15 Genetics of Major Insect Vectors

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15.1 Introduction

15.1.1 Significance and Control of Vector-Borne Disease

Vector-borne diseases are responsible for a substantial portion of the global disease burden causing ~1.4 million deaths annually (Campbell-Lendrum et al., 2005; Figure 15.1) and 17% of the entire disease burden caused by parasitic and infectious diseases (Townson et al., 2005). Control of insect vectors is often the best, and sometimes the only, way to protect the population from these destructive diseases. Vector control is a moving target with globalization and demographic changes causing changes in infection patterns (e.g., rapid spread, urbanization, appearance in nonendemic countries); and the current unprecedented degradation of the global environment is affecting rates and patterns of vector-borne disease in still largely unknown ways.

15.1.2 Contributions of Genetic Studies of Vectors to Understanding Disease Epidemiology and Effective Disease Control Methods

Studies of vector genetics have much to contribute to understanding vector-borne disease epidemiology and to designing successful control methods. Geneticists have performed phylogenetic analyses of major species; have identified new species, subspecies, **cryptic species**, and introduced vectors; and have determined which taxa are epidemiologically important. Cytogeneticists have shown that the evolution of chromosome structure is important in insect vector speciation. Population geneticists have uncovered the complex population structures of insect vector populations. Monitoring gene flow among populations has revealed the geographic coverage needed for control and the source of insects appearing following pesticide applications. Genetic control methods such as the sterile insect technique (SIT) (Krafsur, 2002), or introduction of refractory traits or transgenic symbionts

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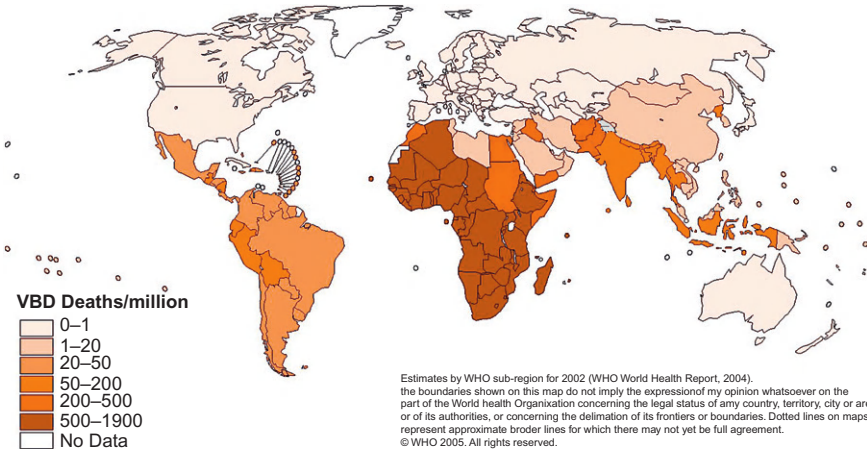

Figure 15.1 Estimates of vector-borne disease deaths per million inhabitants, with permission, copyright WHO.
Source: Available from http://www.who.int/heli/risks/vectors/vector/en/index.html

carrying molecules toxic to pathogens, although still largely theoretical, can add to the arsenal. Molecular genetics and new genome and proteome tools promise advances in understanding the genetic basis of vector capacity including habitat and host preference, innate immunity, drought tolerance, and insecticide resistance, among other phenomena, and the development of new attractants and repellents.

15.1.3 Chapter Overview

In this chapter we review what is known about the genetics of the vectors of three of the most important vector-borne diseases: African sleeping sickness, Chagas disease, and malaria.

15.2 Genetics of Tsetse Flies, *Glossina* spp. (Diptera: Glossinidae), and African Trypanosomiasis

15.2.1 Introduction

Tsetse flies (Diptera: Glossinidae) are among the most important insects in sub-Sahara Africa because they are obligate blood feeders and the vectors of African trypanosomiasis, caused by trypanosomes (Mastigophora: Kinetoplastida: Trypanosomatidae) that kill humans and domestic mammals. It is hard to overestimate the importance of African trypanosomiasis. More than 50 million people are said to be at risk for human African trypanosomiasis (HAT) in 37 countries (WHO, 2006). Trypanosomiasis in domestic animals is termed Nagana, and was estimated to cost African agriculture US $4.5 billion per year (Reinhardt, 2002) via loss of food, dung, and drafting power. Conservative estimates claim approximately

Figure 15.2 Resting tsetse.
Source: Photo courtesy of the DFID Animal Health Program.

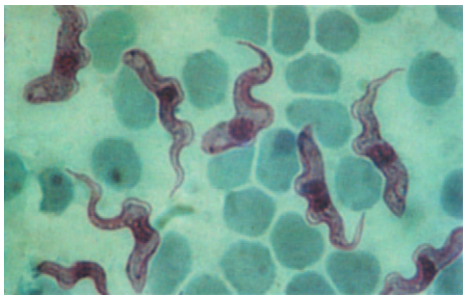

Figure 15.3 *Trypanosoma brucei* in blood.
Source: Photo courtesy of the DFID Animal Health Program.

450,000 HAT cases in 1997 declined to 10,880 by 2007 (Barrett, 2006; WHO, 2006). Simarro et al. (2008) offer a comprehensive epidemiological review.

Tsetse (pronounced "tsee-tsee") flies (Figure 15.2) and trypanosomes (Figure 15.3) have many interesting and unusual biological features. Here I briefly review the principle biological and ecological features of tsetse flies with an emphasis on their population structures and relationships with trypanosomes. Citations are kept to a minimum, with emphasis on recent literature. Much fuller treatments of tsetse flies, trypanosomiasis, and African trypanosomes are available in texts by Buxton (1955), Ford (1971), Jordan (1993), Leak (1998), and Maudlin et al. (2004). Specialist reviews include Gooding and Krafsur (2004, 2005) for genetics, Krafsur (2003, 2009) for population genetics, and Walshe et al. (2009) for tsetse–trypanosome–symbiont interactions.

15.2.2 Systematics and Biology of Tsetse Flies in a Nutshell

The Family Glossinidae

Systematics and unresolved taxonomic problems were reviewed by Jordan (1993) and Gooding and Krafsur (2005). Tsetse flies are assigned to the family Glossinidae (McAlpine, 1989). All extant tsetse flies are classified into a single genus *Glossina* Wiedemann 1830. Four subgenera have been described. *Austenina*, *Nemorhina*, and *Glossina* correspond to the Fusca, Palpalis, and Morsitans species groups, respectively. In general, Fusca group flies are forest inhabitants, Palpalis are riverine and lacustrine (living near lakes), and Morsitans group flies are savanna inhabitants. Subgenus *Machadomyia* consists only of two *G. austeni* subspecies. There is an

unnamed, extinct sister group known from the Florissant shale of Colorado, dating some 38 mya and similar tsetse-like fossils were uncovered in Oligocene strata in Germany indicating formerly a much wider geographic distribution. Thirty-four taxa have been described, consisting of 23 species and 7 species complexes of 17 named subspecies that differ slightly morphologically, if at all. Most subspecies are allopatric. Morphological species identification is not easy.

Species Complexes of Medical Importance: G. morsitans *s.l.,* G. palpalis *s.l., and* G. fuscipes *s.l.*

Three species complexes are geographically widespread and of much medical and economic importance. Some constituent taxa have been examined genetically in *G. morsitans* s.l., *G. palpalis* s.l., and *G. fuscipes* s.l. The most thoroughly examined is *G. morsitans* s.l. and its close relative, *G. swynnertoni*. *G. morsitans* s.l. comprises *G. morsitans morsitans*, *G. m. centralis*, and *G. submorsitans.* Genetic data suggest longstanding isolation. *G. palpalis* s.l. comprises *G. p. palpalis* and *G. p. gambiense*; recent studies suggest incipient speciation in *G. p. palpalis* (Ravel et al., 2007; Dyer et al., 2009). The foregoing taxa are allopatric and hybrid males are sterile, the females typically sterile or semisterile (Gooding, 1993). *G. fuscipes* s.l. consists of *G. f. fuscipes*, *G. f. martini*, and *G. f. quanzensis*. There is no known cross-breeding work on this species complex.

Glossina *Life History*

All *Glossina* are exclusively hematophagous. Reproduction is viviparous by adenotrophic viviparity. In this unusual birthing process, found in several insect taxa, an egg develops into a larva within the mother, the mother deposits the mature larva in an appropriate microhabitat, and the animal pupariates in the soil (Figures 15.4 and 15.5). In tsetse flies, one egg develops at a time within the "uterus" and the subsequent larva develops to maturity within its mother over a 9- or 10-day interval. Adult development from the deposited larva requires about 28 days at tropical temperatures. The youngest larvapositing female is at least 15 days old, so the minimum time necessary to produce two offspring is 25 days. Compensating for slow reproduction are their survival rates, which for adult females typically exceed a mean 97% per day in stable and growing populations. Generation time for savanna species is 45−50 days and population-doubling time is at least 35 days. Hargrove

Figure 15.4 Tsetse larviposition.
Source: Photo courtesy of the DFID Animal Health Program.

Figure 15.5 Tsetse pupae: light are young and dark are older.
Source: Photo courtesy of the DFID Animal Health Program.

(2003, 2004) authoritatively reviewed the essentials of tsetse demography and its consequences for fly population management.

15.2.3 Biogeography

Distribution

Most tsetse fly populations occur within latitudes 12°N to 25°S, about one-third of the African continent (Figures 15.6−15.8). To oversimplify, moisture availability is

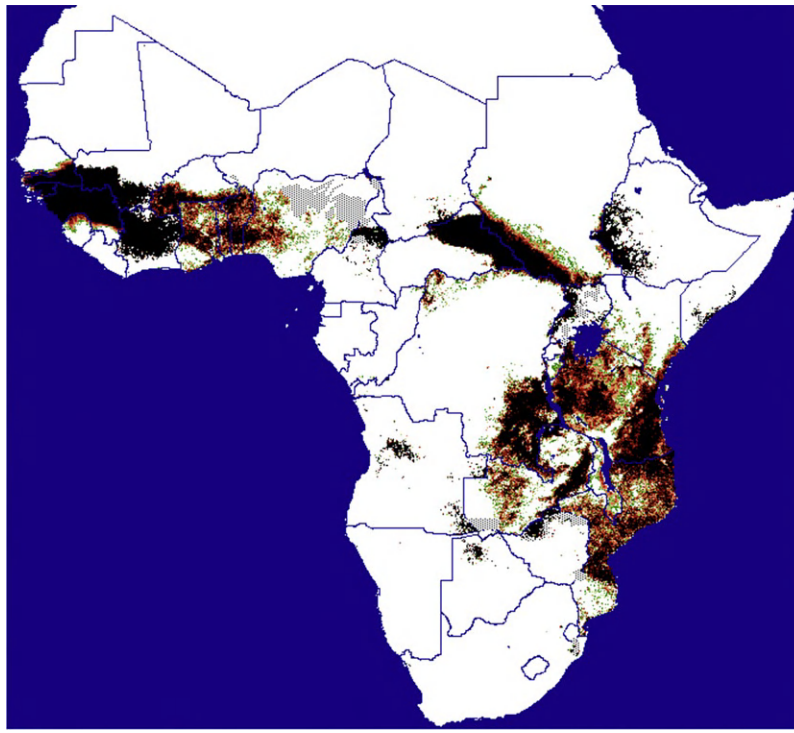

Figure 15.6 Distribution of Morsitans group tsetse flies predicted from satellite imagery courtesy of ERGO Ltd and TALA, Oxford, with permission.
Source: Available from http://ergodd.zoo.ox.ac.uk/.

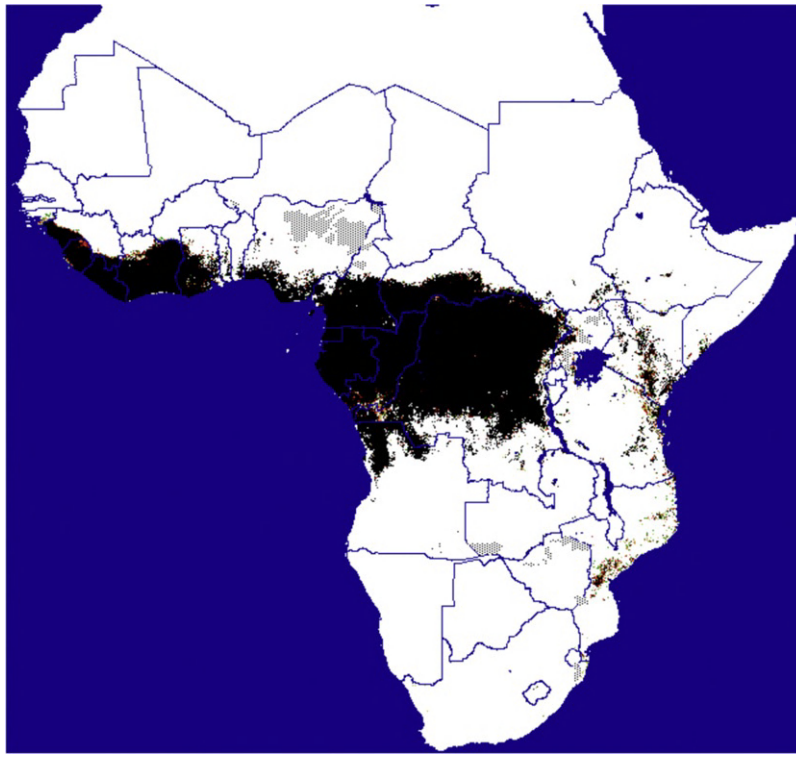

Figure 15.7 Distribution of Fusca group tsetse flies predicted from satellite imagery courtesy of ERGO Ltd and TALA, Oxford, with permission.
Source: Available from http://ergodd.zoo.ox.ac.uk/.

limiting to the north and low temperatures limit southern distribution. Thus tsetse flies are largely confined to sub-Saharan Africa, although relict populations of *G. pallidipes* and *G. palpalis* have been recorded in the southwestern corner of the Arabian Peninsula. They are likely survivors from earlier times when more equable climates prevailed. It would be interesting to examine their genetic affinities. Distribution maps of 30 tsetse taxa are available and correlations of distribution with satellite imagery indices confirm that each has rather specific temperature and precipitation requirements (Rogers and Robinson, 2004).

15.2.4 Genetics and Population Genetics

Cytogenetics

All tsetse flies examined cytologically have two pairs of metacentric autosomes and a sex bivalent: $2N = 4 + XY$. Many also have heterochromatic supernumerary chromosomes that vary in number within and among taxa. Sex chromosome

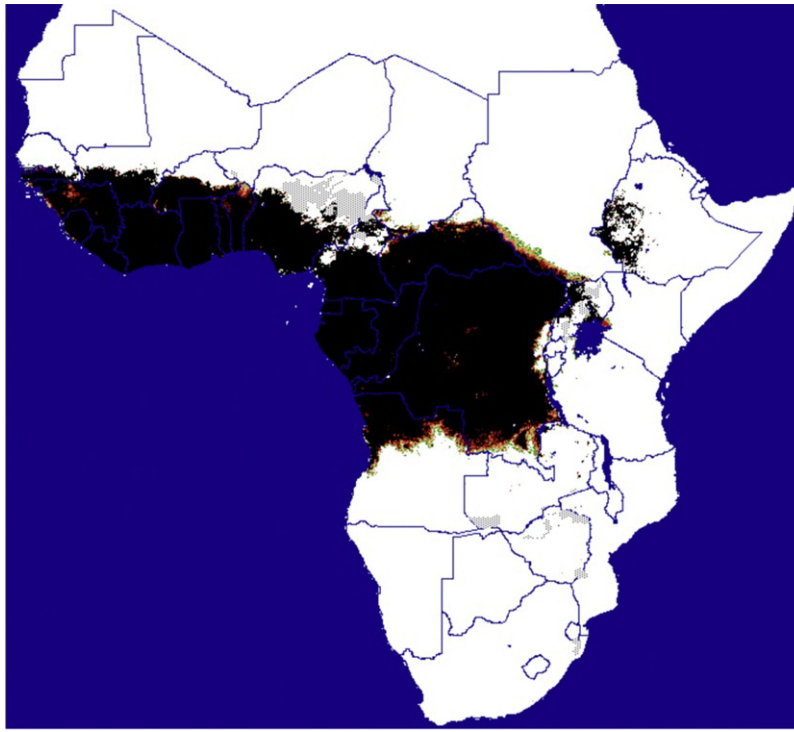

Figure 15.8 Distribution of Palpalis group tsetse flies predicted from satellite imagery courtesy of ERGO Ltd and TALA, Oxford, with permission.
Source: Available from http://ergodd.zoo.ox.ac.uk/.

polymorphisms have been recorded in wild *G. p. palpalis*. Well-banded polytene chromosomes can be obtained from pupal trichogen cells. Examination of polytenes in Morsitans and Palpalis flies has demonstrated that taxa can be separated by pericentric and paracentric inversions (Figure 15.9). Surveys of polytene chromosome **diversity** in natural populations remain to be undertaken and should prove highly informative.

