Urban mutants: Effects of urban-induced mutations on ecology, evolution, and health

Marc T. J. Johnson[1,2], Irtaqa Arif[1,2], Francesco Marchetti[3], Jason Munshi-South[4], Rob W. Ness[1,2], Marta Szulkin[5], Brian C. Verrelli[6], Carole L. Yauk[7], Daniel N. Anstett[8], Warren Booth[9], Aude E. Caizergues[1,2], Elizabeth Carlen[10], Anthony Dant[11], Josefa González[12], César González Lagos[13], Madeleine Oman[1,2], Megan Phifer-Rixey[14], Diana Rennison[15], Michael S. Rosenberg[6], Kristin M. Winchell[16]

[1]Centre for Urban Environments, University of Toronto Mississauga, Mississauga, Canada. [2]Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada. [3]Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada. [4]Department of Biology and Louis Calder Center, Fordham University, Armonk, NY, USA. [5]Faculty of Biology, University of Warsaw, Poland. [6]Center for Biological Data Science, Virginia Commonwealth University, Richmond, VA, USA. [7]Department of Biology, University of Ottawa, Ottawa, ON, Canada. [8]Department of Plant Biology, Department of Entomology, Plant Resilience Institute, Michigan State University, East Lansing, MI, USA. [9]Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. [10]Living Earth Collaborative, Washington University in St. Louis, St. Louis, MO, USA. [11]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA. [12]Institute of Evolutionary Biology, CSIC, UPF. Barcelona, Spain. [13]Departamento de Ciencias, Facultad de Artes Liberales, Universidad Adolfo Ibáñez; Center of Applied Ecology and Sustainability (CAPES), Santiago, Chile. [14]Department of Biology, Drexel University, Philadelphia, PA, USA. [15]School of Biological Sciences, University of California San Diego, La Jolla, CA, USA. [16]Department of Biology, New York University, New York, NY, USA

Author for correspondence: marc.johnson@utoronto.ca

Abstract

Increasing evidence suggests that urbanization is associated with higher mutation rates, which can affect the health and evolution of organisms that inhabit cities. Elevated pollution levels in urban areas can induce DNA damage leading to *de novo* mutations. Studies on mutations induced by urban pollution are most prevalent in humans and microbes, whereas studies of non-human eukaryotes are rare, even though increased mutation rates have the potential to affect organisms and their populations in contemporary time. A wealth of data indicates that most mutations will be neutral or deleterious, and higher mutation rates associated with elevated pollution in urban populations can increase the risk of cancer in humans and potentially other species. Evolutionary theory further suggests the potential for urban-driven increased deleterious mutational loads in some organisms, which could lead to a decline in population growth of a wide diversity of organisms. While beneficial mutations are expected to be rare, higher mutation rates in urban areas could influence adaptive evolution, especially in organisms with short generation times. We explore avenues for future research to better understand the effects of urban-induced mutations on the fitness, ecology, and evolution of city-dwelling organisms.

Mutation is the fundamental evolutionary force that creates all genetic variation. Despite its importance, variation in mutation rates is often overlooked or considered of negligible significance in empirical studies of ecology and evolution, particularly in eukaryotes{Fenster, 2020 #6}. Mutation rates can be influenced by the environment{MacLean, 2013 #11;Fitzgerald, 2017 #10}, and can evolve through time{Lynch, 2016 #9;Bergeron, 2023 #91}. One place where a consideration of mutation rates may be particularly relevant is in cities, where emerging evidence suggests that mutation rates are elevated by pollution{Somers, 2004 #3699;Yauk, 1996 #3746}. Our Perspective considers how elevated mutation rates in urban environments could impact the fitness and health of individuals, which may alter the ecological and evolutionary trajectories of populations.

One of the most consistent differences between urban and non-urban environments that could influence mutation rates is higher chemical pollution. Transportation, industry, wastewater management, home heating, landfills, and pesticide application are some of the activities in urban areas commonly associated with elevated air, water and soil pollution{Organization, 2016 #36;FAO, 2021 #37;UNEP, 2016 #38}. Although less frequent in urban areas, nuclear plants, nuclear testing, and warfare can also result in highly mutagenic ionizing radiation (e.g., Fukushima, Three Mile Island, Hiroshima){Filburn, 2016 #13}. Studies on the mutagenic effects of radiation also provide general insight into how highly mutagenic pollutants can influence organisms in cities. While pollution is not unique to urban areas, the concentration and diversity of pollutants is often highest in cities (Fig. 1), exposing organisms to harmful stressors in unprecedented ways{Organization, 2016 #36;FAO, 2021 #37;UNEP, 2016 #38}.

