Review

Origin and status of *Culex pipiens* mosquito ecotypes

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SUMMARY

The northern house mosquito *Culex pipiens sensu stricto* is one of the most important disease vector mosquitoes in temperate zones across the northern hemisphere, responsible for the emergence of West Nile Virus over the last two decades. It comprises two ecologically distinct forms — an aboveground form, *pipiens*, diapauses in winter and primarily bites birds, while a belowground form, *molestus*, thrives year-round in subways, basements and other human-made, belowground habitats, bites mammals, and can even lay eggs without a blood meal. The two forms hybridize in some but not all places, leading to a complex ecological mosaic that complicates predictions of vectorial capacity. Moreover, the origin of the belowground *molestus* is contentious, with iconic populations from the London Underground subway system being held up by evolutionary biologists as a preeminent example of rapid, *in situ*, urban adaptation and speciation. We review the recent and historical literature on the origin and ecology of this important mosquito and its enigmatic forms. A synthesis of genetic and ecological studies spanning 100+ years clarifies a striking latitudinal gradient — behaviorally divergent and reproductively isolated forms in northern Europe gradually break down into what appear to be well-mixed, intermediate populations in North Africa. Moreover, a continuous narrative thread dating back to the original description of form *molestus* in Egypt in 1775 refutes the popular idea that belowground mosquitoes in London evolved *in situ* from their aboveground counterparts. These enigmatic mosquitoes are more likely derived from populations in the Middle East, where human-biting and other adaptations to human environments may have evolved on the timescale of millennia rather than centuries. We outline several areas for future work and discuss the implications of these patterns for public health and for our understanding of urban adaptation in the Anthropocene.
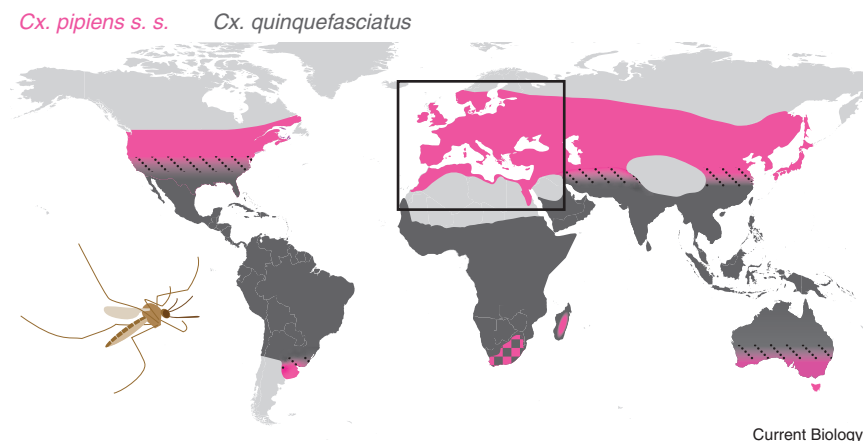
Introduction

In the winter of 1940 to 1941, the Second World War was raging and Londoners faced a sustained, eight-month, Nazi bombing campaign known as The Blitz[1]. During one particularly intense period from September to November, bombs were dropped for fifty-seven nights in a row. Many who had lost their homes or simply wanted a more secure place to sleep sought nightly refuge in the city's extensive underground subway system. Tens of thousands of men, women, and children slept shoulder-to-shoulder on station platforms, escalators, and sometimes in the subway tunnels themselves[1]. The underground system provided life-saving protection from bombs, but the Londoners who took refuge there nevertheless found themselves attacked by a different enemy — mosquitoes. The London Underground had also become home to a belowground ecotype of the widespread, bird-biting mosquito *Culex pipiens sensu stricto*[2]. Unlike their aboveground cousins, these pesky biters were well equipped for life in man-made basements, subways, and cesspits — they were active year-round, could mate in confined spaces, often laid their first clutch of eggs without a blood meal, and voraciously attacked humans[2].

The London Underground mosquitoes, as they became known, captured the attention of the general public and scientific community alike. Evolutionary biologists hypothesized that they had evolved *in situ* within the one hundred year period since the construction of the London Underground and continue to cite these populations as an iconic example of rapid urban adaptation[3–9]. For vector biologists and public health researchers, on the other hand, the discovery merely helped confirm observations that had been trickling in since at least the 1920s of similar belowground populations in other European (and North American) cities[10,11]. The aboveground and belowground ecotypes were eventually named *Cx. pipiens* form *pipiens* and *Cx. pipiens* form *molestus*[11–13], and hybridization between them may have contributed to the emergence of West Nile Virus in the United States and Europe over the past several decades (see Conclusions and outlook). Today, *Cx. pipiens* represents the most intensively studied temperate vector across the Northern Hemisphere[14,15], and while it is made up of two distinct ecotypes at colder northern latitudes in Europe, the dichotomy breaks down elsewhere — leading to a complex ecological mosaic and a rich, but often confusing, literature.

Here, we review what is known about the origin and ecology of this important mosquito and its major ecotypes, focusing in particular on their putative native range across the Western Palearctic — namely Europe, western Asia, North Africa, and the Middle East (Figure 1). We synthesize the observations of early naturalists with more recent genetic and behavioral data to illustrate how above- and belowground ecotypes in the north transition through highly variable yet still structured populations at

middle latitudes to more homogenous intermediate populations in northwestern Africa. We also examine the widely cited theory that belowground mosquitoes in London and other urban areas evolved from their aboveground counterparts *in situ* over the last few hundred years — finding surprisingly little, if any, support for this hypothesis.