Genetic Variability Based on Allozymes

Allozyme, microsatellite, and mitochondrial variation has been assessed in all Morsitans and most medically important Palpalis group taxa (Table 15.1). Allozyme diversities have also been assessed in some Fusca taxa. Allozyme diversities are fairly homogeneous among the taxa examined. Diversity, defined as the mean heterozygosity over loci, is about 6% in tsetse and compares with 10−20% in other Diptera such as *Musca*, *Stomoxys*, *Haematobia*, and *Drosophila*. The best explanation for the difference is that genetic diversity is inversely proportional to **effective population sizes**. Effective population size, N_e, is the harmonic mean number of reproducing individuals in an ideal population that have the same allele

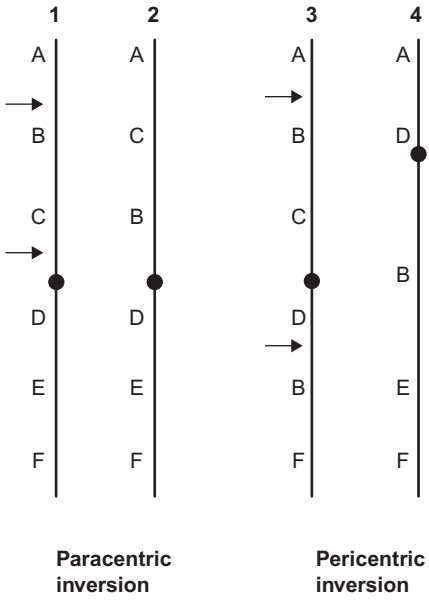

Figure 15.9 Chromosome inversions arise by two simultaneous breaks, indicated by arrows, followed by inversion of the interior segments and rejoins with the "wrong" partners. A paracentric inversion (chromosomes 1→2) occurs in one arm of a chromosome and a pericentric inversion (3→4) includes the spindle fiber attachment region (dark circles) and both arms of a chromosome. Note that pericentric inversions can result in a change in chromosome morphology.

Table 15.1 Allozyme Variation in Tsetse Flies Compared with Other Diptera

Taxa	Number of Loci	Percent Polymorphic	Mean Alleles per Locus	Mean Diversity, H_e	Diversity at Polymorphic Loci
G. m. morsitans	45	20.0	1.4	6.6	29.9
G. m. centralis	31	32.3	1.4	6.0	18.7
G. swynnertoni	34	17.6	1.2	7.2	40.5
G. pallidipes	38	26.3	1.5	6.8	25.4
Musca domestica	68	53.4	2.5	18.3	36.7
Musca autumnalis	50	62.0	2.4	18.6	30.5
Stomoxys calcitrans	38	52.6	1.8	9.6	18.2

frequencies as an actual population under study. *Glossina* population numbers averaged over time and space are much less than the common Diptera with which they have been compared. Diversities at polymorphic allozyme loci, on the other hand, are of the same magnitude as those in other Diptera. There is good empirical evidence in *G. pallidipes* for **balancing selection** that favors allozyme heterozygotes.

Genetic Variability Based on Microsatellite Loci

Microsatellite loci in genomic DNA have been identified in Morsitans and Palpalis group flies (Table 15.2). Many loci are shared across subgenera. Microsatellite diversities, averaged over loci, varied from a low 0.43 in *G. f. fuscipes* up to 0.81 in *G. m. submorsitans*. The mean over 6 taxa and 58 population samples is 0.70

Table 15.2 Microsatellite Diversities and Tests for Random Matings *F* in *Glossina* spp. and the Housefly

	Number of Populations	Number of Loci	Alleles per Locus	Diversity H_e	Within Demes F_{IS}	Among Demes F_{ST}
G. m. morsitans	6	6	11.0 ± 5.6	0.73 ± 0.06	0.03 ± 0.03	0.19 ± 0.05
G. m. morsitans	9	7	28.6 ± 4.5	0.74 ± 0.05	0.17 ± 0.07	0.13 ± 0.01
G. m. centralis	7	7	8.8 ± 3.7	0.70 ± 0.09	−0.12 ± 0.04	0.19 ± 0.04
G. m. submorsitans	7	7	12.7 ± 6.2	0.81 ± 0.04	0.03 ± 0.03	0.17 ± 0.07
G. pallidipes	21	8	26.8 ± 8.7	0.80 ± 0.03	0.07 ± 0.03	0.18 ± 0.02
G. f. fuscipes [a]	8	5	8.2 ± 3.6	0.43 ± 0.07	0.11 ± 0.05	0.22 ± 0.07
Musca domestica	14	7	7.9 ± 1.1	0.86 ± 0.02	0.08 ± 0.02	0.13 ± 0.02

[a](Abila et al., 2008)

Table 15.3 Mitochondrial Diversities and Genetic Differentiation in Wild *Glossina* Species

	Method	Number of Populations	Number of Flies	Number of Haplotypes	Haplotype Diversity, H_S	F_{ST}
G. m. morsitans	SSCP	5	111	25	0.81 ± 0.04	0.09 ± 0.02
G. m. morsitans	ABI 3730	7	96	33	0.81	0.40 ± 0.08
G. m. centralis	SSCP	6	265	7	0.54 ± 0.16	0.81 ± 0.07
G. m. submorsitans	SSCP	7	282	26	0.51 ± 0.12	0.35
G. pallidipes	SSCP	21	624	39	0.42 ± 0.02	0.52 ± 0.001
G. pallidipes	ABI 3730	23	873	181	0.73 ± 0.09	0.47 ± 0.07
G. p. gambiensis	SSCP	13	372	9	0.18	0.68
G. swynnertoni	ABI 3730	8	149	18	0.59 ± 0.10	0.04 ± 0.003
G. f. fuscipes [a]	ABI 3730	22	284	36	0.91 ± 0.008	0.60 ± 0.07

[a](Beadell et al., 2010)

and compares with a mean 0.86 over 14 samples of the cosmopolitan housefly, *Musca domestica*. Here, again, we see that a larger effective population size results in greater diversity.

Genetic Variability Based on mtDNA

Mitochondrial variation has also been assessed in *Glossina*. Examination of cytochrome oxidase subunit I and ribosomal 16S2 loci in large samples of Morsitans and Palpalis group flies disclosed many variants (Table 15.3). Mitochondrial diversity (only 18 haplotypes) was least in *G. swynnertoni* although allozyme variation was about the same as in other taxa. This tsetse fly occupies a relatively small region of north-central Tanzania. Further, it has been subject to extensive control procedures and its range has contracted. Mitochondrial variation is single copy and inherited matrilineally and more sensitive to demographic flux than diploid, nuclear

variation. As already discussed with regard to *G. pallidipes*, natural selection favors allozyme heterozygotes.

Rationale for Diversity Levels Observed

Low diversities in *G. m. centralis* and southern African *G. pallidipes* reflect earlier demographic events. The rinderpest epizootic that began in the late nineteenth century is said to have killed 90% or more of the mammalian population of central and southern Africa with the virtual elimination of Morsitans group flies, that only slowly recovered from undetectably small numbers (Ford, 1971). In *G. pallidipes*, microsatellite and mitochondrial diversities were less in southern Africa than in East Africa and both were strongly correlated with each other, but neither was correlated with allozyme diversity. In contrast to the allozyme diversities, mitochondrial and microsatellite variation was consistent with a severe and prolonged reduction in population sizes in southern Africa. Allozyme variation was conserved, however, and differentiation at allozyme loci among populations was much less than at microsatellite and mitochondrial loci providing a good example of balanced polymorphism, discussed further later.

15.2.5 Population Structure

Breeding structure and gene flow have been examined in *G. morsitans* s.l., *G. pallidipes*, *G. swynnertoni*, *G. f. fuscipes*, *G. p. palpalis*, and *G. p. gambiense*. These are among the chief vectors of African trypanosomes. Some of the foregoing taxa were sampled over much of their geographic ranges and lab cultures of some were compared with genetic data from field samples. Two generalities emerged from the investigations: random **genetic drift** was pronounced in all taxa, leading to highly significant levels of genetic differentiation among conspecific populations. Most population samples were differentiated even when within 25−50 km of each other, and genetic diversity in lab cultures was only mildly attenuated compared with their field cousins, with the possible exception mitochondrial diversity (8 haplotypes, $H_s = 0.36$) in a longstanding *G. austeni* culture. Estimates of genetic differentiation among subpopulations are provided by F_{ST} and its analogs. F_{ST} may be defined as the among-subpopulation variance in gene frequencies as a fraction of the variance among all individuals and is interpreted as departures from random mating among subpopulations. The accompanying tables provide mean estimates of F_{ST} taken over all samples and indicate low levels of gene flow (Tables 15.2 and 15.3). The theoretical, mathematical relationship between genetic differentiation and gene flow is a reciprocal one such that the number of reproducing migrants exchanged among demes is proportional to the inverse of F_{ST}. For most tsetse taxa examined, the mean numbers of reproductive flies exchanged among populations is generally less than one to two per generation, indicative of strong genetic drift. No work has been reported on the breeding structures of vectors *G. brevipalpis, G. tachinoides, G. longipalpis, and G. f. quanzensis*.

Explanations for Population Structure Observed

How may we best interpret the significant levels of genetic differentiation among conspecific tsetse populations? Mark-recapture studies have shown strong propensities to disperse (reviewed by Rogers, 1977; Leak, 1998; Hargrove, 2003). Thus, mean displacement rates of savanna-inhabiting tsetse flies were a mean 252 m d^{-1}. The foregoing rate predicts rapid dispersion and a frontal advance of approximately 5 km y^{-1}, thereby counteracting genetic drift. Theory indicates that numerically little gene flow is necessary to do so (Wright, 1978). Assuming numerically significant rates of dispersion among demes, how may we account for the high levels of genetic drift typically recorded? Spatial variation in natural selection offers an explanation.

Agents of natural selection include prevailing temperature and moisture regimes that govern the distribution of tsetse flies. It has been shown that among regional, conspecific *G. pallidipes* and *G. morsitans* populations, different discriminant models and predictor variables best describe their distributions (Rogers and Robinson, 2004). This provides empirical evidence that spatially separated demes have adapted to their different environments, perhaps accounting for a measure of the genetic differentiation typically observed. Tests of hypotheses were performed on *G. pallidipes* in five ecologically diverse sites in Kenya and Zambia where the prevailing climates substantially differ. Hypotheses tested included homogeneity in among-population thermal tolerances, desiccation tolerances, body water amounts, lipid amounts, and spontaneous locomotory activities. Population responses were nearly homogeneous and little potential for evolutionary change was detected (Terblanche et al., 2006).

Cuticular hydrocarbons and lipids are important in water conservation and sex recognition and could, in principle, provide evidence of local adaptation. The chief sex pheromone in *G. pallidipes* did not differ among flies from Ethiopia, Zimbabwe, and five independently derived lab cultures (Carlson et al., 2000), nor in four diverse Kenya populations (Jurenka et al., 2007). Hydrocarbon quantities differed among populations, but did not correlate with environmental variables.

The foregoing genetic studies indicate that genetic drift in tsetse is typically stronger than gene flow. We have no direct evidence to support selection as an isolating mechanism. Drift is inversely proportional to effective population size, and tsetse maximum reproductive rates are low. For now, it seems we cannot reject the hypothesis that random genetic drift operates principally as a consequence of small effective population sizes and spatial isolation.

15.2.6 Commensals and Symbionts in Tsetse Flies

Three maternally transmitted microorganisms can be found in tsetse (Aksoy, 2000; Walshe et al., 2009, for reviews). These are *Wolbachia pipientis*, an intracellular parasitic Rickettsia that causes **cytoplasmic incompatibility** in some insect species, but cytoplasmic incompatibility has not been demonstrated experimentally in tsetse flies; *Wigglesworthia glossinidia* is an intracellular, obligatory symbiont that produces essential vitamins and other substances absent in the host fly's blood meals. This bacterium has been sequenced and has a small genome a bit less than

700 kb. Studies indicate an association of *W. glossinidia* with vector competence in host flies in the laboratory. *Sodalis glossinidius* occurs in some species and strains of tsetse flies and it demonstrates much genotypic diversity. Its genome has also been sequenced. *Wolbachia* and *Sodalis* incidence vary among natural tsetse species and populations. In natural conspecific tsetse populations, *Sodalis glossinidius* prevalence varies spatially and its presence in fly colonies can favor susceptibility to trypanosome infection. There is empirical evidence from Cameroon of a statistical association of *Sodalis* with trypanosome (*T. brucei* s.l., *T. congolense*) infections (Farikou et al., 2010).

15.2.7 Population Genetics of African Trypanosomes

Trypanosome Systematics of T. b. brucei*,* T. b. gambiense*, and* T. b. rhodesiense

Trypanosome systematics was reviewed by Stevens and Brisse (2004). Only the medically and economically most significant taxa are dealt with here because they are the best known. *Trypanosoma brucei* s.l. consists of *T. b. brucei*, *T. b. gambiense*, and *T. b. rhodesiense*. They cannot be distinguished morphologically. *T. b. brucei* is a widely distributed parasite of ungulates and a chief agent of nagana. It cannot infect people. HAT is caused in east and southern Africa by *T. b. rhodesiense*, and in west and central Africa by *T. b. gambiense*. These cause clinically distinct diseases but both are invariably lethal unless carefully treated by expert medical help. *T. b. rhodesiense* infection is clinically acute; its reservoirs include humans and cattle. Infection with *T. b. gambiense* is chronic and much slower to kill, however, this form accounts for most human cases. In the decade between 1997 and 2006, 97% of reported HAT cases were caused by *T. b. gambiense* (Simarro et al., 2008). Its reservoirs are principally humans but there is tangible evidence for other mammalian hosts (Njiokou et al., 2006). In mammals, trypanosomes undergo continuous antigenic variation, thereby defying host immune responses. Most HAT foci, about 200 in number, are localized and longstanding, but epidemics occur, some of great magnitude (Buxton, 1955; Ford, 1971).

Identification of Different Species of Trypanosome

In addition to *T. b. brucei*, economically significant agents of animal trypanosomiasis include *T. congolense* and *T. vivax*. Identification of trypanosome infection in the field depends on clinical criteria and microscopic diagnosis of trypanosomes, however, lacks adequate sensitivities. There is a useful card agglutination test for *T. b. gambiense* good for mass screening but no such test for *T. b. rhodesiense*. PCR-based specific diagnostic tools are available for experimental uses but sensitive, inexpensive, diagnostic tests for field use are urgently required. Chappuis et al. (2005) reviewed diagnostic options.

Trypanosome Genetic Diversity

Much genetic diversity has been demonstrated within *T. brucei* subspecies; Koffi et al. (2009) used microsatellite loci to demonstrate high levels of genetic differentiation among geographic isolates of *T. b. gambiense* from patients and extraordinary levels of genetic homogeneity in isolates from single patients (i.e., strong linkage disequilibrium and negative F_{IS}), indicative of strong clonal structure. Simo et al. (2010) also demonstrated strong population structure in *T. brucei* among 72 isolates (67 were *T. b. gambiense*) from nine foci in west and central Africa with the same results. Indeed, HAT epidemics usually demonstrate clonal etiologies, including those caused by *T. b. rhodesiense*.

Trypanosome Population Structure

Although trypanosome population structure is typically clonal, superinfections of two or more strains have been found in ~9% of 137 cryopreserved isolates of the three *T. brucei* subspecies from mammalian hosts across Africa (e.g., Balmer and Caccone, 2008). Meiosis and genetic recombination of *T. b. brucei* in *G. morsitans* have been conclusively demonstrated experimentally and take place in the fly's salivary glands (Gibson et al., 2008). In principle, genetic recombination is important epidemiologically because it would allow the evolution of new strains; its frequency and extent among natural populations is unknown but likely to be quite low. For now, genetically and spatially representative sampling of *Trypanosoma brucei* s.l. in the field is technically and operationally problematic because the technology of trypanosome recovery is inadequate and survey costs great.

