of virus infection and dissemination in mosquitoes is temperature-dependent; higher temperature results in shorter extrinsic incubation. Virus infection may harm mosquitoes in some cases, and they become infected for life.

Because the flaviviruses, alphaviruses, and bunyaviruses have RNA genomes and can replicate in both invertebrate and vertebrate hosts, these viruses have a high capacity for rapid evolution into antigenically variable strains of varying virulence. This capacity for change is important, because it may result in emergence of virulent strains; indeed there is evidence for this process (see sections on Chikungunya, O'nyong nyong, and Rocio encephalitis viruses, later).

The literature on the mosquito-borne viruses is voluminous. Extensive reviews are presented in the multivolume series edited by Monath (1988), in Strickland

TABLE 15.3 Relationships of Selected Alphaviruses to Mosquito Vectors and Vertebrate Reservoir Hosts

Virus Species	Vector(s) Reservoirs	Vertebrate
Eastern equine encephalomyelitis	*Culiseta melanura, Aedes sollicitans, Coquillettidia perturbans, Culex nigripalpus, Culex (Melaniconion)* spp.	Birds
Western equine encephalomyelitis	*Culex tarsalis, Culex (Melaniconion)* spp., *Aedes albifasciatus, Aedes melanimon, Aedes dorsalis*	Birds, lagomorphs
Venezuelan equine encephalomyelitis[a]	*Culex (Melanoconion)* spp., *Aedes, Psorophora, Anopheles, Mansonia* spp.	Rodents
Chikungunya	*Aedes aegypti, Aedes africanus*	Primates, incl. humans
O'nyong nyong	*Anopheles* spp.	Humans
Ross River	*Culex annulirostris, Aedes vigilax, Aedes polynesiensis*	Humans, rodents

[a]*See Table 15.4 for elaboration of this virus complex.*

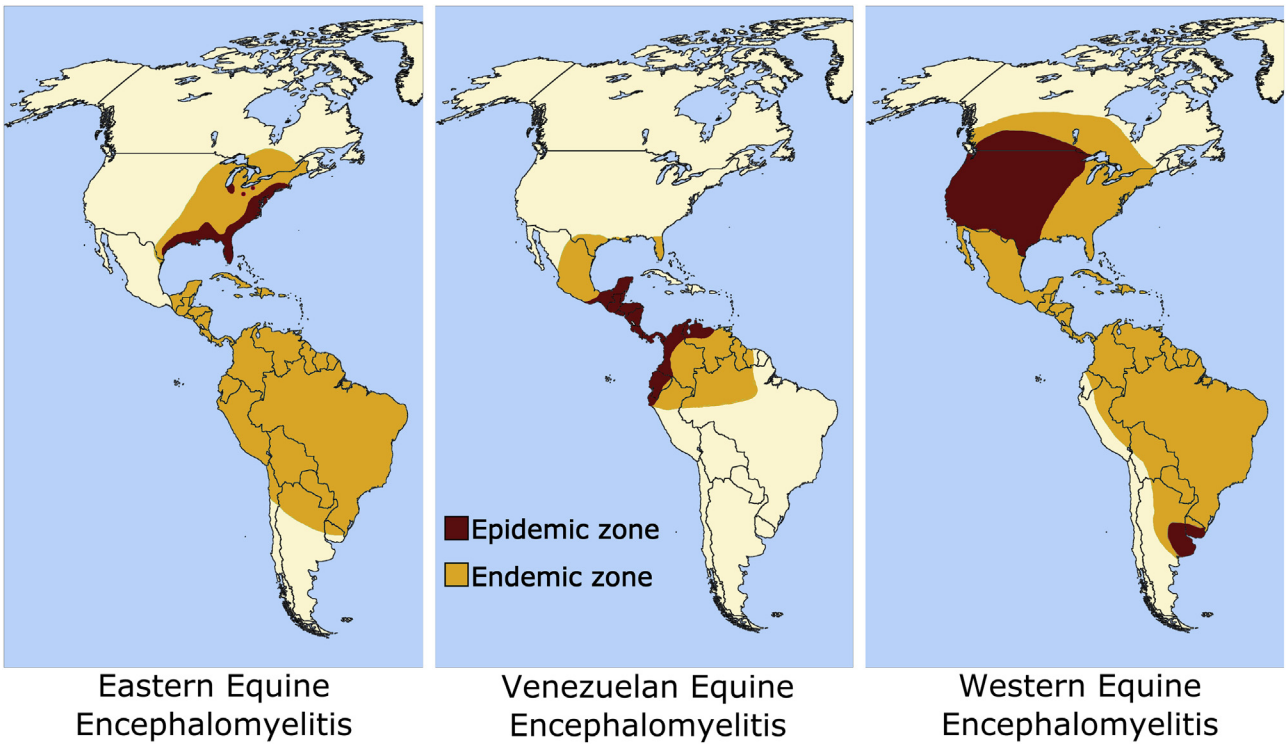

FIGURE 15.21 Endemic distribution and epidemic zones of mosquito-borne alphaviruses that cause encephalitis in the New World. *Reconstructed from Mitchell, 1977, and other sources.*

(1991), in the sections on Togaviridae, Flaviviridae, and Bunyaviridae by Fields et al. (1996), in Reeves (1990), and in Clements (2011), as well as in references mentioned under specific groups later and at the end of this chapter.

Togaviridae (*Alphavirus*)

The Togaviridae, genus *Alphavirus*, contains seven antigenic complexes of viruses involving 37 types, subtypes, and varieties distributed worldwide, 35 of which have been isolated from mosquitoes. Many of these are medically important. Table 15.3 summarizes relationships between primary mosquito vectors and important alphaviruses, whereas Fig. 15.21 shows their distribution in the Western Hemisphere.

Eastern equine encephalomyelitis (EEE) virus. This virus is distributed in South America, Central America, the

Caribbean basin, and eastern North America. Analyses of geographic strains of EEE virus have revealed two varieties: South American and North American/Caribbean. The virus has been isolated from many different states in the United States, but most cases of human or equine disease are in coastal states from Massachusetts to Louisiana, an area of upstate New York near Syracuse, a swamp focus in east-central Ohio, and southern Michigan and part of northern Indiana.

Eastern equine encephalomyelitis virus is one of the most pathogenic among all of the mosquito-borne encephalitis viruses. In humans, disease caused by EEE virus infection results in high morbidity and mortality. The type and severity of illness in humans depend on the age and health status of the individuals. Children, the elderly, immunocompromised individuals, and sometimes apparently healthy adults develop acute encephalitis with high fever, drowsiness, lethargy, vomiting, convulsions, and coma. Mortality rates among clinical cases exceed 50%. Individuals who survive infection often show neurologic sequelae, although some survivors recover completely, sometimes showing rapid and dramatic improvement from coma.

In the eastern United States, EEE virus occurs in a bird−mosquito enzootic cycle in swamps that support the biology of the enzootic vector, *Culiseta melanura*. The swamps comprising EEE foci are characterized in the northern distribution of its range by northern white cedar, black spruce, tamarack, or red maple trees, typical of swamp or bog ecosystems. Larvae of *Cs. melanura* occur in water-filled cavities underneath raised tree hummocks, water-filled depressions formed by uprooted trees, and in holes in bog mats. Adults feed on birds in the swamp and may leave swamps for open areas to locate hosts, returning later to oviposit. *Culiseta melanura* females are highly efficient vectors of EEE virus and transmit it primarily to swamp-dwelling passeriform birds. More than 48 species of wild, native birds have shown evidence of infection. The mechanism of overwintering of EEE virus is unknown. Studies in New Jersey indicate that the virus may recrudesce in resident birds in the spring, whereas other studies incriminate reptiles as overwintering hosts. Still other scenarios suggest that this virus is introduced by migrating birds from southern regions where viral transmission may occur year-round.

In some summers, for reasons possibly related to weather patterns and density of bird and mosquito hosts, EEE virus in its enzootic swamp setting becomes amplified to high levels so that epizootics and epidemics develop. Certain mosquito species function as bridge vectors, especially *Aedes sollicitans* in coastal areas and *Coquillettidia perturbans* in inland areas. They acquire virus infection by feeding on viremic birds, later blood-feeding on mammals and transmitting the virus to them. In Central and South America, EEE virus apparently circulates among rodents and birds through mosquitoes in the *Culex* (*Melanoconion*) group; however, the relationships among vectors and vertebrate hosts involved in enzootic, epizootic, and epidemic cycles of EEE virus in these regions are poorly known compared with in North America.

Epidemics and concurrent epizootics have been documented in the eastern United States since the 1930s, generally involving cases in horses, pheasants, and humans. The virus was first isolated from brains of horses that died during a 1933 epizootic along the eastern coast of the United States, in Virginia, Delaware, and Maryland. Disease in humans was first recognized in 1938 in Massachusetts. Generally, outbreaks involve many horse cases and very few human cases. Nearly all outbreaks involving human cases have occurred along the eastern coast of the United States. From 1964 to 1995, a total of 151 human cases of EEE infection were reported in the United States, with an average of about five cases (range, usually 0 to 14) per year. From 2007 to 2016, there were 70 diagnosed cases, including 28 deaths. The greatest number of human cases occurred in 1959, when 36 were documented, mainly in New Jersey. Morris (1988) reviewed the ecology, epidemiology, and vector relationships of EEE.

Western equine encephalomyelitis (WEE) virus. This virus is a complex of six types and six subtypes. All are mosquito-borne, with the exception of **Fort Morgan virus**, which is associated with cliff swallows and their parasitic cimicid bugs (*Oeciacus vicarius*) in western North America. The other viruses are widely distributed across the high plains of the United States and Canada, in California, and through Central and South America. The WEE virus type that occurs in North America has been responsible for acute encephalitis in horses and humans. An apparently less pathogenic virus, **Highlands J virus**, is closely related to Fort Morgan virus, yet it exists in the same basic North American enzootic cycle as EEE virus.

Disease caused by WEE virus in humans is less severe than that caused by EEE virus, and some infections are unapparent. Symptoms are generally similar, with acute onset of meningitis or encephalitis with headache, fever, drowsiness, and coma and death in severe cases. Fatality rates are in the range of 5% or less. Most morbidity and mortality occur in infants rather than teens, adults, or the elderly. Neurologic sequelae are often evident in survivors.

In North America, WEE virus apparently exists enzootically in at least two different cycles. The primary cycle involves passerine and columbiform birds (especially house sparrows, house finches, blackbirds, orioles, and mourning doves), with *Culex tarsalis* functioning as the enzootic, epizootic, and epidemic vector. Nestling birds, which are more exposed to mosquito bites, may be more

important as virus amplifier hosts than adult birds. The other cycle involves jackrabbits as vertebrate hosts and *Aedes melanimon* or *Ae. dorsalis* as vectors in California, Utah, and Colorado. Ground and tree squirrels also may function as vertebrate hosts in some areas, and in the central United States *Ae. trivittatus* may be a secondary vector. In Central and South America, WEE virus apparently circulates in nature among *Culex* (*Melanoconion*) mosquitoes, although a cycle involving *Ae. albifasciatus* and lagomorphs has been elucidated in Argentina.

Epizootics and epidemics of WEE in North America appear to be related to cumulative summertime precipitation and wintertime snowpack development, both of which can increase populations of *Culex tarsalis* and favor virus transmission. The larvae of this mosquito are often associated with agricultural irrigation and vernal flooding. Areas that normally are dry can produce large populations of *Cx. tarsalis* if irrigation activities result in the accumulation of pools of still water long enough to support larval development. For example, *Cx. tarsalis* populations burgeon after winter rain and vernal snow melt result in inundation of saltwater marshes along the north shore of Salton Sea in southern California (USA). Western equine encephalitis virus is detected in *Cx. tarsalis* populations about 2 months after these populations increase, during March through June. The virus spreads to upland sites to the northwest of the sea, where mosquitoes are produced primarily from poorly maintained irrigation systems. The movement of WEE virus along this corridor is probably due to dispersal of infected *Cx. tarsalis* rather than to movement of birds. The mechanism by which WEE virus overwinters at this site, or is introduced there, is not known.

WEE virus was first isolated in 1930 from the brain of a dead horse in the San Joaquin Valley of California (USA). It later was identified as the causative agent of human disease in a child who died of encephalitis in the San Joaquin Valley of California in 1938. Since that time, WEE virus has been implicated in epizootics in horses and concurrent epidemics in humans, with cases numbering from hundreds to thousands in some instances. Large outbreaks occurred in 1941 and 1975 in the Red River Valley in Minnesota and North Dakota (USA), and Manitoba (Canada), in 1952 in the Central Valley of California, and in 1965 in Hale County in western Texas (USA). There are horse cases almost every summer within the range of the virus, but epizootics do not occur every summer. From 1964 to 1995, a total of 639 human cases of WEE infection were reported in the United States, with an average of about 20 cases (range, 0–172) per year. Reisen and Monath (1988) provided a review of WEE.

Venezuelan equine encephalomyelitis (VEE) virus. This is complex of 12 viruses that cause disease in humans and equids (horses, burros, and mules) and occurs in northern South America, Central America and Mexico, occasionally extending into Texas (Walton and Grayson, 1988). These viruses exist as either enzootic or epizootic varieties and strains, with overlapping or disjunct geographic distributions and with variable vector and vertebrate–host relationships. Table 15.4 shows the classification and vector associations of these viruses.

Many VEE "enzootic" virus subtypes and varieties exist in cycles involving rodents and mosquitoes of the *Culex* (*Melanoconion*) group, such as *Culex ocossa*, *Cx. panocossa*, and *Cx. taeniopus* in Central and South America. Rodents in the genera *Sigmodon*, *Oryzomys*, *Zygodontomys*, *Heteromys*, *Peromyscus*, and *Proechimys* are important vertebrate hosts; birds, opossums, and bats also may be reservoir hosts. The ecology of the epizootic viruses is quite different. A large number of species of mosquitoes in several different genera (see Table 15.4) have been implicated as vectors of the epizootic/epidemic virus strains. Equids attain sufficient viremia to infect these mosquitoes. VEE epidemics can be maintained by mosquito–equid–mosquito transmission, unlike WEE and EEE epidemics, in which equids are for the most part dead-end hosts. Wading birds, particularly green herons, have been incriminated as vertebrate hosts of epizootic strain IA-B in Panama, with the crab hole mosquito *Deinocerites pseudes* functioning as vector. Persistence of epizootic strains of VEE in interepidemic periods is not well understood, thus their emergence in epidemics among equids and humans is difficult to predict.

The single representative of the VEE viruses in the United States, other than during epizootics in Texas, occurs in the Everglades region of southern Florida. Called **Everglades virus**, it is associated with *Culex* (*Melanoconion*) *cedecei* (formerly, *Cx. opisthopus*) as a vector and cotton rats (*Sigmodon hispidus*) and cotton mice (*Peromyscus gossypinus*) as vertebrate hosts. The zoonotic setting is the hardwood hammocks of the Everglades, where mosquito and rodent habitats overlap. Serosurveys of Seminole and Miccosukee Indians in these regions have shown that many Indians have antibodies to VEE virus, but there have been very few cases of human disease attributable to this virus.

Humans infected with an epizootic or certain enzootic strains of VEE virus may show no symptoms, only mild flulike symptoms, or severe encephalitis with acute onset of vomiting, headache, seizures, and fever. Symptoms tend to be most severe in children. During epidemics, the mortality rate is typically less than 1%, although in some epidemics the mortality rate has been considerably higher.

The VEE viruses in Central America and northern South America have been intensively studied because of the history of epidemics among equids and humans in these regions. Outbreaks of VEE have occurred periodically in South America, Central America, and Mexico since the 1930s. The first VEE virus was isolated in 1938 from a dead horse in Venezuela. In 1969, a large outbreak of VEE

TABLE 15.4 Venezuelan Equine Encephalomyelitis Virus

Subtype	Variety	Name	Vector Associations	Geographic Distribution
I	A-B[a]	–	*Aedes, Psorophora, Mansonia, Anopheles, Deinocerites pseudes*	Central America, South America (northern)
	C[a]	–	(same as IA-B)	Central America, South America (northern)
	D	–	*Culex* (*Mel.*) *ocossa* *Culex* (*Mel.*) *panocossa*	Central America, South America (northern)
	E	–	*Culex* (*Mel.*) *taeniopus*	Cental America
	F	–	Unknown	Brazil
II		Everglades	*Culex* (*Mel.*) *cedecei*	United States (southern Florida)
III	A	Mucambo	*Culex* (*Mel.*) *portesi*	South America (northern)
	B[b]	Tonate	Unknown	South America (northern)
	C	–	Unknown	Peru
IV		Pixuna	Unknown	Brazil
V		Cabassou	Unknown	French Guiana
VI		–	Unknown	Argentina

Classification, mosquito associations, and geographic distribution of subtypes and varieties of this virus.
[a]*Virulent to equids and humans and involved in epizootics. The other subtypes and varieties are enzootic.*
[b]*A related variety IIIB (Bijou Bridge virus) is not listed here. It is associated with cliff swallow bugs (*Oeciacus vicarius*; Hemiptera: Cimicidae) in western North America.*

involving both equids and humans in Central America spread northward through Mexico in the next 2 years, moving into Texas (USA) in 1971. Cases continued in Mexico through 1972. There were thousands of both horse and human cases throughout this region during that time, but epizootic virus activity did not occur again there until an outbreak in Venezuela in 1992–1993 and in Chiapas, Mexico, in 1993. More recently, an outbreak of VEE occurred in northern Colombia and Venezuela in 1995, with about 50,000 equines and 75,000 humans infected, including 3000 humans with neurologic symptoms and 300 fatalities. The rapidity of spread of these outbreaks over large geographic areas undoubtedly is due to the role of horses as competent reservoir hosts. Weaver et al. (2004) have presented a comprehensive review of VEE.

Chikungunya (CHIK) virus. This virus, which has caused outbreaks for many years in Africa and parts of India and southeastern Asia (Jupp and McIntosh, 1988), has recently surged in human populations. In 2004, almost half a million human cases were reported in Africa, primarily coastal Kenya. An outbreak starting in 2005 spread across the islands of the Indian Ocean, resulting by early 2006 in 244,000 cases on Réunion, with an attack rate of 35% and more than 200 deaths. From there it spread to India, causing over 1 million cases. Travelers returning to Italy and France from the islands as Réunion, Mauritius, the Comoros, and Seychelles brought the virus with them. This resulted in cases diagnosed in Europe and a focal epidemic involving local transmission. More recently it has spread to the Americas. Overviews of CHIK virus biology, clinical aspects, and global expansion have been presented by Powers and Logue (2007), Morens and Fauci (2014), and Wahid et al. (2017).

CHIK virus generally does not cause the encephalitis-type symptoms characteristic of EEE, WEE, and VEE viral infections but rather a denguelike arthralgic illness with fever, rash, and severe joint pain. Some infections lead to chronic arthritis. Neurologic and other severe complications occurred in an Indian Ocean epidemic, with deaths reported for the first time.

In Africa, CHIK virus infects nonhuman primates such as the vervet monkey (*Cercopithecus aethiops*) and baboon (*Papio ursinus*), and wild primates often carry antibodies to it, suggesting their zoonotic role in its epidemiology. The enzootic vectors include *Aedes africanus*, *Ae. luteocephalus*, *Ae. opok*, *Ae. furcifer*, *Ae. taylori*, and *Ae. cordellieri* in the savanna and forest cycles. Additional reservoirs appear to include rodents and cattle and the mosquito species that feed on them. The virus appears to lack a zoonotic reservoir in Asia and the Indian Ocean region. Humans infected with CHIK virus often develop viremia sufficient to infect *Ae. aegypti* and *Ae. albopictus*, important vectors in urban and suburban areas.

Aedes aegypti is the primary vector of CHIK virus in urban areas of India and Asia, where it causes epidemics of arthralgic disease during rainy seasons. A 1994 epidemic in Vellore, southern India, was characterized by human cases increasing during August and September, reaching a peak in October as the human-biting frequency and viral infection rate of *Ae. aegypti* increased. The epidemic lasted about 5 months and affected 44% of the city's population. This epidemic and others, along with experimental studies, indicate that interrupted feeding by mosquitoes, resulting in partial bloodmeals, may facilitate both mechanical and biological transmission of CHIK virus, thus rapidly amplifying the virus in human populations.

Increases in CHIK virus activity that started along the East African coast are thought to have been caused not by abundant rainfall but rather by several years of drought. Drought promotes the use of water-storage containers around human habitations, producing large numbers of *Ae. aegypti* as vectors.

The epidemics on the Indian Ocean islands and in Italy and France have involved *Ae. albopictus* as the primary vector, rather than *Ae. aegypti*. A small genetic change in the virus apparently has significantly increased the vector competence of this typically more rural and less anthropophilic mosquito (Schuffenecker et al., 2006). The establishment of *Ae. albopictus* in many temperate areas of the world in recent years increases the opportunities for CHIK virus to spread in other human populations. The virus was discovered in the Americas in 2013, first in the Caribbean Islands and subsequently in many countries in North, Central, and South America, from the United States to Argentina. As of 2016, locally transmitted cases in the continental United States have been reported only from Florida and Texas. The relative roles of *Ae. albopictus* and *Ae. aegypti* in the Americas are under investigation and probably vary with locality. There is the opportunity for CHIK to use nonhuman primates in enzootic cycles in the Americas, though these remain to be documented.