Culex pipiens comprises distinct above- and belowground ecotypes in colder northern climates

The northern house mosquito *Culex pipiens sensu stricto* (hereafter *Cx. pipiens*) is one of the most common mosquitoes found breeding in human habitats throughout the temperate northern hemisphere (Figure 1). In the higher latitudes of northern Europe, Asia, and North America, aboveground populations match the canonical description of *Cx. pipiens* form *pipiens* (hereafter *pipiens*) (Figure 2). Like most mosquitoes, females require a blood meal to synthesize eggs, and males require open space to form mating swarms at dusk[16]. Females primarily bite birds[14] and are therefore important vectors of pathogens that circulate within bird populations, including avian malaria and West Nile Virus[17]. Although larvae thrive in human sources of water, such as aboveground water tanks, irrigation ditches, and open sewers, they can also be found in natural environments[14]. Finally, shortening autumn days trigger a reproductive diapause, allowing mated females to survive the long, cold winter at latitudes as high as 60–65°N in Scandinavia and northern Russia[14,18].
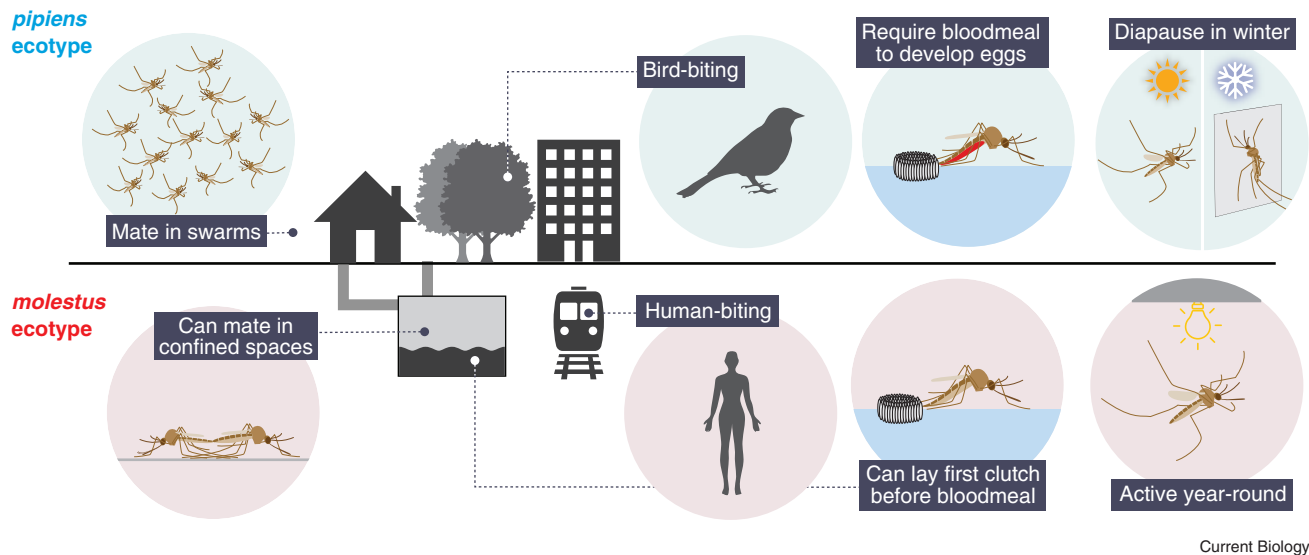
Until the early 1900s, all *Cx. pipiens* populations documented from northern Europe resembled those described above. Medical entomologists then began to make some puzzling observations — *Cx. pipiens* look-alikes breeding in cellars/cesspits and biting people. In 1923, P.G. Shute went to investigate a 'mosquito nuisance' affecting homes in the center of London and found dozens of *Cx. pipiens* females engorged with mammalian blood resting on the walls and ceilings of bedrooms[2]. He could not identify the breeding site. In 1929, E. Roubaud and P. de Boissezon independently published reports of *Cx. pipiens* breeding through the winter in a wine cellar near Paris[19] and the boiler room of a medical dispensary in Toulouse[20]. Similar reports trickled in from other areas of France[21], Germany[22], Denmark[23], the United States[24], and of course the London Underground[2].

The belowground mosquitoes were somewhat variable, but they clearly differed from their aboveground counterparts in four key behaviors[25] (Figure 2). First, they did not diapause in winter. Instead, they could be found breeding throughout the year in their buffered belowground habitat[19]. Second, they did not require open space for swarming and mating. Instead, they could mate in cages as small as 50 cubic centimeters[26]. Males were even observed to approach resting females[27], which is unusual as mosquitoes typically initiate mating during flight[16]. Third, mated females were able to lay their first clutch of eggs without a bloodmeal. Roubaud called this ability 'autogeny'[19], and it allowed belowground populations to thrive without access to animal hosts. However, females were also willing to bite, and bloodmeals allowed them to lay additional, larger egg clutches. When belowground females did bite, they were not only attracted to birds but also voraciously attacked humans and other mammals. It was this last trait that earned them the name *Cx. pipiens* form *molestus* (hereafter *molestus*), originally coined for a human-biting form of *Cx. pipiens* in Egypt[28].