Short vector dispersal distances could explain, in principle, spatially differentiated trypanosome populations. The question of trypanosome adaptation to local vector populations is relevant. Low trypanosome infection rates in tsetse (<1%) are typical because only a small fraction of ingested trypanosomes establish infective forms in the insect's salivary glands and there are multiple mechanisms of tsetse immunity to trypanosome infection (Aksoy and Rio, 2005, and Walshe et al., 2009, for reviews).

15.2.8 Genomics

Glossina *Genomics*

The genome of *G. m. morsitans* is being sequenced via the shotgun approach and its transcriptome made available. Annotated expressed sequence tag libraries in *G. m. morsitans* are available and continue to grow. There is also a bacterial artificial chromosome library (http://bacpac.chori.org). A *G. m. morsitans* gene database is available from http://old.genedb.org/genedb/glossina/, ftp://ftp.sanger.ac.uk/pub/pathogens/Glossina/morsitans/ and http://www.vectorbase.org/index.php. Sequencing the *G. p. palpalis* genome has been proposed (Askoy, 2010). Its genome is *c*. 7000 mb, compared with *c*. 600 mb in *G. m. morsitans* and *c*. 278 mb in *Anopheles gambiae*.

Trypanosome Genomics

Trypanosoma b. brucei has been sequenced (Berriman et al., 2005). Its genome is *c*. 26 mb. Partial shotgun sequencing of *T. b. gambiense* is underway and whole genome shotgun sequencing of *T. congolense* and *T. vivax* has begun. Details and sequences are available at the Wellcome Trust Sanger Institute: http://www.sanger.ac.uk/Projects/G_morsitans. The genomes are now available of tsetse symbiotic enteric bacteria *Sodalis glossinidia* (Toh et al., 2006) and *Wigglesworthia glossinidia* (Akman et al., 2002). The *Wolbachia pipientis* genome is also available (Wu et al., 2004).

The foregoing developments are promising because they greatly facilitate research by providing a framework for functional studies and may lead ultimately to more effective drug chemotherapies for trypanosomiasis and provide important insights into trypanosome morphological differentiation and cellular adaptations to its vector, much of which are poorly understood (Sharma et al., 2009). Additional practical applications for genomic research have been identified (Askoy et al., 2010) and discussed (Walshe et al., 2009).

15.2.9 Tsetse Population Management and Relevance of Genetic Studies

A Brief History of Attempts at Population Management

There are no vaccines for HAT and pharmaceutical treatment is expensive, dangerous, and unavailable to millions of people most at risk. Thus, African trypanosomiasis is best controlled by eliminating its insect vectors. Older methods of tsetse population management included destruction of wildlife that serve as trypanosome reservoirs, removal of vegetation that can harbor tsetse flies, and insecticidal applications. None of these methods taken separately or together have afforded long-term control because of invasion from nearby, untreated populations. Genetic methods have been advocated and tested (see Gooding and Krafsur, 2005, for review and critique). The SIT has been applied experimentally to several tsetse taxa (reviews in Hargrove, 2003; Klassen and Curtis, 2005). In theory, cytoplasmic incompatibility can be used to introduce high genetic loads (leading to reduced reproductive capacity) into a natural population and replace it with innocuous conspecific forms. Current research, elegant in concept and design, involves developing transgenic *Sodalis glossinidius* to express trypanolytic substances and building a *Wolbachia*-infected line of tsetse containing the transformed symbionts (Aksoy et al., 2001; Aksoy, 2003). The *Wolbachia* is then used to introduce cytoplasmic incompatibility when released into an uninfected natural population. Uninfected, wild-type females are at a reproductive disadvantage because they are sterile when mated with *Wolbachia*-infected males. In theory, the engineered, trypanosome-refractory line eventually replaces the wild-type, trypanosome- susceptible tsetse population.

The Promise and Limitations of Genetic Population Management

How applicable are genetic methods of tsetse population management and trypanosomiasis control likely to become? An eradication program utilizing population

reduction by insecticide-laced targets and SIT was carried out against *G. austeni* on Unguja Island (Zanzibar). Sterile male releases, begun in August 1994, were ended in December 1997. *G. austeni* has not reappeared since. Hargrove (2003) authoritatively evaluated the Zanzibar results and concluded that application of SIT on the continent is contraindicated because of its very high dollar costs, poor sterile fly competitiveness, and the availability of other proven cost-effective methods. Examination of the SIT for tsetse flies, via a useful simulation model, has shown the method is economically and ecologically inappropriate even under the best-case scenarios modeled (Vale and Torr, 2005). Replacement of natural vector populations with conspecific nonvectors, while elegant in theory, seems impractical because it depends on: (1) the successful development, production, and release of adequate numbers of reproductively competitive flies carrying *Wolbachia* and genetically transformed commensals, (2) a thorough knowledge of vector-trypanosome ecology throughout the region to be treated, (3) absence of trypanosome strains resistant to the trypanocidal substance(s), and (4) the age structure of target populations will cause lengthy population response times such that long generation times and high survival rates require continuous releases of an engineered tsetse strain over long intervals to expose target population virgins to the engineered released forms. Thus, the financial costs of field application and follow-up are likely to be enormous and difficult to sustain.

Conventional Tsetse Population Management

Applicable conventional methods include reduction of vector survival probabilities and densities via traps and targets, sequential aerial applications of low-volume insecticides, and targeted applications of pour-on insecticides to cattle at risk (Torr et al., 2005). Odor-baited, deltamethrin-impregnated targets and carefully plotted and timed aerial applications of deltamethrin eliminated *G. m. centralis* from the Okavango delta in Botswana during 2001−2002 (Kgori et al., 2006). The tsetse has not been detected since in the treated 15,900 km^2 (6139 mi^2) area even though untreated populations continue in nearby Zambia and Angola.

15.2.10 Further Work Needed

Barriers to developing further scientific knowledge of tsetse fly and trypanosome biology include a severe paucity of laboratory cultures representative of natural populations. Much work on trypanosomes is without reference to their vectors in nature and some may be irrelevant, therefore, to field conditions. Culture of tsetse flies is extraordinarily labor intensive, often difficult, and expensive. Few colonies now exist and most have been extant for many years, subject to inadvertent selection and inbreeding. Of 34 taxa, only nine are in culture: *G. austeni, G. pallidipes*, *G. m. morsitans*, *G. m. centralis*, *G. m. submorsitans*, *G. tachinoides*, *G. p. palpalis*, *G. p. gambiense*, and *G. f. fuscipes*. The pronounced genetic variation among natural tsetse populations argues for geographically more extensive sampling across the geographic range to assess genetic variation and reciprocal crossing of different

lines to assay fertilities and uncover sibling species. Because of the importance of commensal organisms in tsetse physiology and vector capacity, a better understanding of their prevalence, genetic diversity, and geographic distribution is also required. Vector−parasite coadaptations have important epidemiological and economic consequences but how they vary spatially is unknown.

Regarding tsetse fly population management, it is noteworthy that their historical distribution and abundance is unchanged except for their elimination in relatively small areas on the northern and southern margins—southwestern Zambia, northeastern Zimbabwe, northern Nigeria, and the Okavango in Botswana. Proposed genetic approaches to trypanosomiasis control are interesting and related research is yielding important scientific insights, but laboratory elegance alone would seem unlikely to overcome the dynamic nature of tsetse fly populations in their natural habitats. Nevertheless, exploitation of the rapidly accumulating genomic databases may lead eventually to highly efficacious and affordable trypanosomiasis control.

15.2.11 Concluding Remarks

Current knowledge of pathogenic trypanosome genetic variation in the field and corresponding variation in vector tsetse fly species and populations are insufficient to support a thorough understanding of trypanosomiasis epidemiology and epizootiology. Better methods are required for assessing trypanosome prevalence and genetic variation in the field. Comprehensive geographical sampling is required of trypanosome variation vis-à-vis that of their tsetse vectors. The breeding structures of *G. brevipalpis, G. f. quanzensis*, and *G. longipalpis* are unknown and these are the vectors in Mozambique and much of central Africa. The present view that most, if not all, Morsitans and many Palpalis group populations are local may serve to define areas in which systematic vector management schemes may be applied without massive immigration from untreated, conspecific populations. An effective and affordable genetically based area-wide tsetse fly population management is unlikely to be developed in the foreseeable future.

15.3 Genetics of the Triatominae (Hemiptera, Reduviidae) and Chagas Disease

15.3.1 Introduction

Distribution and Importance

American trypanosomiasis is a zoonosis caused by the flagellate protozoa *Trypanosoma cruzi* and is restricted to the New World. The distribution of animal trypanosomiasis is widespread from the southern United States to Patagonia (roughly 40°N to 45°S). Foci of the human disease (also called Chagas disease) range from Mexico to the northern half of Argentina, mostly in poor rural areas where houses are

infested with insect vectors belonging to the sub-family Triatominae (Hemiptera, Reduviidae). Chagas disease is amongst the most serious of the so-called neglected tropical diseases. It can be fatal in its early acute stage, but more usually progresses to a debilitating chronic disease that affects up to 30% of infected people and involves severe cardiac and intestinal lesions (Schofield, 1994). The World Bank (1993) ranked Chagas disease as by far the most serious of the parasitic diseases affecting Latin America, with a considerable social and economic impact far in excess of the combined impact of malaria, leishmaniasis, and schistosomiasis.

After a peak estimate of 24 million people infected and 100 million at risk in the second part of the twentieth century (Walsh, 1984), regional Chagas control initiatives (Southern Cone, Andean, Central American, and Amazonian) have resulted in a dramatic reduction of disease prevalence due to decreased vector transmission and an increase in blood donation screening. A 1990 estimate of 16–18 million people infected has been reduced to a current estimate of ~9 million infected (Schofield et al., 2006).

Vector Transmission

The Triatominae, also known as kissing or conenose bugs, are the sole vectors of American trypanosomiasis (Figure 15.10). A triatomine vector becomes infected with *T. cruzi* by feeding on the blood of an infected person or animal. Following the blood meal, it generally deposits urine and feces on the host skin and the parasite is transmitted through the feces of infected vectors. *T. cruzi* is a stercorian trypanosome, meaning the infective forms develop in the digestive tract of the vector.

Genetic Structure of Trypanosoma cruzi

The genetic structure of *T. cruzi* is predominantly clonal, with restricted recombination, as various cellular strains persist as stable genotypes that can spread through large geographic regions (Tibayrenc et al., 1986; Tibayrenc and Breniere, 1988). Rare genetic exchange, over evolutionary time, has had a profound impact on the adaptation of *T. cruzi* to new environments, vectors, and hosts, including humans (Vallejo et al., 2009). The biological, biochemical, and genetic diversities of

Figure 15.10 Sylvan *T. infestans* caught on a sticky trap (Noireau trap). Note the smaller nymphal stages as well as the larger adult on the right. Photographer, François Noireau.

T. cruzi strains has long been recognized along with their eco-epidemiological complexity, and numerous approaches have been used to characterize the population structure of *T. cruzi*. Recently, a consensus was reached that *T. cruzi* strains should be referred to by six discrete typing units (*T. cruzi* I–VI) (Zingales et al., 2009).

15.3.2 Systematics and Biogeography

Phylogeny of the Triatominae

There is consensus that the Triatominae are derived from predatory Reduviids. On the other hand, their monophyletic or polyphyletic origin is still in dispute (Hypsa et al., 2002; de Paula et al., 2005). According to Lent and Wygodzinsky (1979), all triatomines derived from a single ancestral form, with the defining synapomorphic trait being the bloodsucking habit and associated morphological and physiological adaptations; and this monophyletic hypothesis was supported by results of a comprehensive study of 16S rDNA sequence over a large number of taxa (Hypsa et al., 2002) and recently, by cladistic analyses of Reduviidae based on genes sequences and morphological characters (Weirauch, 2008; Weirauch and Munro, 2009). This hypothesis is difficult to reconcile with the wide geographical distribution and "nest-dwelling" habit of Triatominae, because it implies that a specialized ancestral nest-dweller would subsequently adapt to range of different habitats associated with a range of different hosts (Schofield, 1988). By contrast, several arguments suggest that the Triatominae represent a polyphyletic group derived from different lineages within the predatory Reduviidae. Evidence is adduced from comparative differences between tribes in form (different tribes in Triatominae have a body form that matches that of distinct subfamilies in Reduviidae), and also at the level of salivary gland physiology and parasite susceptibility (Schofield and Galvão, 2009). More recent studies based on DNA sequence showed disruption of the Rhodniini + Triatomini clades by predatory taxa, supporting the idea of polyphyletic origins (de Paula et al., 2005).

The Five Triatominae Tribes

Triatominae are generally classified into 5 tribes and 15 genera and include, at present, some 140 species. One species (*Triatoma rubrofasciata*) is widespread both in the New World and some tropical regions of Asia and Africa. A few others are found only in Asia and India (Galvão et al., 2003). However, most species (~125) occur exclusively in the New World, between latitude 42°N (northeast United States) and 46°S (Argentine Patagonia) (Lent and Wygodzinsky, 1979; Schofield and Galvão, 2009). The species of epidemiological importance belong to the two tribes Rhodniini and Triatomini that seem to be relatively phylogenetically distant (de Paula et al., 2005). Cryptic species, which are thought to arise through morphological convergence of two different species in competition for similar habitats, seem uncommon in Triatominae (Dujardin et al., 2009). They were apparently detected within *Triatoma sordida* (Noireau et al., 1998; Panzera et al., 2006), *R. robustus* (Monteiro et al., 2003), and *Triatoma dimidiata* (Marcilla et al., 2001;

Panzera et al., 2006), although the latter species showed phenotypic differences as well as genetic (Bustamante et al., 2004; Dorn et al., 2007).

The Rhodniini Tribe

The tribe Rhodniini contains two genera, *Rhodnius* and *Psammolestes*. Mitochondrial cytochrome *b* gene (*cyt b*) genealogies tend to support the existence of two lineages within the tribe Rhodniini: the *pictipes* and the *robustus* lineages (Abad-Franch and Monteiro, 2007). The *pictipes* lineage includes species from both the eastern (*pictipes, stali, brethesi*, and some sylvan species) and western sides of the Andes (*pallescens, colombiensis*, and *ecuadoriensis*). The members of the *robustus* lineage, also including the genus *Psammolestes*, are found east of the Andes (Abad-Franch et al., 2009). Within the *robustus* lineage, the species forming the *prolixus* group (*R. prolixus*, *R. robustus*, *R. neglectus*, and *R. nasutus*) are particularly difficult to distinguish. One of these (*R. prolixus*) is a primary domestic vector of Chagas disease (Figure 15.11) while the three others are synanthropic species that can invade or even sporadically colonize man-made ecotopes. The specific status of *R. robustus*, which is virtually indistinguishable from *R. prolixus*, has been clarified by the sequence analysis of a fragment of the mitochondrial *cyt b* gene. *R. robustus* currently includes four cryptic species. *R. robustus* I occurs in Venezuela (Orinoco

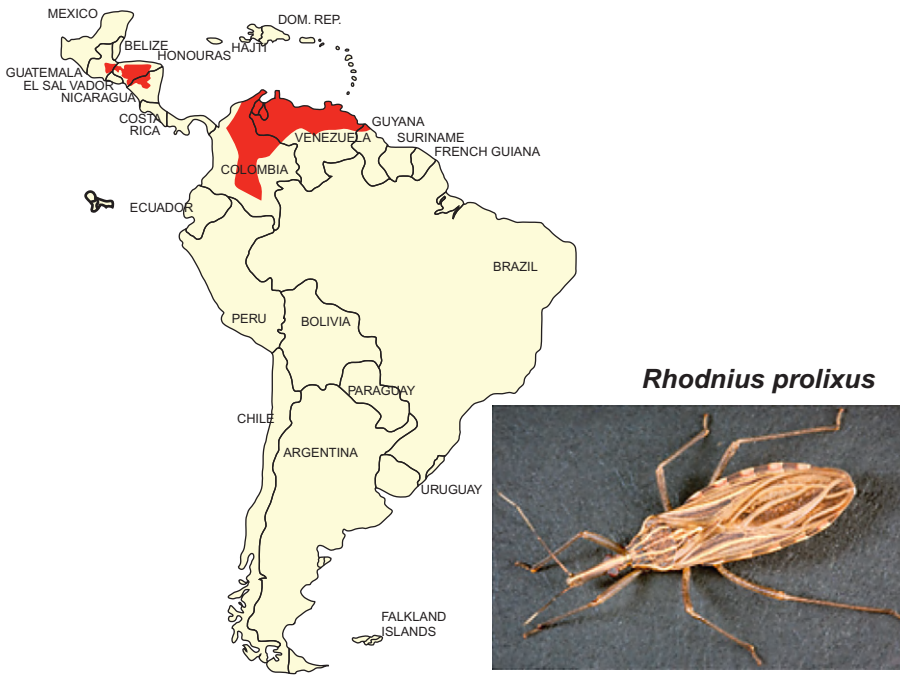

Figure 15.11 Approximate current distribution of *Rhodnius prolixus*.
Source: Data from WHO (1989). Linda Waller, artist; James Gathany, photographer (Centers for Disease Control and Prevention).

region) and is more closely related to *R. prolixus* than to the other three cryptic species found in the Amazon region (Monteiro et al., 2003).