Other Alphaviruses

There are other important alphaviruses that occur endemically and epidemically and cause fever, arthralgia, and other symptoms in humans.

O'nyong-nyong (ONN) virus. This is an antigenic subtype of CHIK virus that is transmitted among humans by *Anopheles* species in widespread parts of Africa. The vectors are the same ones that transmit human malaria parasites (i.e., *An. gambiae* and *An. funestus*). A large epidemic occurred from 1959 through the 1960s, infecting about 2 million people in Uganda, Kenya, Tanzania, Malawi, Zambia, and Mozambique. The virus, whose name comes from an Acholi African word meaning "weakening of the joints," was first isolated from humans during this time and was later isolated from *An. funestus* in 1974. Only isolated cases had occurred since then, until an epidemic in Uganda in 1997. Neither a vertebrate animal host nor the mechanism of persistence of ONN virus between these epidemics is known. The reservoir host is probably humans. A closely related virus, called **Igbo Ora**, also is transmitted by *Anopheles* mosquitoes and occurs in parts of West Africa, where it was associated with an outbreak of CHIK-like disease in the Ivory Coast in 1984. ONN and Igbo Ora are the only arboviruses causing human disease that have *Anopheles* mosquitoes as the primary vectors.

Sindbis (SIN) virus. This virus is distributed widely in eastern Europe, Scandinavia, the former Soviet Union, Asia, Africa, the Middle East, and Australia. The virus is a member of the WEE virus complex. It was originally isolated from *Culex univittatus* in Egypt in 1952 and has been associated with a human disease of rash, fever, and muscle and joint pain in Uganda, South Africa, and Australia. Birds are vertebrate hosts. A subtype of SIN virus is **Ockelbo**, which is distributed in Sweden, Finland, and northern Russia. It is transmitted by *Culiseta ochroptera*, and possibly *Culex* and *Aedes* spp., and has been associated with human disease similar to that caused by SIN virus.

Ross River (RR) virus. This virus occurs in Australia, Fiji, and the Cook Islands, where it causes an illness known as **epidemic polyarthritis**, consisting of fever, rash, and arthralgia (Kay et al., 1988). In Australia, RR virus is transmitted by *Aedes camptorhynchus*, *Ae. Vigilax*, and *Culex annulirostris*, whereas in the islands the vectors are *Ae. aegypti* and *Ae. polynesiensis*. The virus was first isolated from *Ae. vigilax* in 1963. The vertebrate reservoir hosts are uncertain, but in Australia they appear to be marsupials. RR virus ecology and distribution were reviewed by Russell (2002). **Barmah Forest virus** is another mosquito-borne alphavirus in Australia that causes symptoms similar to RR virus.

Mayaro (MAY) virus. This virus occurs in the Caribbean and parts of South America. In humans the illness is similar to CHIK. The virus was first isolated from febrile patients in Trinidad in 1954. Marmosets are the reservoir hosts of the virus, and *Haemagogus* mosquitoes are vectors.

Flaviviridae (*Flavivirus*)

The Flaviviridae, genus *Flavivirus*, contains eight antigenic complexes plus many unassigned viruses involving 70 types, subtypes, and varieties distributed worldwide. Some of these have mosquitoes as vectors while others are associated with ticks or with rodents or bats. The flavivirus

TABLE 15.5 Relationships of Selected Flaviviruses to Mosquito Vectors and Vertebrate Reservoir Hosts

Virus Species	Vector(s)	Vertebrate Reservoirs
Yellow fever	*Aedes aegypti*	Humans in urban environments
	Aedes africanus	Monkeys
	Aedes bromeliae	Monkeys
	Aedes furcifer	Monkeys
	Aedes luteocephalus	Monkeys
	Aedes metallicus	Monkeys
	Aedes taylori	Monkeys
	Aedes vittatus	Monkeys
	Haemagogus spp.	Monkeys
	Sabethes spp.	Monkeys
Dengue	*Aedes aegypti*	Humans
	Aedes albopictus	Humans (monkeys?)
	Aedes niveus group	Monkeys
	Aedes africanus	Monkeys
	Aedes furcifer	Monkeys
	Aedes taylori	Monkeys
	Aedes luteocephalus	Monkeys
	Aedes opok	Monkeys
	Aedes scutellaris	Humans
	Aedes polynesiensis	Humans
	Aedes pseudoscutellaris	Humans
	Aedes rotumae	Humans
Japanese encephalitis	*Culex tritaeniorhynchus*	Birds, pigs
	Culex gelidus	Birds
	Culex vishnui complex	Birds
St. Louis encephalitis	*Culex pipiens*	Birds
	Culex quinquefasciatus	Birds
	Culex tarsalis	Birds
	Culex nigripalpus	Birds
Murray Valley encephalitis	*Culex annulirostris*	Birds
West Nile	*Culex* spp.	Birds
	Culex univittatus	Birds
	Culex modestus	Birds
Zika	*Aedes aegypti*	Humans
	Aedes albopictus	Humans
	Aedes africanus, Ae. luteocephalus	Monkeys

Continued

diseases include some of the most dangerous and historically significant infections of humans. Table 15.5 shows the mosquito—vector and vertebrate—host associations of some of the more important mosquito-borne flaviviruses. Among them are yellow fever, dengue, Japanese encephalitis, St. Louis encephalitis, Murray Valley encephalitis, West Nile, and Ilheus viruses.

Yellow Fever (YF) virus

This disease is caused by yellow fever (YF) virus and occurs over broad portions of lowland equatorial Africa and South and Central America (Fig. 15.22), as either isolated cases or epidemics (Monath, 1988). Yellow fever virus exists principally in two epidemiological forms: an enzootic form, maintained in monkey populations by forest mosquitoes in a sylvan cycle and responsible for most of

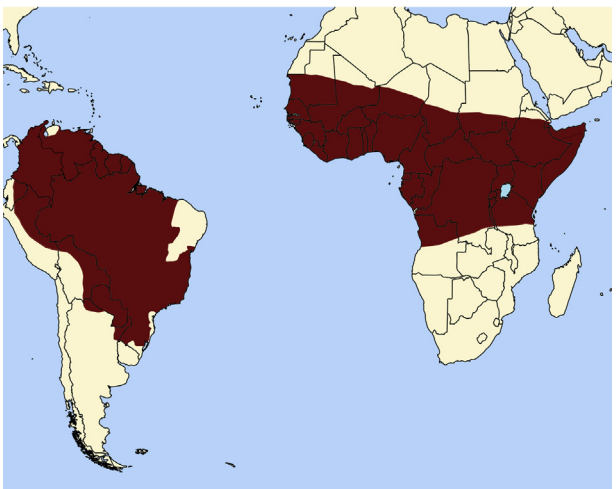

FIGURE 15.22 Geographic distribution of human yellow fever cases. *Based on data from the Centers for Disease Control and Prevention, USA, and the World Health Organization.*

FIGURE 15.23 *Aedes aegypti* female feeding on blood. This is the primary vector of dengue, urban yellow fever, Chikungunya, and Zika viruses. The "lyre" pattern on the thorax is distinctive. *Photograph by Robert G. Hancock.*

the isolated human cases (**jungle yellow fever**), and an epidemic form, spreading rapidly through human populations by the domestic form of *Aedes aegypti* (Fig. 15.23) in an urban cycle. The urban vector is particularly efficient because it readily enters and typically rests in houses, feeds almost exclusively on humans, and oviposits in artificial containers. It frequently takes two or three bloodmeals per gonotrophic cycle to supplement its energy reserves in lieu of sugar-feeding.

In previous centuries, **urban yellow fever** affected subtropical and temperate regions of North America with devastating effects. It remains a serious cause of mortality, particularly in village settings in tropical Africa, and is a constant threat in South America. There are sporadic, annual cases and occasional epidemics in Africa and 50–300 cases of jungle YF annually in South America. Jungle YF occurred in Brazil (1932), Panama, and northward into Central America (1948–1955) and several episodes (including some urban transmission) in Trinidad (1954, 1959, 1978–79). Epidemics in Africa have involved diverse areas, including Ethiopia (1960–1962), the Gambia (1978), Ghana (1979–1980, 1983, 1993, 1996), Benin (1996), Burkina Faso (1983), Nigeria (1986, 1994), and Kenya (1992–1993). Substantial outbreaks have occurred as recently as 2016 in Angola and the Democratic Republic of the Congo, and 2017 in Brazil. The global prospect for continuing YF epidemics was given by Barrett and Higgs (2007).

Yellow fever is a hemorrhagic disease. Mortality in infected humans ranges from 5%–75% or greater. After infection by mosquito bite, there is an incubation period of three to 6 days, followed by sudden high fever (>104°F [40°C]), headache, nausea, and pain. Humans are viremic during this initial acute phase for only about 3 days. The virus is viscerotropic and causes parenchymal cell necrosis in the liver, resulting in elevated blood bilirubin levels and jaundice—thus the name "yellow fever." Jaundice leads to systemic toxemia. Hemorrhage manifests as bleeding gums, easy bruising, and sloughing of the stomach lining, causing a characteristic **black vomit**. Delirium and coma often precede death.

The causative agent of YF is the prototype virus of the Flaviviridae and the first arbovirus to be associated with human disease. Although the disease was first recognized in the New World in the 17th century, the virus was isolated first in 1927 in Ghana, Africa, when the blood of a man with YF was inoculated into rhesus monkeys. The virus apparently originated in Central Africa among monkeys (e.g., *Cercopithecus* spp., family Cercopithecidae) and mosquitoes dwelling in the forest canopy. In this environment, the vector is the monkey-feeding *Aedes africanus*, a relative of *Ae. aegypti*. There also is evidence for infection with chimpanzees (*Pan troglodytes*, family Pongidae), baboons (*Papio*, family Cercopithecidae), and bushbabies (*Galago*, family Lorisidae). Generally, Old World monkeys do not suffer mortality from infection. In western Africa, where YF virus also is enzootic, the likely sylvatic vectors are *Aedes vittatus*, *Ae. metallicus*, *Ae. furcifer*, *Ae. taylori*, and *Ae. luteocephalus*, which develop in tree holes and sometimes other natural containers and rock pools.

Human disease occurs when humans enter the habitat where the mosquito–monkey cycle is ongoing and are bitten by infected mosquitoes. Alternatively, infected monkeys or baboons sometimes enter human habitat on raids in gardens or banana plantations, bringing YF virus to mosquitoes at the interface between forest and human habitation. The African mosquito *Aedes bromeliae* (formerly *Ae. simpsoni*), the larvae of which develop in water-filled leaf axils of plants such as banana, occupies this interface and frequently has become involved in forest-edge and rural transmission of YF virus to humans. *Aedes bromeliae* sometimes has served as the primary vector in massive rural epidemics. Thus, it functions both as a bridge vector between sylvatic and rural cycles and as an interhuman vector in that peridomestic environment. It was the principal vector during the 1960–1962 epidemic in Ethiopia, in which about 100,000 people became infected and 30,000 died.

The spread of both the domestic form of *Aedes aegypti* and YF virus from Africa to other parts of the world apparently occurred within the past 400 years. Trading and slaving ships, with their potentially virus-infected cargoes of slaves and with water barrels as a mosquito development site, greatly facilitated spread and establishment of both vector and virus. The need for slave labor in the newly established sugar cane-molasses-rum economy of the Caribbean region undoubtedly promoted movement of YF virus about the New World. Often, crews became ill with YF while their ships were in transit. Epidemics regularly

occurred in port cities both in West Africa and in coastal South America, North America, and the Caribbean. *Aedes aegypti* also moved into the Arabian peninsula and Indian subcontinent, and then to Asia and the Pacific region, probably via dhow traffic along sea trade routes. Yellow fever has not become established in Asia, even though vector-competent mosquitoes and humans occur there.

Yellow fever epidemics have occurred in the New World over a period of three centuries, beginning in the mid 1600s. Epidemics occurred in such places as Barbados and Trinidad (Caribbean), Havana (Cuba), Yucatan (Mexico), Guadeloupe (Caribbean), and Guayaquil (Ecuador) and in Charleston (South Carolina), Mobile (Alabama), Pensacola (Florida), and other areas of the United States as far north as Boston and New York. In the 1700s and 1800s, epidemics continued in ports of tropical and temperate America, including an outbreak in Philadelphia (Pennsylvania) in 1793, where some 4000 deaths occurred in a population of 55,000. A large outbreak in Haiti in 1802 decimated the French military force there, causing Napoleon to abandon his New World ambitions, contributing to the Louisiana Purchase by the US government. New Orleans (Louisiana) had regular epidemics of YF from 1796 through 1905. The shipping blockade enforced by the navy on the federalist side of the US civil war (1861−1865) prevented YF in New Orleans during that period. Probably, the blockade stopped importation of infected ship crews. An epidemic in the Mississippi River valley in 1878, extending north as far as Gallipolis, Ohio, caused over 13,000 deaths. The last epidemic in the United States was in New Orleans in 1905, involving 3402 cases and 452 deaths.

As YF virus invaded the New World, it became established in a mosquito−monkey cycle in forested parts of Central and South America. In the sylvatic cycle there, the vectors are forest canopy−dwelling mosquitoes, particularly *Haemagogus* spp. and *Sabethes chloropterus*, whose larvae develop in tree holes. New World Monkeys in the family Cebidae are highly susceptible to infection. During epizootics, howler monkeys (*Alouatta* spp.), squirrel monkeys (*Saimiri* spp.), spider monkeys (*Ateles* spp.), and owl monkeys (*Aotus* spp.) may show considerable mortality in their populations. However, capuchin monkeys (*Cebus* spp.) and woolly monkeys (*Lagothrix* spp.) circulate mosquito-infective viremias, but they do not die from infection. Therefore, in this cycle noticeable die-offs of monkeys may or may not precede sylvatic transmission of YF virus to humans. Sylvan YF in the Americas can lead to urban outbreaks when humans enter the tropical forests where transmission is ongoing, become infected by mosquito bite, then return to villages or cities. If *Aedes aegypti* is present in these settlements, it can initiate an epidemic of urban yellow fever.

The history of the discovery that YF virus is transmitted by mosquitoes is intriguing. The discovery is particularly important because YF was the first arbovirus to be recognized as a mosquito-borne agent, and mosquito control measures imposed quickly afterward caused a dramatic reduction in this devastating disease. Although suspicion that the agent causing YF might be transmitted by mosquitoes can be traced to several independent sources in the 1800s, it was the intuition of the Cuban physician **Carlos Finlay** (Fig. 1.2A), followed by the research activities of the United States Yellow Fever Commission in Havana, Cuba, in 1900, that resulted in experimental evidence that *Aedes aegypti* was the vector. This commission was comprised of the US Army officers **Walter Reed** (Fig. 1.2B), **James Carroll**, **Jesse Lazear**, **Aristides Agramonte**, and others. After consulting with Finlay, this team carried out a series of experiments that demonstrated the transmissibility of the agent from infected to uninfected humans by mosquito bite, after a suitable incubation period in mosquitoes. During this work, James Carroll allowed himself to be bitten by an infected mosquito and later developed YF, but he recovered. Jesse Lazear was accidentally bitten, and he died of the disease.

The team's findings stimulated **William Gorgas**, a physician in the United States Army, to impose a control program against *Ae. aegypti*, resulting in the elimination of urban YF in Havana and, soon after, in the Panama Canal Zone. Later, the Rockefeller Foundation sponsored teams of biomedical scientists and public health practitioners to begin intensive studies of YF by establishing a research center in Guayaquil, Ecuador, in 1918. This foundation eventually established research institutes in Brazil, Nigeria, Uganda, the United States, and elsewhere, and supported research and disease control programs in many sites. For a time, the causative agent of YF was thought to be a spirochete. Within 10 years of isolation of the virus in 1927, an attenuated, live vaccine was produced that was shown to provide excellent protection against infection. **Max Theiler** was awarded the Nobel Prize in Medicine for his efforts in this regard. In many areas of South America, antimosquito programs resulted in virtual elimination of urban outbreaks, although sylvan transmission continued. Despite these early advances, cases of jungle and rural YF, and epidemics in Africa, continue to occur. The reintroduction and resurgence of *Ae. aegypti* populations throughout Central and South America and the Caribbean islands in recent decades, and the immense growth of cities that provide a habitat for it, have created an increased potential for urban epidemics. Recent ongoing outbreaks have been reported in Democratic Republic of the Congo (2012), Angola (2015), Uganda (2016), Nigeria (2017), and Brazil (2017), among others.

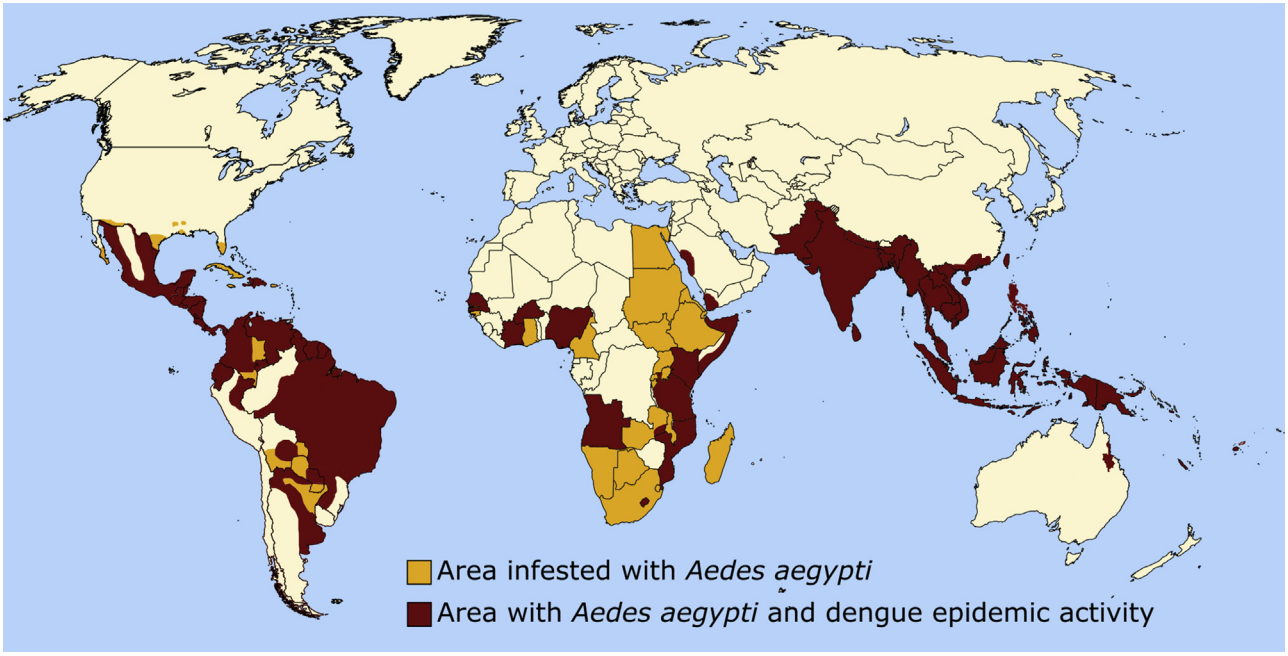

FIGURE 15.24 Geographic distribution of countries reporting human dengue fever cases and infestations with *Aedes aegypti* mosquitoes. *Based on data from the Centers for Disease Control and Prevention, USA, and Barcellos and Lowe, 2014.*

Dengue (DEN) virus

This disease is caused by **dengue (DEN) virus**, represented by four closely related serotypes called dengue 1, 2, 3, and 4 (Gubler, 1988). The disease in humans is either classic dengue fever or the more **severe dengue hemorrhagic fever (DHF)** or **dengue shock syndrome (DSS)**. It has been estimated recently that about 390 million dengue infections occur per year, worldwide. The DEN viruses are transmitted by mosquitoes, principally *Aedes aegypti*. The current distribution of DEN viruses includes Southeast Asia, the south Pacific, the Caribbean basin, Mexico, Central America, and South America. However, epidemics of DEN have occurred elsewhere in the past, including the United States, Japan, Australia, Greece, and both eastern and western Africa. Fig. 15.24 shows its current distribution and the regions with projected risk for occurrence, given the appropriate climate and availability of vectors. Dengue is commonly reported as an introduced disease in the continental United States, but indigenous transmission has occurred in Texas several times, and in both Texas and Florida as recently as 2013. All four serotypes now occur in the Western Hemisphere. In addition, to large-scale epidemics in the Americas, there have been recent, large outbreaks involving dengue hemorrhagic fever in Africa, China, Taiwan, India, Maldives, and Sri Lanka. In the hyperendemic areas of southeastern Asia, such as Thailand and the Philippines, the severe forms of disease have become more common and appear in epidemics at 3- to 5-year intervals.