Early authors noted subtle morphological differences between *pipiens* and *molestus* ecotypes[10,29], but they are considered morphologically indistinguishable today[12]. They are also capable of mating and producing fertile offspring in captivity[10], begging the question of how they remain distinct in nature. Microhabitat segregation and selection against migrants are likely contributors. For example, long, cold winters confine the non-diapausing *molestus* to its buffered belowground environment[30], while lack of bird hosts and open space for mating should prevent *pipiens* from successfully breeding belowground. As we will see, however, residual reproductive isolation appears to be present even when both are active aboveground, suggesting the presence of additional barriers.

The distinction between ecotypes breaks down further south

While populations of *Cx. pipiens* in northern Europe usually fit neatly into above- and belowground ecotypes, it has long been clear that the situation is more complex further south. Closed belowground environments in southern Europe are still consistently occupied by mosquitoes that fit the canonical description of *molestus*[31–33] (Figure 2), but aboveground populations run the

Figure 2. *Cx. pipiens* comprises two ecotypes in colder, northern latitudes.
The *pipiens* ecotype lives aboveground, while *molestus* is confined to man-made belowground habitats. The schematics summarize several striking behavioral and physiological differences.
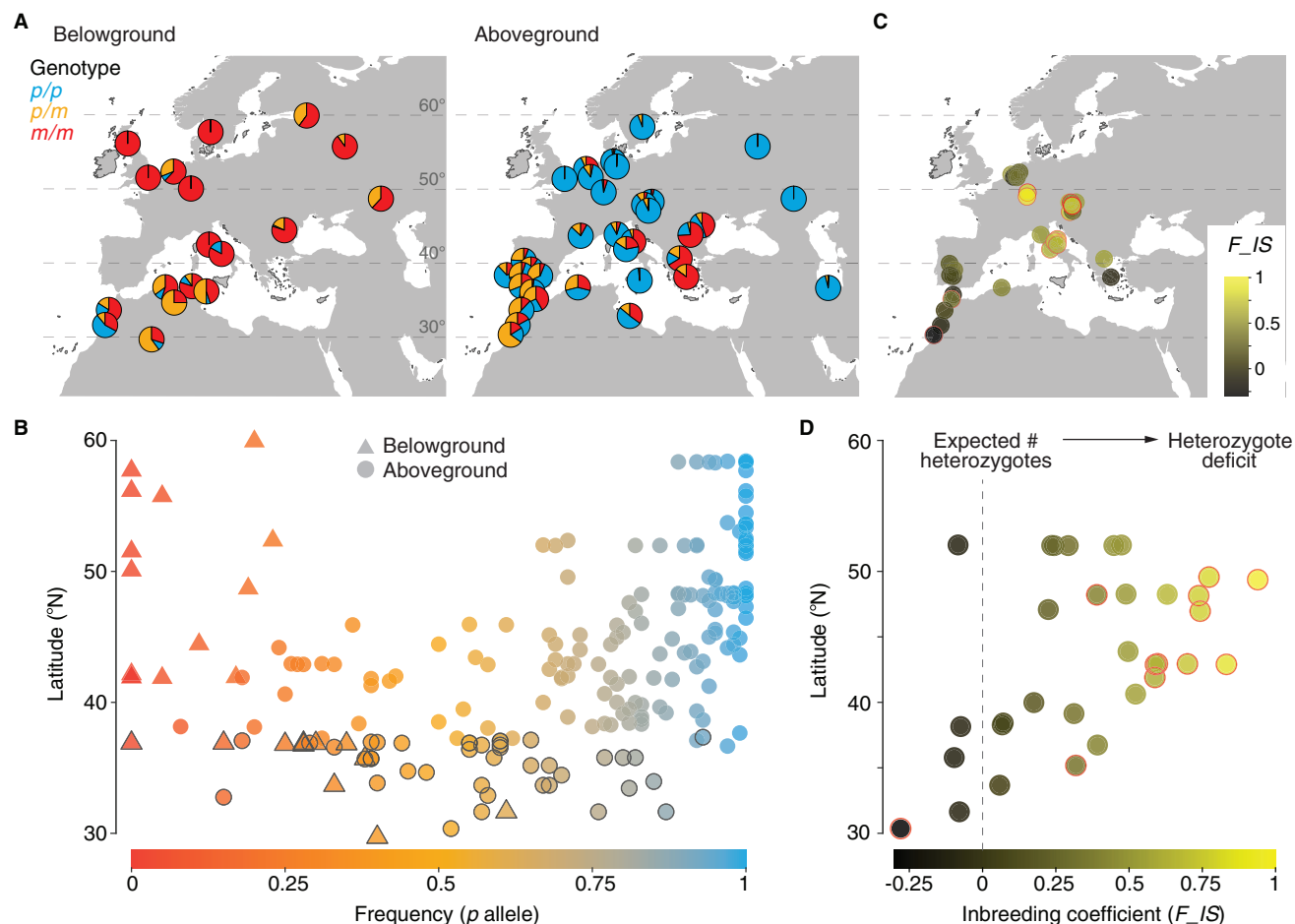
gamut from *pipiens*-like to *molestus*-like to many things in between. The earliest hints in the literature come from 19th century descriptions of new human-biting 'species' from Croatia[34] and Italy[35], which have since been subsumed under the *Cx. pipiens* umbrella[13,29,36]. These mosquitoes lived aboveground, but readily entered homes and were said to be far more bloodthirsty than the 'common' *Cx. pipiens*. Later, as belowground populations were being described in the north, southern populations characterized by all four key *molestus* behaviors were found breeding aboveground in rural areas of Greece and Malta[27,29]. Nevertheless, most populations from southern Europe were more *pipiens*-like or exhibited a mix of traits that put them somewhere in between the two northern extremes[11,37,38]. One strain from southern France, for example, was willing to bite humans and lacked diapause, but could lay only a few (if any) eggs without a blood meal and mated in modestly-sized, but not very small, cages[38]. Even further south in northwestern Africa, this type of intermediate behavior appeared to be the norm[39–41].