The Triatomini Tribe

Two genera belonging to the Triatomini tribe (*Panstrongylus* and *Triatoma*) are of epidemiological importance. The phylogeny of *Panstrongylus* is currently under discussion, although there is evidence supported by cladistic, molecular, morphometric, and cytogenetic studies, suggesting the existence of northern and southern clades (Patterson et al., 2009). Only the placement of *P. megistus* is discordant. Indeed, it is largely southern in its distribution and is unusual cytogenetically, having only 18 autosomal chromosomes compared to the 20 autosomes of other *Panstrongylus* (Crossa et al., 2002). *Triatoma* is the most numerous genus of Triatominae, with 80 formally recognized species (Schofield and Galvão, 2009). North American, Central American, and Caribbean species as a whole seem genetically separate from the South American species, that is, in parallel to the northern and southern clades of *Panstrongylus* species.

The "*Infestans*" Subcomplex

The subcomplex *infestans* sensu (Schofield and Galvão, 2009) includes the species *infestans*, *delpontei,* and *platensis*. *T. infestans* was and still remains the most important and widespread vector of Chagas disease (Figure 15.12) in the Southern

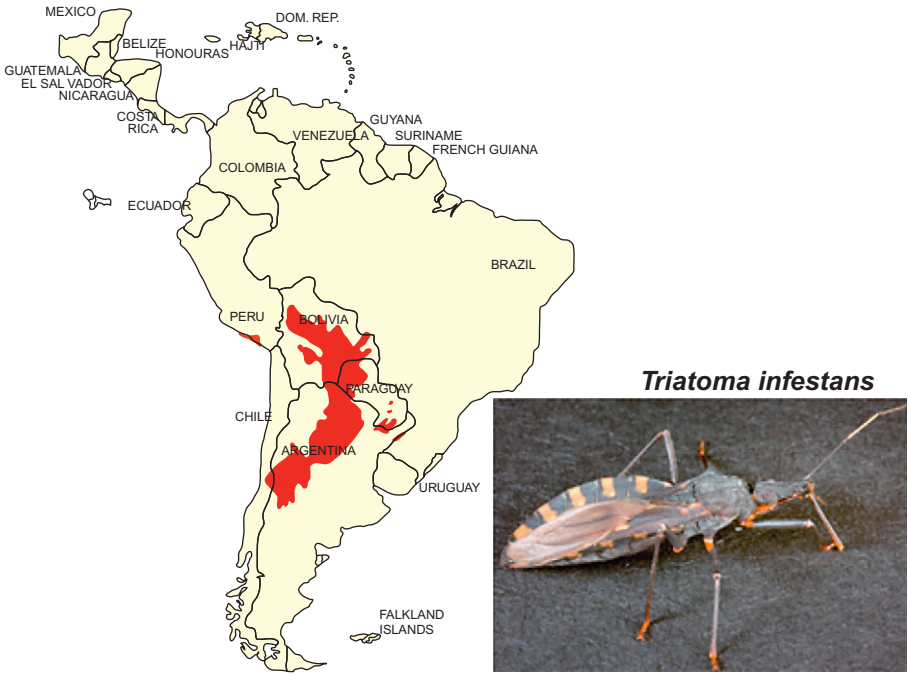

Figure 15.12 Approximate current distribution of *Triatoma infestans*.
Source: Data from Schofield (2006). Linda Waller, artist; James Gathany, photographer (Centers for Disease Control and Prevention).

Cone countries, whereas the latter two species are closely associated with bird nests. These three species are very closely related and have the same diploid chromosome number 2N = 22 (20 autosomes + XX/XY). They also have several cytogenetic traits that differ from all other triatomines, including large autosomes, C-heterochromatic blocks, and meiotic heteropycnotic chromocentres formed by autosomes and sex chromosomes (Panzera et al., 1995). All evidence indicates that, within this subcomplex, *T. platensis* is the closest relative to *T. infestans* (Bargues et al., 2006). The status of *T. infestans* and *T. platensis* as two distinct species is almost entirely based upon their ecological niche separation.

T. dimidiata, the Major Vector in Mesoamerica

T. dimidiata has become the most important vector of Chagas disease in Mexico, Central America, and northern Andean countries of South America, since control activities to eliminate *R. prolixus* have made substantial progress (Figure 15.13). *T. dimidiata* has extensive phenotypic, genotypic, and behavioral diversity in sylvan, peridomestic, and domestic habitats across its geographic range. Recent studies strongly suggest that this taxon, which has been historically regarded as a single species, includes a cryptic species and perhaps as many as three subspecies distributed in specific geographic areas with different epidemiological importance (Marcilla

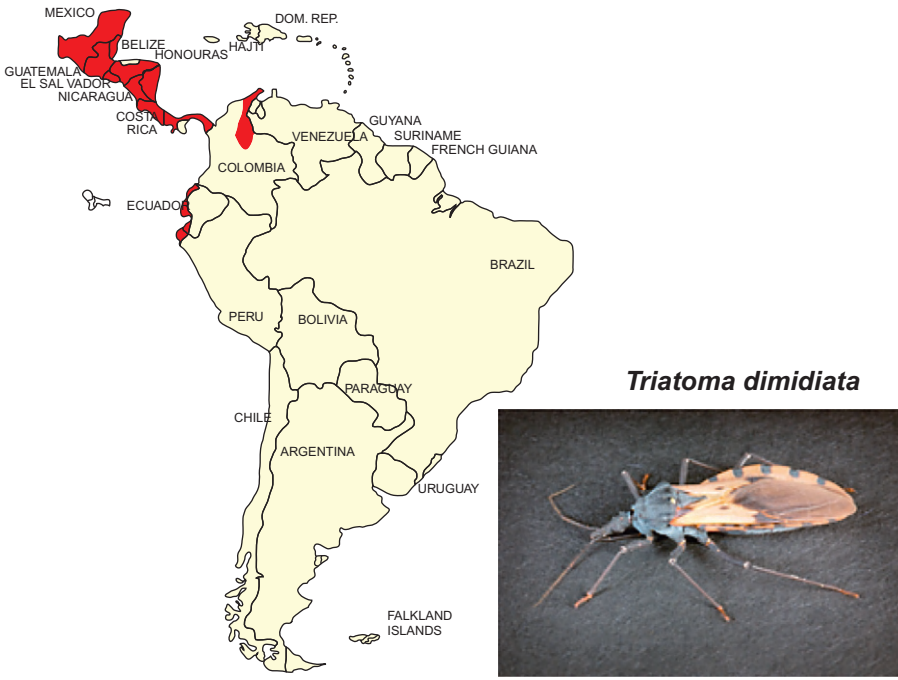

Figure 15.13 Approximate current distribution of *Triatoma dimidiata*.
Source: Data from WHO (1989). Linda Waller, artist; James Gathany, photographer (Centers for Disease Control and Prevention).

et al., 2001; Panzera et al., 2006; Dorn et al., 2007, 2009; Bargues et al., 2008). These current distinctions between taxa have major implications for transmission capacity and vector control.

15.3.3 Basic Biological and Ecological Features

Life Cycle of the Triatominae

The life cycle of the Triatominae is composed of egg and five nymph stages, which will reach sexual maturity from a few months (*R. prolixus*, *T. infestans*) to more than 1 year (*T. dimidiata*, *P. megistus*). All the stages feed on vertebrate blood but deprived of access to blood, some species can feed on other arthropods, a relic ancestral trait (Schofield, 1988). Bugs may survive for more than a month without access to food, depending on other environmental conditions, such as temperature. In most Triatominae, including strictly sylvan ones, it is worth noting a relative lack of host specificity in the feeding habits. Exceptions are observed in some ornithophylic species (*Psammolestes* spp. and *T. delpontei*) or the tropicopolitan *T. rubrofasciata* that feed preferentially on rodents.

Life History of the Triatominae

During the day, in sylvan as well as in the domestic environment, the Triatominae remain motionless, hidden inside their refuges. At nightfall, they may walk or fly (adults) to look for blood (Figueiras and Lazzari, 2000). Beyond the locomotor capacity, it is important to distinguish two dispersion modes in the Triatominae: active and passive dispersion. Both walking and flying constitute active dispersion. Flight orientation is apparently random, but it seems that during its flight the bug can be attracted to light. Passive dispersion is transportation of generally immature stages by an animal host or with the familiar objects carried or worn by the human host. This latter mode of dispersion is the most important to explain the territorial expansion of the main vectors (*T. infestans*, *R. prolixus*, *T. dimidiata*) (Schofield, 1988).

As a general but not absolute rule, the habitat of the Triatominae offers shelter and easy access to a blood source with a stable availability of hosts. According to Schofield (1988) and Gaunt and Miles (2000), each of the three most epidemiological important genera of Triatominae is virtually always associated with a particular habitat. So, species of the genus *Rhodnius* are primarily associated with palms, the genus *Panstrongylus* has predominantly evolved in burrows and tree cavities, and the genus *Triatoma* in terrestrial rocky habitats or rodent burrows. If this assumption is generally true for the genera *Rhodnius* and *Panstrongylus*, it is more questionable for the genus *Triatoma,* in which species may be found exclusively in trees or in both habitats (arboreal and terrestrial) in different localities (e.g., for *T. infestans*, *T. sordida,* and *T. guasayana*, Andean populations live in rockpiles and lowland populations in hollow trees).

Effects of Anthropogenic Change and Domicilation

Environmental disturbance caused by humans and resulting in damage to triatomine habitat may lead to changes in insect habitats, breeding behavior, and host preference. These disturbances and subsequent starvation are certainly the main causes of flight dispersal, which can lead to settlement in artificial structures. Domesticity is the key determinant of vector capacity in the Triatominae. Only the species adapted to human dwellings, which represent less than 5% of the total number of species, are actively contributing to transmission. The distinction between intrusion, domiciliation, and domestication may help in defining the epidemiological importance of populations or species of Triatominae (Dujardin et al., 2000). Intrusion occurs when adult specimens of sylvan species are present inside human dwellings, probably attracted by light or introduced by passive carriage. In this situation, there is no evidence of colonization (eggs, nymphs, exuviae are not found). Domiciliation represents a tentative adaptation to the house and corresponds to the finding of eggs and exuviae, and small colonies of adults and nymphs in the house (which means the complete cycle of the insect is occurring inside the house). For example, this situation has been described for *Triatoma sordida* in Bolivia (Noireau et al., 1997). The definition of domestication includes the above criteria for domiciliation, with an additional one related to the geographic scope. It is no longer a local, geographically restricted observation, but concerns a more widely extended territory with obvious arguments supporting migration by passive carriage. It is important to recall that the existence of "domesticated" species does not exclude the existence of sylvan foci. Wild populations of *T. infestans* and *R. prolixus* were recorded in Bolivia and Argentina, and Venezuela, respectively (Noireau et al., 2005; Fitzpatrick et al., 2008). More research is needed to understand the factors allowing a species to reach a high level of adaptation to the domestic habitat. We know that the adaptation systematically reduces the size of the insect (Schofield et al., 1999; Caro-Riaño et al., 2009). Another sort of plasticity, that is behavioral and related to habitat selection, might also factor into the adaptation. In the sylvan environment, species can exclusively inhabit a particular microhabitat (e.g., rockpiles or trees). However, when they invade the peridomestic/domestic environment (Figure 15.14), they can adapt to different habitats and occupy substrates that they do not colonize in the sylvan environment (de la Fuente et al., 2008). In order to provide an initial operational evaluation of each species, it may be useful to adopt a classification system based on a scale describing the degree of adaptation: (1) restricted to the sylvan environment, (2) infestation of peridomestic structures, (3) intrusion into houses, (4) domiciliation, and finally, (5) domestication; and the epidemiological importance (proved or putative vector vs. species without a documented role in the transmission of *T. cruzi* to humans).

MacArthur and Wilson (1967) distinguished "r" and "K" demographic strategies defining populations occupying unstable or stable habitats, respectively. If the "r" strategy is typically the one of mosquitoes, the "K" strategy is congruent with domestic Triatominae that are relatively large insects, produce small quantity of descendants (about 100 eggs during the life in a female of *T. infestans*), have an

Figure 15.14 House and peridomestic area (rock walls, granery) in Bolivian Andes. Photographer, François Noireau.

extended developmental cycle, poor active dispersal capacity, and are timid feeders. Triatominae do not exhaust the resources of their environment; rather seem to opt for an optimal use. Finally, they would probably be unable to recover or to escape to other richer environments after a catastrophic mortality (Rabinovich, 1974). Thus, extinction would be the fate of "K" strategist when confronted with an adverse environment, as observed for the domestic species of Triatominae, which have been targeted by international vector control programs (Schofield and Dias, 1999).

15.3.4 Triatominae Genetics

Cytogenetics

Triatomine chromosomes are holocentric; that is, there is not a single identifiable centromere, but rather the centromere function is spread out along the entire chromosome (see review of triatomine cytogenetics in Panzera et al., 2010). Therefore, even if a chromosome is fragmented, the fragments will be appropriately aligned and segregated during mitosis and meiosis. All but three species of triatomines examined cytologically contain 20 autosomes. The majority of the species also contain a single sex bivalent, XY for males and XX for females ($2N = 20 + XY$). However, in some species the X chromosome is fragmented (in both males and females) into two or (rarely) three fragments. For example, a *T. dimidiata* male contains 23 chromosomes, consisting of 20 autosomes, plus an X fragmented into

two pieces (males, $2N = 20 + X_1X_2Y$); and in *T. vitticeps* the X is in three pieces (e.g., females) ($2N = 20 + X_1X_1X_2X_2X_3X_3$). The fragmented X is found in nearly all North American *Triatoma* species, whereas most South American species have the XY system. Variation in the amount and location of heterochromatin has been observed, most notably with a significantly greater amount of C-banded heterochromatin in Andean *T. infestans* as compared to non-Andean (Panzera et al., 2004). In the Andean, thought to represent the ancestral form, C-heterochromatic blocks are present on the majority of the 22 chromosomes, whereas in non-Andean specimens, heterochromatic regions are restricted to only 4−7 autosomes. This additional heterochromatin correlates with ~30% more DNA in the specimens from the Andes. *T. infestans* is thought to have lost DNA during the dispersal process from an origin in the Bolivian Andes. Chromosomal differences were also important in uncovering a cryptic species in *T. dimidiata* (Panzera et al., 2006). The power of chromosome diversity analysis in resolving taxonomic and phylogenetic questions is just beginning to be realized. Recent advances such as fluorescent in situ hybridization and comparative genome hybridization will add to this power.

