

Chapter 12

Evolution Today: The Mosquitoes of the London Underground

Public use of the London Underground began on January 10, 1863. That date, or perhaps some earlier date when the tunnels were being readied for traffic, marks the beginning of the path toward the formation of a new species of mosquito. We often wonder how long it takes to form a new species; Darwin speculated timescales on the order of tens of thousands to hundreds of thousands of generations. The mosquitoes of the London Underground show that if conditions are right, the process can be much faster. I chose this example because it happened on Darwin's home turf and postdates the publication of the *Origin*. I will not pass judgment on whether these mosquitoes should now be defined as a distinct species, but will instead show that they have moved far down the path toward forming a reproductively isolated network of populations, which is the currently accepted definition of a species. My emphasis here is the same as Darwin's in the *Origin*: we are concerned with the process of speciation rather than naming of species.

Culex pipiens is the most widespread mosquito in the world. It is found on all continents except Antarctica. It is a vector of diseases, including West Nile virus and St. Louis encephalitis. It breeds wherever it can find stagnant pools of water, such as in untended birdbaths, forgotten buckets in the backyard, discarded automobile tires, clogged rain gutters, or wherever else fetid, stagnant water accumulates. It does very well in polluted urban settings. It lays rafts of eggs that float on the water, then hatch into wriggling larvae that feed on microbes. They emerge as adults around ten days later.

Females seek victims from whom they obtain a blood meal, then use the nutrients to form new batches of eggs. Anywhere they find a good supply of stagnant water and hosts for blood meals, a few of these mosquitoes can quickly multiply into swarms.

In London, *C. pipiens* is found as a surface form (*C. pipiens pipiens*) and as a subterranean form (*C. pipiens molestus*). The adult surface mosquitoes get their blood primarily from birds. They mate in large swarms in open areas. They live in a seasonal environment, which means they have a seasonal diapause, a time when they stop reproducing, store fat, and hide away for the winter in sheltered areas that stay warm enough to keep them from freezing.

The mosquitoes that ventured into the London Underground found excellent, unoccupied pools of stagnant water to lay their eggs in, but encountered a very different habitat for the adults. There were no birds to feed on, so they instead began feeding on mammals, primarily rats and humans. Their mating occurred in closed areas. Their environment had no seasons and was always fairly warm, so they lost their seasonal diapause and remained active all year long. They also evolved the ability to produce a clutch of eggs without first getting a blood meal, perhaps because their larval environments were rich but their prospects of finding a blood meal as adults were poor. While their presence was generally only an issue for rats and the few who worked in the tunnels, these subterranean mosquitoes added to the misery of the Londoners who sought refuge in the Underground during the nighttime bombings of World War II.

In the 1990s, British geneticists Katharine Byrne and Richard Nichols became interested in the origin of the Underground mosquitoes and in their relationship to those found on the surface (Byrne and Nichols 1999). Subterranean (*molestus*) mosquitoes can be found in similar sheltered habitats, such as caves and sewers, throughout western Europe and in other parts of the world. These populations share some of the attributes of the London Underground mosquitoes, so it is possible that some of them made their way to the London Underground. On the other hand, it may be that the resident surface mosquitoes invaded the London Underground on their own and independently adapted to the subterranean environment.

Byrne and Nichols used electrophoresis, the same method used by Highton to study *Plethodon* salamanders, to compare the Underground and surface populations. They sampled and quantified genetic variation at twenty loci in mosquitoes from seven sites dispersed throughout the more than 110

miles of tunnels, ranging from Shepherd's Bush in the west to Finsbury Park in the northeast to Elephant and Castle in the southeast. They also sampled from twelve surface populations found in gardens and ponds close to the location of the Underground sites. Comparing results, they found that all the alleles in the Underground populations could also be found in the surface populations. If mosquitoes from elsewhere had colonized the Underground, then we would expect those long-distant colonists to have brought some unique alleles into the Underground gene pool that were not seen in the surface population from London. The absence of such foreign alleles suggests that the original Underground mosquitoes were colonists from the surrounding countryside and not long-distance migrants from the mainland. The thought of a mosquito flying across the English Channel may seem silly, but people can inadvertently transport mosquitoes. For example, the paradisaical qualities of Hawaii once included having no mosquitoes, but people accidentally transported them there as unwanted baggage.