The advent of genetic approaches such as allozymes and microsatellites corroborated the variability of Mediterranean populations[31–33,42–47]. Moreover, by allowing rapid assessment of many individuals, they confirmed that this variation is still partially structured by habitat. More or less pure *molestus* and *pipiens* can still often be found in canonical belowground and aboveground environments[31–33,42]. But gene flow is present, and many populations are genetically mixed — especially in intermediate habitats such as belowground sites with access to the open air and aboveground urban or suburban habitats[31–33,42,48,49]. A microsatellite study from 2004 included northern and southern European samples in the same analysis, providing some of the first holistic evidence that genetically 'pure' aboveground and belowground individuals prevail in the north (UK, Sweden, Germany, and northern France), while individuals with mixed ancestry are common in the south (southern France, Italy)[43].

Importantly, Mediterranean populations don't just vary from place to place or habitat to habitat — there is also widespread structure within populations. Multi-locus studies indicate that *molestus*-like and *pipiens*-like individuals can be caught at the same trapping sites with fewer than expected intermediates[45,47]. In Portugal, the *molestus*-like individuals were more common inside animal shelters and human dwellings than outdoors (another potential level of microhabitat segregation), but most samples included a mix[45]. The widespread detection of heterozygote deficits at single loci[32,33] also points to extensive structure within aboveground populations in southern Europe.

What reproductive barriers are responsible for lingering structure at lower latitudes where mild winters allow both ecotypes to thrive aboveground? A remarkable field study from 1981 points to mating behavior[31]. Italian researchers released tens of thousands of genetically identifiable aboveground '*molestus*' from urban Rome in a rural area where *pipiens*-like mosquitoes were dominant. They then monitored the genetic make-up of mating swarms for months thereafter. Males swarming near the ground were almost always derived from the introduced *molestus*, while those captured two meters up near the foliage of trees were the local *pipiens*. Hybrids were rare, both in the swarms and at breeding sites, even three to four months after the initial release. The ability of *molestus* to mate in confined spaces (Figure 2) may thus reflect a generalized change in swarming behavior that limits hybridization in open air environments[31,50–52].

Another possible source of reproductive isolation between *pipiens* and *molestus* is cytoplasmic incompatibility caused by the bacterial endosymbiont *Wolbachia pipientis*[53,54]. Almost all *Cx. pipiens* individuals in nature are infected with *Wolbachia* (but see Dumas *et al.*[55]), and when a male and female carry bacteria from incompatible genetic groups, mating can result in partial or complete embryonic lethality. There are at least four genetic groups of *Wolbachia* segregating in *Cx. pipiens* in Europe and North Africa[56], though not yet any evidence that they separate

Figure 3. Genetic data illustrate a latitudinal gradient from distinct northern ecotypes to intermediate southern populations.
Compilation of published CQ11 genotype data for up to 214 diverse *Cx. pipiens* populations (see Table S1 and Supplemental methods for details). (A) Genotype frequencies for 18 belowground populations (left, N≥8 individuals each) and 35 geographically representative aboveground populations (right, N≥30 except two Russian populations N = 7–12). *p*, *pipiens* allele; *m*, *molestus* allele. (B) Allele frequencies for 214 populations (N≥5 individuals each). Symbols with black outline indicate North Africa. (C,D) Inbreeding coefficient F_{IS} for 32 aboveground populations with N≥30 and minor allele frequency≥0.05. Red outlines mark significant departures from Hardy-Weinberg equilibrium, with positive and negative values indicating a heterozygote deficit and excess, respectively. Pies/dots in (A) and (C) are jittered from their exact locations for visibility. Taken together, these data suggest that genetically distinct aboveground and belowground ecotypes in northern Europe break down into highly variable, structured populations at middle latitudes and more reliably intermediate, well-mixed populations in North Africa. However, it is important to keep in mind that single-locus data may not be representative of genome-wide patterns.