Dengue is characterized by fever, rash, severe headache, and excruciating pain in muscles and joints, earning it the name **breakbone fever**. Clinical disease develops 5–8 days after bite of an infected mosquito. Often the disease runs a mild course of about a week, leading to complete recovery. However, DEN can become severe in cases of DHF and DSS, both of which generally occur in children and may be fatal. These manifestations were first observed as complications of dengue fever in 1954 and are characterized by blotchy rash, bleeding from the nose and gums (Fig. 15.25), and shock.

The increased frequency of DHF and DSS in Southeast Asia and parts of the Caribbean and Latin America have

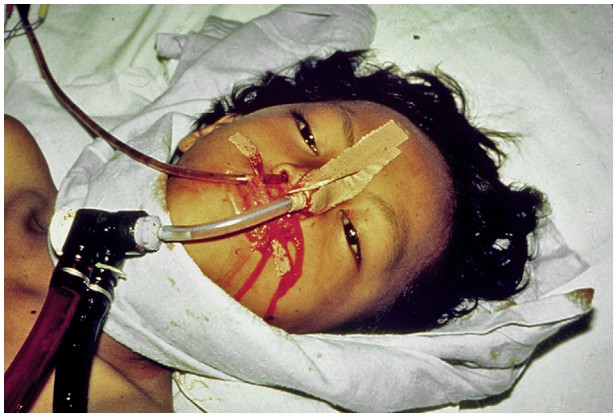

FIGURE 15.25 Dengue hemorrhagic fever (DHF), showing external bleeding from mucous membranes. *Photograph by Duane J. Gubler.*

stimulated a debate in the biomedical community regarding the mechanisms by which DHF emerges during epidemics of classic dengue fever. One hypothesis is that there are variable forms of the different serotypes of DEN viruses, some of which are more pathogenic than others. Another idea is that people of different races differ in propensity to develop severe symptoms and that the virus has been spreading into more susceptible populations. Still another hypothesis is that, as human populations in tropical cities in Asia and the Americas increase and as DEN epidemics in general increase in frequency, there are simply more cases with the noticeable manifestations of DHF and DSS. The fourth hypothesis, and the one currently viewed as most likely, is that prior exposure to one serotype, followed by exposure to another serotype within a critical period of 5 years, leads to the development of hemorrhagic fever and, in some cases, shock syndrome. The more recent epidemics taking place in various parts of the world, especially in the Americas, indicate that DEN has replaced YF as the major urban, epidemic flavivirus of importance, worldwide.

The main epidemic vector of DEN viruses, *Aedes aegypti* (Fig. 15.23), is ideal, because it commonly rests indoors, feeds preferentially on humans, has a tendency to take supplementary bloodmeals, and often moves from one residence to another as it oviposits. The larvae develop in such vessels as water barrels and jars, potted-plant containers, cemetery urns, and discarded tires. The close proximity of these larval habitats to human dwellings further facilitates *Ae. aegypti*—human contact and allows large mosquito populations to develop. To the extent that larval sites are hand-filled, DEN transmission is independent of rainfall patterns. Indeed, in some places where water stores are replenished independently of rainfall, epidemics may occur in the hot, dry season when temperatures are higher and the extrinsic incubation period of the viruses in the mosquitoes is shortened. However, in areas where breeding containers depend primarily on rainwater, DEN epidemics occur during rainy seasons.

Other vectors of DEN viruses are *Aedes albopictus* (Fig. 15.26) in rural areas of Southeast Asia and *Ae. polynesiensis*, *Ae. scutellaris*, *Ae. pseudoscutellaris*, and *Ae. rotumae* in the Pacific region. The role of the newly introduced *Ae. albopictus* in the New World as a vector of DEN viruses remains to be determined. In peninsular Malaysia, a series of studies showed that all four serotypes of DEN virus circulated between monkeys and mosquitoes of the *Ae. niveus* group, suggesting the possibility of an enzootic sylvatic cycle. Similarly, studies in West Africa provide equally strong evidence that monkey populations there maintain a sylvatic form of DEN type 2 virus. Where monkeys and humans are loosely associated in forested areas and human habitations are intermixed with patches of forest, this strain of the virus has been isolated from humans, monkeys, and arboreal mosquitoes such as *Ae. africanus*, *Ae. opok*, *Ae. luteocephalus*, *Ae. furcifer*, and *Ae. taylori,* which appear to transmit the virus indiscriminately among monkeys and humans. Thus, DEN may have a zoonotic reservoir and system of transmission in West Africa similar to that of YF and CHIK viruses in the same region. But in this case the enzootic and endemic systems probably remain distinct and separate from one another.

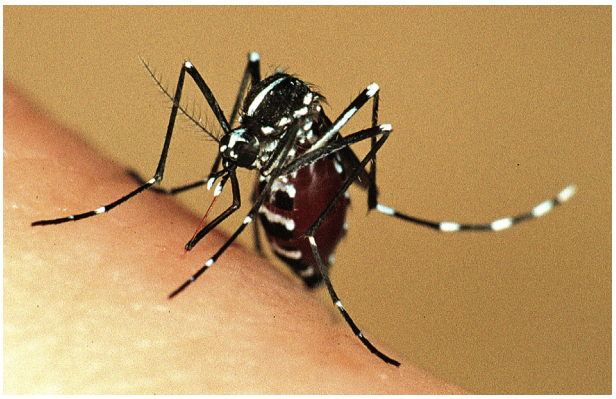

FIGURE 15.26 Asian tiger mosquito (*Aedes albopictus*), female feeding on human. It transmits Chikungunya and and dengue viruses in some locations. The median longitudinal silver stripe on the thorax is its most prominent identifying feature. *Photograph by Woodbridge A. Foster.*

Humans generally are considered to be the only vertebrate host of the endemic/epidemic form of the virus in situations where monkeys do not occur, such as congested urban slums in huge tropical cities including Bangkok, Manila, Jakarta, Caracas, and Guayaquil. Thus, a mosquito—human—mosquito cycle of DEN transmission by *Ae. aegypti* is the usual means of virus maintenance and epidemic spread. Transovarial transmission of some of the DEN viruses has been demonstrated in *Ae. aegypti* and *Ae. albopictus*, and therefore mosquitoes may be reservoirs, particularly in periods of low-level transmission among humans. A multiauthored book on dengue, Gubler et al. [eds] (2014), presents a comprehensive treatment of DEN virus and the disease.

Japanese Encephalitis Virus Complex

Another important group of mosquito-borne flaviviruses is the Japanese encephalitis virus antigenic complex, including West Nile, Japanese encephalitis, St. Louis encephalitis, and Murray Valley encephalitis viruses. Some authorities refer to this complex as the **West Nile antigenic complex**. These viruses occur in widely separated geographic regions but show similarities in the nature of their enzootic cycles. Each has *Culex* vectors (Table 15.5) and birds as vertebrate reservoirs. In the case of Japanese encephalitis virus, pigs often serve as amplifying hosts. Fig. 15.27 shows the worldwide distribution of this complex of viruses. The most important of these in terms of human morbidity and mortality is Japanese encephalitis

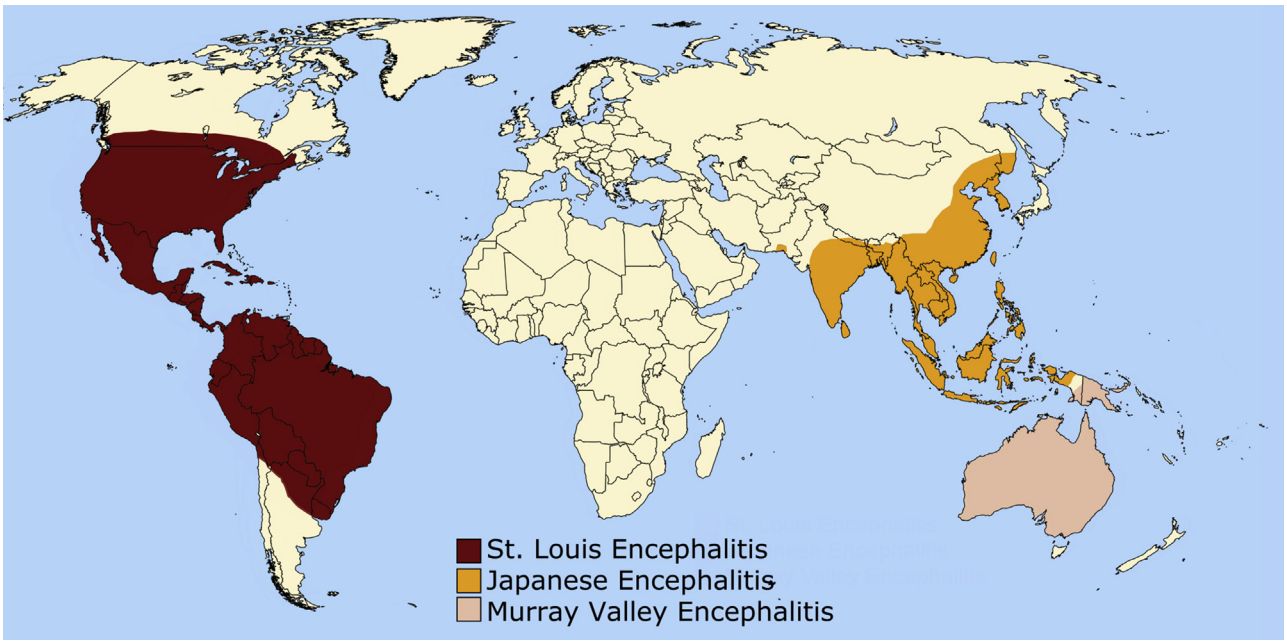

FIGURE 15.27 Geographic distribution of mosquito-borne flaviviruses causing encephalitis. *Reconstructed from Mitchell, 1977, the Centers for Disease Control and Prevention, USA, and other sources.*

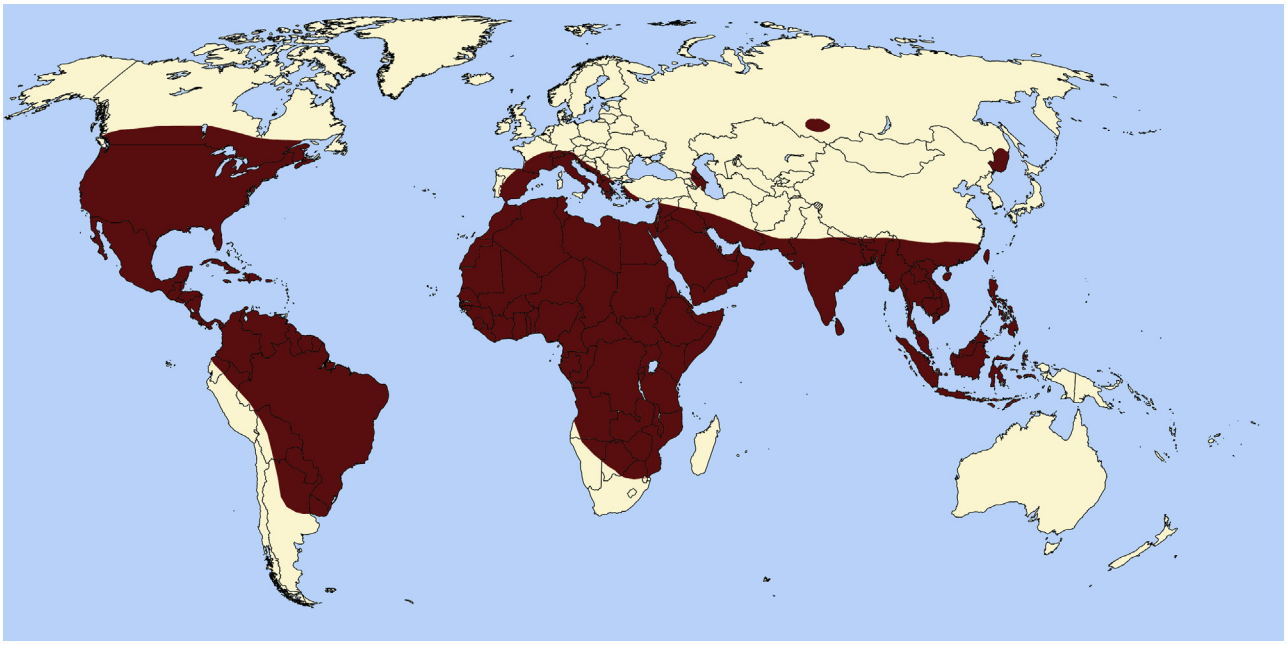

FIGURE 15.28 Geographic distribution of West Nile virus, a mosquito-borne flavivirus. *Based on data from the Centers for Disease Control and Prevention, USA.*

virus. However, all cause human illness of varying severity, depending on the virus and age and health of the person.

West Nile (WN) Virus. This virus was first isolated from the blood of a febrile man in Uganda in 1937. The primary mosquito vectors are *Culex* spp., particularly *Cx. pipiens* and *Cx. univittatus*. Birds are the vertebrate reservoirs and amplifying hosts during epidemics. WN virus is widely distributed in Africa, the Middle East, Europe, parts of the former Soviet Union, India, Indonesia, and North and South America (Fig. 15.28). It has been the cause of endemic and epidemic fever, myalgia, and rash, especially in children in the Middle East, and has caused encephalitis in some instances. Seroprevalence of antibody

to WN virus in Egypt can exceed 60% in adults. Epidemics have occurred in Israel (1950s), southern France (1962), South Africa (1974, and 1983−1984), Romania (1996) and other eastern European countries, and most recently in North America. A review of the epidemiology of WN virus is presented by Kramer et al. (2008).

In 1999, WN virus was introduced through unknown means into New York City, USA, and was linked to human encephalitis in 61 confirmed cases (mostly in the city borough of Queens); there were seven fatalities. Initially, the outbreak was thought to be due to St. Louis encephalitis. However, an unusual feature of the outbreak was the large numbers of birds that died, especially crows (*Corvus brachyrhynchos*). The vectors are believed to have been *Cx. pipiens* and other *Culex* species, although WN virus later was isolated from other mosquito genera as well. A serosurvey conducted by the CDC in the Queens area indicated that as many as 1,250 people had been infected during the course of the outbreak, and that about 240 of these people (19%) may have experienced clinical illness due to infection.

The virus successfully overwintered in the New York area, despite efforts to control *Culex* mosquitoes, and spread to adjoining northeastern states in 2000. By 2001 it had spread into the South and Midwest regions of the United States and Canada. In the Midwest human cases and high bird mortality peaked in 2002. By that time the first human case had already occurred on the West Coast and virus was isolated from birds and mosquitoes. The following year, infections peaked in the South, and highest numbers of human cases occurred in the prairie and Rocky Mountain States, followed by a high incidence of cases in California during 2004 and 2005. The highest virus activity in the United States was recorded in 2003, with more than 9862 human cases of WN, including 264 deaths. Large fluctuations in incidence have occurred since that year. During the period 2002 to 2007, the virus also spread steadily southward into Mexico, the Caribbean islands, Central America, and finally South America as far as Argentina. However, WN virus activity remains remarkably low in much of Latin America. In the United States, total clinical cases between 1999 and 2016 were 46,086, with 2017 deaths. Epidemiological studies indicate that about 80% of human infections are asymptomatic. The great majority of clinical cases are classified by CDC as "West Nile fever," with fewer than 5% presenting "West Nile neuroinvasive disease," including these categories: encephalitis, meningitis, and acute flaccid paralysis (sometimes called WN poliomyelitis).

In the North American outbreaks over 200 species of birds suffered mortality from WN virus infections, with corvids (crows and jays), raptors, and exotic species being particularly vulnerable. About 40% of infected equines (horses, donkeys) died. Bird species vary widely in their tolerance of infections and in their ability to generate high and prolonged viremias. Jays, grackles, crows, house finches, and house sparrows are all highly competent in the latter regard, making them good reservoir hosts.

FIGURE 15.29 *Culex quinquefasciatus* females laying eggs in form of floating rafts; this species is a vector of filariasis, West Nile virus infection, and St. Louis encephalitis, among other diseases. *Photograph by Woodbridge A. Foster.*

Many mosquito species are capable of supporting and transmitting WN virus. In the United States *Culex* species are the principal vectors that maintain it in bird populations: particularly *Cx. pipiens* in the Northeast and upper Midwest, *Cx. quinquefasciatus* (Fig. 15.29) in the South, and *Cx. tarsalis* in several western regions. Mosquitoes that feed on both birds and mammals, such as *Coquillettidia perturbans* and *Aedes vexans*, may act as bridge vectors, transmitting the virus from birds to equines and humans. However, many *Cx. pipiens* populations feed readily on both birds and mammals and can likewise, serve as bridge vectors.

Studies in northeastern North America have shown that a small proportion of infected *Cx. pipiens* adults can pass WN virus to their offspring by transovarial transmission. Thus diapausing adults emerging in the fall may carry the virus through the winter in their hibernacula and transmit it to birds the following spring.

Japanese encephalitis (JE) virus. Japanese encephalitis is a severe disease of acute encephalitis, with children and the elderly primarily affected and with mortality rates reaching over 25% of those with overt disease. Many infections are asymptomatic or mild. Survivors often show neurologic sequelae. The disease is distributed throughout the rice-growing areas of Asia, from Japan south to Papua New Guinea and west to India and Nepal (Fig. 15.27). In Japan, JE epidemics have occurred in August and September in many different years since its discovery there. The virus first was isolated from the brain of a human who died of encephalitis in 1935, then from brain tissue of a

horse in 1937, and from *Culex tritaeniorhynchus* in 1938. Numbers of cases have declined in Japan, Korea, and Taiwan, because of vector control, vaccination, and changes in agricultural practices (Burke and Leake, 1988).

The enzootic cycle of JE virus involves *Cx. tritaeniorhynchus* and several other *Culex* spp. including *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. fuscocephala*, and *Cx. gelidus*, all of which are associated with rice culture. Wading birds such as herons are important enzootic reservoirs. Pigs are important amplifier hosts in rural, rice-growing areas where swine are kept. Japanese encephalitis is probably the most important of the mosquito-borne encephalitis diseases, owing to its epidemic nature, widespread distribution, and large number of humans who acquire infection, die, or recover yet suffer neurologic sequelae.

St. Louis encephalitis (SLE) virus. This virus was identified as the causative agent of disease during an outbreak of encephalitis-like illness in Paris, Illinois (USA), in 1932 and St. Louis, Missouri (USA), in 1933. It was isolated from a patient with encephalitis in the Yakima Valley of Washington (USA) in the early 1940s and found to be a frequent cause of human illness in the Central Valley of California in the 1930s and 1940s. This virus is distributed widely in North America and also occurs in parts of Mexico, Central America, the Caribbean, and South America to Argentina (Fig. 15.27). The encephalitic illness caused by SLE virus shows a bimodal age distribution, with children and elderly people most frequently affected. Attack rates during epidemics range from 5 to 800 per 100,000 population, depending on location, year, strain virulence, and population immunity due to earlier epidemics. In the eastern United States, mortality rates have ranged from about 3% to 20% of laboratory-diagnosed cases, but in the western United States mortality rates are lower.

In North America, three enzootic cycles of SLE virus have been described. In the eastern United States, north of Florida but including Texas, the primary vectors are *Culex pipiens* and *Cx. quinquefasciatus* (Fig. 15.29). The former mosquito occurs in a more northerly distribution, whereas the latter is more southerly, with a hybrid zone at about the latitude of Memphis, Tennessee (USA). Females of both species feed on birds. In addition, *Cx. quinquefasciatus* females frequently feed on mammals as the summer progresses. Whether these mosquitoes alone or other vectors function in transmission to humans depends on the abundance of these two species and other competent vectors. House sparrows are important vertebrate amplifier hosts in peridomestic settings. In the western United States, a mosquito–bird–mosquito cycle similar to that of western equine encephalomyelitis virus, involving *Cx. tarsalis*, has been elucidated. In addition, in California both *Cx. pipiens* and *Cx. quinquefasciatus*, and possibly *Cx. stigmatosoma*, function secondarily as either enzootic or epidemic vectors. In Florida, *Cx. nigripalpus* apparently is the enzootic, epizootic, and epidemic vector of SLE virus. In Latin America and in the Caribbean basin, SLE virus has been isolated from many different species of *Culex*, *Sabethes*, *Mansonia*, *Wyeomyia*, and other genera, and from a wide variety of birds and mammals. In these areas, human SLE generally is rare. The mechanism of virus overwintering in North America is not well known. There is some evidence of virus persistence in overwintering, diapausing female mosquitoes.

