Cities serve as testbeds for evolutionary change

Urban living can pressure flora and fauna to adapt in intriguing ways. Biologists are starting to take advantage of this convenient laboratory of evolution.

Carolyn Beans, *Science Writer*

Every student of evolution knows the story of the peppered moth. The species comes in two colors: one a peppered white, the other black. During Britain's industrial revolution, hungry birds spotted the lighter morph in soot-coated forests surrounding cities. Meanwhile, the rarer and better camouflaged darker morph avoided becoming lunch and carried the darker gene variant to a higher frequency in the population. When pollution cleared, the lighter morph again became more common. Although the methodology of the original peppered moth research came into question in the late 1990s (1), subsequent research confirmed its findings (2).

Yet despite this classic case of natural selection under urban conditions, so iconic that the peppered moth adorns the logo of the Society for the Study of Evolution, biologists have mainly chosen to study evolution in places with less human disturbance. "Most people didn't think that cities were really interesting biologically, that they were kind of anti-life," says evolutionary ecologist Marc Johnson, who directs the Centre for Urban Environments at the University of Toronto Mississauga.

That mindset is changing as Johnson and others use cities as powerful testbeds for evolutionary mechanisms. Because species often live both within and beyond city borders, researchers can directly compare populations to pinpoint whether and how these mechanisms respond to the urban environment. They can also compare the same species' responses across different cities. "That's why a lot of people are getting excited about this," says Johnson. "They like the idea of these replicated studies on a global level."

Indeed, researchers are beginning to team up for multicity studies in multiple countries. And in a 2017 review of urban evolution research, Johnson and coauthor Jason Munshi-South (an evolutionary biologist at Fordham University) listed 192 studies, more than half published in the last 5 years (3). Johnson's team discovered that to better tolerate the cold, urban clovers in Canada evolved to lose the genes responsible for an herbivore defense. Other researchers found that a lizard species evolved longer limbs and stickier feet to navigate Puerto Rican cities, an ant gained heat tolerance in the urban heat island of Cleveland, and the gene pools of New York City rats have diverged between uptown and downtown.

Researchers are studying white clover in cities across the globe to learn more about how urbanization shapes evolutionary change. Image credit: Marc Johnson (University of Toronto Mississauga, Mississauga, Canada).

But the field is young, and many questions remain. How commonly do species truly evolve to settle into urban abodes as opposed to accommodating urban life with the tools they already have? What factors determine why one species evolves and another doesn't? And do species repeatedly evolve in the same ways across different cities? The answers could further illuminate the mechanisms and limits of evolution within cities and beyond.

Evolving Priorities

Some urban evolutionary biologists initially entered the field in search of species to study closer to home. For Johnson, it was something of an adaptation to new circumstances. "I've spent most of my life trying to get out of cities. Once I became a professor and

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The Puerto Rican crested anole evolved longer limbs and stickier feet to better traverse urban terrain. Image credit: Kristin Winchell (Washington University in St. Louis, St. Louis).

had a young family, it was harder to go away." So he looked for evolution on his home turf.

Johnson, like many evolutionary biologists, was interested in how the mechanisms of evolution—natural selection, gene flow, genetic drift, and DNA mutation—each contribute to an evolutionary change in a population of plants, animals or microbes. He had good reason to suspect that cities might influence these mechanisms.

"Humans have touched every part of this planet either directly or indirectly," says Johnson. "But cities are different." Aspects of city living such as intensified noise, light, and heat could change which traits natural selection favors. Infrastructure such as buildings and roads could block gene flow—the movement of genes from one population into another that occurs when individuals migrate or release genetic material such as seeds or pollen. And because urban populations of plants and animals are often smaller and tucked into isolated patches of green space, they may be more susceptible to genetic drift—random fluctuations in the frequency of gene variants within a population. Although more studies are needed, some preliminary evidence even suggests that pollution in cities could act as a mutagen, increasing the rate of DNA mutation (4, 5).

Johnson tests how natural selection, gene flow, and genetic drift shape the evolution of white clover (*Trifolium repens*), a tiny weed that blankets city roadsides, suburban lawns, and rural pastures. By studying the clover across this urban-to-rural gradient, he can search for evolutionary shifts associated with city life.

Johnson focuses on one particular white clover trait: the ability to make hydrogen cyanide. Individual white clovers wielding this chemical defense are better at warding off insects and other herbivores. Those that can't produce the defense better tolerate freezing temperatures.

In a recent study, Johnson and his team explored how urbanization influences the frequency of hydrogen cyanide producers in 20 cities in southern Ontario (6). In each city, they tested for hydrogen cyanide production in clovers along a transect from the city center to rural surroundings. The researchers discovered that urban white clovers were more likely to lack the herbivore defense, with hydrogen cyanide producers increasing in frequency by about 0.6% with every kilometer of distance from an urban center.