mosquitoes with *pipiens*- and *molestus*-like ecology. The effect of *Wolbachia* variation on genetic structure in natural populations remains an active area of research.
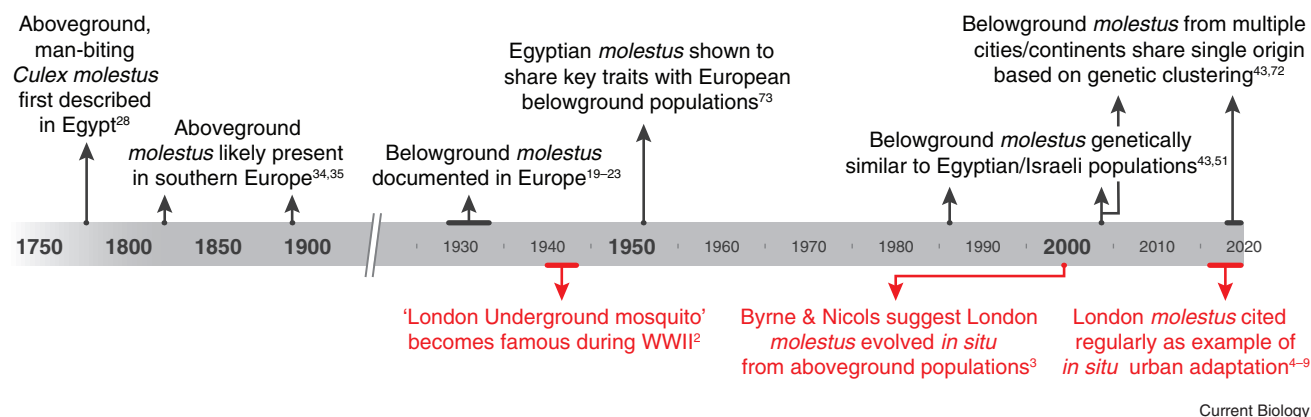
In summary, the complex patterns of variation observed within *Cx. pipiens* across the Mediterranean basin likely reflect a variable mosaic of selection on behavior and physiology across urban–rural, indoor–outdoor, and aboveground–belowground environments, alongside the presence of significant (but incomplete) reproductive barriers.

Compilation of genetic studies highlights a striking latitudinal gradient

The studies described above point to a latitudinal gradient across Europe and North Africa; differentiation between above- and belowground populations gradually decreases from north to south, presumably because the winters become less severe and

non-diapausing *molestus*-like mosquitoes are more and more able to survive aboveground[11,49]. To better illustrate this pattern, we synthesized genetic data from 214 diverse *Cx. pipiens* populations scattered across the region (Figure 3, Table S1 and Supplemental methods). Our synthesis is made possible by the widespread use of a simple, PCR assay based on sequence variation flanking a microsatellite locus called CQ11. CQ11 shows more or less fixed differences between canonical *pipiens* and *molestus* populations in Europe and North America[57]. As a single locus, it is not reliable for classifying individual mosquitoes in places where gene flow occurs. However, a flurry of recent studies has used it for the rapid and economical diagnosis of populations as *pipiens*-like, *molestus*-like, or something in between.

Northern populations (>45°N) fall into two discrete classes, as expected, with most belowground and aboveground individuals

Figure 4. The theory of *in situ* evolution in the London Underground mosquito rose to prominence despite contradictory evidence in the broader scientific literature.
Black lettering describes research on diverse European and Middle Eastern populations. Red lettering describes research and popular attention paid to London Underground mosquitoes. The notoriety of London populations generated a popular narrative of rapid, *in situ*, urban adaptation that is at odds with genetic and ecological data linking them to previously described taxa in both Europe and the Middle East.

being homozygous for the *molestus* and *pipiens* alleles, respectively (Figure 3A,B). Further south, along the European coast of the Mediterranean (35–45°N), aboveground populations become extremely variable, showing nearly every possible configuration of genotype and allele frequencies (Figure 3A,B). Importantly, many aboveground sites at middle latitudes harbored fewer heterozygotes than one would expect in a well-mixed population at Hardy-Weinberg equilibrium (Figure 3C,D). This can be seen in the uniformly positive (and often significant) inbreeding coefficients between 40°N and 50°N (Figure 3D) and hints at the lingering reproductive isolation between aboveground *pipiens*- and *molestus*-like individuals noted in previous work.

At the lowest latitudes, along the North African coast of the Atlantic and Mediterranean, mosquitoes are more reliably intermediate (Figure 3A,B). Few sampling localities yield the extreme allele frequencies characteristic of the two canonical ecotypes (Figure 3B), and heterozygote frequencies are largely as expected for well mixed, unstructured populations (Figure 3C,D). Even here, however, the *molestus* allele is slightly more frequent belowground (Figure 3B).

Taken together, these patterns produce a V-shape in a plot of allele frequency by latitude, with below and aboveground populations lining up along the left and right sides of the 'V' (Figure 3B). The frequency of the *pipiens* allele is negatively correlated with latitude belowground (N = 26, Pearson's $r = -0.61$, $P = 9.3*10^{-4}$) but positively correlated with latitude aboveground (N = 188, Pearson's $r = 0.56$, $P = 2.2*10^{-16}$).

Continent-scale patterns of variation at the CQ11 locus are consistent with those seen in smaller multilocus datasets and mirror regional variation in behavior (see previous section). It is nevertheless important to remember that selection acting on individual loci can render single-locus data unreliable for inferring population-scale phenomena. More work is also required to understand the connection between genotype and phenotype at the individual level. We know little about the genes underlying ecotype-specific behaviors (though some key genomic regions have been implicated[58,59]), and while CQ11 genotypes roughly
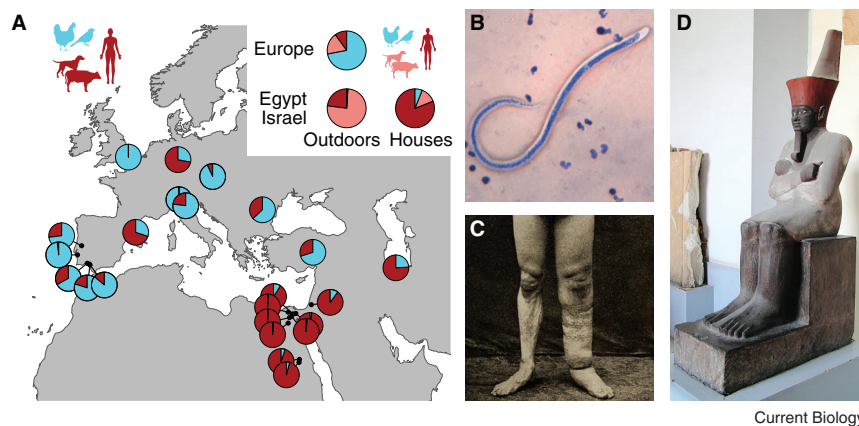
predict individual behavior in some mixed populations[32,44], they are in no way prescriptive.