The history of SLE in North America has been that of epidemics, either local or widespread, with intervening years when there was apparently no virus activity or epidemics, and either no or a few isolated human cases. The first epidemic, in the early 1930s in St. Louis, Missouri, was accompanied by hot and dry weather, which favored the development of populations of mosquitoes of the *Culex pipiens* complex, the larvae of which develop in water rich in sewage or other organic matter. The epidemic involved about 1,100 human cases and 200 deaths. Since that time, there have been some 50 outbreaks of SLE in the United States. Cases during three recent decades are shown by state in Fig. 15.30. Human cases also have occurred in Manitoba and Ontario, Canada. These epidemics have been both rural and urban. A very large outbreak occurred in 1975, involving 30 states and the District of Columbia, with over 1800 cases reported. More recently, epidemics have occurred in such disparate locations of the United States as Pine Bluff (Arkansas), Florida, Los Angeles (California), Houston (Texas), New Orleans (Louisiana), and Grand Junction (Colorado). A total of 4,437 human cases of SLE infection were reported to the CDC from 1964 to 1995, with an average of 139 cases per year (range, 4–1967). From 2007 to 2016, there were 95 diagnosed cases and 5 deaths. Monath (1980) and Tsai and Mitchell

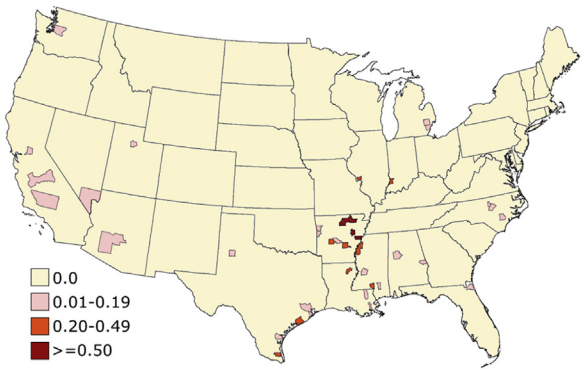

FIGURE 15.30 Historical distribution of human cases of St. Louis encephalitis virus (SLE) neuroinvasive disease (encephalitis and/or meningitis) in the United States; average annual incidence by county of residence per 100,000 population, 2007 to 2016. *Based on data from the Centers for Disease Control and Prevention, USA.*

(1988) have reviewed the ecology and public health significance of SLE.

Murray Valley encephalitis (MVE) virus. This virus has been associated with encephalitis-type illness in humans in eastern and western parts of Australia and in New Guinea (Marshall, 1988) (Fig. 15.27). Mortality rates vary greatly during outbreaks, ranging from 18% to 80%, with long-term neurological effects occurring in 30%−50% of survivors. Epidemics of encephalitis in Australia in 1917, 1918, 1922, and 1925 were probably caused by MVE virus. It was isolated from the brain of a human in 1951 and from a pool of *Culex annulirostris* in 1960. Later periods of transmission were detected in Australia in 1956, 1971, 1974, 1978, 1981, and 1984. A resurgence of virus activity and human infections (17) occurred in 2011, the first outbreak of significance since 1974. The enzootic cycle involves *Cx. annulirostris* as vector and birds as vertebrate reservoir and amplifier hosts. Epidemics appear to be associated with excessive rainfall, which allows an increase of mosquito populations to high densities and the immigration of wading birds. **Kunjin virus** is closely related to MVE virus. It is also mosquito-borne, but it is associated rarely with human disease in Australia.

Zika (ZIK) virus. This virus was first isolated from a sentinel rhesus monkey in Zika Forest, Uganda, in 1947, and soon after in the mosquito *Aedes africanus* in that forest. Human cases were first documented in Africa in 1952. However, the first major outbreak in humans occurred much later, in Micronesia in 2007. It has now spread through nearly all of equatorial Africa, to southern Asia, including Pakistan, India, Bangladesh, and nearly all of Southeast Asia and to many of the South Pacific Islands. More recently it also has been introduced into the Americas, first appearing in Brazil in 2015 and spreading through mainland South, Central, and North America, from central Argentina, through the Caribbean Islands, to the southern United States (Fig. 15.31). Worldwide, cases numbered in the hundreds of thousands by 2016. Within the United States local transmission had been documented by 2016 in limited localities of southern Florida and Texas. As large proportions of vulnerable human populations in the Americas become immune following infection, it is expected that ZIK's incidence will stabilize or decline, as already observed in Brazil between 2015 and 2017. Current figures are available from the CDC Website.

Typical ZIK infection in humans is manifested as a mild febrile illness, including headache, fever, rash, and sometimes joint pain. Death is uncommon. It lasts about 1 week, with many human infections being asymptomatic. However, it has the potential to cause more insidious consequences, apparently due to a single mutation in the virus (Yuan et al., 2017). It was first linked to Guillain-Barré syndrome, a neurological disease. Subsequently, an association was noticed between ZIK and fetal microcephaly and to postinfection neurological disorders in children and adults. These sequelae, particularly the resulting thousands of microcephalic babies infected in utero that have appeared in Brazil and other Latin American countries,

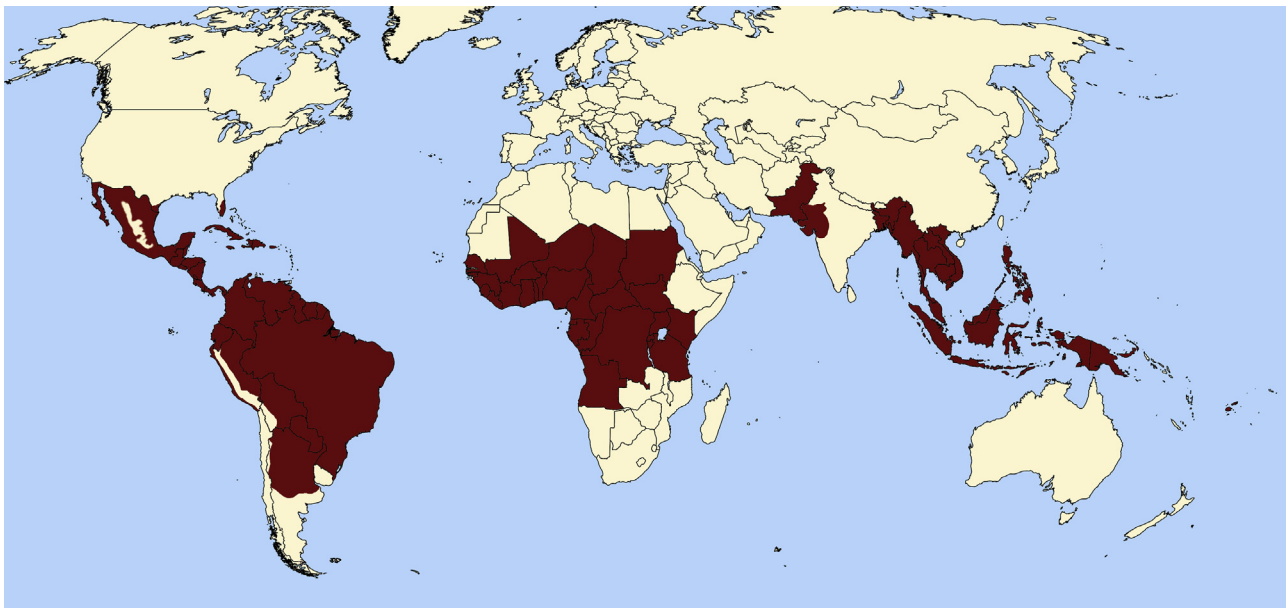

FIGURE 15.31 Approximate global distribution of human infection and likely transmission of Zika virus, as of early 2017; transmission is expected to be rare or absent in deserts and in mountains above 2,000 m; locally acquired cases occurred in southern Florida and Texas, USA. *Based on data from the Centers for Disease Control and Prevention, USA, and individual national reports from some countries.*

have made this a disease more alarming than dengue and CHIK virus infections that also threaten subtropical and temperate regions.

Transmission of ZIK appears to be achieved primarily by the same daytime biting, container-breeding peridomestic *Aedes aegypti* and *Ae. albopictus* that deliver DEN and CHIK viruses to people. As with those viruses, in most instances *Ae. aegypti* appears to be the main vector, because of its stronger preference for human hosts and utilization of water associated with houses. *Ae. albopictus* is less narrow in its blood-feeding tendencies, lowering its efficiency as a vector between humans. But it presents more of a threat in cooler climates because of its ability to undergo a winter egg diapause. Countering that threat is the higher animal-biting rate of *Ae. albopictus*, the shorter warm season, and the greater avoidance or protection from mosquito bites that humans use in temperate countries. Evidence suggests that most animals do not serve as alternate hosts, and therefore that zoonotic reservoirs for ZIK are unlikely in temperate and urban areas. However, the role of primates as reservoirs in subtropical and tropical countries is likely, considering its isolation from various primates in Africa, and more recent detection in monkeys and marmosets in Brazil (Favoretto et al., 2016). One very unusual characteristic of ZIK virus transmission is its ability to be sexually transmitted between humans, particularly from men to women. This means that infected, and even asymptomatic, males pose a risk to females and their offspring during pregnancy. For a review of ZIK virus and a perspective on recent ZIK outbreaks, see Weaver et al. (2016), Shuaib et al. (2016), Fauci and Morens (2016), and Aliota et al. (2017).

Other Flaviviruses

There are many other mosquito-borne flaviviruses of importance to human health, including Rocio virus and Ilheus virus.

Rocio (ROC) virus. This virus occurs in Brazil (Iversson, 1988). It appeared suddenly in São Paulo State in 1975 and caused an encephalitis-type illness in about 1,100 cases over the following 5 years, with some fatalities. Most cases occurred among young, male agricultural workers. Human infections have occurred sporadically since that time, most recently in 2010. The vector and vertebrate host relationships for this virus are poorly known, although *Psorophora ferox* and *Aedes scapularis* have been incriminated as vectors, based on virus isolation in the field and laboratory transmission experiments.

Ilheus (ILH) virus, which is closely related to Rocio virus, occurs in Trinidad, Panama, and parts of South America. It has been associated with about 10 documented human cases of illness and has been isolated from mosquitoes in eight different genera, mostly from *Psorophora* spp.

Bunyaviridae (*Orthobunyavirus* and *Phlebovirus*)

The Bunyaviridae include the genera *Orthobunyavirus* and *Phlebovirus*. The viruses in the genus *Orthobunyavirus* comprise a complex and diverse group of more than 50 virus species distributed worldwide. Mosquitoes or biting midges serve as vectors, and small mammals, ungulates, or birds are the vertebrate hosts. *Phlebovirus* contains 37 viruses, most of which have phlebotomine sand fly vectors. It also includes the important mosquito-borne Rift Valley Fever virus.

Among the 30 virus species associated with mosquitoes and with human or animal diseases in the genus *Orthobunyavirus* are 14 serotypes in the species California encephalitis virus (Grimstad, 1988; Eldridge, 1990; Fauquet et al., 2005). Table 15.6 lists these virus serotypes and indicates their geographic distribution, mosquito vectors, and vertebrate host relationships. Characteristically they are transmitted by *Aedes* spp., but other genera such as *Culiseta* and *Anopheles* may be involved. Nine occur only in North America. The others are distributed in various places worldwide. The viruses in this species were isolated and described during the period from 1943 through the 1970s, culminating in a monograph by Calisher and Thompson (1983). Some of them are described next.

California encephalitis (CE) virus. This is the prototype virus for the complex of the same name and was the first of the California serogroup viruses to be associated with human disease, involving three cases in California (USA) in 1943. It was isolated from *Aedes melanimon* and *Culex tarsalis* at that place and time. Extensive studies of the mosquito and vertebrate—host relationships of this virus in California have shown that *Ae. melanimon* and *Ae. dorsalis* are the principal vectors and that the virus is transmitted transovarially by these species. Serologic surveys have implicated jackrabbits, cottontail rabbits, California ground squirrels, and kangaroo rats as vertebrate hosts. The virus also has been isolated in New Mexico, Utah, and Texas (USA), and in Canada. However, since the time of original discovery, CE virus only rarely has been associated with human disease.

La Crosse encephalitis (LAC) virus. This is the most important human pathogen in the California serogroup, causing an acute, febrile illness in children. Most cases are subclinical or mild, but some progress to severe encephalitis and, rarely, death. In 1964, LAC virus was isolated from preserved brain tissue of a child who had died of

TABLE 15.6 California Encephalitis Virus (*Orthobunyavirus*, Bunyaviridae): Mosquito Associations, Vertebrate Hosts, and Geographic Distribution of the Serotypes of This Virus Species

Serotype	Vector Associations	Vertebrate Associations	Geographic Distribution
California encephalitis	*Aedes melanimon* *Ae. dorsalis*	Lagomorphs	United States (western, southwestern)
Inkoo	*Aedes* spp.	Lagomorphs?	Finland
La Crosse	*Ae. triseriatus*	Sciurid rodents, foxes	United States (eastern)
Snowshoe Hare	*Ae. stimulans* group *Ae. canadensis* *Culiseta inornata*	Lagomorphs	United States (northern), Canada
San Angelo	*Aedes, Anopheles, Psorophora*?	Unknown	United States (southwestern)
Tahyna	*Ae. vexans, Cs. annulata*	Lagomorphs	Europe, Tajikistan, Azerbaijan
Lumbo	*Ae. pembaensis*?	Unknown	East Africa
Melao	*Ae. scapularis*?	Unknown	Trinidad, Brazil, Panama
Jamestown Canyon[a]	*Cs. inornata* *Ae. communis group* *Ae. provocans* *Ae. abserratus* *Ae. intrudens* *Ae. stimulans group* *Anopheles* spp.	Deer	United States, Canada
South River		Deer?	United States (northeastern)
Keystone	*Ae. atlanticus, Ae. tormentor*	Lagomorphs, cotton rats	United States (coastal, eastern)
Serra do Navio	*Ae. fulvus*	Unknown	Brazil (Amapa state)
Trivittatus	*Ae. trivittatus*	Lagomorphs	United States
Guaroa	*Anopheles* spp.	Unknown	Panama, Colombia, Brazil

[a]*Jerry Slough virus is a strain of Jamestown Canyon virus in the western United States.*

encephalitis in 1960 in the vicinity of La Crosse, Wisconsin (USA). It currently is distributed in the eastern United States, including the midwestern states bordering the Great Lakes, east to New York and Pennsylvania, south to West Virginia and North Carolina, and west to Texas. However, most human cases occur in West Virginia, Wisconsin, Illinois, Indiana, and Ohio.

The disease tends to be highly focal within its known range, such that particular regions or towns are known to be endemic. Prevalence varies regionally. In the United States, there were 2,245 cases of LAC reported to the CDC from 1964 to 1995, with an average of 70 per year (range, 29−160). In Ohio, where cases are particularly well documented, there was an average of 26 cases per year between 1963 and 1995. La Crosse encephalitis probably is underreported to public health agencies.

The principal vector of LAC virus is the Eastern tree hole mosquito, *Aedes triseriatus*. The virus is transmitted both horizontally to sciurid rodents, particularly chipmunks and squirrels, and vertically from female mosquitoes to their progeny. The discovery of transovarial transmission of LAC virus was one of the first documentations of this phenomenon in mosquitoes and revealed an overwintering mechanism for LAC virus. It also demonstrated that vertebrate reservoirs were not always essential to the persistence of mosquito-borne viruses in nature and that the mosquito itself could be a reservoir host. Thus an infected female is able to transmit the virus at its first blood-feeding without previously having taken an infectious bloodmeal. Another important finding was that *Ae. triseriatus* males, infected transovarially, transferred LAC virus to females via mating (i.e., venereal transmission).

Epidemiologic investigations of cases of encephalitis or aseptic meningitis of unknown origin often reveal LAC encephalitis in areas where previously it was unknown. Such investigations almost always reveal populations of *Aedes triseriatus* in the immediate vicinity where infection was thought to occur, such as backyards or wooded areas where children play. Water-filled artificial containers, particularly discarded tires, have become important habitats for *Ae.*

triseriatus larvae and provide a link between the sylvan La Crosse cycle and humans. In Ohio and New York State, LAC virus also has been isolated repeatedly from *Aedes canadensis*; however, the role of this mosquito as a vector to humans and its role in an enzootic cycle are not well understood.

Snowshoe Hare (SSH) virus. This pathogen is closely related to La Crosse virus, but its ecology is very different. It originally was isolated from the blood of a snowshoe hare in Montana in 1958. Lagomorphs (hares and rabbits) are the enzootic vertebrate hosts. Snowshoe hare virus is distributed in the northern parts of the United States and in Canada, where it has been isolated from a variety of *Aedes* spp. and from *Culiseta inornata*. Even though SSH virus is very similar antigenically to La Crosse virus, human disease rarely has been documented except in Ontario, Quebec, and Nova Scotia (Canada), where 10 cases of an encephalitis-like illness have been attributed to SSH virus.

Keystone (KEY) virus. This was first isolated in 1964 from a collection of blood-fed *Aedes atlanticus* and *Ae. tormentor* in Florida (USA). It is not considered to be a human pathogen. It occurs along the eastern seaboard of the United States where it has been isolated from *Ae. atlanticus*, *Ae. tormentor*, *Ae. infirmatus*, and other mosquitoes. Transovarial transmission of the virus has been demonstrated for *Ae. atlanticus* in the field. Gray squirrels and cottontail rabbits in northern coastal areas, and cottontail rabbits and cotton rats in Florida and Texas (USA), have been identified as vertebrate hosts.

Trivittatus (TVT) virus. This was first isolated from *Aedes trivittatus* in North Dakota (USA) in 1948. It also has been isolated from other mosquitoes, including *Ae. infirmatus* in the southeastern USA, where *Ae. trivittatus* is absent. Transovarial transmission has been demonstrated in the latter species. Trivittatus virus shows a widespread distribution in the eastern half of the USA. Cottontail rabbits are vertebrate hosts.

Jamestown Canyon (JC) virus. This was originally isolated from *Culiseta inornata* in Colorado (USA) in 1961. Since that time it has been isolated in both Canada and the United States from *Aedes*, *Culiseta,* and *Anopheles* species in regions from Alaska east to Ontario and New England, south to Maryland, and in western and southwestern states, including California. The principal vectors are *Aedes* spp. with univoltine life cycles (i.e., snow pool and spring species). An antigenic strain known only from California (USA) is **Jerry Slough virus**, which is transmitted by *Culiseta inornata*. In the eastern USA, a JC-related serotype is **South River virus**. Transovarial transmission of JC virus has been demonstrated in some mosquito species. Its vertebrate hosts are large wild ungulates, especially deer. JC virus has been associated with encephalitis-type illness in humans in Ontario, New York, and Michigan.

Tahyna (TAH) virus. This is distributed widely in Europe and parts of western Asia. It has been associated with human febrile and central nervous system illnesses in France, the former Czechoslovakia, and Tajikistan. Foci are now known from Finland south to Tajikistan. Although the prevalence of infection in humans is poorly known, serosurveys in the Rhine River valley of Germany documented antibody to TAH virus in up to 23% of humans living in the area. In the former Czechoslovakia, TAH virus was implicated in 1% febrile illnesses of children in an endemic area, and 20% of central nervous system illnesses. This virus was first isolated from *Aedes vexans* and *Aedes caspius* in Slovakia in 1958. The mosquito vectors are *Ae. vexans*, *Ae. caspius*, and *Culiseta annulata*. Hares and pigs are vertebrate reservoir hosts. **Lumbo virus** is a variety of TAH and occurs in parts of Africa.

Rift Valley fever (RVF) virus. This pathogen (Fig. 15.20) is classified with viruses in the genus *Phlebovirus* in the Bunyaviridae (Meegan and Bailey, 1988). It is distributed in eastern Africa north to Egypt, and in parts of West Africa, where it has been associated with large outbreaks of acute illness in livestock (see "Veterinary Importance" later). Humans may become infected by mosquito bite, or more commonly by contact with virus-contaminated blood or through inhalation of virus in aerosols during slaughter of livestock. Humans rarely die of infection but develop an illness including fever, headache, myalgia, retinitis, and in rare cases liver involvement. Large epizootics and epidemics have occurred in South Africa (1950–1951, 1953), Zimbabwe (1968–1969), Egypt (1977–1978, 1993), Mauritania (1987), and Kenya and Somalia (1997–1998). These outbreaks generally involved thousands to hundreds of thousands of cases in livestock. The epidemic in Egypt in 1977–1978 probably involved some 200,000 human cases and 600 deaths. The epidemic in Mauritania involved about 1,000 human cases and 50 deaths. In late 2006, an epidemic of RVF virus began in Kenya and spread to Somalia and Tanzania, causing 1062 confirmed human illnesses and 315 deaths, a higher case-fatality rate than in previous outbreaks.

A variety of mosquito vectors have been associated with RVF virus, including *Culex pipiens* in the Egyptian outbreak. In parts of the eastern African savanna, an enzootic cycle of RVF virus has been identified in and around *dambos*, low-lying temporary wetlands. Prolonged rainfall floods these areas and allows development of large populations of *Aedes mcintoshi* and other *Aedes* spp. These mosquitoes maintain RVF virus through transovarial transmission and transmit it to domestic and wild ungulates

that come to the dambos for water, functioning as enzootic vectors. As amplification ensues, epizootic vectors such as *Culex theileri* become important in transmission to domestic livestock. RVF has been reviewed by Linthicum et al. (2016).

Malaria

Malaria is one of the most widespread and prevalent of infectious human diseases. It is caused by sporozoan protists that infect blood tissues and other organs of the body, primarily the liver. The organisms are transmitted by *Anopheles* mosquitoes. The word "malaria" derives from the Italian *mala aria*, or "bad air." Another term for malaria is **paludism** from the French *paludisme* and the Spanish *paludismo*. Both of these words are derived from the Latin *palus*, meaning "swamp." The connotation is that malaria was contracted through association with swamps and inhaling the bad air emanating from them. In English-speaking countries, malaria was called **ague** from the Old French *agu* ("sharp"), from the context of the Latin *febris acuta*, or acute fever. Ague referred to the cyclic fevers and chills, or **paroxysms**, which are characteristic of malaria.