Population Genetics

Genetic Diversity in Triatomine Populations

Variability of triatomine populations has been assessed by allozymes (reviewed in Dujardin et al., 2002), nuclear and mitochondrial DNA sequence, and by microsatellite markers. In general, two main vectors in South America, *T. infestans* and *R. prolixus*, show less genetic diversity than Central and North American species by isozyme analyses (Table 15.4). This lower genetic diversity is also seen with very low levels of variability in *R. prolixus* mtDNA (*cyt b*, Table 15.5); with only three haplotypes observed in 41 specimens (Monteiro et al., 2003) or 14 haplotypes/~550 specimens (Fitzpatrick et al., 2008). *T. infestans* also shows a similar *cyt b* diversity (7 haplotypes/62 specimens, Giordano et al., 2005), whereas Central American *T. dimidiata* shows high (15 *cyt b* haplotypes/58 specimens (Blandon-Naranjo et al., 2010)) or extremely high levels (21 haplotypes/23 specimens, Dorn et al., 2009); however, the latter is from a region containing the cryptic species and so likely reflects interspecific variability. A single North American population of *T. sanguisuga* showed the astonishing number of 37 haplotypes/54 specimens; a possible cryptic species in this population is being investigated (de la Rua, et al., unpublished data). Nuclear (ITS2) sequence data also supports higher diversity in *T. dimidiata* populations as compared to *T. infestans* and *R. prolixus* (Table 15.5). This may be due to the fact that over most of their range *T. infestans* and *R. prolixus* are exclusively domestic; it has been suggested that domestication results in a diminished genetic repertoire perhaps due to founder effects and genetic drift in isolated populations (Schofield et al., 1999). However, some studies report no difference in allele frequencies (allozymes, *T. infestans*, Dujardin et al., 1987) or haplotype diversity (*cyt b, R. prolixus,* Fitzpatrick et al., 2008) between domestic and sylvatic populations, and in other studies, a higher haplotype diversity was noted in domestic as compared to sylvatic populations (*cytochrome oxidase* sequence, *T. infestans*, Piccinali et al.,

Table 15.4 Isozyme Diversities of Triatomine Populations

North and Central American Species	*n*	Number of Loci	% Polymorphic	Mean Diversity, H_e	Reference
T. barberi Mexico	35	17	18	0.038	Flores et al. (2001)
T. dimidiata Mexico	18	16	50	0.187	Flores et al. (2001)
T. longipennis Mexico	39	17	53	0.109	Flores et al. (2001)
T. pallidipennis Mexico	31	17	29	0.072	Flores et al. (2001)
T. picturata Mexico	28	17	41	0.124	Flores et al. (2001)
South American Species					
R. prolixus Colony, Venezuela	90	22	22	0.06	Harry et al. (1992)
R. prolixus Colony, Venezuela	181	19	10.6	0.017	Harry et al. (1993)
R. prolixus Colombia	41	10	9.1	0.011	Lopez et al. (1995)
R. prolixus Colombia	8	17	0	0.0	Dujardin et al. (1998b)
R. prolixus Colony, Venezuela	12	12	8.0	0.01	Monteiro et al. (2002)
T. infestans Bolivia	37	19	15.7	0.04	Dujardin and Tibayrenc (1985a)
T. infestans Peru	NS	19	5	$H_o = 0.015$	Dujardin and Tibayrenc (1985b)
T. infestans Chile	36	18	15.3	$H = 0.178$	Frias and Kattan (1989)
T. infestans Bolivia	177	19	16	$H = 0.049$	Dujardin et al. (2002)
T. infestans Colony, Argentina	50	17	52.9	0.074	Garcia et al. (1995)
T. infestans Bolivia and Uruguay	1044	24	4–13	0.050	Pereira et al. (1996)
T. infestans Bolivia, Peru, Uruguay, and Brazil	1154	19	5	0.049	Dujardin et al. (1998a)

Table 15.5 Mitochondrial and Nuclear Diversities in Triatomine Populations

North and Central American Species	Method	Number of Populations	Number of Insects	Number of Haplotypes or [a]Mean Alleles per Locus	Mean Diversity, H_S or H_e	Reference
T. dimidiata Costa Rica	*cyt b*	7	58	15	NS	Blandon-Naranjo et al. (2010)
T. dimidiata Mexico and Guatemala	*cyt b*	NS	23	21[b]	NS	Dorn et al. (2009)
T. dimidiata Costa Rica	16S rDNA	7	58	6	NS	Blandon-Naranjo et al. (2010)
T. dimidiata 7 countries	ITS2	47	113	24[c]	$H_S = 0.822$[ʃ]	Bargues et al. (2008)
T. dimidiata Mexico and Guatemala	ITS2	26	47	11[c]	NS	Dorn et al. (2009)
T. dimidiata Mexico	4 ML	12	295	10.3[ab]	$H_e = 0.805$[‡]	Dumonteil et al. (2007)
T. sanguisuga United States	*cyt b*	1	54	37	$H_S = 0.978$	de la Rua, et al., unpublished data
South American Species						
R. prolixus 4 countries	*cyt b*	12	41	3	NS	Monteiro et al. (2003)
R. prolixus Venezuela	*cyt b*	33	551	14	$H_S = 0.432$	Fitzpatrick et al. (2008)
T. infestans Brazil, Bolivia, and Argentina	*cyt b*	9	33	4	NS	Monteiro et al. (1999)
T. infestans Bolivia	*cyt b*	30	62	7	$H_S = 0.448$	Giordano et al. (2005)
T. infestans Peru, Bolivia, Uruguay, Argentina	*co* I	54	244	37	$H_S = 0.766$	Piccinali et al. (2009)

(*Continued*)

Table 15.5 (Continued)

North and Central American Species	Method	Number of Populations	Number of Insects	Number of Haplotypes or [a]Mean Alleles per Locus	Mean Diversity, H_S or H_e	Reference
T. infestans Argentina	16S rDNA	16	130	18	$H_S = 0.030$	Segura et al. (2009)
T. infestans Argentina	12S + 16S rDNA	5	40	13	$H_S = 0.538$	Garcia et al. (2003)
T. infestans Paraguay	ITS2	7	7	2	NS	Marcilla et al. (2000)
T. infestans 7 countries	ITS2	31	35	5	NS	Bargues et al. (2006)
T. infestans 7 countries	ITS1, 5.8S, ITS2	31	35	7	$H_S = 0.298$	Bargues et al. (2006)
R. prolixus Venezuela	9–10 ML	33	555	3.1[a]	$H_e = 0.476$	Fitzpatrick et al. (2008)
T. infestans Argentina	10 ML	1	34	10.7[a]	$H_e = 0.753$	García et al. (2004)
T. infestans Argentina	10 ML	19	598	6.8[a]	$H_e = 0.588$	Pérez de Rosas et al. (2007)
T. infestans Argentina	10 ML	21	352	17[a]	$H_e = 0.72$	Marcet et al. (2008)
T. infestans Bolivia	9 ML	6	111	7.3[a]	$H_e = 0.656$	Richer et al. (2007)
T. infestans Bolivia	10 ML	6	238	5.6[a]	$H_e = 0.432$	Pérez de Rosas et al. (2008)
T. infestans Bolivia	10 ML	23	253	14.5[a]	NS	Pizarro et al. (2008)

ML = microsatellite loci, H_S = haplotype diversity, H_e = expected heterozygosity, H_o = observed heterozygosity, H = heterozygosity, NS = not stated.
[a]Mean number of alleles per locus.
[b]From area with cryptic species present.
[c]Cryptic species removed.

2009). An alternative hypothesis is that low genetic variability reflects rapid and recent expansion due to passive dispersal (Dujardin et al., 1998b). *T. dimidiata* and other Central and North American species are widespread in sylvan, peridomestic, and domestic habitats. Survival in these very diverse habitats may have resulted in distinct selective pressures, thus maintaining genetic diversity. Interestingly, the strong selective pressure of pesticide application can result in diminished genetic variability (Garcia et al., 2003) or no effect or even an increase in the genetic variability in a population (Pérez de Rosas et al., 2007, 2008). The authors speculate that the reduction in population may not be as severe as expected (i.e., less genetic bottleneck effect) or that the high variability may be due to genetic drift in subpopulations, where each subpopulation retains a different combination of alleles. If that is followed by rapid growth of the population, it may be preserving the genetic diversity.

Importance of Population Genetic Studies for Taxonomy and Control

Effective control methods require correct identification of the target population, and genetic studies have been important in taxonomic clarification in the triatomines, including an understanding of the epidemiological importance of sylvan populations. For example, genetic studies demonstrated that domestic *R. prolixus* is not a derivative of the sylvan *R. robustus* but, in fact, is a separate species, diminishing the concern that *R. robustus* might be epidemiologically important (Lyman et al., 1999). To date genetic studies have not identified cryptic species in *T. infestans*. However, it is a **polytypic species**, meaning that genetically and morphologically distinguishable forms have been described, which when mated result in fertile offspring. In addition to the cytogenetic differences in the Andean compared to non-Andean *T. infestans*, these taxa can be distinguished by nuclear (with one exception, Bargues et al., 2006) and mtDNA (Giordano et al., 2005) with a significant F_{ST} between Andean and non-Andean populations. Interestingly, despite all these differences, offspring resulting from laboratory crosses between the two taxa are fertile (Panzera et al., 2004). Distinct chromatic *T. infestans* variants have also been identified. A "dark morph" was indistinguishable from other *T. infestans* by allozyme analysis, however, differs in nuclear (Bargues et al., 2006) and mtDNA sequence (Monteiro et al., 1999). Another dark variant found in Argentina, first identified as *T. melanosoma*, is now considered a subspecies of *T. infestans, T. i. melanosoma*, based on several characteristics (Monteiro et al., 1999; Bargues et al., 2006). Recent mtDNA analyses suggest a divergent taxon in *T. infestans* in Argentina (Piccinali et al., 2009). *T. dimidiata* also appears to be a polytypic species, with the number of divergent taxa still under investigation (reviewed in Dorn et al., 2007). Genetic studies also revealed a cryptic species in *T. dimidiata* (Marcilla et al., 2001) as well as in *T. sordida*, *T. brasiliensis*, *R. robustus,* and *R. ecuadoriensis* (reviewed in Abad-Franch and Monteiro, 2005). Studies are underway to understand the epidemiological importance of the distinct taxa. The finding of these morphologically similar but genetically divergent cryptic species has led to the suggestion that triatomines show "morphological plasticity," that is, rapid morphological change in response to environmental selection that may reflect convergent evolution rather than shared ancestry (Dujardin et al., 1999).

Hybrids, Cryptic Species, and Introgression in the Triatominae

Natural hybrids between species also occur in triatomines such as *T. infestans* and *T. platensis* (Abalos, 1948) and *T. dimidiata* and the cryptic species from the Yucatan Peninsula, Mexico (Herrera-Aguilar et al., 2009). In the former case the two species occupy distinct ecological niches and the rare hybrids are fertile over many generations. In contrast, for *T. dimidiata*, populations are sympatric, however, decreased fitness of hybrids may be what is maintaining >5 million year separation of the species. (The time of separation is based on a molecular clock using ITS2 divergence rates; Bargues et al., 2008.) **Introgression** of mtDNA was suggested because of similar mtDNA sequence in *T. infestans* and *T. platensis* (Garcia and Powell, 1998). Discordant phylogenies based on nuclear and mtDNA provide stronger evidence of introgression between *R. prolixus* and *R. robustus* (Fitzpatrick et al., 2008), *T. platensis* and *T. delpontei* (Mas-Coma and Bargues, 2009), *T. dimidiata* and the cryptic species (Herrera-Aguilar et al., 2009), and *Mepraia spinolai* and *M. gajardoi* (Calleros et al., 2010).

Population Structure

Studies investigating gene flow among populations have been important in understanding the geographic coverage needed for successful control, the source of triatomines appearing following pesticide treatment, the role of peridomestic and sylvan populations in human transmission; and would be important to understand the potential spread of an introduced genetically modified symbiont. Triatomines are generally poor flyers; however, some species can fly up to 1 km and even wingless nymphs can walk tens of meters (Núñez, 1987). Passive transport by humans and perhaps even migratory birds (via eggs or nymphs) has been important in spreading and mixing populations. The overall results of population genetic studies in *T. dimidiata* and *T. infestans* show that at larger geographic scales (populations >50 km apart) there is generally a gradient of allele frequency differences among populations consistent with an "**isolation by distance**" model (Wright, 1943). At smaller geographic scales, the picture is more complicated, varies geographically, and sometimes is affected by the pesticide application history.

Where *R. prolixus* is present in both domestic and sylvan ecotopes, shared mtDNA haplotypes and a nonsignificant F_{ST} using 10 microsatellite loci demonstrated gene flow between these populations and indicated that sylvan *R. prolixus* poses a risk for reinfestation of treated houses (Fitzpatrick et al., 2008).

Population Structure of *T. dimidiata* Across its extensive range, *T. dimidiata* shows quite different behaviors ranging from living exclusively in domestic and **peridomestic habitats**, to seasonally entering homes, to living exclusively in sylvan areas. Therefore, substantially different degrees of migration and gene flow might be expected among different populations. For domestic *T. dimidiata* populations, there appears to be high gene flow among houses within a village and among nearby villages (up to 27 km distance) by RAPD-PCR ($F_{ST} = 0.025$ and 0.019, respectively, Table 15.6) giving an estimated 9.7−12 mating migrants per generation (Nm) (Dorn et al., 2003). With the same technique Ramirez et al. (2005)

Table 15.6 Subdivision of *T. dimidiata*, *R. prolixus*, and *T. infestans* Populations by Microsatellite Analyses

Species/ Country	Number of Loci	Number of Insects per Population	Geographic Distance	F_{ST}	Reference
Among villages					
T. dimidiata Mexico	4	11–34	<280 km	0.06[a]	Dumonteil et al. (2007)
T. dimidiata Guatemala	8	28–30	<13 km	0.07	Stevens, et al., unpublished data
T. infestans Argentina	10	20–37	<30 km	$\theta = 0.135$	Pérez de Rosas et al. (2007)
T. infestans Argentina	10	28–70	<220 km	$\theta = 0.169$	Pérez de Rosas et al. (2008)
T. infestans Argentina	10	12–99	<31 km	0.02–0.2	Marcet et al. (2008)
T. infestans Bolivia	10	9–78	<100 km	0.06	Pizarro et al. (2008)
Among houses					
T. dimidiata Guatemala	8	2–7	NS	0.07–0.27	Stevens, et al., unpublished data
R. prolixus Venezuela	9–10	14	NS	−0.02	Fitzpatrick et al. (2008)
T. infestans Argentina	10	11–24	NS	$\theta = 0.05–0.07$	Pérez de Rosas et al. (2007)
T. infestans Argentina	10	9–30	<2.5 km	0.02–0.16	Marcet et al. (2008)
T. infestans Argentina	10	15–31	<1.3 km	0.10	Pérez de Rosas et al. (2008)
T. infestans Bolivia	10	3–14	<1.5 km	0.07	Pizarro et al. (2008)
Among ecotopes					
R. prolixus Venezuela	9–10	12–39	Among ecotopes	0.002–0.2	Fitzpatrick et al. (2008)
T. infestans Argentina	9	6–31	<1.1 km, among sylvan	0.002–0.02	Richer et al. (2007)
T. infestans Bolivia	9	6–32	<1.1 km sylvan vs. domestic or peridomestic	0.03, 0.11	Richer et al. (2007)
T. infestans Bolivia	10	36–42	<750 m domestic vs. peridomestic	0.03	Pizarro et al. (2008)

[a]From area with cryptic species present.

showed movement of at least two or three mating migrants per generation ($F_{ST} = 0.07$) among nearby (within 10−200 m) domestic, peridomestic, and sylvan (cave) populations. Also by RAPD-PCR, nearly half the individuals within a single house were unrelated, again supporting substantial migration by *T. dimidiata* (Melgar et al., 2007). In localities where *T. dimidiata* seasonally enters houses, results of microsatellite analyses showed a high gene flow among nearby houses ($F_{ST} = 0.037$), villages ($F_{ST} = 0.055$, within 250 km), and between the forest and houses ($F_{ST} = 0.01-0.03$, 1−280 km), for an Nm = 5−25 (Dumonteil et al., 2007). However, the cryptic species was later discovered in this area, so these results need to be confirmed with the identity of the samples known. More recently, in an area of domestic *T. dimidiata*, microsatellite analyses showed a weak but statistically significant genetic structure among populations within 12 km ($F_{Sg} = 0.07$ or about three mating migrants per generation between towns) and among houses ($F_{ST} = 0.07-0.27$) (Stevens et al., unpublished data). Twenty-four genetic clusters were spread across seven villages, and most insects were only distantly related to others in the same house, again supporting substantial movement among houses and villages. This high migration among nearby houses, villages, and even sylvan locations by *T. dimidiata* means that simply treating individual houses or even villages with pesticides is not likely to be effective (Figure 15.15).