The contentious theory of *in situ* adaptation in the London Underground

As explained above, *molestus* populations from the London Underground became famous during WWII (Figure 4). Early authors mused over a potential local origin[2], but they received little (if any) research attention over the subsequent fifty years. Then, in 1999, Byrne and Nicols published an influential allozyme study confirming that London Underground mosquitoes were reproductively isolated from their aboveground counterparts and hypothesizing that they evolved *in situ*[3] (Figure 4). They suggested that aboveground *pipiens* colonized the 'Tube' not long after it was constructed in the mid-1800s. Physically separated from their aboveground ancestors, the colonists then quickly evolved human biting, the ability to mate in confined spaces, and other key *molestus* behaviors as an adaptation to their concrete, subterranean home. Today, evolutionary biologists frequently hold up the London Underground mosquito as a likely example of rapid urban adaptation[4–9] (Figure 4), and the popular science media treats the theory as fact[60–65]. What evidence is there to support these claims? And if unsupported, where, when, and why did *molestus* first evolve?

Byrne and Nicols analyzed genetic variation at twenty allozyme loci in London populations. Belowground mosquitoes from eight Tube stations were relatively homogenous and more closely related to each other than to nearby aboveground mosquitoes. This is the main result of the paper, supporting the conclusion that London Underground populations are reproductively isolated and share a single origin. The authors then go on to suggest that they evolved *in situ* based on the fact that all but one of the specific allozyme alleles common belowground could also be readily found aboveground in London. While this pattern is certainly consistent with *in situ* evolution, it is also consistent with the idea that *molestus* migrated to London from another part of the species' range. Fixed genetic differences can take thousands to millions of years to accumulate

Figure 5. Cx. pipiens populations from the Middle East are mammal-biting, consistent with their recent and possibly ancient role in disease transmission.
(A) Published host-choice data for 27 aboveground Cx. pipiens populations (Table S2 and Supplemental methods). Pies placed on the map show fraction of bloodmeals taken from birds (light blue) or mammals (maroon) in nature. Inset summarizes data for outdoor and indoor house collection sites separately, with mammal meals further divided into human and non-human fractions. Mosquitoes from aboveground populations in Egypt and Israel consistently bite mammals, including humans. (B) Wuchereria bancrofti is a human-specific filarial worm that was prevalent in Egypt and transmitted by Cx. pipiens during the 20th century[76]. Photo shows blood smear with ~0.3 mm long larva. (C) W. bancrofti infections can cause swelling in the legs known as elephantiasis. (D) A statue of the pharaoh Mentuhotep II with an enlarged left leg is one of several ancient Egyptian artifacts that suggest W. bancrofti infections have been prevalent in the Middle East for millennia[77]. Images in (B) courtesy of Oregon Public Health Laboratory, (C) courtesy of the College of Physicians of Philadelphia Historical Medical Library, and (D) from Djehouty/Wikimedia Commons (CC BY-SA 4.0).
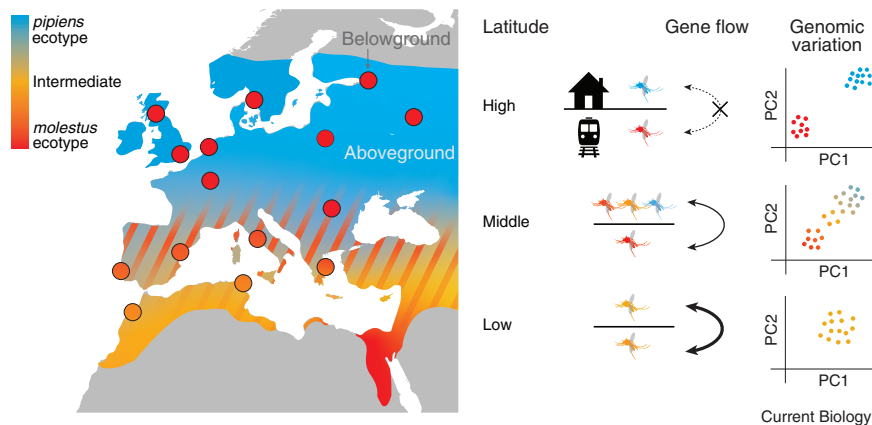
between diverging taxa, even when geographically distant, and may be restricted to narrow parts of the genome directly involved in key traits[66]. This is especially true when selection acts on pre-existing variants[67] and/or gene flow is ongoing[68]. Moreover, as explained above, molestus is not confined to London, and subsequent genomic analyses show that belowground mosquitoes from locations as distant as the UK, Spain, Portugal, Germany, Belarus, Kyrgyzstan, and the USA form a single genetic cluster[43,69–71] (but see Aardema et al.[72]). Most molestus populations are thus likely to share a common origin, and there is no particular reason to believe that this origin is the London Underground.