The organisms that cause human malaria are protozoans of the genus *Plasmodium*, family Plasmodiidae (Order Haemosporidida, Class Haemosporidea, Phylum Sporozoa). They are obligate intracellular parasites. There are four species of exclusively human malaria: *P. falciparum*, causing **malignant tertian malaria**; *P. vivax*, causing **benign tertian malaria**; *P. malariae*, causing **quartan malaria**; and *P. ovale*. The first two species are widespread in the tropics, whereas *P. vivax* also occurs in some temperate areas. *Plasmodium malariae* also is distributed widely but less commonly, and *P. ovale* is rare, occurring mainly in Africa. Recently, a fifth species of *Plasmodium* that causes **quotidian malaria**, *P. knowlesi*, has been discovered to be relatively common in the human population of some countries of Southeast Asia (28%–84% of malaria cases in Malaysian Borneo). Heretofore this species had been known to occur almost exclusively in monkeys, with only occasional human infections (see Collins, 2012). Evidence indicates that similar *Plasmodium* anthropozoonoses occur in Venezuela and Brazil, involving the primate malarias *P. brasilianum* (Lalremruata et al., 2015) and *P. simium* (Brasil et al., 2017), respectively. Primate malarias are discussed later in this chapter.

Currently, 1.6 billion people are at direct risk of malaria infection via mosquito bite. Globally, in 2016 an estimated 216 million cases occurred, including 445,000 deaths. Of that total, 194 million cases and 407,000 deaths occurred in Africa (WHO World Malaria Report, 2017). Travelers may become infected during visits to endemic areas. Malaria currently occurs in and parts of northern Africa and most of southern Africa; the Middle East to Iran, Afghanistan, and Pakistan; India and Sri Lanka; parts of China, and Southeast Asia, including Indonesia, the Philippines, Irian Jaya, New Guinea; and Latin America from Mexico through Central America to most of the northern half of South America (Fig. 15.32).

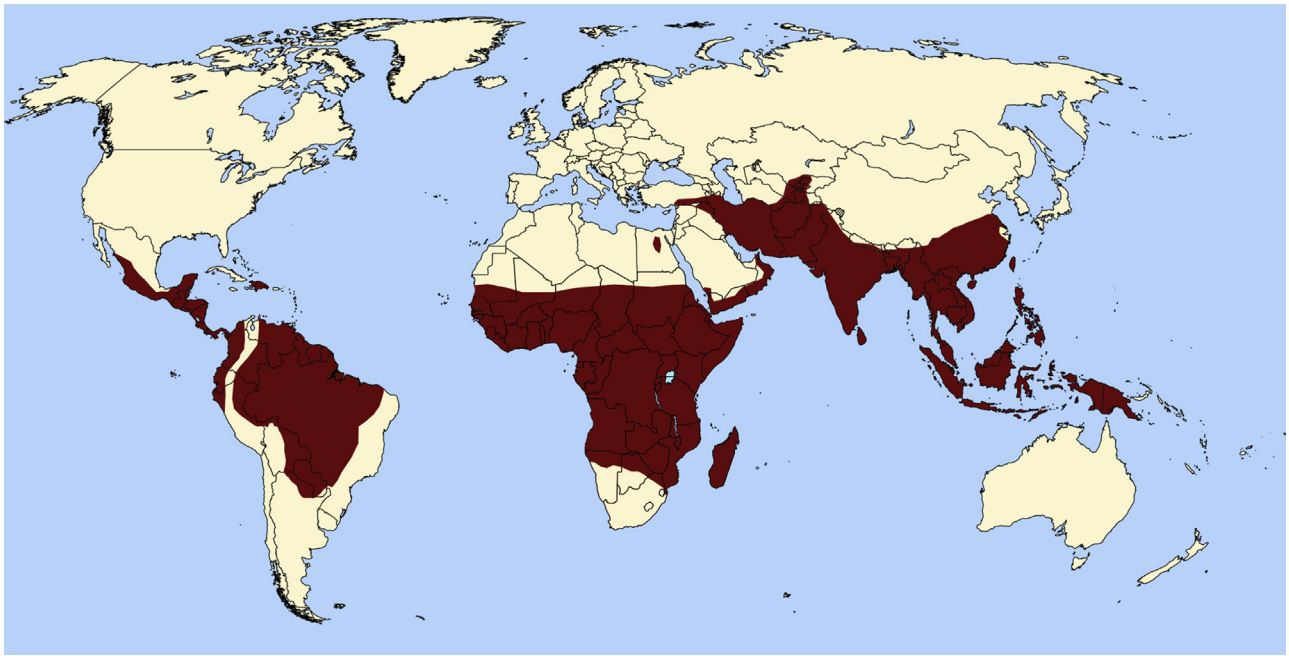

FIGURE 15.32 Geographic distribution of human malaria. *Based on data from the Centers for Disease Control and Prevention, USA.*

The degree of endemicity, or disease prevalence, depends on a variety of factors, including the species of malaria present, environmental and social factors, and species of vectors present. Human malaria is thought not to have occurred in the Western Hemisphere prior to the period of European exploration, colonization, importation of African slaves, and establishment of intercontinental trade.

The literature on human malaria and its vectors is voluminous. A comprehensive source is Gilles and Warrell (1994). Other sources include Boyd (1949), Macdonald (1957), Molineaux and Gramiccia (1980), Wernsdorfer and McGregor (1988), and Strickland (1991).

Plasmodium Life Cycle

The complex life cycle of *Plasmodium* spp. involves both sexual and asexual reproduction. The sexual phase (**gametogony**) begins in the blood of the human host and is completed within the lumen of the midgut of the mosquito. The first phase of asexual reproduction (**sporogony**) occurs on the outer wall of the midgut. The second phase of asexual reproduction occurs first in the liver and later in the blood of the human host. The process in both sites is termed **schizogony** or **merogony**. Sporogony is often referred to as the **exogenous phase** of malaria parasite development, because it occurs outside of the human host. Conversely, merogony is often referred to as the **endogenous phase** of development within the human host. These two developmental phases are depicted in Fig. 15.33 and discussed in more detail later.

An *Anopheles* female becomes infected with malarial parasites when she ingests blood containing red blood cells that are infected with **gametocytes**, specifically the sexual microgametocyte and macrogametocyte stages of the parasite. A microgametocyte bursts from its host red blood cell within the bloodmeal in the midgut lumen of the mosquito, where it extends four to eight flagella-like forms called **microgametes** in a process termed **exflagellation**. A macrogametocyte sheds the erythrocytic membrane and transforms into a single mature macrogamete. One microgamete locates and fertilizes a macrogamete, forming a diploid zygote. The zygote transforms into a motile **ookinete**. The ookinete passes through the peritrophic membrane, then through the midgut epithelial cell membrane, and forms an **oocyst** between the midgut epithelial cells and the basement membrane of the epithelium. A single, malaria-infected *Anopheles* may have few to hundreds of oocysts, depending on the original number of gametocyte-infected red blood cells in the bloodmeal and on the number of macrogametocytes that become fertilized. During sporogony, the encysted parasite becomes haploid again and undergoes multiple mitoses until the oocyst contains thousands of motile **sporozoites**. The oocyst bursts, releasing sporozoites, which make their way to the salivary glands and penetrate the secretory cells. The sporozoites

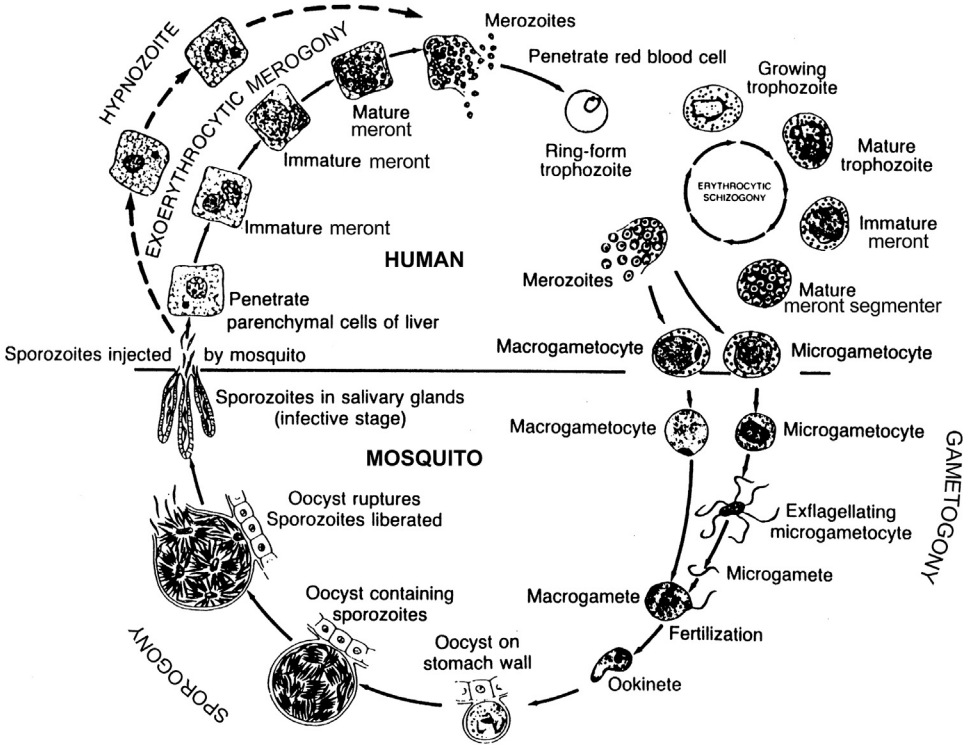

FIGURE 15.33 Life cycle of *Plasmodium vivax* in the human and *Anopheles* hosts. *Modified from Strickland, 1991.*

accumulate within these cells, with some also passing into the salivary ducts. The mosquito then is infective. The sporozoites enter a human host when the mosquito probes and the salivary gland cells release saliva into the skin prior to ingesting blood. The period of time between ingestion of gametocytes and infection of the salivary glands with sporozoites is the extrinsic incubation period.

In the human host, sporozoites migrate in the blood to the liver within minutes of entering subdermal capillaries. They invade liver parenchymal cells where they typically form a **primary tissue meront**. In the case of *P. vivax* and *P. ovale* they may form a **hypnozoite**, a quiescent or resting stage of the parasite. Inside each meront, **merozoites** develop through the process of exoerythrocytic merogony. The meront then bursts, releasing merozoites into the blood stream, where they circulate and invade red blood cells. The merozoites form meronts in the red blood cells, where they produce more merozoites through a process called erythrocytic merogony. An invasive merozoite, once inside a red blood cell, transforms into a **trophozoite**, which uses hemoglobin for nutrients, then into a segmenter form distinguished by dark dots of heme in the red blood cell, and finally into a mature meront, which produces still more merozoites. Merozoites released from infected red blood cells invade new red blood cells where the process of erythrocytic merogony begins anew. Other invasive merozoites form microgametocytes or macrogametocytes within the blood cells, which will mature into microgametes and macrogametes if they are ingested by *Anopheles* mosquitoes during blood-feeding.

Clinical Disease

Malaria is characterized by sudden paroxysms of fever and chills that recur at highly predictable intervals, often in the afternoon. Other acute symptoms include headache, lethargy, fatigue, and profuse sweating after each bout of fever. After infection of erythrocytes by merozoites, erythrocytic merogony leads to the synchronous rupture of the erythrocytes and release of new merozoites, toxins, and heme digestion products. This event in the circulatory system prompts each episode of chills and fever. The next episode occurs in 24, 48, or 72 h, depending on the species of *Plasmodium*.

Malarial infections in humans can result in severe illness and sometimes death. However, the particular symptoms, including timing and severity, vary with the species of *Plasmodium*. The most severe form of malaria is caused by *P. falciparum*, in which merozoites invade both young and old red blood cells. Over time, repeated reinvasions and mass destruction of red blood cells may lead to high parasitemia, severe anemia, and anoxia of tissues. In some cases, hemolysis results in a condition of hemoglobinuria, called **blackwater fever** when the urine contains hemoglobin and turns reddish-brown. Toxins from dead red blood cells stimulate macrophages to produce chemicals such as tumor necrosis factor and other cytokines, which cause characteristic malaria symptoms such as fever. In falciparum malaria, infected red blood cells stick to the vascular epithelium of capillaries in organs including the brain, impeding blood flow and causing a serious and sometimes lethal condition called **cerebral malaria**. Because of this affinity for internal organs, only very young trophozoites and gametocytes are common in peripheral blood. Malaria caused by *P. falciparum* is called **malignant tertian malaria** because of the severity of symptoms and because of the typical 48-h interval between paroxysms. The term "tertian" for a 2-day cycle originated from counting the day when the paroxysm occurs as the first day, so that the next paroxysm occurs on the third. Left untreated, nonfatal infections with *P. falciparum* last 5 months or more, depending on the immune status of the individual.

Plasmodium vivax malaria is called **benign tertian malaria** because symptoms are less severe than *P. falciparum* malaria, and death rarely occurs. Paroxysms occur on a 48-h cycle. In this type of malaria, the merozoites invade only immature red blood cells, called reticulocytes, which typically comprise less than 6% of the total red blood cell count in circulation. Thus vivax malaria, compared to falciparum malaria, has less severe symptoms of anemia and toxemia, making death unlikely. The infected red blood cells do not stick to the epithelial lining of capillaries as they do in falciparum malaria. Vivax malaria can evolve into chronic infection with development of an enlarged spleen, or **splenomegaly**. However, persons infected with other malarias also may have enlarged spleens, as these organs work to replace red blood cells lost to infection. The hypnozoite stage of *P. vivax* provides a mechanism for the parasite to overwinter in humans in temperate areas with short transmission seasons. The period between infection and onset of symptoms can last up to 9 months. Untreated infection persists in the body for many months to many years, with relapses recurring at irregular intervals after initial infection and acute onset of disease.

Plasmodium ovale is a less common tertian malaria with milder symptoms. Its course of infection is similar to that of *P. vivax*. Two subspecies of *P. ovale* have been detected by genetic means, *P. ovale curtisi* and *P. ovale wallikeri*. These appear to be distinct nonrecombinant lineages and may be valid species. Though they are sympatric in many regions and morphologically identical, certain biological and clinical differences are emerging.

Plasmodium knowlesi, a species previously known only as one of the monkey malarias, morphologically similar to *P. malariae*, has a 24-h or quotidian cycle of paroxysms. The high frequency of asexual reproduction in red blood cells results in hyperparasitemia, making it especially

virulent if left untreated. Still another monkey malaria species that recently has been detected in a Brazilian human population is *P. simium*, which is closely allied to *P. vivax*.

Plasmodium malariae, which causes **quartan malaria**, differs from *P. vivax* and *P. falciparum* in that the parasites invade only mature erythrocytes. Therefore, symptoms can be more severe than in vivax malaria in the acute phase. However, infections tend to develop more slowly and become chronic. Malaria caused by *P. malariae* has a 72-h erythrocytic cycle. The term *quartan* refers to the 4 days included within one cycle, with a 3-day interval from the beginning of the first paroxysm to the beginning of the next. Recrudescences of *P. malariae* may occur in individuals up to 50 years after initial infection, owing to low levels of parasitemia that increase under periods of immunosuppression. Its characteristics (including morphological, biological, and molecular-genetic attributes) are identical to *P. brasilianum*, indicating that it is probably the same species and occurs naturally in monkey populations in South America (Lalremruata et al., 2015; Rayner, 2015).

After a person is bitten and inoculated with sporozoites, and exoerythrocytic merogony commences in the liver, symptoms do not appear until days to weeks later (up to a month in *P. malariae*), when erythrocytic merogony begins in the blood. In *P. vivax* and *P. ovale*, if the sporozoites develop into hypnozoites in the liver cells, relapses are possible long after inoculation and initial onset of symptoms, with an intervening period of no apparent symptoms of infection. For *P. falciparum* and *P. malariae*, there are no persistent exoerythrocytic stages of the parasites and relapses do not occur. However, infection with *P. malariae* may recrudesce years after initial infection owing to persistent erythrocytic infections. Therefore, in human malaria there is a clear distinction between relapse and recrudescence of infection. The course of infection of malaria in humans varies with many factors, including history of past exposure and presence of antibodies; age, health, and nutritional status; and genetic resistance factors such as the sickle-cell anemia trait, Duffy-negative blood type, certain hemoglobin types such as hemoglobin S and fetal hemoglobin; and deficiency of the erythrocytic enzyme glucose-6-phospate dehydrogenase.

Mosquito Vectors and Epidemiology

Many different species of *Anopheles* mosquitoes are competent vectors of malaria organisms (Table 15.7). However, most *Anopheles* spp. are not, because of variation in host-selection patterns, longevity, abundance, and vector competence. In North America, *Anopheles quadrimaculatus*, which forms a complex with four more localized but nearly identical species (Reinert et al., 1997), is the principal vector of malaria in the eastern two-thirds of the continent. It develops along the edges of permanent pools, lakes, and swamps that provide relatively clean, still, sunlit water, with lush emergent vegetation, marginal brush, or floating debris that provides partial shade and protection from wave action. In western North America, *An. freeborni* is the main vector, an inhabitant of clear water in open, shallow, sunlit pools, ponds, ditches, and seepage areas that are partially shaded by vegetation. *Anopheles hermsi* also is a vector in California.

Other important vectors include *An. albimanus* in Central America, *An. darlingi* in South America, *An. gambiae* (Fig. 15.34) and *An. funestus* in Africa, *An. culicifacies* in Asia, and *An. dirus* in Southeast Asia. *Anopheles gambiae* is considered the most important of all, because of its involvement in such large numbers of malaria cases and deaths, mainly in Africa. This species lives in close association with humans, on which it primarily feeds, and can complete a gonotrophic cycle in only 2 days. Larvae develop in a wide variety of sunlit surface pools during the rainy seasons, many of which are associated with human activity. These include borrow pits, roadside ditches, wheel ruts, and the hoof prints of domestic animals. Larval development normally takes only about 1 week.

Malaria has been viewed in the context of stable or unstable transmission, reflecting in part the attributes of the *Anopheles* population that affect its vectorial capacity. These include density, longevity, tendency to feed on humans, and duration of the extrinsic incubation period of the parasite in the vector. **Stable malaria** is most often associated with *P. falciparum* infection in highly endemic settings. It is characterized by low fluctuations in parasite incidence in human and vector populations, high prevalence, and high seroprevalence for antibodies. Epidemics are unlikely under these conditions, even though transmission continues at high rates. In such settings, vectors tend to be highly anthropophagic, exhibit greater longevity, and have relatively low, stable densities but still exhibit considerable seasonal variation. **Unstable malaria** tends to be associated with *P. vivax* infections in endemic settings of high fluctuation in disease incidence. Vectors tend to be zoophagic, have seasonally profound variation in population densities, have low or nondetectable field infection rates, and may have shorter longevity than do those in stable malaria settings. Epidemics can occur in conditions of unstable malaria if environmental changes favor increased vector–human contact—such as during civil strife, following water projects such as dams or irrigation schemes, or when a new vector species is introduced into an area.

The epidemiological implications of infection of humans in Southeast Asia with *P. knowlesi* have not yet been fully investigated. Because the same species is maintained in monkeys, this form of malaria may be considered an anthropozoonosis. If so, even complete interruption of transmission between humans will fail to

TABLE 15.7 ***Anopheles*** **Vectors of Human Malaria Parasites in 12 Epidemiologic Zones**

Malaria Epidemiologic Zone	*Anopheles* Vectors
North American	Subgenus *Anopheles*: *freeborni, punctipennis, quadrimaculatus* Subgenus *Nyssorhynchus*: *albimanus*
Central American	Subgenus *Anopheles: aztecus, pseudopunctipennis, punctimacula* Subgenus *Nyssorhynchus: albimanus, albitarsis, allopha, aquasalis, argyritarsis, darlingi*
South American	Subgenus *Anopheles*: *pseudopunctipennis, punctimacula* Subgenus *Nyssorhynchus: albimanus, albitarsis, aquasalis, argyritarsis, braziliensis, darlingi, nuneztovari* Subgenus *Kerteszia*: *bellator, cruzii*
North Eurasian	Subgenus *Anopheles*: *atroparvus, messeae, sacharovi, sinensis* Subgenus *Cellia*: *pattoni*
Mediterranean	Subgenus *Anopheles*: *atroparvus, claviger, labranchiae, messeae, sacharovi* Subgenus *Cellia*: *hispaniola, pattoni*
Africo-Arabian	Subgenus *Cellia*: *hispaniola, multicolor, pharoensis, sergentii*
Africo-Tropical	Subgenus *Cellia: arabiensis, christyi coluzzii, funestus, gambiae, melas, merus, moucheti, nili, pharoensis*
Indo-Iranian	Subgenus *Anopheles: sacharovi* Subgenus *Cellia: annularis, culicifacies, fluviatilis, pulcherrimus, stephensi, superpictus, tesselatus*
Indo-Chinese Hills	Subgenus *Anopheles*: *nigerrimus* Subgenus *Cellia: annularis, culicifacies, dirus, fluviatilis, maculatus, minimus*
Malaysian	Subgenus *Anopheles*: *campestris, donaldi, letifer, nigerrimus, whartoni* Subgenus *Cellia: aconitus, balabacensis, dirus, flavirostris, leucosphyrus, ludlowae, maculatus, minimus, philippinensis, subpictus, sundaicus*
Chinese	Subgenus *Anopheles*: *anthropophagus, sinensis* Subgenus *Cellia: pattoni*
Australasian	Subgenus *Anopheles*: *bancrofti* Subgenus *Cellia*: *annulipes, farauti, karwari, koliensis, punctulatus, subpictus*

Subgenera, species, and geographic distributions are given.
From Macdonald, 1957.