Population Structure of *T. infestans* Results using allozyme analyses show gene flow between nearby (~20−50 km) *T. infestans* domestic, and domestic and sylvan populations with a smaller panmictic unit observed in some localities (Dujardin et al., 1987, 1998b; Brenière et al., 1998). With higher resolution microsatellite markers, geographic variation in the degree of subdivision of populations was again observed, and in certain areas pesticide application appeared to affect population subdivision. In most localities movement was quite limited as there was significant genetic differentiation among villages and houses within a village (Pérez de Rosas et al., 2007, 2008; Marcet et al., 2008) (Table 15.6). The number of genetically related clusters identified is close to the number of villages studied in several studies (8 clusters in 10 villages, Marcet et al., 2008; 5 in 5 villages, Pizarro et al., 2008; 7 clusters in 6 villages, Pérez de Rosas et al., 2008) and generally most clusters were localized to just one or a few villages. Taken together results indicate that *T. infestans* shows limited gene flow, likely due to limited migration.

Results of most, but not all, studies indicate gene flow among nearby ecotopes for *T. infestans* and *R. prolixus* (Dujardin et al., 1987; Monteiro et al., 1999; Giordano et al., 2005; Fitzpatrick et al., 2008; Table 15.6).

So overall, *T. dimidiata* populations present more diversity than do those of *T. infestans* or *R. prolixus*. *T. dimidiata* also shows more migration than does *T. infestans* based on the differentiation among populations (by F_{ST}) and the number and distribution of genetically related clusters in villages.

Implications for Control

These results have important implications for control. With a long life span and low genetic diversity, one might hope to avoid development of insecticide

Figure 15.15 Fumigation using residual pesticides in Guatemala. Photographer, Patricia Dorn.

resistance in *T. infestans*. Unfortunately, insecticide resistance has been noted in several populations, perhaps partly due to inadequate pesticide application (Gonzalez Audino et al., 2004; Picollo et al., 2005; Toloza et al., 2008). Although not presently known, it is likely just a matter of time before resistance will also appear in the more diverse *T. dimidiata*.

Understanding the amount of gene flow among populations can inform the geographic coverage needed for control. The low gene flow detected among most domestic *T. infestans* populations made it an excellent target and is probably the reason for the success of the Southern Cone Initiative, which has achieved the remarkable average reduction of 94% in Chagas disease incidence in the Southern Cone countries (WHO, 2002). Application of residual pesticides in houses has also been extremely effective in the elimination of most populations of the exclusively domestic *R. prolixus* in Central America (Yamagata and Nakagawa, 2006). However, where sylvan and peridomestic *T. infestans* populations occur in addition

to domestic, gene flow among the three ecotopes can at least partially explain the control failures in the Gran Chaco region (northern Argentina, Bolivia, and Paraguay), along with emergence of resistance to pyrethroids (Noireau et al., 2005). Understanding the source of insects appearing after pesticide treatment, if they are not simply resistant, is enormously important to designing effective control strategies. Population genetic studies have identified nearly all "reinfestants" as survivors or migrants from nearby peridomestic sites (Dujardin et al., 1996; Garcia et al., 2003; Pérez de Rosas et al., 2007; Pizarro et al., 2008). Peridomestic sites, with their extremely heterogeneous microhabitats, are particularly challenging for control. Gene flow among sylvan and domestic *R. prolixus* in Venezuela and the substantial migration seen among *T. dimidiata* populations means that simply spraying houses is unlikely to be effective for these species. Indeed, even in areas where sylvan *T. dimidiata* populations are unknown, reinfestation confounds control efforts (Yamagata and Nakagawa, 2006). Where peridomestic and sylvan populations are present, methods such as the use of screens and house improvements will be necessary to control transmission (Ferral et al., 2010; Monroy et al., 1998). And certainly if the genetically modified gut symbiont, developed to kill *T. cruzi* in the triatomine gut (CRUZIGUARD), is to be applied in the field (Beard et al., 2002), the migration pattern of the target vector population needs to be understood to predict the potential spread of the symbiont.

15.3.5 Further Work Needed

Genetic studies have been important in understanding the Triatominae at all levels: phylogenetic, taxonomic, and population. Results have contributed to an understanding of the epidemiology of Chagas disease and provided important information toward the design of effective control strategies. However, much remains to be done.

There is a need to reevaluate phylogenetic relationships and taxonomy of the Triatominae that were previously based on morphology and isozymes using new phenotypic and genetic markers and sampling methods that avoid biases. Phylogenetic and taxonomic questions remain at the tribe (Alberproseniini, Bolboderini, Cavernicolini, Linshcosteini), genus (*Meccus, Mepraia, Eratyrus*) and species level (*T. dimidiata* [and the phyllosoma complex], *T. sordida, T. brasiliensis*). Species classification originally based on phenotypic characters, which is now in some cases contradicted by genetic data, needs to be revised. Species of Triatominae that occupy substantial geographical ranges may display clinal variation (revealed by genetic differences) along a major latitudinal axis. If populations of such species are sampled over a wide area, clinal variation can be assessed. On the other hand, if populations are sampled much more focally, the sampled subsets may be considered in isolation—and receive specific designation—when they may in fact represent components of an unknown continuous population (Schofield and Galvão, 2009). Correct identification of organisms is critical to effective control

and to implying shared behavioral traits. As divergent taxa are uncovered, it is important to understand their epidemiological importance.

Of particular interest is to understand what forces (geological, ecological, anthropogenic) have resulted in the subdivision and maintenance of genera, species, and divergent taxa. Combining new tools in genetics, global information systems, and mathematical modeling will make it possible to address these questions and potentially predict future distribution of vector populations, including secondary vectors showing synanthropic tendencies. Human activities such as control efforts, global travel, and those resulting in deforestation and climate change are likely to have a major impact on future vector and Chagas disease distribution.

The diversity of triatomine chromosome structure, even within a species, makes it a model system for understanding chromosome structure and its evolution, and the role of heterochromatin. Comparative genomics and proteomics are in their infancy in triatomine biology. Completion of the *R. prolixus* genome: http://genome.wustl.edu/genomes/view/rhodnius_prolixus/#sequences_maps will provide many new tools for identifying important genes and markers. A *Triatoma* species genome should be added to this toolbox. Comparative studies promise advances in understanding the genetic basis of vector competence/capacity, reproductive isolation among sympatric species, as well as genes and proteins involved in hematophagy, habitat preference especially the important process of domestication, pesticide resistance, and identification of genes/haplotypes/proteins that are common to triatomines or perhaps important for species-specific behavior. These tools can also help to unravel vector and parasite interactions and coevolution. Perhaps as in other vectors, parasite infection could affect vector behavior in ways that increase transmission, or passage through particular triatomine species could affect parasite virulence. Where molecular markers provided geneticists with dramatically increased resolution over isozyme markers, development of new tools such as single nucleotide polymorphisms (SNP) assays and whole genome sequencing will provide a new leap in resolution over current molecular methods used in triatomines.

15.3.6 Concluding Remarks

So, 100 years after its discovery, Chagas disease remains the most serious of the parasitic diseases affecting Latin America. It is now evident that we will miss the World Health Organization's goal of elimination of Chagas disease by 2010. Intergovernmental control initiatives have been enormously successful at interrupting transmission caused by domestic triatomines. However, substantial challenges remain such as vectors with more varied habitats, emergence of insecticide resistance, and demographic and habitat changes that have resulted in the spread of Chagas to urban and nonendemic regions. New hope for eventual elimination comes from an integrative approach combining fields such as genetics with Global Information Systems and mathematical modeling, moving the field from descriptive to predictive, and prevention rather than reaction.

15.4 The *Anopheles gambiae* Complex

15.4.1 Introduction

Background and Brief History of Anopheles gambiae *Complex Classification*

The *Anopheles gambiae* species complex was initially described as containing six cryptic species: *A. gambiae* s.s. Giles, *A. arabiensis* Patton, *A. bwambae* White, *A. melas* Theobald, *A. merus* Dönitz, and *A. quadriannulatus* Theobald. The status of these species was established via the demonstration of F_1 hybrid sterility among crosses between different *A. gambiae* s.l. populations (Davidson, 1956, 1964a,b; Davidson and Hunt, 1973). Subsequent studies revealed that these six species could be distinguished on the basis of fixed differences in chromosomal inversions (Davidson et al., 1967; Davidson and Hunt, 1973; Coluzzi et al., 1979). Keys to distinguish species on the basis of allozyme differences were later developed (Mahon et al., 1976; Miles, 1979). Species in the complex are distributed throughout sub-Saharan Africa (Figure 15.16). Two additional species were later described: *A. comorensis* Brunhes, le Goff and Geoffroy based on subtle morphological features (Brunhes et al., 1997) and *A. quadriannulatus* species B Hunt, Coetzee and Fettene based on hybrid male sterility in crosses with *A. quadriannulatus* (Hunt et al., 1998). Of the eight species, two, *A. gambiae* s.s. and *A. arabiensis*, have the broadest geographic distribution and are the most important vectors of human malaria (Gillies and De Meillon, 1968; Coetzee et al., 2000). *A. gambiae* has been the most studied with respect to molecular and population genetics, and its whole genome sequence was published in 2002 (Holt et al., 2002). Natural populations of *A. gambiae* s.s. have an extremely complex genetic structure that has been the subject of a great deal of research. Despite these efforts the population genetics of this species remains poorly understood.

15.4.2 Levels of Population Genetic Structure in A. gambiae

The ideal gene pool in population genetics is a panmictic population, a homogeneous, randomly mating group of individuals that remains the same through time. To the extent that real populations depart from this they are said to be structured. *A. gambiae* s.l. is structured in at least 3 ways: (1) temporal: there are seasonal variations in population size and composition; (2) geographical: they mate locally, with little migration among villages; and (3) nondimensional: even within the same location and time, mating is nonrandom.

Temporal Structure

There are seasonal differences in abundance and composition of *A. gambiae* s.l. For example, in Banambani, Mali, *A. arabiensis* and *A. gambiae* s.s. are present in large numbers during the rainy season, with a progressive increase of *A. gambiae* s.s. during the rainy season and *A. arabiensis* in the drier months. The bulk of evidence suggests that they are present, but simply in low numbers (Holstein, 1954). One

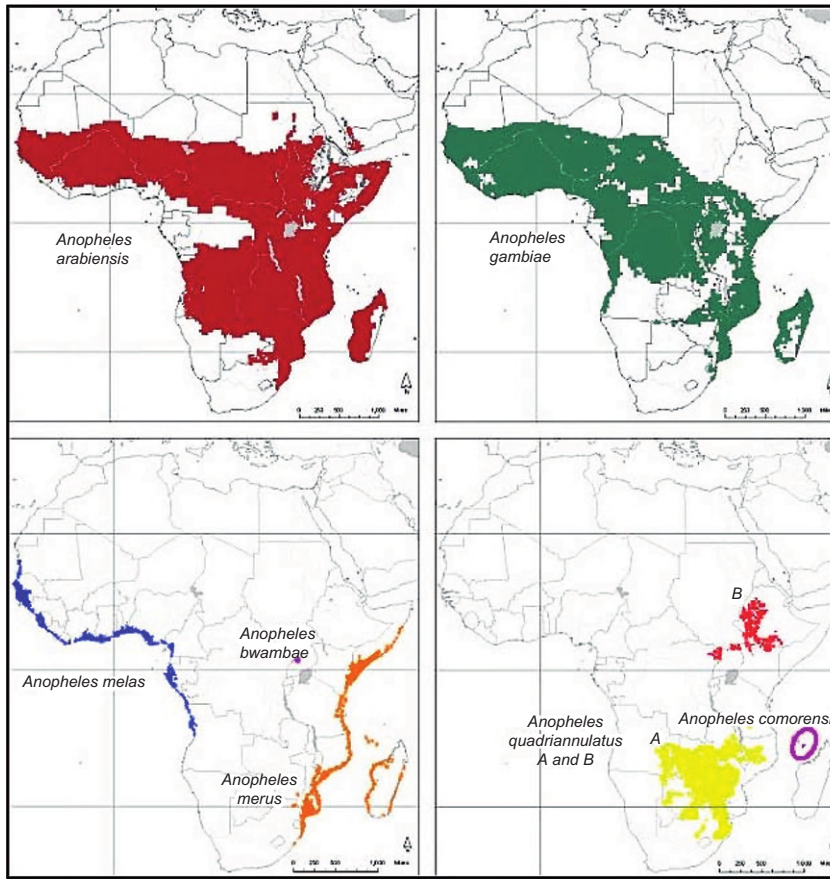

Figure 15.16 Geographic distribution of species in the *Anopheles gambiae* complex. *Source*: Adapted from Ayala and Coluzzi (2005).

cannot, however, absolutely exclude the possibility that they go locally extinct and are re-colonized from neighboring areas where permanent water is available (Taylor et al., 1993). The pattern varies somewhat from place to place, and is especially different in irrigated areas (Diuk-Wasser et al., 2005, 2006).

Geographic Structure

The geographic structure seems to be complex and is poorly understood through much of the species range. Populations of *A. gambiae* s.l., as with many species in the *Anopheles*, carry high levels of polymorphism in the form of **paracentric chromosome inversions**. Because inversion frequencies are certainly subject to selection, most recent attention has been focused on using microsatellite DNA variation, which is assumed to be neutral with respect to selection, to describe the genetic

structure of populations. Utilizing gene frequencies at 9 microsatellite loci, Lehmann et al. (1999) showed that the Rift Valley in eastern Africa imposes a huge barrier to gene flow among populations of *A. gambiae* s.s. there. Lehmann et al. (2003) subsequently conducted a more extensive study that included 16 sites in 10 countries spanning continental Africa. A cluster analysis based on F_{ST} values based on gene frequencies for 11 microsatellite loci revealed a major subdivision among *A. gambiae* populations in Africa. They identified a northwestern (NW) population group, containing populations in Senegal, Ghana, Nigeria, Cameroon, Gabon, Democratic Republic of Congo, and western Kenya, and a southeastern (SE) group including populations in eastern Kenya, Tanzania, Malawi, and Zambia (Figure 15.17). Differentiation between these two population groups was relatively high ($F_{ST} > 0.1$). Genetic differentiation among populations within the two groups was substantially lower and a significant relationship between genetic distance and geographic distance was observed, consistent with an isolation by distance model of population structure.

The classic studies of Coluzzi et al. (1979) and the comprehensive survey of Mali by Touré et al. (1998) have shown widespread geographic variation for chromosome inversion frequencies in populations of *A. gambiae* s.s. and in *A. arabiensis*. Lehmann et al. (1996) and Lanzaro et al. (1998) demonstrated geographic structure for microsatellite DNA for populations in Mali, and Besansky et al. (1994) (with Lehmann et al., 2000) showed the same to be true of mtDNA.

Nondimensional Structure

There is extensive nonrandom mating among genetically distinct subpopulations of *A. gambiae* s.s., known as chromosomal and **molecular forms** (described later). Taylor et al. (2001) measured the amount of gene flow among these populations and between species in Mali in a variety of ways. Their measures of gene flow between forms in Mali seem internally consistent. However, the amount of hybridization between forms varies considerably from location to location. Because some forms are more persistently present than others, and even absent at some locations, the amount of crossing will vary from place to place. There are apparently intrinsic factors that also play a role in the degree of between-form hybridization.