Despite the fact that cities are often warmer than surrounding regions, Johnson's previous work had suggested that some city centers in northern climes actually have colder minimum winter temperatures than nearby natural areas, in part because there's less insulating snow cover. It seemed likely, then, that these cooler city temperatures act as a selective pressure pushing white clover to lose the herbivore defense in favor of cold tolerance.

But adaptation by natural selection isn't the only possible explanation. The random fluctuations of genetic drift give any gene variant a chance of being lost. Hydrogen cyanide production requires two genes and a specific allele, or gene variant, of each. With either missing, the plant can't produce the defense.

Johnson and his team extracted DNA from plants along urban–rural gradients to test whether genetic diversity decreased in urban populations, a telltale sign of genetic drift. But they found no clear differences. And they found ample evidence of another evolutionary mechanism: gene flow. Gene flow can increase a population's genetic diversity but also hinder its ability to adapt if gene variants that are beneficial in one location get pushed out by other versions flowing in from elsewhere. Johnson's team found that urban clovers adapted despite regular gene flow. "What this suggests," he says, "is that cities could be a powerful force for evolutionary change by natural selection."

Surviving the Urban Heat Island

For the acorn ant (*Temnothorax curvispinosus*), the response to urban temperatures at the other extreme may be more complex. These "very convenient little ants that live inside acorns" inhabit both urban and rural areas, says evolutionary ecologist Sarah Diamond of Case Western Reserve University. Diamond studies whether the ants' temperature tolerance is influenced by the urban heat island effect—where structures like asphalt roads and concrete sidewalks retain and exude heat, raising the city's ambient temperature several degrees above that of the surrounding countryside.

Diamond is particularly interested in untangling whether city ants better tolerate extreme heat simply because they grew up in a hotter place—which would be a "plastic" response to new conditions, but within their original capacities—or because they've actually evolved a greater ability to handle higher temperatures. The answer could help fine-tune models of ecosystem responses to climate change.

In a 2017 study, Diamond's team found evidence for both phenomena (7). When they brought ant colonies from urban Cleveland and nearby rural areas back to the lab, the offspring of both urban and rural ants tolerated higher temperatures when they were reared in hot rather than cold laboratory conditions. However, in both the hot- and cold-reared groups, ants with city roots tended to tolerate higher temperatures better still than ants with rural roots, suggesting a genetic basis for increased heat tolerance in the urban ants.

Even so, the researchers wondered whether these results could be generalized to other cities. The team repeated the study, this time collecting colonies from urban and rural sites across Cleveland, as well as Cincinnati and Knoxville, TN (8). They found that Cleveland ants produced the same results as before, and so did Knoxville ants, although only those reared at warmer temperatures. But there was no difference in heat tolerance between urban and rural Cincinnati colonies.

There are many possible reasons why the ants in Cincinnati didn't evolve, says coauthor and evolutionary ecologist Ryan Martin, also of Case Western Reserve University. One is that they just didn't need to. There's less of a difference between urban and rural temperatures in Cincinnati than in the other two cities. "The plastic response may be sufficient to cope with the relatively small increase in urban temperature," says Martin.

An Uptown–Downtown Divide

Urban living has also left an imprint on the gene pool of one of the most infamous city dwellers, the brown rat (*Rattus norvegicus*). Munshi-South uses genetics to study how the New York City landscape influences the genetic structure of brown rat populations. "Genetic structure is simply measurable genetic differences between groups of organisms," explains Munshi-South. "It does not imply that they have adapted to be different from one another. The important point is that the groups are following separate evolutionary trajectories." This could potentially set the stage for future adaptive evolution in each group.

In a 2018 study, Munshi-South discovered rats in the city's Manhattan borough are divided into Uptown and Downtown tribes, possibly because they don't cross Midtown, a mostly commercial area that may offer fewer food resources (9).

To test whether urban infrastructure regularly divides populations in other cities, he invited rat researchers from New Orleans; Vancouver; and Salvador, Brazil to bring rat tissue samples to his lab for genetic analysis. With more than 1,200 rats sampled across four cities, the researchers screened each animal's genome for SNPs—places where a single letter in a genetic sequence can vary. More closely related individuals will usually share more-similar SNP profiles, so researchers were able to map distinct populations across the cities.

The results, also published in 2018, suggest the urban landscape commonly impedes gene flow in rat populations (10). Just as in Manhattan, the rat populations were divided—in Salvador and Vancouver by roadways and in New Orleans by waterways.