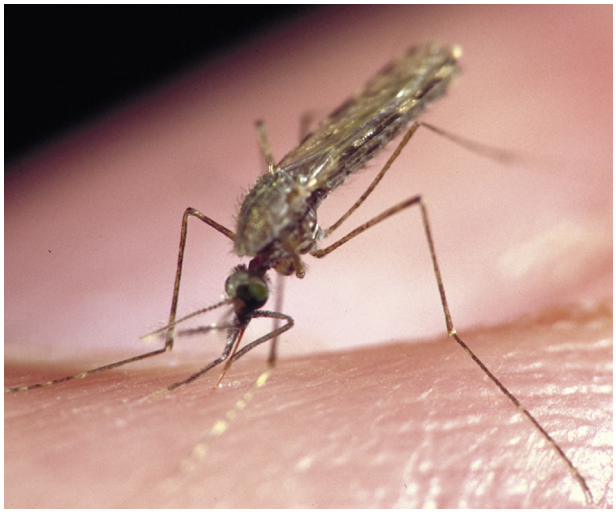

FIGURE 15.34 *Anopheles gambiae* female feeding on blood. This is a major vector of human malaria in Africa, where most cases occur. *Photograph by Woodbridge A. Foster.*

eradicate the parasite, as in the case of YF. The same implications apply to *P. malariae* and *P. simium*, both of which also appear to be anthropozoonoses (i.e., human malarias with monkey reservoirs).

Historical Perspective

After the development of the germ theory of disease by **Louis Pasteur**, the French Algerian physician **Charles Louis Alphonse Laveran** examined and described malarial organisms in the red blood cells of his patients in 1870. This finding, along with the work of **Patrick Manson** (Fig. 1.1A) on filarial nematodes and mosquitoes in China, inspired **Ronald Ross** (Fig. 1.1C), then a physician in British colonial India, to examine the hypothesis of mosquito transmission of malaria parasites in the 1890s. His persistent and careful experimentation and observation with both human and bird malarias, using *Anopheles* and *Culex*

mosquitoes, respectively, provided conclusive proof that mosquitoes transmit *Plasmodium* spp. via bite. In concurrent research, **Giovanni Batista Grassi** and colleagues demonstrated transmission of *Plasmodium falciparum* by *Anopheles maculipennis*—complex mosquitoes in the environs of Rome, Italy. Ross was awarded the Nobel Prize for Medicine in 1902.

Malaria was formerly endemic in many temperate areas of the United States, particularly in the south and southeast. Malaria became epidemic after the Civil War, as malaria-infected soldiers returned to their homes and brought the infection with them to their local communities. Malaria was an important rural disease in the eastern and southern states, California, and other areas of the United States through the 1930s, but gradually disappeared by the 1940s. This was due to a combination of antimosquito measures, improved medical care, a higher standard of living, and transformation of marshes and swamps to agricultural land largely through organized ditching efforts. Changes in life style because of technological advances such as window screens and the invention of the radio, television, and air conditioning also contributed to the decline in malaria. Boyd (1941) reviewed the history of malaria in the United States, and to a brief degree elsewhere in the New World.

Roughly 1000 cases of malaria are introduced into the United States each year. In addition, cases involving local or indigenous transmission occur sporadically, including outbreaks in California (1988, 1989, 1990), Florida (1990, 1996), Michigan (1995), New Jersey (1993), New York (1993), and Texas (1995). These incidents were due to introductions of infected humans into areas with competent *Anopheles* vectors. However, **airport malaria** has occurred near major international airports (e.g., London-Heathrow and Paris-DeGaulle) where infected mosquitoes have been imported on aircraft from endemic regions.

Filariasis

Filariasis is the infection of vertebrate tissues by filarial nematodes or roundworms (Phylum Nematoda, Order Spirurida, Superfamily Filarioidea, Family Onchocercidae). Mosquito-borne filarial nematodes are associated with acute and chronic human disease, termed **lymphatic filariasis**, which is widespread in tropical and subtropical regions (Grove, 1990). The three causative agents of lymphatic filariasis are *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*.

The areas of the world endemic for lymphatic filariasis (Figs. 15.35 and 15.36) include parts of western, central, and southern Africa; parts of northeastern South America (principally Brazil, Surinam, and French Guyana), the Dominican Republic, and Haiti; southern and eastern India, southeastern Asia, eastern China, and southern Japan; the Malay archipelago, Indonesia, the Philippines, Irian Jaya and Papua New Guinea; and many island groups of the south Pacific Ocean, including Melanesia, Micronesia, and Polynesia. Within the United States, filariasis was locally endemic in Charleston, South Carolina, but the disease disappeared there in the late 1930s. It disappeared at about

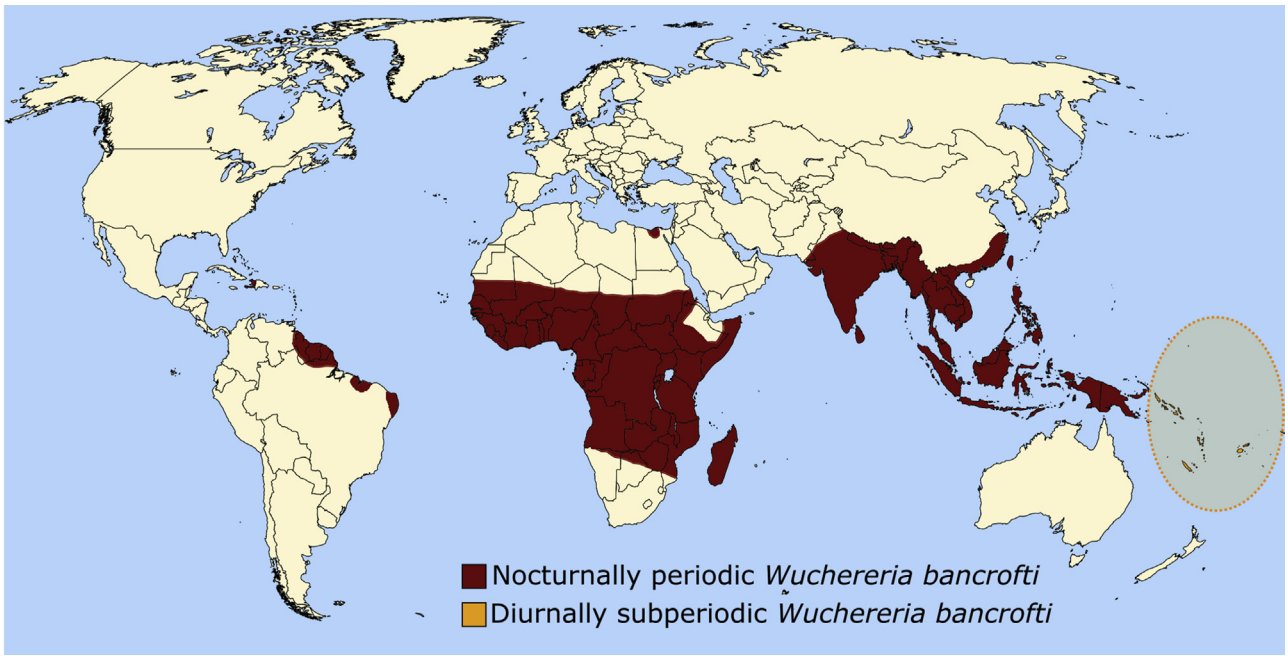

FIGURE 15.35 Geographic distribution of human lymphatic filariasis caused by *Wuchereria bancrofti*, showing both nocturnal and diurnal periodicity. *Based on data from the Centers for Disease Control and Prevention, USA, and the World Health Organization.*

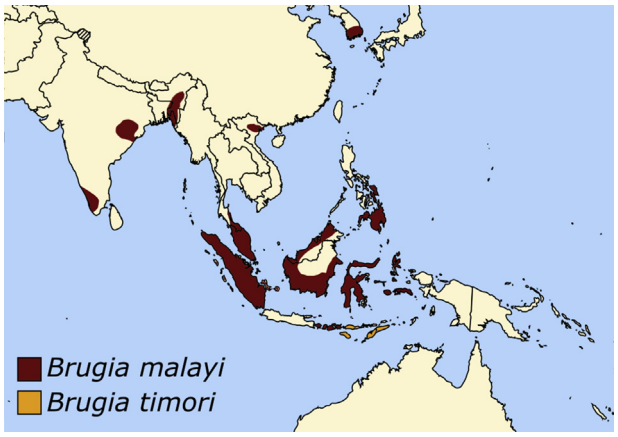

FIGURE 15.36 Geographic distribution of human lymphatic filariasis caused by *Brugia malayi* and *B. timori*. *Reconstructed from Strickland, 1991, and other sources.*

the same time from northern Australia. Lymphatic filariasis no longer occurs in most regions of the Mediterranean basin and on the Arabian Peninsula. Transmission continues, however, along the Nile River and in the Nile Delta of Egypt. Cano et al. (2014) presents a global perspective on its distribution and geographic limits.

Within the area of current distribution, there are an estimated 905 million people at risk of contracting lymphatic filariasis, and some 128 million active infections. Of these, about 115 million are caused by *W. bancrofti*, the causative agent of **Bancroftian filariasis**, which is widespread in both the Old World and New World tropics (Fig. 15.35). Another 13 million cases are caused by *B. malayi*, the causative agent of **Brugian filariasis** or **Malayan filariasis**, which is restricted to southeastern Asia (Fig. 15.36). About 43 million people have chronic symptoms of elephantiasis, hydrocele, or lymphedema (see later). Another Brugian filariasis, **Timorian filariasis**, is caused by infection with *B. timori* and occurs in localized foci among southern islands of Indonesia.

Filarial Life Cycle

Infection with filarial nematodes in humans begins when infective third-stage larvae enter the skin at the site of the mosquito bite, molt twice, and migrate to the lymphatic vessels and lymph nodes, particularly of the lower abdomen. There, the nematodes develop to the adult stage. Female worms (80−100 mm long at maturity for *W. bancrofti* and about half of that length for *B. malayi*) release active, immature worms called **microfilariae** into the peripheral circulatory system. A female can release 50,000 or more microfilariae each day. Microfilariae of *W. bancrofti* are 250−300 µm long and about 7−9 µm wide; those of *B. malayi* are somewhat shorter and thinner. Presence of microfilariae in the blood is called **microfilaremia** and first appears about 6 months to 1 year after adult worms become established in the lymphatic system. An infected human may be microfilaremic for longer than 10 years. The density of microfilariae in peripheral blood is highly variable, but it can range from one to more than 500 microfilariae per 20 mm^3.

The appearance of microfilariae in the peripheral blood has a 24-h cycle (i.e., they exhibit **diel periodicity**). If microfilariae completely disappear from the peripheral circulation at some time during the day, they are said to be **periodic**. If the microfilariae fluctuate in density during a 24-h period but are detectable at all times, they are said to be **subperiodic**. In most areas, the microfilariae appear only at night and are transmitted by mosquitoes that have night-biting habits. These are the **nocturnally periodic** forms of *W. bancrofti* and *B. malayi*. Both *W. bancrofti* and *B. malayi* also have nocturnally subperiodic and diurnally subperiodic forms, though nocturnally subperiodic Bancroftian and diurnally subperiodic Brugian forms have very restricted distributions. In all the subperiodic forms, the microfilariae appear in the peripheral blood of the human host mainly in the evening and night (nocturnally subperiodic) or mainly during the daytime (diurnally subperiodic). These nematodes are associated with two or more species of mosquito vectors, which differ in their typical biting times and whose combined diel patterns of human-biting density are matched by the periodicity of microfilariae. Both nocturnally periodic *W. bancrofti* and nocturnally periodic *B. malayi* are now considered to be strictly human pathogens. However, *B. malayi* in its subperiodic form is a zoonosis, with both leaf monkeys and humans as reservoirs, and with domestic cats and other carnivores also implicated as hosts. *Brugia timori* is nocturnally periodic and has no animal reservoir.

The development of *Wuchereria bancrofti* and *Brugia malayi* in mosquitoes is similar. Microfilariae ingested with the mosquito bloodmeal usually shed their outer, sheathlike membrane as they penetrate through the midgut epithelium. Some microfilariae retain the sheath during penetration. The microfilariae move to the indirect flight muscles of the thorax, penetrate individual cells, and transform to a short **sausage stage**, the L1 or first-stage larva. They molt to more slender L2 or second-stage larvae and then again to the elongate, filariform L3 or third-stage infective larvae (about 1.5 mm long). These larvae leave the thoracic flight muscles, traverse the hemocoel of the mosquito, enter the lumen of the mouthparts, and eventually arrive at the apex of the proboscis. When the mosquito blood-feeds, the L3 larvae exit through the cuticle of the labium, crawl onto the skin, and enter through the hole made by the mosquito during feeding. Therefore, technically the transmission method may be said to be contaminative, rather than inoculative. Heavy infections of larval nematodes can be fatal to the mosquito.

Many species of mosquitoes are refractory to filarial development, owing to genetic factors and to their ability to mount adequate immune responses, whereas other species are susceptible to parasite infection and support the development described earlier. The relationships between *W. bancrofti* and certain mosquito species exhibit evidence of local adaptation. For example, in West Africa, *Culex quinquefasciatus* is not a competent vector of *W. bancrofti*, whereas *Anopheles gambiae* is competent there. By contrast, in India, *Cx. quinquefasciatus* is a competent vector and most *Anopheles* species are not. Thus, there is geographic variation in susceptibility of mosquitoes to filarial nematodes.

Clinical Disease

Generally, a case of human infection does not occur after the bite of a single, infective mosquito. Rather it results after accumulation of hundreds to thousands of such infective bites, under conditions in which there is a high probability of parasite maturation and mating. Humans may show disease symptoms without having microfilaremia, or may have microfilaremia without showing signs of disease. Lymphatic filariasis has both acute and chronic manifestations in the human host. The disease may cause chronic debilitation in untreated cases. **Acute lymphatic filariasis** is characterized by episodes of fever, swelling, pain, and inflammation in the affected lymph nodes and lymph vessels, a condition called adenolymphangitis. The episodes may last for several days and incapacitate the affected individual because of the local and systemic effects. Over time, deep abscesses may develop at the sites of inflammation. Dermal ulcers may form through the skin over these sites, and secondary bacterial infection may ensue.

In the **chronic phase**, which often occurs years after onset of acute symptoms, the pathology may involve accumulation of lymphatic fluid (lymphedema) in the limbs, breasts, vulva, and scrotum, resulting in swelling and enlargement. In the scrotum, this condition is termed hydrocele. The grotesque distentions and thickening, folding, and nodulation of the skin, notably, the lower limbs, is a condition called **elephantiasis** (Figs. 15.37 and 15.38). Bacterial and fungal infections at affected sites exacerbate these conditions. Appearance of lymph fluid in urine may occur as a consequence of the disruption of the abdominal lymphatic vessels and leakage of lymph fluid into the urinary tract, causing urine to appear whitish, a condition called **chyluria**. In contrast with *W. bancrofti*, infection

FIGURE 15.37 Human lymphatic filariasis in two Tahitian women, 60-year-old (left) and 30-year-old (right), in the early 1940s; both individuals after enlargement of legs reduced 30%–50% of their former size by tight bandaging for 6 months, enabling them to walk again. *Photograph by S. Allen Edgar.*

FIGURE 15.38 Human lymphatic filariasis in Tahitian man, with extreme case of elephantiasis involving notably, the left arm, left leg, and scrotum; photograph taken during World War II. *Photograph by S. Allen Edgar.*

with *B. malayi* is not associated with scrotal distension, but rather involves only the limbs. Hypersensitivity to parasite-associated antigens also may be part of the syndrome of lymphatic filariasis. It is mediated through elevated IgE and IgG4 antibodies and is characterized by increased production of eosinophils, coughing, and shortness of breath. This type of filarial disease is called **tropical eosinophilia**.

Lymphatic filariasis has important social implications in communities where it is endemic. Acutely affected individuals are often feverish and in pain, and they may have difficulty working and thus suffer economic loss. Hard work may bring on attacks of filarial fever, which require rest for recovery. In a study conducted in Ghana, West Africa, the episodes of acute adenolymphangitis lasted about 5 days, with 3 days of incapacitation; they occurred during those months of the year when peak agricultural work was required and when mosquito transmission of infective-stage larvae was highest (Gyapong et al., 1996). Likely, chronically infected individuals are immunologically sensitized to their worm infections, and exposure to new L3 larvae results in hypersensitive reactions such as adenitis. Chronically affected individuals with symptoms of scrotal hydrocele and elephantiasis may have difficulty in their social and personal lives and may suffer incontinence and impotence. Although hydrocele can be treated through fluid aspiration from the scrotum, the gross distention associated with elephantiasis is difficult to remedy even with surgery.

Mosquito Vectors and Epidemiology

The filarial nematodes that cause lymphatic filariasis have evolved associations with mosquitoes in the genera *Culex*, *Mansonia*, *Aedes*, and *Anopheles* (Table 15.8). This probably has occurred through a process of adaptive radiation from the original *Anopheles* vectors in Southeast Asia. A very likely scenario for *W. bancrofti* is that it arose as a human pathogen in forested regions of Indonesia and perhaps other parts of Southeast Asia, where the *Anopheles umbrosus* group serves as vectors of *Wuchereria kalimantani*. This filarioid nematode is a parasite of the silvered leaf monkey (brow-ridged langur), *Presbytis cristata*. Similarly, *B. malayi* infection in humans probably evolved from subperiodic *B. malayi* infections among leaf monkey species (*Presbytis* spp.), with *An. hyrcanus* as the vector. Human infection and new disease foci probably arose in forests and along forest ecotones with these same *Anopheles* vectors. Through time, as people developed agricultural systems and moved to other regions, both *W. bancrofti* and *B. malayi* adapted to these new settings and the competent mosquito vectors there. In some parts of Southeast Asia, members of the *Aedes niveus* group are important vectors of the subperiodic form of *W. bancrofti*, including *Ae. niveus* in Thailand; this system may have been the origin of the subperiodic strains that radiated into the Pacific regions.

Transmission of *W. bancrofti* occurs in both urban and rural areas. The primary mosquito vector in urban areas is *Culex quinquefasciatus*. It is the most important vector of nocturnally periodic *W. bancrofti* in the Americas and parts of Africa and Asia, particularly India. It feeds opportunistically at night on both mammals and birds. This mosquito occurs abundantly in areas with poor sanitation, open sewers, untreated waste water, and pit latrines, which provide the high organic content and low oxygen characteristic of the larval habitat. For this reason, *Cx. quinquefasciatus* is often more abundant during parts of the year when water stagnates from lack of rain. In the Nile River Delta of Egypt, *Culex molestus*, a name applied to the autogenous variant of *Cx. pipiens,* is the primary vector. In rural settings, nocturnally periodic Bancroftian and Brugian filariases are transmitted by *Anopheles* mosquitoes, which are also nocturnally active. Often, the same *Anopheles* species that transmit *W. bancrofti* or *B. malayi* in an area also are responsible for local malaria transmission (e.g., *An. darlingi* in South America and *An. gambiae* and *An. funestus* in parts of West and East Africa, respectively, for Bancroftian filariasis; and *An. sinensis* in rice-growing areas of China, for Brugian filariasis).

Nocturnally periodic Brugian filariasis occurs in rural parts of southern India, Malaysia, the Philippines, and Indonesia; there the nocturnally active *Mansonia annulifera* and *Ma. uniformis*, and also *Anopheles* species, are vectors near rice fields and open swamps. The nocturnally subperiodic form occurs in swamp forest areas of Southeast Asia and Indonesia, involving *Ma. bonneae* and *Ma. dives,* which are nocturnally active but also feed during the day within the swamp forests. All of these *Mansonia* species are associated with particular kinds of plants, where the larvae and pupae attach to their submerged roots and stems. In the Pacific region, where many island groups are endemic for *W. bancrofti*, the primary vectors are day-biting *Aedes* spp., but nocturnal biters also are involved, and the form of the parasite is diurnally subperiodic. *Anopheles barbirostris* is the vector of *Brugia timori*.