15.4.3 Polytene Chromosomes and Chromosomal Forms of A. gambiae *s.s.*

Structure and Methods

Discernable banding patterns in polytene chromosomes of late fourth instar larval salivary gland cells (Coluzzi, 1966; Coluzzi, 1968; Coluzzi and Sabatini, 1968, 1969) and in nurse cells of developing ovules in half gravid females reviewed in Coluzzi et al. (2002) served a primary role as a diagnostic technique to separate members of the *A. gambiae* complex. The full chromosome compliment that polytenizes consists of one sex chromosome (X) and 4 autosomal arms (2R + 2L and

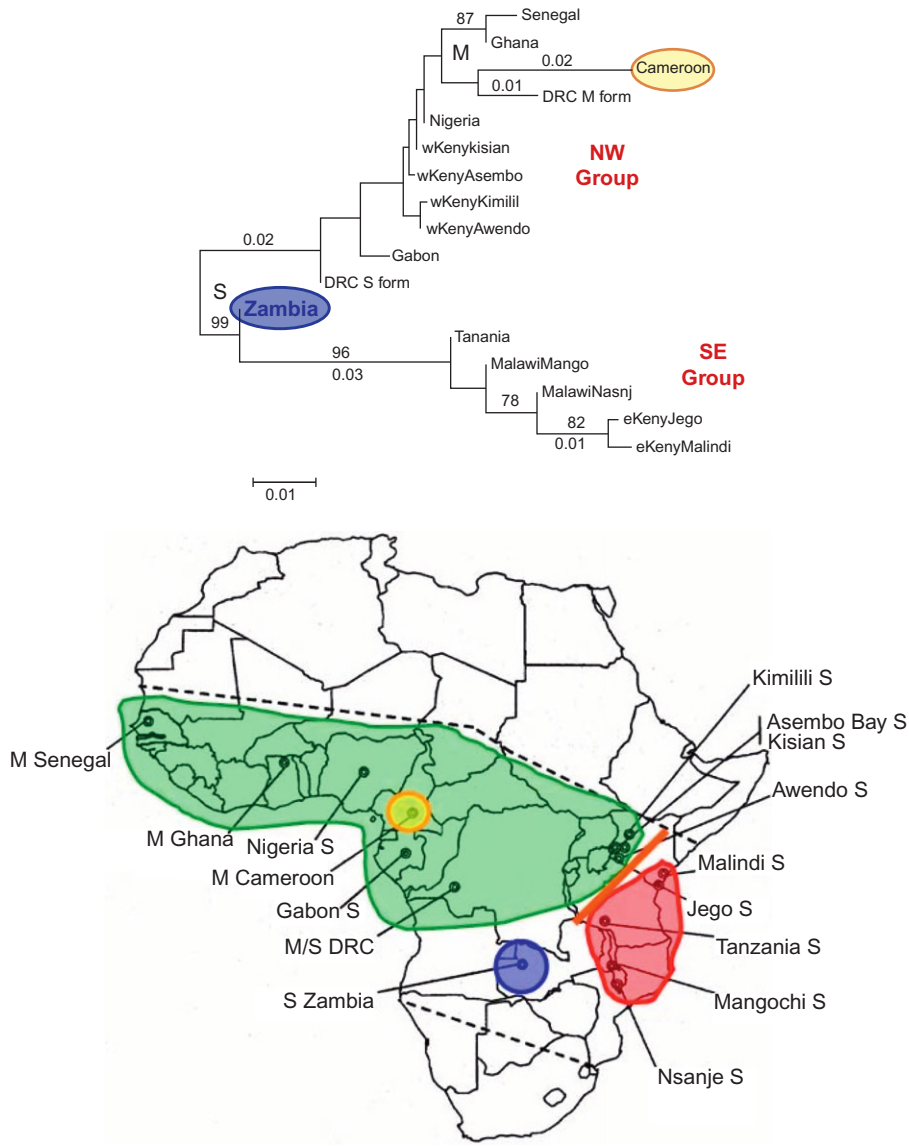

Figure 15.17 Top. Unrooted neighbor-joining population tree based on mean F_{ST} across nine microsatellite loci. M and S populations are denoted at the bases of the clades. The Northwest and Southeast population groups are indicated. Fractions denote branch length (over 0.01) and integers denote biologically significant bootstrap support values. Bottom: Map roughly indicating the boundaries of the different population groups. The orange line separating the Southeast group (in red) from the Northwest group (in green) represents the location of the Great Rift Valley. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this book.)
Source: Figures adapted from Lehmann et al. (2003).

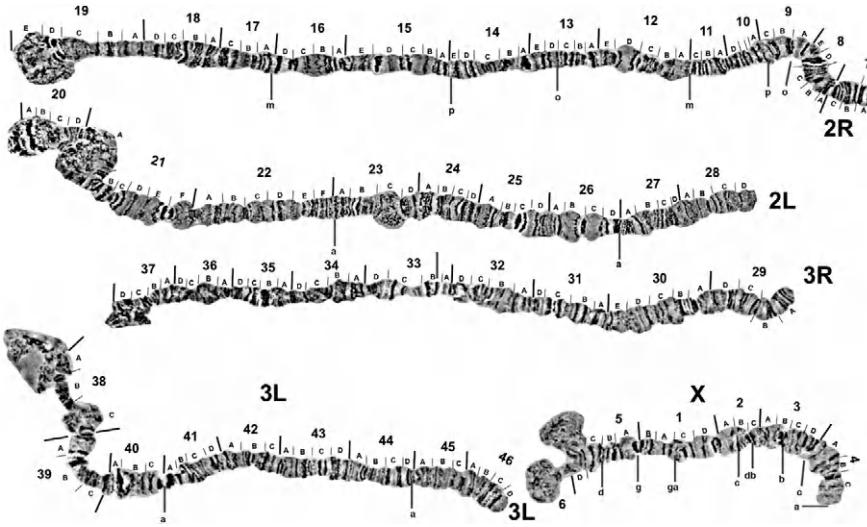

Figure 15.18 Photomap of full polytene chromosome complement of *A. gambiae* s.s. Forest-M form (collected from Tiko, Cameroon) depicting positions of breakpoints of major inversions.

3R + 3L). All members of the complex except for *A. quadriannulatus A* and *B* can be distinguished by fixed paracentric chromosome inversion differences on the X, 2R, 2L, and 3L chromosome arms (bold in Figure 15.18; see Figure 15.9 for an explanation of pericentric and paracentric inversions).

Details of methods used to prepare mosquito polytene chromosomes, originally derived from those used in *Drosophila,* appear in a number of publications (Coluzzi and Sabatini, 1967; Hunt, 1973). Comprehensive methods for salivary gland and nurse cell polytene chromosome extractions, spreading and staining are also posted online at the MR4 website, in *Anopheles* protocols chapters 5.6 and 5.7 (http://www.mr4.org/Portals/3/Methods_in_Anopheles_Research.pdf).

These days, salivary gland chromosomes are seldom used because nurse cell chromosome spreads are of higher quality. Drawings depicting banding patterns of partial and full compliments of salivary gland and ovarian nurse cell polytene chromosome with locations of divisions, subdivisions, and positions of the primary paracentric inversion breakpoints of *A. gambiae* s.l. are available in numerous publications (Coluzzi and Sabatini, 1967; Green, 1972; Coluzzi et al., 2002; della Torre et al., 2002). A photomap of the full chromosome compliment with positions of divisions, subdivisions, and major inversion breakpoints is provided in Figure 15.18. To circumvent the need for actual examination of chromosome spreads to identify inversions, PCR-based (molecular karyotyping) assays have been developed that can identify individuals in any developmental stage and in both sexes that carry inversions 2La (White et al., 2007) and 2Rj (Coulibaly et al., 2007). Complications in interpreting the results using the 2La PCR, due to

polymorphism within the region being amplified, have been reported (Ng'habi et al., 2008).

Chromosome Inversion Polymorphism

Multiple polymorphic inversions within all species except for *A. quadriannulatus* B, *A. merus*, and *A. comorensis* have been recorded (Touré, 1985; Coluzzi et al., 2002). Inversion polymorphisms are particularly high in *A. gambiae* s.s. (Pombi et al., 2008) and a number of subpopulations termed "**chromosomal forms**" based on the inversions that characterize them have been described (Coluzzi et al., 1985; Touré et al., 1998). These include the Bamako, Bissau, Forest, Mopti, and Savanna forms (Table 15.7). The Savanna form has the broadest distribution occurring throughout sub-Saharan Africa, the Mopti form predominates in drier habitats in West Africa, the Forest form occurs in wetter habitats in both East and West Africa, the Bamako form occurs in habitats along the Niger River in West Africa and the Bissau form is restricted to West Africa (della Torre et al., 2002). There is general agreement that inversions represent coadapted gene complexes that allow individuals carrying them to occupy different ecological niches. The nonrandom distribution of inversion breakpoints along the chromosomes (Figure 15.18; Pombi et al., 2008) and the distribution of inversion frequencies throughout the geographical ranges of the species strongly suggest that at least some of the inversions are the product of selection that allow different species and, in the case of *A. gambiae*, s.s., populations to survive and exploit a wide variety of habitats (Touré et al., 1998; Coluzzi et al., 2002; Lee et al., 2009). The best example is the strong association of inversions 2La and 2Rb with aridity with the frequency of these inversions being high in dry areas and even increasing in frequency during the dry season at places that experience distinct wet and dry seasons (Touré et al., 1998; Lee et al., 2009). This has led to the term "**ecophenotype**" being frequently applied to describe chromosomal forms of *A. gambiae* s.s. (Coluzzi et al., 1977). It has furthermore been suggested that the chromosomal forms are to some extent reproductively isolated and represent distinct species or **incipient species** that have evolved or are evolving via a process described as "**ecotypic speciation**" (Coluzzi et al., 1977; Manoukis et al., 2008). Studies of the distribution of the knockdown insecticide resistance gene (*kdr*) in sympatric Bamako-Savanna populations in Mali revealed the gene is present in the Savanna form, but absent in sympatric Bamako populations, which was taken as strong support that the two are reproductively isolated and the authors concluded that the two represent "incipient species" (Fanello et al., 2003). However, in a later study, also based on populations in Mali, the *kdr* gene was in fact found in both Bamako and Savanna forms, even from collections as far back as 1996, suggesting some level of gene flow between the two (Tripet et al., 2007).

Using the chromosomal form concept to define discreet, reproductively isolated populations is problematic because there is substantial overlap in inversions that define them, probably due to some level of contemporary gene flow. This creates ambiguities in assigning individuals to form, diminishing the utility of the

Table 15.7 Fixed and Most Common Polymorphic (floating) Paracentric Inversions Observed in the Seven Members of the *Anopheles gambiae* Complex

Chromosome	*Arabiensis*	*Bwambae*	*Melas*	*Quadriannulatus B*	*Quadriannulatus A*	*Gambiae*	*Merus*
X	bcd/bcd e/+	+/+	+/+	+/+	+/+ f/+	ag/ag	ag/ag
2R	a/+ b/+ bc/+ be/+ bf/+ d^1/+ s/+ br/+ q/+	l/+	m/m n/+ n^1/+ m^1/+	+/+	i/+	+/+ (Sav, Mop)[a] b/+ (For, Sav) cu/+ (Sav) bcu/+ (Sav) jcu/jbcu (Bam) bc/u (Mop) j/+ (Sav) d/+ (Bis) jbd/+ (Sav) bd/+ (Sav) jb/+ (Sav) bcd/+ (Mop) bk/+ (Sav)	op/op
2L	**a/a**	+/+	a^2/+	+/+	+/+	a/+	a^1/a^1
3R	a/+	b/+	c/+ e/+ e^1/+	+/+	+/+	+/+	+/+
3L	+/+	a/a	a/a	+/+	+/+	+/+	+/+

[a]Chromosomal forms: Bamako (Bam), Bissau (Bis), Forest (For), Mopti (Mop), and Savanna (Sav).

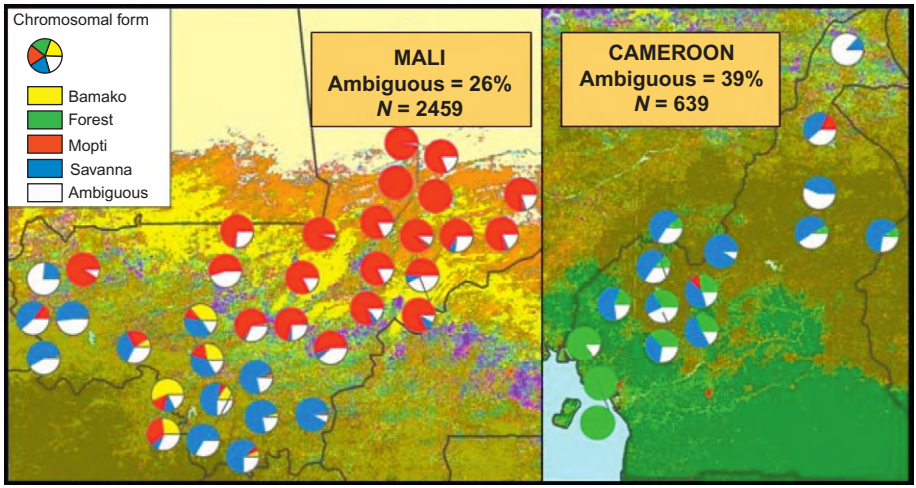

Figure 15.19 The distribution of chromosomal forms of *A. gambiae* s.s. at 36 sites in Mali (left) and 15 sites in Cameroon (right). The chromosomal form concept originated with observations made in West Africa and is based largely on populations in Mali. Even in Mali it is difficult to apply the concept to all individuals and at all locations. We found that 26% of individuals sampled in Mali and 38% from Cameroon could not be classified into chromosomal form.
Source: VectorBase, http://www.vectorbase.org/PopulationData/

chromosomal form concept in defining the reproductive boundaries among populations. For example, in a recent survey of populations in Mali we found that 26% of 2,459 individuals could not be assigned to a chromosomal form and in Cameroon 39% of 632 individuals could likewise not be assigned (Figure 15.19, data available at VectorBase, http://www.vectorbase.org/PopulationData/).

15.4.4 Molecular Diagnostics and the M and S Molecular Forms of A. gambiae *s.s.*

Identification of five of the eight species in the *A. gambiae* complex (the exception are *A. bwambae*, *A. quadriannulatus B,* and *A. comorensis*) was greatly simplified with the development of a PCR diagnostic based on fixed differences in the intergenic spacer (IGS) sequence of the ribosomal gene (rDNA) family (Scott et al., 1993). This breakthrough allowed rapid and accurate identification of species in the complex without the severe restrictions inherent in cytological examination of polytene chromosomes, which is labor intensive, requires a significant level of training, and is restricted to adult females at a specific stage of ovarian development. A similar molecular approach was utilized in an attempt to develop a diagnostic for the chromosomal forms of *A. gambiae* s.s. Favia et al. (1997) first found diagnostic RFLPs also within the rapidly evolving noncoding regions of the rDNA.

They identified 10 nucleotide residues that differ between the Mopti and the Savanna or Bamako chromosomal forms in a 2.3 kb fragment of the 5' end of the rDNA IGS region, which is located on the X chromosome (Favia et al., 2001). These findings were notable because they were the first fixed molecular genetic differences found between chromosomal forms of *A. gambiae* s.s. and they led to the development of a PCR-based diagnostic to differentiate Mopti individuals carrying the M-form of rDNA from Bamako and Savanna individuals carrying the S-form of rDNA (Favia et al., 1997). The diagnostic was developed using samples from Mali and among those early samples there were a few equivocal cases where karyotyping did not match the molecular diagnostic (Favia et al., 1997; della Torre et al., 2001). The diagnostic was also used to identify between-form hybrid-like karyotypes. M/S hybrids produced in the laboratory did yield clearly distinguishable hybrid patterns (of course only in females since the rDNA is located on the X chromosome). Surprisingly, however, field collected individuals carrying "hybrid" karyotypes did not produce results consistent with their being hybrid, but rather produced either M or S patterns (Favia et al., 1997). This observation supports the notion that certain karyotypes, thought to be fixed in one chromosomal form or another, are in fact shared, occurring commonly in one form and rarely in another, the result of ancestry and/or ongoing gene flow. The M/S diagnostic now forms the basis of recognizing two distinct subpopulations of *A. gambiae* s.s., known as "molecular forms" (M and S). Understanding the relationship between these two forms has been the focus of an intense and ongoing research effort. The S form has the broadest distribution occurring throughout sub-Saharan Africa, whereas the M form occurs throughout West and parts of Central Africa but, with the exception of a single site in northern Zimbabwe, is absent from eastern Africa (Figure 15.20; della Torre et al., 2005).

There is good correspondence between the M molecular form and the Mopti chromosomal form in Burkina Faso and Mali, however, the Bamako and Savanna chromosomal forms cannot be distinguished (both are of the S molecular form). The association of M and S molecular forms and chromosomal forms breaks down at other locations in West Africa. For example, in western Senegal and Gambia the association between the Savanna chromosomal form and S molecular form does not hold (della Torre et al., 2005) and the Forest form contains both M and S individuals.