Byrne and Nichols also found that there was much less genetic variation in the Underground mosquitoes than in those on the surface. All the underground populations were genetically more similar to one another than they were to the surface populations. This is the pattern we would expect if a small number of surface mosquitoes moved into the Underground to establish the new populations. There may have been only a single successful colonization event. A small number of colonists would carry only a small amount of genetic variation into their new environment. Byrne and Nichols's conclusion was thus that the founders of the Underground population were most likely to have been a few colonists from the surface that moved into the new habitat provided by the tunnels and spread throughout the Underground as the tunnel network expanded.

Byrne and Nichols collected egg rafts from the breeding sites of the Underground and surface populations, raised the young to maturity, then performed crosses between different Underground populations and between Underground and surface mosquitoes. The different populations of Underground mosquitoes readily bred with one another and produced viable eggs. The hatchlings grew up and proved to be as fertile as their parents. They all thus behaved as if they were different populations of the same species.

Every pairing of a female from the Underground with a group of males from the surface failed to produce eggs. Each time a pairing failed, the female was then mated to males from the Underground and, each time, produced viable offspring; the genotypes of the babies confirmed that an

Underground male was the father. This means that all the Underground females were fertile, so their failure to mate with males from the surface indicated some form of premating reproductive isolation.

Darwin envisioned organisms in nature as always producing a surplus of offspring that are in turn always probing the environment for new opportunities. Byrne and Nichols found evidence for this perennial struggle for existence in the form of surface and Underground populations that were each probing the other's habitat for new opportunities. The Oval Station in the Underground was a site where surface mosquitoes appeared to be establishing a new beachhead for invading the Underground. They were found in a flooded service tunnel at the bottom of a shaft that opened to the surface. This was an Underground site, but the mosquitoes in that shaft were genetically like those on the surface. They required a blood meal to produce eggs. They were also reproductively isolated from the other Underground populations, since all but one pairing between them and mosquitoes from other Underground populations failed to produce offspring. Males from the Oval Station did succeed in mating with one of the Underground females they were paired with. Her raft of eggs produced viable offspring, but the offspring did not produce offspring of their own. Byrne and Nichols also found a surface population of mosquitoes in Beckton, southeast London, that was invading houses and biting people. The genotypes of these mosquitoes grouped them with the Underground populations, which suggests that they had recently emerged from the Underground and established a surface population. They did not produce eggs in captivity, so no mating trials were done on them.

Byrne and Nichols were careful to say that their goal was not to determine whether the Underground and surface populations represented different species, and they did not offer conclusions about how these populations should be classified. I agree with their caution, since it is the process that is important, not what we call the two forms. The important results are that the data in hand argue that the Underground population was derived from a small number of colonists from the surface, and that these colonists spread throughout the tunnel system. They have clearly moved far down the path toward becoming reproductively isolated from surface mosquitoes, or toward the discontinuity that defines a species. Since the Underground represents a newly formed habitat of known age, we can also conclude that all this happened between the publication of the first edition of the *Origin* in 1859 and the mid-1990s, when Byrne and Nichols collected

their samples. This is far less time to form a species, by orders of magnitude, than imagined by Darwin.

Why did the Underground mosquitoes evolve so quickly toward reproductive isolation from the surface mosquitoes? One obvious mechanism is “disruptive selection,” or selection for different phenotypes in the two environments. If each environment demands very different adaptations, then any time a surface and Underground mosquito met and mated, they would produce offspring that were not well suited to either environment. The list of specific adaptations to the Underground is long. The two forms feed on different hosts. Mosquitoes use chemical cues to track down hosts, so there may be differences in the cues that each type of mosquito uses to locate the source of a blood meal. The Underground mosquitoes live in an environment that is warm enough for them to be active year-round, so they no longer have a seasonal diapause; the surface mosquitoes have an obligatory diapause, which is necessary to survive the winter. Byrne and Nichols suggest that Underground mosquitoes that colonize the surface, as they appear to have done in Beckton, will likely be wiped out every winter because they lack the adaptations for diapause. Underground mosquitoes can produce eggs without a blood meal, while surface populations cannot. This adaptation evolved perhaps because the mosquitoes are less certain to encounter hosts in the Underground than on the surface. I imagine that the root of this adaptation is in the larval life stage, since the larvae would first have to pupate, then emerge as adults with sufficient reserves to support the development of eggs. This adaptation would give the Underground mosquitoes a big advantage over any new surface mosquitoes that invaded the Underground and required blood meals to reproduce, if blood meals were hard to obtain.