If not London, then where did molestus first evolve? A persistent narrative thread in the literature dating back over 200 years points to Egypt and surrounding areas (Figure 4). We have so far avoided mention of Egyptian populations because they have received little recent attention. However, the name molestus originated in Egypt's Nile delta, when, in 1775, the Swedish explorer and Linnaeus-disciple Peter Forskål described Cx. molestus — a new 'species' morphologically similar to Cx. pipiens that "bothers sleepers at night" and is "difficult to avoid unless with well-closed curtains"[12,28]. The name was later somewhat controversially adopted for northern belowground populations based solely on their shared human-biting behavior[10,38,40]. However, subsequent work confirms that Egyptian mosquitoes not only tend to bite humans and other mammals (Figure 5A), but also mate in confined spaces, remain active year-round, and show at least low levels of autogeny[73]. Decades-old allozyme data[51] and more recent microsatellite work[43] also document a genetic link between molestus in Europe and aboveground populations in Egypt, Israel, and Jordan. Byrne and Nicols were aware of these data, and although they favored in situ evolution, they carefully presented a Middle Eastern origin as an alternative in the introduction of their paper. Surprisingly, however, this possibility was overlooked in subsequent evolutionary reviews and syntheses[4–9].

The presence of molestus in Egypt and surrounding areas in recent times doesn't necessarily mean that it originated there, but several additional lines of evidence make this idea plausible. First, unlike the situation in Europe, molestus appears to be the only form present in Egypt and Israel. There is no evidence for cryptic genetic structure[51], and aboveground mosquitoes are just as likely to behave like molestus as those found breeding belowground[74]. An Egyptian origin thus alleviates the mental acrobatics required to imagine initial divergence in sympatry. Second, epidemiological data suggest that molestus has been present in Egypt for millennia. Wuchereria bancrofti is a filarial worm that only infects humans and causes lymphatic filariasis[75] (Figure 5B). Although recently eliminated from Egypt, it was recognized as a major public health problem throughout the 1900s, with Cx. pipiens mosquitoes serving as the primary vector[76]. Most people with lymphatic filariasis have no symptoms, but some develop severe swelling in the legs or other extremities known as elephantiasis (Figure 5C). Interestingly, ancient Egyptian papyrus and pharaonic sculptures indicate that this condition, and thus most likely mammal-biting Cx. pipiens, were common in Egypt as long ago as 2000 B.C.[77] (Figure 5D; but note the possibility that Anopheline mosquitoes[78] contributed to transmission in ancient times).

Finally, the long history of agriculture and dense human civilization in Egypt and surrounding areas provide a novel 'human' niche in which one can easily imagine key molestus behaviors (Figure 2) first emerging. The presence of abundant humans and domestic animals may have favored mammal-biting, and the presence of these hosts in houses and animal shelters at night (when Cx. pipiens is most active) may have favored the ability to enter and mate in confined spaces. Breeding year-round is likely ancestral within the species[79]. The only molestus trait that cannot be easily linked to ancient aboveground habitats is autogeny. Indeed, only a minority of molestus females from contemporary Egyptian and Israeli populations can lay eggs without a bloodmeal[73,74]. Ancient irrigation ditches and cesspits may have provided the kind of nutrient-rich larval habitat permissive for initial emergence of this behavior, but autogenous egg clutches are smaller than those laid after a bloodmeal, and the trait is likely under strong selection only when hosts are scarce[33,80]. We therefore hypothesize that autogeny was further selected in modern, belowground habitats. Many authors have shown that the trait responds quickly to the hard selection imposed by lack of vertebrate hosts, as long as at least

Figure 6. Summary of ecogenetic variation within *Cx. pipiens* across the western Palearctic.

The available data suggest a latitudinal model in which aboveground populations (represented by background map color) and belowground populations (represented by circles) are reproductively isolated in the north, where they correspond to the canonical *pipiens* (blue) and *molestus* (red) ecotypes. Gene flow increases as one moves south, generating a complex ecological mosaic with variable aboveground populations at middle latitudes (hatching), and a genetically and ecologically intermediate form in the wetter, western parts of North Africa (orange). Schematics to the right show the types of mosquitoes typically found above- and belowground in each latitudinal band and expected patterns of genomic variation (hypothetical principal components analyses of multilocus data). Belowground *molestus* populations from the north (red circles) are behaviorally and genetically similar to Egyptian populations (solid red background color). *molestus* may have originally evolved thousands of years ago in association with early Egyptian or other Middle Eastern agricultural societies, before moving north and eventually colonizing urban belowground habitats during modern times.

a few females in the starting population can lay eggs without a bloodmeal[40].

Ultimately, hypotheses surrounding the origin of *molestus* must be tested with better genomic data — ideally whole genome sequences from a large sample of individuals, including representatives from Egypt and other ecologically and geographically diverse populations. How old is *molestus*? Where and in what context did it likely first emerge? If *molestus* originated thousands of years ago in the Middle East, when did it spread north and come into contact with *pipiens* in southern Europe? When and from where did it colonize modern belowground habitats? Humans are clearly capable of spreading *molestus* from place to place in modern times[81–84], but might some *molestus* populations be independently derived via the reassortment of genetic variation present in admixed aboveground populations[49]? The idea that *molestus* evolved *de novo* in the London Underground within the last 200 years is almost certainly false, but there are still many interesting questions to answer surrounding its ultimate origin and spread.