The endemicity of mosquito-borne filariasis depends on a high and steady rate of transmission of infective-stage larvae in the human population. In endemic areas, the inoculation rate (parasite transfer rate) can range as high as hundreds of infective bites and thousands of larval inoculations per person per year. The estimated number of L3 larvae transmitted per person per year is called the annual transmission potential. With each infective bite, only a few L3 larvae actually enter the skin. These larvae must then develop further and move to a person's lymphatic system, where mature male and female nematodes mate and initiate microfilarial production. Accumulation of thousands of infective bites over months or years eventually results in an

TABLE 15.8 Mosquito Vectors of Filarioid Nematodes of Humans: Geographic Distribution, and Associations With Periodicity of Microfilaremia

Geographic Region	Filarioid Species	Periodicity	Mosquito Vectors
Neotropical	*Wuchereria bancrofti*	Nocturnally periodic	*Anopheles aquasalis, An. bellator, An. darlingi, Aedes scapularis, Culex quinquefasciatus, Mansonia titillans.*
Afrotropical	*Wuchereria bancrofti*	Nocturnally periodic	*Anopheles funestus, An. gambiae, An. arabiensis, An. bwambae, An. melas, An. merus, An. nili, An. pauliani, Culex quinquefasciatus.*
Middle Eastern	*Wuchereria bancrofti*	Nocturnally periodic	*Culex molestus.*
Oriental	*Wuchereria bancrofti*	Nocturnally periodic	*Anopheles anthropophagus, An. kweiyangensis, An. nigerrimus, An. letifer, An. whartoni, An. aconitus, An. flavirostris, An. minimus, An. candidiensis, An. balabacensis, An. leucosphyrus, An. maculatus, An. philippinensis, An. subpictus, An. vagus, Aedes niveus, Ae. togoi, Ae. poicilius, Culex bitaeniorhynchus, Cx. sitiens complex, Cx. pallens, Cx. quinquefasciatus, Mansonia uniformis.*
	Wuchereria bancrofti	Nocturnally subperiodic	*Anopheles sinensis complex, Aedes harinasutai.*
	Brugia malayi	Nocturnally periodic	*Anopheles barbirostris, An. campestris, An. donaldi, An. anthropophagus, An. kweiyangensis, An. nigerrimus, Aedes togoi, Mansonia uniformis, Ma. bonneae, Ma. dives.*
	Brugia malayi	Nocturnally Subperiodic	*Anopheles sinensis complex, Mansonia uniformis, Ma. bonneae, Ma. annulata, Ma. indiana, Ma. dives.*
	Brugia timori	Nocturnally periodic	*Anopheles barbirostris.*
West Pacific	*Wuchereria bancrofti*	Nocturnally periodic	*Culex pallens, Aedes poicilius, Ae. togoi.*
	Brugia malayi	Nocturnally subperiodic	*Aedes togoi.*
Papuan	*Wuchereria bancrofti*	Nocturnally periodic	*Anopheles bancrofti, An. punctulatus, An. farauti, An. koliensis, Culex annulirostris Cx. bitaeniorhynchus, Mansonia uniformis*
South Pacific	*Wuchereria bancrofti*	Nocturnally subperiodic	*Aedes samoanus.*
		Diurnally subperiodic	*Aedes fijiensis, Ae. oceanicus, Ae. vigilax* group, *Ae. futunae, Ae. polynesiensis, Ae. pseudoscutellaris, Ae. tabu, Ae. tongae, Ae. upolensis.*
	Brugia malayi	Nocturnally periodic	*Aedes oceanicus.*
		Nocturnally subperiodic	*Aedes oceanicus.*

infection of mature worms in a human, who normally then will have a microfilaremia and possibly, chronic disease. Most microfilariae entering the circulatory system are never ingested by a mosquito, while those that are ingested become infective-stage larvae only in a competent vector, and only if the individual mosquitoes survive the extrinsic incubation period. Furthermore, many infective-stage larvae fail to reach a new human host or fail to mature if they do. The inefficiency of transmission and parasite perpetuation is compensated by the prodigious production of microfilariae and the long life of adult worms.

Historical Perspective

Association of infection with filarial nematodes and lymphatic filariasis was first established in the late 1800s. Our understanding of the natural history of lymphatic filariasis is related intimately to the initial discovery of a

link between human pathogens and insect vectors. During 1877 to 1878, **Patrick Manson** (Fig. 1.1A), working in China as a medical officer for the Chinese Imperial Customs Service, conducted experiments on the development of filarial nematodes. Manson had already discovered that the microfilariae occurred in the peripheral blood only at night. He speculated that this was timed to coincide with the night-time biting activity of mosquitoes. After feeding mosquitoes (*Culex quinquefasciatus*) on his gardener, who had a microfilaremia, and then dissecting the mosquitoes on successive days, he found that the worms developed within the mosquitoes into longer, different forms. He speculated that the mosquito functioned as a kind of "nurse" for the filarial worms, so that when a mosquito died on the water after laying an egg raft, the worms entered the water and later infected a person drinking it. At that time, it was not known that mosquitoes could bite more than once during their lives, so the principle of transmission by bite was not established. Yet, the idea that mosquitoes can function as intermediate hosts for a human pathogen is founded on Manson's experiments.

VETERINARY IMPORTANCE

Aside from their importance as vectors of disease agents of animals, mosquitoes are a cause of irritation, blood loss, and allergic reactions. They not are only annoying but also disrupt normal behavior of livestock and companion animals. Large swarms may cause livestock to discontinue feeding and to seek relief. Increased scratching behavior may result in skin abrasions, hair loss, and secondary infection with bacteria at the bite and scratch sites. For cattle, mosquito bites can result in decreased weight gains and milk production and prompt producers to alter pasturing practices. Deaths of cattle due to anemia and stress have been reported.

Mosquito-Borne Viruses of Animals

Mosquito-borne viruses affecting domesticated animals include the groups of alphaviruses that are associated with the equine encephalitides (EEE, WEE, and VEE), all of which cause an acute encephalitis with high fever in equids (i.e., horses, donkeys, mules). The history, distribution, vector relationships, and vertebrate reservoir hosts of these viruses were discussed earlier in the section "Public Health Importance." Other mosquito-borne viruses of veterinary significance include JE virus, Rift Valley fever virus, Wesselsbron virus, fowlpox virus, and myxomatosis virus. Equine infectious anemia (EIA) virus, a lentivirus in the family Retroviridae, may be mechanically transmitted by mosquitoes, but its more important mechanical vectors are larger biting flies (deer flies, horse flies, and stable flies).

FIGURE 15.39 Horse dying from infection with Eastern equine encephalomyelitis virus in Michigan outbreak in 1980. *Photograph by Harold D. Newson.*

Eastern Equine Encephalomyelitis (EEE) virus. This virus is an important cause of mortality of horses and other equids, caged pheasants, whooping cranes, and emus. It occurs in endemic areas of the United States in Texas, along the Gulf Coast and Atlantic Seaboard to Massachusetts, and at inland sites in upstate New York, Ohio, Michigan, Indiana, Georgia, and Alabama. Horses rapidly succumb to infection after a short incubation period of 2–5 days. They exhibit abnormal behavior and high fever, then drop to the ground and lapse into coma before death (Fig. 15.39). Few horses survive infection involving these acute symptoms. Viral infection in the brain shows characteristic lesions in nervous tissue, accompanied by perivascular cuffing with macrophages; viral antigen is detectable in neurons (Fig. 15.40). In pheasant flocks, often a single infected, sick bird will be pecked by other birds, thus transmitting the virus directly to healthy birds without mosquito bite. During such occurrences, called **epiornitics**, thousands of pheasants in a single outdoor pen may die, yet

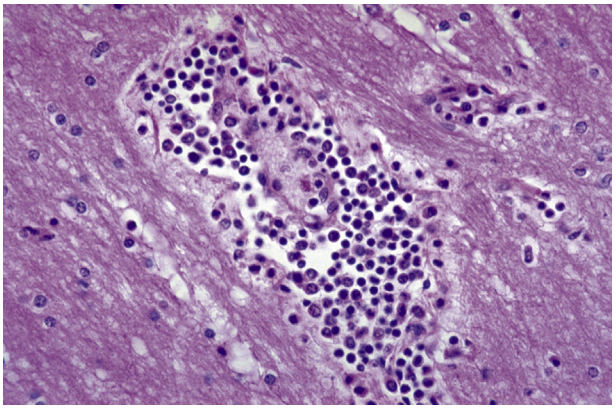

FIGURE 15.40 Section of horse brain (cerebrum) infected with Eastern equine encephalomyelitis virus, showing neutrophil invasion around capillary. *Photograph by Jonathan D. Patterson.*

none of the pheasants in adjacent pens become infected. Aside from equids and exotic birds, EEE viral infection has been reported in young dogs and pigs.

Cases of EEE in horses in cool temperate climates tend to occur in mid to late summer and early fall, whereas in milder climates horse cases begin to occur earlier in the spring and summer. In the tropics and subtropics, cases may occur year-round. Horse deaths due to EEE viral infection are an important indicator of virus activity promoted by bridge vectors in endemic areas. Rapid, differential diagnosis of horse cases is crucial if these animals are to be used as sentinels for potential transmission of EEE virus to humans.

Western Equine Encephalomyelitis (EEE) Virus. As with EEE virus, the primary epidemic host of significance for WEE virus is the horse. Human cases are rare. Since the first isolation of WEE virus from the brain of a dead horse in 1930 in the San Joaquin Valley of California (USA), this virus has been implicated in epizootics in horses, with cases numbering from hundreds to thousands in some instances. Large outbreaks occurred in 1941 and 1975 in the Red River Valley in Minnesota, North Dakota, and Manitoba (USA and Canada), in 1952 in the Central Valley of California, and in 1965 in Hale County in western Texas (USA). There are horse cases almost every summer within the range of the virus, but epizootics do not always occur. For both EEE and WEE viruses, immunization has reduced the frequency of horse cases.

Venezuelan Equine Encephalomyelitis (VEE) Complex. Encephalomyelitis in equids caused by viruses of the VEE complex occurs in northern South America, Central America, and Mexico. The epizootic viruses are transmitted by many species of mosquitoes (see Table 15.4) among horses, burros, and mules. These animals develop a viremia sufficient to infect the mosquitoes. Consequently, VEE epidemics can be maintained by transmission between mosquito and horses, differing in this regard from WEE and EEE, for which horses are largely dead-end hosts. An epidemic strain of VEE virus was first isolated in 1938 from a horse in Venezuela. In 1969, a large outbreak of VEE involving both equids and humans in Central America spread northward in the succeeding 2 years through Mexico, and in 1971 across the border into Texas (USA) (PAHO, 1972). Cases continued in Mexico through 1972. There were thousands of horse cases throughout this region during that time. Epizootic virus activity did not occur again in the region until an outbreak in Venezuela in 1992 and 1993 and in Chiapas, Mexico, in 1993. Another outbreak of VEE occurred in northern Colombia and Venezuela in 1995. The rapidity of spread of these outbreaks over large geographic areas is undoubtedly due to the role of both horses and birds as competent reservoir hosts. It is expedited by the evacuation of horses, already infected but not yet ill, away from an epizootic area.

Japanese Encephalitis (JE) Virus. Encephalitis caused by JE virus occurs in widespread parts of Asia, including Malaysia and Indonesia. In Japan, epizootics and epidemics have occurred in August and September in many years since the discovery of this disease in 1935 in that country. The virus was isolated from brain tissue of a horse in 1937. Japanese encephalitis virus causes acute infection in horses and swine. It is particularly an economic problem because of the importance of swine as a food source and market commodity in rural Asia. Pigs develop viremia sufficient for mosquito transmission, therefore serving as important amplifying hosts, and may develop encephalitic symptoms. Transplacental infection causes stillbirth and abortion. Infected boars may become sterile.

Rift Valley Fever (RVF) virus. This pathogen has caused epizootics of acute illness, elevated rates of abortion, and death in cattle, goats, and sheep in Egypt and parts of Africa. Outbreaks in Egypt and Mauritania were particularly noteworthy. The virus is both viscerotropic and neurotropic in these animals. Their viremias are of sufficient titer to infect mosquitoes. Disease outbreaks generally have involved thousands to hundreds of thousands of livestock cases, causing substantial economic losses, but these losses are rarely counted accurately.

Wesselsbron (WSL) Virus. This is a flavivirus with distribution in parts of Africa, Madagascar, and Thailand. It causes a disease similar to that of RVF in sheep and goats and also causes a mild illness in cattle. Infected ewes may abort their fetuses, and lambs suffer high mortality. Humans infected with Wesselsbron virus may develop a febrile illness with rash, fever, and myalgia. The virus is transmitted by *Aedes* spp., including *Ae. mcintoshi* and *Ae. circumluteolus* in South Africa.

Fowlpox (FP) virus. This virus belongs to a group of poxviruses that infect vertebrates and invertebrates and are classified within the family Poxviridae. Among the poxviruses are those in the bird-infecting genus *Avipoxvirus*, such as fowlpox, canarypox, and pigeonpox viruses. Mosquitoes may mechanically transmit the avipox viruses by contamination of mouthparts and subsequent transfer of infectious virions to noninfected birds. Fowlpox is an important disease of domestic fowl, particularly chickens. It causes development of papules along the comb and beak. While probing these papules, mosquitoes may contaminate their mouthparts with virions. If disturbed during feeding, they may move to another animal to feed, thus transferring the virus to a new host. Another form of fowlpox virus is transmitted directly by droplets of pus-containing the virus.

Myxoma (MYX) virus. This is a leporivirus and the causative agent of **myxomatosis**, an enzootic disease of lagomorphs in parts of South America and the western United States. It is transmitted mechanically by bite of arthropods, principally by mosquitoes and fleas, depending on the region. These viruses produce dermal, vascularized tumors. When vectors probe these tumors, the mouthparts become contaminated with virus particles. Later, if the mosquitoes probe another uninfected lagomorph, that animal may become infected. Natural infections of myxoma virus occur without acute disease in rabbits of the genus *Sylvilagus* in South America and California (USA). However, Old World rabbits (*Oryctolagus cuniculus*) are highly susceptible to infection and generally die. Outbreaks of acute disease among domesticated Old World rabbits have been documented in South America and California.

Myxoma virus was introduced into Australia in the 1950s as a means of controlling introduced European rabbits, a pest in that country. The virus spread rapidly through the rabbit populations via mechanical transmission by mosquitoes, fleas, and other means, and greatly reduced rabbit populations there. The mosquito vectors in Australia are *Culex annulirostris*, *Anopheles annulipes*, and *Aedes* spp.

Nonhuman Malarias

Many *Plasmodium* spp. infect animals other than humans, including reptiles, birds, rodents, and nonhuman primates.

Reptilian Malarias

The malarias of reptiles, formerly called saurian malarias, are caused by a group of 29 *Plasmodium* species. They infect a wide range of lizards and some snakes in 15 families (Telford, 1994). Vectors are biting midges, phlebotomine sand flies, and *Culex* mosquitoes. Haemoproteid and leucocytozooid malarias also occur in reptiles, but their vectors have not been established.

Avian Malarias

Malarial infection of birds is widespread geographically (Van Riper et al., 1994). Parasites in three common genera of hemosporine blood parasites of birds (*Hepatocystis*, *Haemoproteus*, and *Leucocytozoon*) are transmitted by biting midge, louse fly, and black fly vectors, respectively. The avian malarias in the genus *Plasmodium* are all mosquito-borne. *Plasmodium* spp. that infect birds have been important research models for studying malaria. Indeed, the original observations by Ronald Ross on the role of mosquitoes as malaria vectors were made with bird malaria.

Currently, about 30 species of avian *Plasmodium* are recognized. However, the taxonomic status of some species is uncertain and others remain to be described. Among the important species that cause disease in domestic fowl or wild birds are *P. gallinaceum* (sometimes called **chicken malaria**), *P. hermansi* (a parasite of wild and domestic turkeys in the United States), *P. relictum*, *P. lophurae*, *P. cathemerium*, *P. circumflexum*, and *P. elongatum*. As with human malarias, there is variation in life cycles and pathogenesis of the avian malarias. This variation is related to intrinsic qualities of the species and to variation in susceptibility among host species, age, and general health status.

A bird becomes infected after inoculation of sporozoites from an infective mosquito. Merogony occurs in bone marrow, endothelial cells, and in the erythrocytes. In acute infections, these parasites may cause severe anemia, damage to bone marrow tissues, and other pathology that may result in death. Younger birds tend to be more susceptible to overt illness than older birds.

Although *Anopheles* mosquitoes can be competent laboratory vectors for some bird malarias, field and laboratory data show that culicines in the genera *Culex*, *Culiseta*, and *Aedes* are the natural vectors. In Africa, *Aedes aegypti* is an important local vector of *P. gallinaceum* to chickens. The impact of bird malaria on natural bird populations is poorly known. It was introduced into Hawaii (USA) along with exotic birds and *Culex* mosquitoes and is thought to be responsible for the reduction and extinction of native bird populations there. Bird malaria occasionally has been documented as the cause of morbidity and mortality among penguins in zoos.

Rodent Malarias

The 12 *Plasmodium* spp./ infecting rodents, called rodent or **murine malarias**, all occur in Africa and Asia. The vectors are assumed to be *Anopheles* mosquitoes, but in most cases the vector species is unknown. *Plasmodium berghei*, *P. vinckei*, *P. yoelli*, *P. chabaudi,* and *P. aegyptensis* parasitize African murine rodents. The first two are transmitted by *Anopheles dureni* in Zaire, and *P. vinckei* is transmitted by *An. cinctus* in Nigeria. *Plasmodium atheruri* infects the African brush-tailed porcupine (*Atherurus africanus*) and is transmitted by *An. smithii. Plasmodium anomaluri*, *P. landauae*, and *P. pulmophilum* occur in African flying squirrels (*Anomalurus* spp.); *An. marchadyi* is the probable vector of the *P. atheruri*. The three species of *Plasmodium* found in Asian flying squirrels are *P. booliati*, *P. watteni*, and *P. incertae*. The significance of rodent malarias to the health and population dynamics of their natural hosts is largely unknown, although the prevalences of infection can be high. They have become important laboratory models for human malaria, particularly in host immunological responses, drug screening studies, and vaccine development. Cox (1993) provides a succinct review of the rodent malarias.

Primate Malarias

The nonhuman primate malarias are caused by a group of 25 *Plasmodium* spp., many of which are closely related to the human malarias (Collins and Aikawa, 1993). Seven of them infect lemurs in Madagascar and are poorly known. All 18 others have life cycles similar to those of the human malarias. Most have a tertian periodicity, but two species (*P. brasilianum* and *P. inui*) are quartan and one (*P. knowlesi*) is quotidian (i.e., has a periodicity of 1 day). *Plasmodium knowlesi* appears to have become a frequent parasite of humans, as well. Probably all are transmitted by *Anopheles* mosquitoes, but for 10 of them the vector species are unknown.

Of the 18 well-known primate malaria species, 13 occur in southern or southeastern Asia, where macaques, langurs (leaf monkeys), gibbons, and orangutans are the vertebrate hosts. These plasmodia include *P. pitheci* in the orangutan, the first nonhuman primate malaria to be described; *P. knowlesi*, a macaque parasite that has become an important laboratory model for development of human vaccines; and *P. cynomolgi*, a parasite of macaques and langurs that serves as an important model for human *P. vivax* malaria. The vectors of these Asian primate malarias include *Anopheles hackeri*, *An. dirus*, *An. balabacensis*, *An. elegans*, and *An. introlatus*.

Three primate malarias occur in Africa, where *Plasmodium gonderi* infects mangabeys and mandrills, and *P. reichenowi* and *P. schwetzi* infect chimpanzees and gorillas. Their natural vectors are unknown. Two *Plasmodium* species infect nonhuman primates in South America. *Plasmodium simium* infects howler monkeys and woolly spider monkeys in Brazil. It is similar to the human parasite *P. vivax* and infects humans under natural conditions. *Plasmodium brasilianum* infects a wide range of New World monkeys in the family Cebidae, including howler monkeys, spider monkeys, woolly spider monkeys, titis, capuchins, woolly monkeys, bearded sakis, and squirrel monkeys. It appears to be identical to the human parasite *P. malariae*, suggesting that the latter species also should be considered an anthropozoonosis and the name *P. brasilianum* be retired (Lalremruata et al., 2015; Rayner, 2015). Both South American species are transmitted by *Anopheles cruzii*, which also is an important vector of human malaria in parts of South America. The larvae inhabit water-filled leaf axils of bromeliad plants at heights of 5 m or more, where the adults are likely to encounter arboreal primates.

Many species of *Anopheles* are competent laboratory vectors of primate malarias, including *An. stephensi*, *An. maculatus*, *An. gambiae*, and *An. dirus*, which serve as vectors of human malaria. *Plasmodium knowlesi* infects humans experimentally and naturally, and can be transmitted by the bite of *An. dirus* to other humans. *Plasmodium cynomolgi* has infected laboratory workers, and experimental studies showed that mosquito transmission from monkeys to humans, and from humans to humans, can occur. *Plasmodium brasilianum* also infects humans and may be synonymous with *P. malariae*. Human malaria due to infection with *P. brasilianum* and *P. simium* possibly occurred as a zoonosis in the New World prior to the arrival of Europeans, with *An. cruzii* acting as the vector. Alternatively, these two simian parasites might be derived from human *Plasmodium* species to which they are closely related. Coatney et al. (1971) reviewed the infectivity of nonhuman primate malarias to humans, and Collins and Aikawa (1993) reviewed the primate malarias.