A second possible mechanism for the rapid evolution of reproductive isolation is that it is a by-product of the evolution of reproductive behavior. Adaptation to the Underground could have altered mating behavior or mechanisms of mate choice in a way that made it less likely for the Underground mosquitoes to mate with surface mosquitoes. Byrne and Nichols specify that surface and Underground mosquitoes form mating swarms in different types of settings. The surface mosquitoes swarm in open areas, while the Underground mosquitoes breed in confined spaces. This difference in behavior by itself could help to reduce the gene flow between the two populations. However, the way the two forms were mated in Byrne and Nichols’s experiments would have circumvented such differences in mating

sites, so there must be something else that contributes to their premating isolation than just where they choose to mate.

Byrne and Nichols reviewed other studies of the *pipiens* and *molestus* forms from throughout Europe, the Middle East, and North Africa and found that *pipiens* and *molestus* from northern latitudes tend to be more distinct from one another than those from southern latitudes. The northernmost *molestus* and *pipiens* populations show genetic differences between neighboring populations similar to what we see in London. At midlatitudes, in the northern Mediterranean, the differences are less extreme, and genetic studies yield evidence of some gene flow between the two forms. At more southern latitudes, in the Middle East and North Africa, there are few differences between the two forms. One key change in the southernmost populations is that the surface mosquitoes are more like the underground mosquitoes in that neither group has a diapause, probably because of the warmer winters and reduced differences in winter and summer day length. Byrne and Nichols propose that the increasing severity of winters in the northern parts of the range and the split between diapausing surface populations and nondiapausing subterranean populations is a key factor that separates the two forms in northern latitudes. Subterranean mosquitoes breed year-round, while surface mosquitoes have a short, summertime breeding season, so there is only a short period of overlap when they could crossbreed. Furthermore, any subterranean mosquitoes that invaded the surface habitat too late for summer mating would not likely survive until the next mating season. Lacking the ability to diapause, they would be exterminated come winter.

I feel the burden of Coyne and Orr's critical gaze as I consider the implications of these results. What might the alternative explanations be? The main alternative is that the mosquitoes that moved into the London Underground were migrant *molestus* mosquitoes from somewhere else and arrived with a full suite of adaptations to the Underground. The best available counterargument is the genetic data. Unique alleles among the twenty sampled loci have been found in foreign populations, but none of them appear in the London Underground. All alleles in the underground are a subset of what is seen on the surface. Further, their genetic similarity supports the theory that the Underground population could have been derived from only a few and perhaps just a single successful colonization event from one of the surface *pipiens* populations found near the Underground. Likewise, other *molestus* mosquitoes from other habitats throughout their multicontinental range may also

represent the independent invasion of a subterranean environment and the independent evolution of adaptation to that environment. We have seen this pattern before. In chapter 6, I reported on work by Culver and associates on the small amphipod crustaceans inhabiting different cave systems in the southeastern United States and by Verovnik and associates on isopod crustaceans from different cave systems in Albania and Italy. The cave forms were all well adapted to their new environment, with reduced eyes but enhanced antennae for navigating in an environment without light. Both sets of authors also found that each cave population was genetically most similar to the nearby surface population, rather than to other cave populations, so each represented an independent colonization of and adaptation to the cave environment. (Although the genetic data on foreign mosquitoes seem to rule out foreign *molestus* migration to the London Underground, one other source of migrants seems possible. One could imagine that the Underground mosquitoes are descendants of some other local *molestus* population that got a head start in adapting to a subterranean habitat before the Underground—perhaps, say, the dungeons under the Tower of London.)

A second alternative explanation is that even if the mosquitoes of the London Underground represent an independent colonization event and a very young, fast drive toward speciation, some of the genetic building blocks that caused their adaptation to the Underground may not be unique. Byrne (1996, in Byrne and Nichols 1999) discovered what might be a “pre-adaptation” to life in the Underground. She identified an allele of one enzyme that is rare on the surface but occurred at a frequency above 90% in the Underground. Chevillon and colleagues performed an analysis of the distribution of the same allele in *molestus* and *pipiens* populations from the French Alps and found evidence of selection in favor of this allele each time a population adapted to the underground environment, but selection against it in the surface populations. They suggest that this substitution of one allele for another at this genetic locus could contribute to the evolution of the ability to reproduce without obtaining a blood meal. In this way, a population of invading mosquitoes could establish a beachhead in a subterranean environment. The allele may simply be a rarity found in surface (*pipiens*) populations, but it may also be more common in the *pipiens* populations than it used to be, because of occasional matings between *molestus* and *pipiens* forms wherever they come into contact with one another.