The Relationship Between Chromosomal and Molecular Forms of A. gambiae *s.s.*

In summary, the M and S molecular forms are associated with chromosomal forms only in some locations, and so therefore they largely fail as a diagnostic for chromosomal form. However, the significance of the M and S forms of *A. gambiae* goes well beyond their utility as proxies for identifying chromosomal forms. M and S forms occur in sympatry at many sites in West and Central Africa, and typically there is a high degree of reproductive isolation between the two forms. Hybridization between the forms occurs rarely (<1%) in Mali (Tripet et al., 2001; Edillo et al., 2002) and reproductive isolation between M and S appears to be

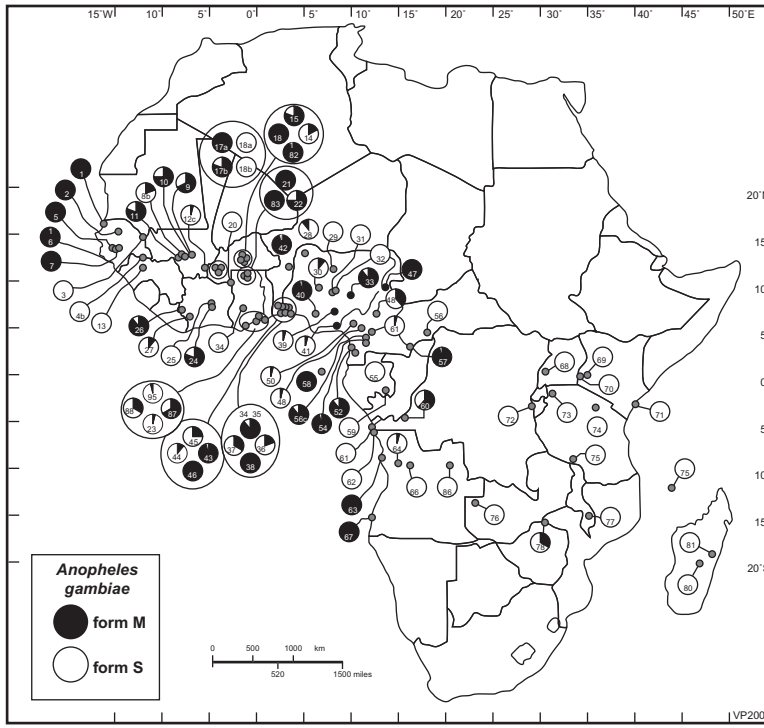

Figure 15.20 Relative frequencies of M-form and S-form of *A. gambiae* s.s. at 87 sites in 24 African countries, with permission, copyright Elsevier.
Source: From della Torre et al. (2005).

complete in Cameroon (Wondji et al., 2005). The M and S "alleles" are based on two base pair substitutions in the IGS sequence of the rDNA gene family on the X chromosome. Studies aimed at describing genetic differentiation between the M and S form populations revealed that microsatellite DNA differentiation was exceptionally high in a region of the genome proximal to the centromere on the X chromosome, near the M/S locus (Wang et al., 2001; Lehmann et al., 2003). High levels of M/S form divergence in this portion of the X chromosome was substantiated through detailed examination of the region using microsatellites and DNA sequencing (Stump et al., 2005a,b; Slotman et al., 2006). Studies aimed at describing genome-wide divergence between M and S using the Affymetrix *Plasmodium*/*Anopheles* Genome Microarray (Turner et al., 2005; White et al., 2009; White et al., 2010) likewise revealed the same X chromosome region, but also revealed divergence on chromosomes 2 and 3. These regions of divergence have been considered to represent "**islands of speciation**" because it is thought that they contain genes that are directly involved in reproductive isolation. It appears that the M and S "alleles" are linked to genes located within these "islands of speciation" and that two largely reproductively isolated populations of *A. gambiae* (M form and S form)

exist in nature. The M and S forms are commonly referred to in the literature as "incipient species" (della Torre et al., 2001, 2002, 2005; Fanello et al., 2003; Stump et al., 2005b; Manoukis et al., 2008; White et al., 2010, and many others). But there are problems that suggest that the M and S form concept does not represent the full level of complexity in the genetic structure of *A. gambiae* s.s.

Geographic Variation in the Association of Chromosomal Forms and Molecular Forms of *A. gambiae* s.s.

Although the M and S forms are largely reproductively isolated in many places where they occur together, this is not true everywhere. In the Gambia M/S hybrids were identified from a number of sites at frequencies as high as 16.7% of the *A. gambiae* s.s. individuals sampled (Caputo et al., 2008) and in Guinea-Bissau hybrids were recovered in 24% of the individuals assayed (Oliveira et al., 2008). These results suggest that in localities covering a relatively large geographic area the linkage between the M and S alleles and those genes that directly affect reproductive isolation has broken down. Therefore the notion of an M form and an S form that are largely reproductively isolated (incipient species) is an oversimplification. As described above in Cameroon, the M and S forms appear to be completely isolated reproductively, whereas in Mali the reproductive barrier between them appears to be "leaky" (e.g., some hybridization occurs). A comparison of the M form in Mali and the M form in Cameroon has revealed that the two are very different genetically, in fact, divergence between these two is higher than the level of divergence between the M and S forms (Figure 15.21). This

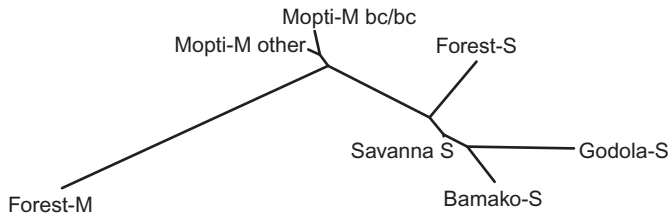

Figure 15.21 Unrooted phylogenetic tree (neighbor-joining) of seven groups of *An. gambiae* identified by a Bayesian analysis (using *Structure* software), these include Forest-M = M form individuals with the standard karyotype (no inversions) collected from the villages of Tiko and Mutengene, Cameroon; Mopti-M bc/bc = M form individuals homozygous for the 2R b/c inversions, collected from the villages of Nara, Banikane and Dire in Mali; Mopti-M other = M form individuals with inversion polymorphism excluding the bc/bc karyotype, collected from the villages of Banikane, Nara, Banambani and Kela in Mali; Forest-S = S form individuals with the standard karyotype, collected from Tiko, Mutengene, Foumbot and Ndop in Cameroon; Savanna-S = S form individuals with typical Savanna form inversion polymorphism including 2R b, c and u, collected from Mutengene and Foumbot in Cameroon and Pemperena, Kela and Banambani in Mali; Godola-S = S form individuals with the 2Rbcd karyotype. The tree is based on pair-wise F_{ST} values derived from allele frequencies at 20 microsatellite loci on chromosomes 2 and 3. Distances between all branches are significant. *Source*: From Lee et al. (2009).

observation has led to a recognition of two, distinct M form groups, the Mopti-M form, which is polymorphic with respect to the 2R b, c, and u chromosome inversions and the Forest-M form which lacks inversions on chromosome 2R (Slotman et al., 2007; Lee et al., 2009). It is likely that additional subdivision of both the M and S forms will be resolved, for example, in places like the Gambia and Guinea-Bissau where the M/S alleles are apparently less tightly linked to speciation genes.

15.4.5 *Significance of* A. gambiae *Population Genetics to Malaria Transmission and Control*

Ultimately malaria control efforts in Africa will have to be conducted on a large geographical scale. Although the development of a strategic plan for such an undertaking will require coordinating efforts along political boundaries, the success of such an effort will depend largely on identifying regions of operation based on biologically meaningful boundaries. Sub-Saharan Africa includes a wide variety of ecological zones. It is not surprising that the mosquito, *Anopheles gambiae* s.s., with a distribution across the continent, is highly diverse. The success of malaria control strategies aimed at controlling or manipulating its vectors will have to include knowledge of diversity in vector populations, how this diversity is distributed in time and space and the forces limiting gene flow and maintaining diversity among populations.

Studies of the population genetics of *A. gambiae* and of vector species in general have resulted in numerous and significant contributions to our understanding of the biology of this group of organisms. The earliest contributions focused on clarifying the taxonomic status of populations. Species in the genus *Anopheles* have frequently evolved without acquiring obvious morphological differences. As described above, cryptic or sibling species within the *A. gambiae* complex were initially described on the basis of male sterility in hybrid individuals resulting from between-species mating experiments. Conducting crosses to detect hybrid sterility as a method for routine identification has obvious limitations. Efficient and readily applicable methods were soon developed including the development of effective cytotaxonomic and later molecular tools that are used to distinguish species in the complex. However, many questions remain regarding the taxonomic status of populations, especially within *A. gambiae* s.s., and the resolution of these problems will be achieved by applying population genetics approaches to the problem.

Analyses of the genetic structure of *A. gambiae* s.l. populations have contributed to understanding the distribution of phenotypic variation in the complex and at the subspecific level. The description of *A. quadriannulatus* as a distinct species and recognition that it is primarily zoophyllic provided an explanation for variation in host preference (Gillies and De Meillon, 1968). The association of specific chromosome inversions in *A. gambiae* s.s. with endo- and exophily (Coluzzi et al., 1977) and with habitat preferences with respect to aridity (Coluzzi et al., 1979; Touré et al., 1998; Lee et al., 2009) have been established. The evolution of insecticide resistance in populations of *A. gambiae* s.s. poses a serious challenge to current malaria control programs that rely on impregnated bednets and indoor spraying for

vector control (N'Guessan et al., 2010). The differential distribution of resistance genes, such as *kdr*, among M and S form populations establishes the importance of recognizing population structure to insecticide resistance monitoring (Chandre et al., 1999; Weill et al., 2000; Tripet et al., 2007).

The availability of the *A. gambiae* s.s. whole genome sequence (Holt et al., 2002) has ushered in the advent of **population genomics** in vector biology. The promise of establishing the relationship between phenotype and genotype is attainable through the powerful new approach of **association mapping**. Early work aimed at identifying genes directly responsible for phenotypes of interest involved the use of laboratory strains selected for those phenotypes (Sinden, 2004; Aguilar et al., 2005). Recently it has been pointed out that this approach has serious limitations and that studies based on natural populations provides far more useful information (Tripet et al., 2008; Boëte, 2009). It is well known that the presence of population structure can result in "spurious associations" between a phenotype and markers that are not linked to any causative loci (e.g., Lander and Schork, 1994; Ewens and Spielman, 1995; Pritchard and Rosenberg, 1999; Kang et al., 2008). This becomes a problem when these subpopulations are not recognized so that a sample being used in an association mapping study consists of a mixture of individuals originating from two or more diverged subpopulations.

The movement of genes, including the use of gene drive vehicles (e.g., transposable elements), from one lineage or population to another depends on mating between an individual carrying the gene and one which does not. Although designs for novel approaches to target vector populations of mosquitoes are interesting and potentially useful, the population genetics component is very poorly understood. Critically, most conceptual models for genetic control assume that the mosquito population into which a refractory gene system is to be released represents a single, randomly mating unit. We have summarized the evidence that natural populations of *A. gambiae* are subdivided by barriers to reproduction and that gene flow via migration among geographic populations is limited. Field studies designed to estimate levels and patterns of gene flow within and among natural vector populations are needed to provide a foundation for predicting the potential utility of new molecular-level approaches, and for designing field trials to evaluate their efficacy under natural conditions in Africa.

15.4.6 Conclusions

With respect to current concepts toward describing the genetics of populations of *A. gambiae* s.s.:

- The chromosomal form concept is only valid in a very restricted geographical region.
- The distribution of at least some chromosome inversions supports the idea that they represent "coadapted gene complexes."
- The chromosome inversions themselves do not appear to mediate reproductive isolation.
- Reproductive isolation among molecular forms appears to be associated with relatively small genomic "islands of divergence" located near the centromere on the X chromosome and possibly other "islands" located on chromosomes 2 and 3.

- The M/S molecular form concept is valid only where these "alleles" are linked to "speciation genes," probably on the X chromosome. This linkage appears to be absent in some places (e.g., Guinea-Bissau, the Gambia) and in some populations (e.g., Savanna and Bamako populations).
- Natural populations of *A. gambiae* s.s. are structured into subpopulations that are to varying degrees reproductively isolated. The boundaries among these populations are poorly defined by the current concepts of chromosomal and molecular form. It is possible, perhaps likely, that some of these populations represent species or incipient species, however, current concepts of molecular and chromosomal forms fail to define them.

Glossary

Allopatric a description of taxa occupying distinctly different geographic ranges.

Association mapping it is a method of gene mapping that utilizes historic linkage disequilibrium (linkage) to associate phenotypes to genotypes.

Balancing selection maintenance of a polymorphism or polymorphisms above the frequency established by its mutation rate. Heterozygote advantage is one manifestation of balancing selection, meaning that a heterozygote has a fitness advantage over the corresponding homozygotes.

Chromosomal forms reproductively isolated populations of *A. gambiae* s.s. characterized by different paracentric inversion chromosome arrangements on the right arm of chromosome 2.

Cryptic species those species that are identical or nearly identical in appearance but can be discovered by genetic divergence as indicated by mating incompatibilities or sterile matings in interspecific crosses.

Cytoplasmic incompatibility sterile matings between *Wolbachia*-infected males and uninfected females. Females carrying reproductive parasite *Wolbachia* are fertile when mated with uninfected or *Wolbachia*-infected flies. In this way the maternal host lineages with *Wolbachia* can displace uninfected, maternal lineages if their Darwinian fitnesses are adequate.

Diversity at diploid genetic loci, it is heterozygosity averaged over loci. For mitochondrial genomes, which are single copy and therefore haploid, it is the probability that two randomly chosen individuals of a species have different haplotypes. Mathematically, diversity h of a nuclear or mitochondrial polymorphism is, $h = 1 - \Sigma x_i^2$, where x is the frequency of allele (or haplotype) i. Diversity over r loci is $H = \Sigma h/r$.

Ecophenotype a phenotype, in the case of *A. gambiae* s.s. chromosomal of molecular form, that shows a strong association with regional and seasonal environments.

Ecotypic speciation ecological and adaptive divergence among populations leading to reproductive isolation and speciation.

Effective population size and gene flow effective population size N_e is the hypothetical number of reproducing organisms in an ideal population (i.e., a population obeying Hardy−Weinberg assumptions) that corresponds with the population under investigation. It is unrelated to census numbers but always much less. Its relationship to gene flow is the number of effective migrants, $N_e m$, exchanged per generation among demes-$N_e m = (1 - F_{ST})/4F_{ST}$ for diploid loci.

F_{ST} and F_{IS} inbreeding coefficients estimated by variance statistics. Biologically, F_{ST} measures departures from random mating among demes (subpopulations). It estimates genetic differentiation and is the among-deme variance in allelic frequencies as a

fraction of the variance among all individuals in a population. F_{ST} is inversely related to gene flow. F_{IS} measures departures from random mating within demes. F statistics can also be defined as correlations between uniting gametes: F_{IS} is the correlation between gametes in an individual relative to its subpopulation; F_{ST} is the correlation between random gametes in a deme relative to the correlation of pooled gametes in the whole population.

Genetic bottleneck a great reduction in effective population size. Genetic diversity becomes reduced in proportion to the magnitude of population reduction and the number of generations over which the bottleneck occurs. Recovery from a bottleneck, in terms of increased genetic diversity, requires tens of thousands of generations in the absence of inward gene flow.

Genetic drift change in gene frequencies from one generation to the next caused by random sampling. Its magnitude is inversely proportional to effective population size.

Incipient species populations evolving toward complete reproductive isolation, and therefore distinct biological species status,

Introgression introduction of genetic material from another species or variant into a population by hybridization followed by repeated backcrossing.

Islands of speciation discrete segments of a genome that are highly diverged and thought to contain genes affecting reproductive isolation between individuals within different populations.

Isolation by distance the probability of mating between two individuals decreases with increasing distance between them, resulting in a direct relationship between geographic and genetic distance.

Molecular forms populations of *A. gambiae* s.s. that differ with respect to specific sequence in a region of the intergenic spacer segment of the ribosomal gene, as visualized using diagnostic PCR methods.

Monophyletic describes a group of organisms that includes the most recent common ancestor of all those organisms and all the descendents of that common ancestor.

Paracentric chromosome inversion an inversion of a segment of a chromosome that does not include the centromere.

Peridomestic habitats the area surrounding houses (e.g., wood piles, animal corrals, fences, walls).

Polyphyletic describes a group of organisms derived from two or more parental lineages.

Polytypic species a species that contains several variant forms, especially geographically or temporally differentiated subspecies or varieties, which would normally interbreed if present in the same time and place.

Population genomics sampling of numerous, or all, variable gene loci within a genome to infer those evolutionary forces responsible for observed patterns of variation.

Sylvan refers to wild areas, as in "sylvan habitats."

Synapomorphic a description of a shared, derived trait found among two or more taxa and their most recent common ancestor, whose ancestor in turn does not possess the trait.

Transgenic an organism that contains foreign genes that were introduced into its genome.

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