The team is now building habitat-suitability maps that predict in more detail how urban features may influence rat movements. "We take variables that describe city landscapes," explains Munshi-South, "the age of buildings, types of sewers, the amount of roads, the percent green space cover, human socioeconomics." They input all of this data into a model that predicts how these features influence the genetically distinct populations they've already identified. If the prediction is accurate, they could potentially use the model to forecast rat movements in other cities without having to genetically profile local rats.

The results, says Munshi-South, could inform pest management. "That is the hope," he says. "That this fine-grain understanding of how rats form these local populations will allow [public health departments] to target rats at the right spatial scale."

Adaptive Compromise

For some urban dwellers, success can come down to survival of the fastest. Evolutionary biologist Kristin Winchell, a postdoctoral researcher at Washington University in St. Louis, studies urban adaptation in a lizard long recognized as a champion of rapid evolution (11). "The cool thing about urban environments is it's a natural experiment," says Winchell. "We're not doing any manipulation, so it's a more realistic capturing of natural selection as a whole."

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In a 2016 study, Winchell explored whether the Puerto Rican crested anole, *Anolis cristatellus*, adapted to urban life (12). In forests, crested anoles often perch on tree trunks. In Puerto Rican cities, they regularly cling to surfaces such as buildings, glass windows, and metal fences. But the team noticed that some anoles slipped and fell on these surfaces and wondered if natural selection favored traits that would allow the lizards to cling more adeptly.

Winchell and her team caught crested anoles in the Puerto Rican cities of Mayagüez, Ponce, and San Juan, as well as in forests beyond each urban area. The researchers measured traits that could be important for perching on city structures, such as limb length and the number of modified scales, known as subdigital lamellae, on the underside of lizards' toes that help them cling to surfaces. Compared with forest lizards, urban lizards had longer limbs and more lamellae, differences the team confirmed were genetic, rather than a plastic response to growing up on city perches.

Still, Winchell wondered exactly how this adaptation benefited lizards. To find out, she created lizard racetracks—runways covered in bark, painted concrete, or aluminum sheeting, whose inclines she could set to 37° or 60°—then clocked lizards on the tracks. Her results, published last year, showed that both urban and rural lizards performed poorly on steep tracks and that having longer forelimbs correlated with more slipping and slower sprint speeds (13). But on the more gradual incline, urban lizards were the clear winners, regardless of the surface material.

Winchell now thinks natural selection may primarily favor longer limbs for speedily traversing the ground from one urban perch to the next while also favoring more lamellae to help compensate for the disadvantage of longer forelimbs when actually on an urban perch. "The urban habitat is a lot more open," explains Winchell. "They can't jump between perches like they can in the natural environment."

The Limits of Urban Adaptation

Despite clear and repeatable examples of urban evolution, researchers still can't say how commonly city species evolve to embrace urban landscapes. In a recent meta-analysis of experimental and observational studies reporting more than 1,600 phenotypic changes across species, urban ecologist Marina Alberti of the University of Washington and her team conclude that animal and plant traits shift more rapidly in urban areas (14). But most studies in the analysis didn't measure natural selection or identify an underlying genetic basis to the change. "Phenotypic change doesn't necessarily translate to genetic change," says Alberti. Some species, for example, may have the flexibility to respond plastically to cities.

Other city residents may thrive without changing at all. "Raccoons do great in cities. Are they evolving?" questions Martin. "They might have characteristics that make them good already." In other cases, urban species may be just getting by. Winchell found that another anole species primarily perches on city trees and other vegetation (15). "If that type of habitat is lost or becomes more isolated," she says, "that species is probably not going to persist in the urban environment."

Evolutionary biologists need more tests of adaptation caused by clear selective pressures to determine which factors influence whether it happens and how consistently. To date, the majority of urban evolution studies have focused instead on the more neutral mechanisms of gene flow and genetic drift, partly because these are often easier to measure than natural selection and partly because of the specific questions that interested particular researchers.

Munshi-South says the field is broadening as more people enter it. "I think now you're going to start to see a diversification and a lot more effort to understand adaptation," he says. Indeed, the National Science Foundation recently awarded a $500,000 grant to Alberti, Johnson, Munshi-South, and others to build a global network of researchers who will develop multicity studies to explore how urbanization drives evolutionary change and the potential for that change to influence entire ecosystems.

Johnson is also taking his white clover project global. More than 550 collaborators are repeating his study in more than 180 cities across the world. "To the best of my knowledge, this is the largest scale and largest collaborative evolutionary project ever attempted," he says. Initially selected for convenience, Johnson now sees the urban white clover as a robust model for understanding how urbanization affects evolution more generally. "We can answer evolutionary questions," he says, "at a scale and with replication that we never thought was imaginable."

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