Conclusions and outlook

For better or for worse, *Culex pipiens* mosquitoes have been capturing the attention of scientists and the general public for centuries. They are the most common mosquitoes in human habitats across the temperate Northern Hemisphere and display a level of ecogenetic variation that alternately inspires and frustrates even the most astute entomologists, vector biologists, and public health officials. We have reviewed what is known about this variation across the western Palearctic, clarifying a latitudinal gradient in ecology, microhabitat segregation, and gene flow (Figure 6). In northern Europe and Asia, the species comprises two divergent ecotypes, with *pipiens* aboveground and *molestus* in man-made belowground environments. Harsh winters and strong divergent selection likely limit gene flow and allow the two to maintain a remarkable suite of divergent behaviors (Figure 2). As one moves south into the Mediterranean basin, gene flow increases. There are still two genetic clusters corresponding to *pipiens*- and *molestus*-like individuals, but they are less extreme and less restricted to their respective above- and belowground habitats. Aboveground mosquitoes are particularly

diverse and sometimes display a mix of traits that makes them difficult to categorize. Finally, gene flow is high at low latitudes, with little to no difference between microhabitats in North Africa. Populations in the wetter, western parts of North Africa are intermediate, but those in Egypt and surrounding areas are genetically and behaviorally similar to northern *molestus*. Indeed, several lines of evidence suggest that *molestus* first evolved in association with early agricultural societies in the Middle East, before migrating north and eventually colonizing northern belowground habitats like the London Underground during modern times (Figures 4 and 5).

While gross ecological patterns are beginning to come into focus, many questions remain unanswered, and the increasing threat of West Nile Virus and other mosquito-borne pathogens makes continuing research efforts essential[85,86]. West Nile Virus is a bird virus for which humans are dead-end hosts[87]. Spillover therefore requires mosquitoes that are willing to bite both birds and mammals. Research in US cities suggests that hybridization between *pipiens* and *molestus* produces such bridge vectors and may drive West Nile Virus epidemics in major urban areas[43,88,89]. This idea has motivated much of the recent CQ11 genotyping work, and we hope that our synthesis of these data (Figures 3 and 6) will help public health officials across the western Palearctic better predict the potential for West Nile Virus transmission in their region. One should keep in mind, however, that CQ11 genotypes are only loose correlates of behavior and middle latitude populations are remarkably heterogeneous. Some of this heterogeneity is linked to local variation in urbanicity[32,47,48] (but see Vogels *et al.*[90]), but it remains difficult to predict exactly where or in what situations one will find *pipiens*, *molestus*, both, or something in between. Progress will likely require grappling with genome-wide patterns of genetic variation and standardizing the collection of behavioral data across studies and countries. Integrating behavioral and genomic data with knowledge of human population density, land use, and microclimate for a large sample may then clarify the key environmental factors that shape ecological diversity.

Understanding the environmental determinants of *Cx. pipiens* diversity is important because it can help us predict changes under future climate and land use regimes[91]. It is widely accepted

that the geographic distributions of many animals and plants, including mosquitoes, will shift as the planet warms[92,93]. The latitudinal nature of the patterns reviewed here (Figure 6) suggests that the dynamics and ecology of preexisting *Cx. pipiens* populations will also change. Warmer winters may expand the latitudinal zone within which *molestus* can survive aboveground, increasing hybridization between ecotypes in northern areas where they currently remain distinct. Such changes would be invisible at the morphological level, but nevertheless have potential consequences for disease transmission.

Although beyond the scope of the current review, the genetics and ecology of *Cx. pipiens* mosquitoes outside the western Palearctic region is also complex, in part due to interactions with related species[13,72,79]. Notably, both ecotypes of *Cx. pipiens* hybridize extensively with their tropical sibling *Cx. quinquefasciatus* when they come into contact in Asia, Australia, and the Americas (Figure 1). *Cx. quinquefasciatus* females require a bloodmeal to lay eggs, but are reminiscent of *molestus* in other ways, including in their willingness to bite humans and mate in captivity. This raises the possibility that gene flow or ancestral variation may have contributed to the evolution of *molestus* behavior. The only place where the temperate and tropical sibling species are fully reproductively isolated is in southern Africa, where local *Cx. pipiens* populations may actually represent a third, possibly ancestral species[55]. Understanding the evolutionary history (and future) of *pipiens* and *molestus* ecotypes will likely require consideration of this broader evolutionary context.

Finally, our review of the origin of London *molestus* has interesting implications for understanding urban plant and animal communities. The London Underground mosquito is frequently held up as an iconic example consistent with rapid urban adaptation. It is not exactly clear how this idea grew such long legs, but the weight of the evidence instead suggests that *molestus*' success in modern cities is largely the result of ancestral traits that first evolved in a different time and place — on the timescale of millennia rather than centuries. Whole genome sequences from geographically diverse populations will be critical for testing this hypothesis and putting dates on key evolutionary events[94]. Interestingly, *Aedes aegypti* is another vector mosquito that likely first arose in association with newly sedentary human societies in the Holocene, but is now taking advantage of the modern urban niche[95]. The extent to which ancestral traits (*versus* newly evolved adaptations) underlie the success of contemporary urban taxa is an important outstanding question in urban ecology and evolution[8,96]. We expect future work on *molestus* to continue to inform this relatively new field and to provide a captivating conduit for communicating these ideas to the general public.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.01.062.

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