Even though such genetic variation that facilitates adaptation to subterranean habitats may exist in surface mosquitoes, this does not change the

fact that the London Underground mosquitoes still represent the rapid evolution of reproductive isolation. The idea of a preexisting surface variation just clarifies how one particular mechanism might facilitate such rapid evolution. It is a special case of our more general understanding of how such adaptation to a new environment occurs. The most general explanation for how organisms adapt is the same as the one proposed by Darwin, which is that all natural populations of organisms possess differences between individuals in all traits. The suggestion that a population may be using genetic variation that resulted from adaptations made by past populations to environments similar to those confronting the current population is really just a new variation on this old theme. The adaptation of mosquitoes to subterranean habitats may represent independent events in that each case is an invasion of the new habitat by the local population, but the actual adaptation may involve, in part, a reassembly of traits that evolved under similar circumstances but in a different place and time.

The mosquitoes of the London Underground are not the only example we have of organisms invading a new habitat with the aid of genetic building blocks derived from other populations that adapted to similar environments long ago. I present one additional example here because it emphasizes the potential rapidity with which reproductive isolation can evolve, plus offers a more general message about what we have learned over the past few decades regarding the genetic diversity of species in nature. This example also highlights the ways in which we can now integrate modern molecular genetic methods with the study of evolution.

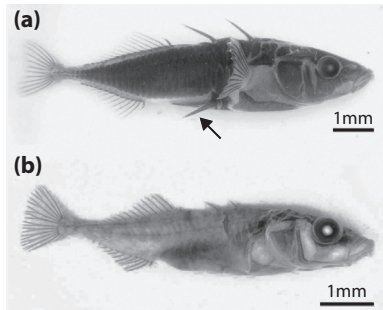
Three-spine sticklebacks (*Gasterosteus aculeatus*) are small, primarily marine fish that migrate into freshwater environments to breed. When the glaciers that capped the northern latitudes of the earth retreated around ten thousand years ago, they left behind a diversity of vacant freshwater habitats, including rivers, lakes, and marshes that were similar, in principle, to the vacancies created by the construction of the London Underground. The sticklebacks invaded thousands of these new environments and often evolved into permanent freshwater populations. Many of them also evolved morphologies that were distinct from those of their marine ancestors (fig. 15). The most obvious changes were a reduction of armor. The marine forms are well armored as a defense against fish predators. They have bony plates on their sides, spines along their backs (hence the name “stickleback”), and a pelvic girdle arrayed with stiff pelvic spines. If you look at them head-on,

the spines form a triangle: the dorsal spines point up, while the pelvic spines point down and to each side. The bony plates on the sides bridge the distance between the dorsal and pelvic spines so that the whole structure is a stiff ring studded with spikes, a configuration that causes these fish to stick in the throat of would-be predators and sometimes be spit out again. However, the typical marine predators are often absent in freshwater environments, and all or part of the armor has disappeared in many of the freshwater sticklebacks. That said, there is considerable diversity among freshwater populations in the degree to which the armoring has been lost.

Colosimo and colleagues (2005) found that an allele at a single gene strongly influenced the freshwater sticklebacks' loss of the lateral bony plates that run along the sides of their marine ancestors. This allele was found in freshwater populations of sticklebacks throughout their range and was also found in the marine populations. They estimated that the allele originated around 2 million years ago. The pattern of its continuing occurrence in different places means that there may have been a single, ancient event in which sticklebacks adapted to a freshwater environment and that some of the alleles associated with this adaptation became enriched in the marine populations because of some continued interbreeding between ma-

Figure 15
Marine vs. Freshwater Sticklebacks (*Gasterosteus aculeatus*)

The upper fish is typical of the marine members of this species, with distinct dorsal spines and a spine on the pelvic girdle (indicated with arrow). The other bony armor has been stained so that it shows up as dark gray to black in this photograph. The flanks of the body of the marine form are almost entirely plated with armor. Freshwater sticklebacks vary in their amount of armoring. The one pictured here has reduced dorsal spines, no pelvic spine or even a pelvic girdle, and few lateral plates. The fact that marine and freshwater sticklebacks readily interbreed in captivity has greatly facilitated research that characterizes the genes that control the expression of these traits.