Dog Heartworm

Dog heartworm is caused by the mosquito-borne filarial nematode *Dirofilaria immitis*, a member of the family Onchocercidae (Boreham and Atwell, 1988). Adult *D. immitis* occupy the right ventricle of the canine heart and the pulmonary arteries (Fig. 15.41). The worms are 12–31 cm long and form aggregations of up to 50 or more individuals. In large aggregations, infection may extend to the right atrium. Contrary to popular belief, heartworm disease in dogs is not simply a consequence of a heavy worm burden in the ventricle resulting in impedance of blood flow. Rather, it is the result of deleterious changes in the endothelium and integrity of the walls of the pulmonary arteries, leading to pulmonary hypertension and right ventricular hypertrophy. These pathologic changes cause decreased cardiac output to the lungs, weakness, lethargy, chronic coughing, and ultimately congestive heart failure. Dogs may die if left untreated.

The **life cycle** of *Dirofilaria immitis* involves canids and mosquitoes. Dogs become infected by the bite of a mosquito whose labium carries third-stage larvae. These larvae break out of the labium while it is bent during feeding and are

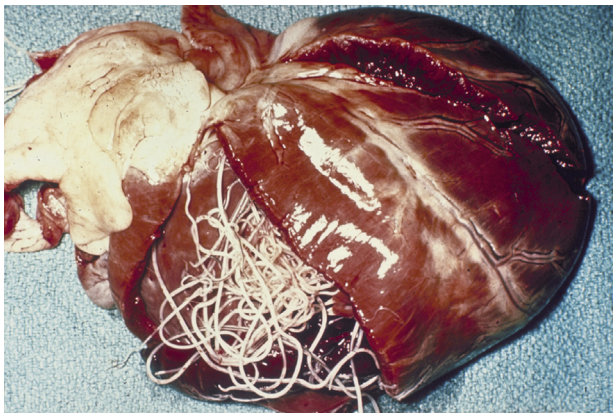

FIGURE 15.41 *Dirofilaria immitis* adults in right ventricle of dog heart. *Photograph from Harold D. Newson.*

deposited onto the dog's skin, along with a small droplet of mosquito hemolymph from the ruptured labium. Only about 10% of the larvae successfully enter the skin, generally through the hole made by the mosquito's fascicle. They remain in situ subcutaneously, where they molt to fourth-stage larvae. The larvae then migrate to other subcutaneous, adipose, or muscle tissues and molt again to a fifth-stage larva. These worms, now approximately 18 mm long, enter the venous circulation and become established in the heart and pulmonary arteries. Generally, the fifth-stage larvae reach the heart at about 70–90 days after infection.

In the heart and pulmonary arteries, the fifth-stage larvae develop into sexually mature adults. After mating, at 6–7 months, the females begin to release into circulation the microfilariae, active embyronic life stages about 300 μm long and 7 μm wide. The microfilaremia varies considerably, from 1,000 to 100,000 microfilariae per milliliter. It is nocturnally subperiodic, with peak concentrations occurring in the peripheral blood in the evening. Some dogs never develop microfilaremia, even though they support *D. immitis* adults and may have patent disease. These dogs are said to have occult infections.

Mosquitoes become infected with *Dirofilaria immitis* when they imbibe blood from a microfilaremic dog. In an average bloodmeal of 5 μL, a mosquito may ingest between five and 500 microfilariae. Within 48 h of ingestion, microfilariae migrate posteriorly in the midgut lumen to the Malpighian tubules and then into the distal cells of these tubules, where they develop intracellularly to "sausage forms" or first-stage larvae, taking about 4 days at 26°C. Some remain trapped in the midgut. If more than a few begin to develop in the tubules, the mosquito is likely to be killed. The first-stage larvae molt to the second-stage at about 8–10 days after ingestion. As they continue to grow they cause swelling and distention of the Malpighian tubules. At 12–14 days after ingestion, they molt to the third stage (Fig. 15.42). These filariform larvae break out of the Malpighian tubules and migrate through the hemolymph to the head and base of the mouthparts, then into the interior of the labium. The mosquito is then infective. The rate of these developmental processes is temperature dependent and varies with factors affecting competence for parasite development.

Vectors of *Dirofilaria immitis* differ with geographic region; many mosquito species in several genera are competent to transmit it. Grieve et al. (1983) listed 20 species field-caught in the United States, in the genera *Aedes*, *Psorophora*, *Anopheles*, and *Culex*, in which infective-stage larvae of *D. immitis* have been detected.

Other Filarial Nematodes of Animals

Other species of *Dirofilaria* infect mammals. These include *Dirofilaria ursi*, a bear parasite transmitted by the black fly *Simulium venustum*, and *D. roemeri*, a wallaroo (a type of small kangaroo) parasite transmitted by the horse fly *Dasybasis hebes*; and the following mosquito-transmitted *Dirofilaria* species: *D. repens* in canids; *D. carynodes* and *D. magnilarvatum* in monkeys; *D. scapiceps* in rabbits; *D. tenuis* in raccoons; and *D. subdermata* in porcupines.

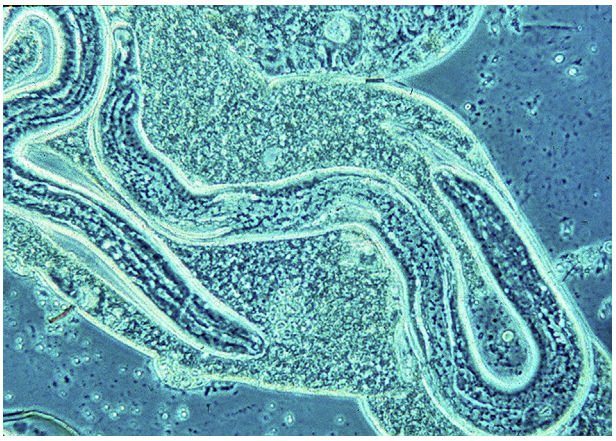

FIGURE 15.42 Filariform larva of *Dirofilaria immitis* within a Malpighian tubule of *Ae. aegypti*, prior to rupturing the tubule and migrating to the labium, where transfer to a dog occurs during blood-feeding. *Photograph by Bonnie Buxton.*

In addition to *Dirofilaria* spp., a large number of filarial nematodes in other genera of the Onchocercidae infect wild and domestic animals. Vectors include mosquitoes and a wide range of other blood-feeding Diptera, lice, fleas, mites, and ticks. The mosquito-borne onchocercid nematodes include species in the following genera; *Aproctella*, *Breinlia*, *Brugia*, *Cardiofilaria*, *Conispiculum*, *Dirofilaria*, *Deraiophoronema*, *Folyella*, *Loiana*, *Molinema*, *Pelecitus*, *Oswaldofilaria*, *Saurositus*, *Skrjabinofilaria*, *Waltonella*, and *Wuchereria* (Lavoipierre, 1958; Hawking and Worms, 1961; Bain and Chabaud, 1986; Anderson, 1992). *Brugia pahangi* of jirds (*Meriones*) is an important laboratory organism for studies on filariasis. *Brugia malayi* develops in the peritoneal cavity of gerbils, providing a laboratory infection model.

PREVENTION AND CONTROL

The four overlapping aims of mosquito control are to prevent mosquito bites, keep mosquito populations at acceptable densities, minimize mosquito–vertebrate contact, and reduce the longevity of female mosquitoes. All of these actions minimize the annoying and harmful effects of bites and blood loss, and they interrupt pathogen transmission. The eradication of either mosquito species or their associated diseases is no longer viewed as a viable objective, except in small, isolated regions or in the case of recent invasions. Two exemplary failures of the eradication approach, on a grand scale, were the World Health

Organization's global malaria eradication program and the Pan American Health Organization's attempt to eradicate *Aedes aegypti* from the Western Hemisphere. A notable exception was the successful elimination of the African immigrant *Anopheles gambiae* from Brazil. The more realistic objective of modern mosquito control programs is integrated pest management to reduce mosquito abundance and disease prevalence, using prudent combinations of methods.

Personal protection is the most direct and simple approach to prevention. Outdoor exposure can be avoided during peak mosquito activity, and window screens can prevent mosquito entry into houses and animal shelters. Head nets reduce annoyance and prevent bites about the face and neck. Bed nets, impregnated with synthetic pyrethroid and strung over beds at night, repel mosquitoes and kill those that land on the nets. Impregnated mesh suits with hoods work similarly, and can be worn over clothing. Other insecticidal devices create a repellent smoke or vapor that reduces mosquito attack in the immediate vicinity. Chemical repellents applied to skin or clothing prevent mosquitoes from landing or cause them to leave before probing. The most common one is *N,N*-diethyl-*meta*-toluamide, or DEET.

Organized control programs provides efficient, area-wide mosquito management at local, regional, or national levels. In the United States, mosquito programs typically are county-level abatement districts. These focus on the control of nuisance and vector species, but often also participate in surveillance for mosquito-borne disease pathogens. National organizations are usually parts of ministries of health and coordinate their disease and vector control efforts at that level. Especially in developing countries, there is now increasing emphasis on community cooperation, low technology, sustainability, and the integrated use of a variety of control tools that are adapted to local customs, conditions, and resources. Bed nets treated with synthetic pyrethroid insecticides have emerged as important community protection devices for malaria control and are now available in long-lasting, wash-durable formulations. They have been distributed in mass campaigns in countries of sub-Saharan Africa, resulting in reductions in malaria morbidity and mortality in large sections of some countries.

Habitat modification is a traditional and reliable tool in mosquito management. Adult resting places can be rendered unsuitable by harborage alteration. Changes in larval habitat that prevent oviposition, hatching, or larval development are called source reduction. Water is altered or eliminated in a variety of ways. This includes plastic foam beads that provide a floating barrier over latrine water, underground sewage lines, land drainage through ditches or underground tile pipes, waste tire shredding, trash-container disposal and natural container elimination, lids for water-storage barrels, vegetational changes in ponds, altered flow of tidal water through salt marshes, and water-level manipulation in reservoirs and rice fields. Each method is designed to interfere with specific features of a mosquito's natural history. Through appropriate application of ecological principles and an intimate knowledge of mosquito behavior and life cycles, desirable natural wetlands and newly created ones can be modified to minimize mosquito production while benefiting other wildlife.

Biological control of mosquitoes by predators or parasites has been studied extensively and has been reviewed by Chapman (1985), Beaty and Marquardt (1996), Hemingway (2005), and Floore (2007). Aerial predators, such as dragonflies, birds, and bats, receive much attention but do not specialize in adult mosquitoes and have little if any effect on their densities. Most efforts have been directed at the larval stage. Aquatic predators, both naturally occurring and introduced, include the mosquito fish (*Gambusia affinis*) and killifish (*Fundulus* spp.). Other fish, such as grass carp, e.g., *Tilapia* and *Cyprinus*, remove aquatic vegetation that provides harborage for larvae. Invertebrate predators include the predatory mosquito *Toxorhynchites*, several families of aquatic bugs and beetles, predatory copepods, hydras, and turbellarian flatworms; however, only copepods have been implemented with substantial success.

There have been attempts to develop the use of parasites and pathogens of mosquito larvae as control agents, including the nematode *Romanomermis culicivorax*; protozoans such as the ciliates *Lambornella* and *Tetrahymena*; the gregarine sporozoan *Ascogregarina*; and the microsporidian *Nosema*. Fungal pathogens include *Coelomomyces*, *Lagenidium*, *Culicinomyces*, and *Metarrhizium*. Viruses pathogenic to larvae include the iridescent viruses, densonucleosis viruses, polyhedrosis viruses such as the baculoviruses, and entomopox viruses. Generally, the above-mentioned parasites or pathogens of mosquito larvae are still in experimental stages of development, or they have limited effectiveness and have not been used routinely in operational programs.

An exception is the bacterium *Bacillus thuringiensis israelensis*, or *Bti*, which has been developed into commercial formulations since its original discovery in 1975. It is used extensively in mosquito control programs. Larvae die when they ingest crystalline, proteinaceous toxins produced by the bacterial cells during sporulation. The bacterium *Bacillus sphaericus* has a similar mode of action but is more specific. It is particularly effective against *Culex* larvae, and is more persistent in water, and more tolerant of water with a high organic content, than is *Bti*.

Genetic control, a category of biological control using a wide variety of genetic methods, has been successful against several insect pests; its practical application to mosquitoes, however, to halt pathogen transmission, is only recently gaining widespread traction. There are two

hypothetical approaches: (1) self-limiting systems; release of sterilized males or incompatible (female-killing) strains, resulting in attrition of the natural population, and (2) self-sustaining systems; replacement of natural vector populations with strains that are poor vectors or are refractory to the pathogens they formerly transmitted. The appeal of both approaches is that their effects are minimally disruptive to the environment and are species specific, because they depend on mate-seeking for their implementation. The special attraction of the replacement approach is that it is potentially self-sustaining, requiring no further input. However, it requires a drive mechanism to spread the refractory property through the mosquito population. Achieving driver-gene stability and selective propagation through males (to avoid enhancing the female population), among other developments and obstacles, have been reviewed by Rai (1996), Wood (2005), and Alphey (2014).

The intracellular bacterium *Wolbachia* has played a significant role in several of these potential genetic approaches to **vector manipulation**, including both self-limited and self-sustaining types. In addition, to its sex ratio—distorting and gene-drive features, *Wolbachia* interferes with the vector's competence for transmitting pathogens such as dengue virus.

The most prominent innovation among the **sterile-male techniques** is to release males carrying a dominant lethal gene or a genetic system that allows the altered males to sire only male offspring, which then continue spreading the gene in the dwindling natural population of females. For vector replacement, progress has been most rapid in developing transgenic *Anopheles* spp. that are refractory to infection by malaria parasites, transgenic *Aedes aegypti* that are incompetent to transmit dengue virus, and experimentation with gene-drive systems that allow their altered genomes to invade populations.

Chemical control is achieved with insecticides against either larvae or adults. **Larvicides** are placed in water where larvae develop, or where water will accumulate and provide habitat for larvae. Formerly used larvicides included inorganic compounds such copper arsenate, fuel oil, and organochlorine chemicals such as DDT and dieldrin. Currently, categories of registered larvicides are light mineral oils, organophosphates, and insect-growth regulators. Rapidly degradable oils spread over the water surface, penetrating the tracheal systems of larvae and pupae and suffocating them. Organophosphates, such as temephos, malathion, and chlorpyrifos, function as nerve poisons. The insect-growth regulator methoprene is a mimic of juvenile hormone and interferes with metamorphosis and emergence. The specific kind and formulation (dust, powder, water-soluble liquid, emulsion, oil-soluble liquid, granule, pellet, briquet) of the larvicide recommended depends on the biology of the target mosquito, the kind and size of habitat, the method of application, the chemical composition of the water, and the presence of nontarget organisms that might be adversely affected. Some can be formulated for slow release from a carrier. These may be applied to dry ground, releasing the active ingredient when inundated.

Adulticides are applied to surfaces where adults will rest or in the air where they fly. Residual insecticides applied to resting surfaces may retain their toxicity for days to months. They were central to the global malaria eradication program, in which DDT spraying of the inner walls of human dwellings at 6-month intervals killed all mosquitoes landing on these walls before or after taking blood. In areas where the vectors bit humans primarily indoors, this effectively interrupted most malaria transmission until mosquito populations developed resistance to the insecticide or when programs were abandoned. This approach is still used widely in some areas. Residual adulticides also can be used outdoors on vegetation or structures that serve as harborages. They tend to have short-term effects, because sunlight, wind, and rain cause the insecticide to degrade. A novel use for ivermectin, a systemic antiparasitic compound used widely against nematodes such as those causing river blindness (onchocerciasis) and nematode infections of livestock, appears to be its deleterious effects on mosquitoes such as *An. gambiae* in Africa when it feeds on the blood of the treated host. As a means of reducing mosquito populations or their vectorial capacity, this approach is being considered.

Adulticides intended for direct contact between airborne droplets and the mosquito are of two types: **thermal fogs** and low or **ultra-low volume (ULV) sprays**. Both can be applied from hand-carried equipment, motor vehicles, or aircraft. Thermal fogging involves mixing an insecticide with a combustible liquid such as kerosene. The mixture is heated, creating a fog of insecticide that drifts through the area to be treated. The ULV approach involves special nozzles and pumps that dispense fine droplets of insecticide, forming a mist that passes through the target area. Currently, insecticides registered for use in fogs and low-volume sprays are organophosphates, carbamates, pyrethrins, and synthetic pyrethroids. **Resistence to insecticides** is an important consequence of their use and has developed in many mosquito populations. The mechanisms of physiological resistance have been well characterized biochemically and genetically. **Behavioral resistance** also can develop. This is typically a change in adult feeding or resting behavior, so that mosquitoes no longer contact insecticide residues. Liu (2015) has reviewed the subject of mosquito resistance to insecticides.

Surveillance, which is at the core of effective mosquito control programs, determines mosquito distribution and abundance and degree of pathogen activity. The goal is to provide data so that control agencies can take action to prevent mosquito-related problems from occurring.

Unfortunately, there have been few control programs establishing action thresholds for mosquito density or infection rate, the levels of threat at which controls should be initiated. More often, action is based on human perception of a pest problem, conditions similar to past experience with disease outbreaks, or first detection of pathogen activity. Surveillance strategies and techniques for mosquito-borne encephalitis viruses have been presented by Moore et al. (1993) and Moore and Gage (1996). Bruce-Chwatt (1980) and Sasa (1976) reviewed traditional techniques for detecting malaria and filarial parasites, respectively, and several new ones are in use. Methods that make use of geographic information systems (GIS) and decision support systems (DSS) to predict, prevent, and control vector-borne diseases (e.g., dengue, malaria, and West Nile virus) have been evaluated by Eisen and Eisen (2011).

Control of Pathogen Transmission

Although all methods of reducing vector populations can lower the incidence of mosquito-borne disease, quantitative models of the dynamics of disease transmission have become important tools for setting realistic control objectives. They allow programs to focus efforts on parts of the pathogen-transmission system most vulnerable to attack. Useful references on this subject are Ross (1911), Macdonald (1957), Molineaux and Gramiccia (1980), Fine (1981), Koella (1991), and Dye (1992).

The Ross-Macdonald equation describes the case reproduction rate, the total number of new cases of a disease arising from a single infective case in a totally susceptible population. The vectorial capacity equation expresses that function on a daily basis using entomological parameters. Although the vectorial capacity measure is not epidemiologically comprehensive, it allows a comparison of the relative importance of different vectors and provides estimates of critical vector density, the adult mosquito density below which the case reproduction is less than one and the disease should die out. It also illustrates, mathematically, that even small changes in the interval between bites on susceptible hosts (which is squared) or in the longevity of vectors (which changes exponentially) cause large changes in transmission rates. The latter relationship has been critical in mounting effective disease-control operations, which target older females, rather than just female density in general.

More complex models sometimes show good agreement between predicted and observed results in extensive field studies of malaria (Molineaux and Gramiccia, 1980; Koella, 1991) and may become useful in establishing action thresholds. A simple and direct measure of transmission is the entomological inoculation rate (Onori and Grab, 1980), which is the product of the vector's human-biting rate and proportion of vectors that are infective.

Vaccines and drugs are important tools in protecting or treating humans and other animals susceptible to mosquito-borne disease. They serve not only to protect the individual but also to reduce transmission to others. Vaccines are available for several arboviral diseases, including yellow fever and Japanese encephalitis for humans and eastern, western, and Venezuelan encephalitis for equids. These vary in the duration of protection they provide. An experimental human vaccine against eastern equine encephalitis has been produced. None currently exists for the dengue viruses. Human malaria vaccines are under development, and some field trials have achieved limited success, but their wide-scale efficacy remains uncertain. The three kinds of malaria vaccines being considered use antigens from sporozoites, blood stages, or gametes; the last kind is called a transmission-blocking vaccine because the human antibodies take effect against stages that form within the midgut of the mosquito.

Among **drugs**, there exists a wide spectrum of antimalarials used for prophylaxis, therapy, or both. The most commonly used chemoprophylactic is chloroquine, against which there is now widespread resistance in *Plasmodium falciparum* and some *P. vivax* populations. Mefloquine is prescribed as a prophylactic for areas with resistant populations. Artemesinin, a drug derived from a plant long used by Chinese herbalists, is now widely used in derivative form in combination with other antimalarial compounds to treat uncomplicated malaria cases where resistance is widespread. Strickland (1991) presented a detailed review of malaria chemotherapy and chemoprophylaxis.

For lymphatic filariasis, diethylcarbamazine (DEC) is the standard chemotherapy, which reduces microfilaremia but does not kill adult worms. Advanced disease manifestations (e.g., elephantiasis) cannot be reversed, except by surgery, but sustained mass treatment of human populations can drive transmission to zero. This was achieved in parts of China in 1 year by the use of DEC-fortified cooking salt. Owing to the longevity of adult worms, mass treatment for 5–10 years is necessary to completely break the infection cycle in a community. Ivermectin and albendazole are two other drugs showing efficacy in lymphatic filariasis cases. Both DEC and ivermectin are used as chemoprophylaxis against dog heartworm infections.

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