rine and freshwater fish. The next advance of the glaciers would have wiped out these ancient freshwater populations, but the genetic tool kit that had enabled them to adapt to freshwater environments could have remained as rare alleles in the marine populations. There have been several advances and retreats of glaciers over the past 2 million years. Each retreat would have opened up new freshwater environments that could be invaded by sticklebacks; each advance would have wiped out those freshwater populations. During each retreat phase, fish that retained the some of the genetic building blocks their ancestors had used to adapt to freshwater habitats of the past could invade some of the new freshwater habitats. By this hypothesis, each freshwater invader begins with a marine phenotype and independently evolves a freshwater phenotype as it adapts to its new environment, but at least some of the genes that contribute to these local adaptations do so with alleles derived from long-extinct freshwater populations that persisted in the gene pool of marine sticklebacks.

We now have many other examples of organisms that have moved into new habitats, experienced strong disruptive selection as they adapted to their new environment, and are now at least partly reproductively isolated from their ancestral population. Apple maggot flies (*Rhagoletis pomonella*) normally lay their eggs on the fruits of hawthorns (genus *Crataegus*). In the 1860s, in the Hudson Valley of New York, it was discovered that some of them had shifted to laying eggs on apples that had been introduced from Europe. There is now a distinct “apple” race of these flies that is reproductively isolated from its hawthorn-preferring ancestors. Copepods (*Eurytemora affinis*) have moved from their marine environment into freshwater canals and reservoirs, Yucca moths (*Prodoxus quinquepunctellus*) adapted to an introduced species of yucca, and pea aphids (*Acyrtosiphon pisium*) adapted to different monocultures of crop plants, to name a few more. The impressive (and still growing) body of research on all these organisms and others supports the conclusion that local adaptation can be rapid and that it can in turn lead to the evolution of reproductive isolation, sometimes on timescales that are on the order of tens to hundreds of years. It also tell us that adaptation and speciation are going on now, right under our noses, rather than being something that happened in the past or happens so slowly that it is not detectable. All that is required for us to see speciation in progress is to look for it in the appropriate fashion.

One important message common to all these examples is that they illustrate the complexity of species in nature. Species are typically subdivided

into many local populations that are adapted to local conditions. The extent of local adaptation will be a product of how strong selection is and the degrees to which local populations are isolated from each other. Recent studies that couple molecular genetic techniques with the study of local adaptation, as in Byrne and Nichols's study of the mosquitoes of the London Underground or Colosimo and colleagues' study of sticklebacks, have shown again and again that species in nature should be thought of as fluid mosaics of populations that are becoming locally adapted, sometimes with the aid of similar adaptations attained by long-extinct populations that adapted to similar environments. These locally adapted populations often go extinct, but sometimes, when the opportunity arises, establish a new beachhead in previously unoccupied territory. Some local beachhead populations are now verging on becoming reproductively isolated as they adapt to their new environment. Most such forays fail as the local population disappears or is pulled back into the fold of its species of origin by gene flow, but some cross the threshold of reproductive isolation to become new species. Once that threshold is crossed, an irreversible discontinuity has been formed. This modern view of a species as a fluid mosaic of differentiated populations is consistent with Darwin's view of the constant struggle for existence and the principle of divergence, which together cause the formation of distinct populations, varieties, and—on rare occasions—new species.

Most of these examples also contain a message about the influence of human activities on nature. Many people are now preoccupied with human-caused changes to the environment and the march of vast numbers of species toward extinction. Sadly, this is the dominant theme of our human legacy. However, as we are changing the environment we are also creating new environments that can become opportunities for some species. Most of the examples presented above are associated with recent changes caused by humans: the digging of the London Underground and creation of a new subterranean habitat; the introduction of new host plants (apples, Yucca) into North America; the digging of canals and reservoirs that provide new opportunities for invasion by marine organisms; and the cultivation of crops in monocultures, creating a discrete patchwork of alternative environments. At the same time that we are causing extinction, we are also acting as a potent agent of natural selection by changing the environment so rapidly and in so many ways. Extinction results when change is too rapid and too large for adaptation to be an option, but sometimes organisms can keep pace with human impact on the environment. Sometimes we create

new opportunities and habitats that serve as blank slates for invasion and local adaptation and, in rare cases, the formation of new species.

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