Urban chemical pollution can cause physiological and genotoxic stress to organisms that may result in mutations. Such pollution is known to result in respiratory illnesses in humans{Seaton, 1995 #14}, reduced photosynthesis and cell damage in plants{Seyyednejad, 2011 #18}, higher mortality in fishes and amphibians{Casey, 2005 #17}, and decreased fledgling success in birds{Chatelain, 2021 #16}. Exposure to some pollutants can damage DNA and induce *de novo* mutations (hereafter simply called

68 “mutations”){Claxton, 2007 #209;White, 2004 #56;Humans, 2015 #30;Marchetti, 2020 #68} (Box 1,
69 Table 1). While carcinogenic pollutants are known to cause somatic mutations, the fitness effects of
70 these mutations and the prevalence of pollution-induced germline mutations are poorly understood
71 outside of lab settings. Moreover, whether urban-induced higher mutation rates lead to an increased
72 number of deleterious mutations, population decline, or accelerated adaptive evolution, has not been
73 considered until now{but see \Bromham, 2015 #39}.
74 Studies of the effects of urbanization on evolution have focused on genetic drift, gene flow, and
75 natural selection, while the potential for elevated mutation rates in cities to influence the ecology and
76 evolution of populations is virtually unexplored and of high priority for future research{Diamond, 2021
77 #4452;Johnson, 2017 #3562;Szulkin, 2020 #320;Verrelli, 2022 #4482}. Our goal is to provide a
78 forward-looking Perspective of the potential for elevated mutation rates in cities to influence the
79 ecology and evolution of populations. We begin by reviewing urban pollutants and the damage they
80 cause to DNA. Next, we consider how pollution affects somatic and germline mutations and the
81 potential importance of these mutations for ecology and evolution. While urban pollution can affect all
82 organisms living in cities, most existing examples come from research on humans. We consider the
83 effects of pollution on human and nonhumans throughout this paper, and we use the extensive literature
84 on humans as a model to understand the wider ecological and evolutionary impacts of evolution for all
85 organisms. These wider implications beyond humans are particularly important because although cities
86 reduce and homogenize species diversity, urban habitats still harbour substantial biodiversity
87 {Aronson, 2014 #351;Knapp, 2012 #350;Rogers, 2023 #352}, and many of these species in cities are
88 of conservation concern or play fundamental ecosystem roles{Lambert, 2020 #353}. We end by
89 discussing gaps in current research and directions for future research. Our findings have potentially
90 large and hitherto overlooked implications for: (1) the health of both human and wild organisms, and
91 (2) the persistence of biodiversity in a rapidly urbanizing world.
92

Urban pollutants and damage to DNA

Air, water, and soil in cities are consistently associated with a diverse mixture of pollutants (Table 1, Box 1). The sources of most outdoor air pollutants in cities are combustion by-products from transportation, power generation, home heating and cooking, and industry{Leung, 2015 #34;Programme, 2017 #289}. These by-products include pollutants such as PAH, NOx, sulphur dioxide (SO_2), CO, and various metal species (e.g., Hg, Cu, Pb, Sn). These compounds can interact with or bind to PM, which can then be deposited in soil{Baensch-Baltruschat, 2020 #33;Nirmalkar, 2021 #32;Humans, 2015 #30;Humans, 2010 #35}. Soil can also become contaminated with genotoxicants from industrial by-products, manufacturing, mining, and road salting{Programme, 2017 #289}. Air pollutants, soil leaching, run-off, and sewage all contribute to water pollution{Martínez-Bravo, 2019 #28}, which can lead to elevated levels of pesticides{Nagy, 2014 #27;Annabi, 2019 #26}, polychlorinated biphenyls (PCBs){Agudo, 2016 #25}, pharmaceutical products{Chowdhury, 2020 #22;Isidori, 2005 #24;Metzler, 1998 #21}, and microplastics{Tagorti, 2022 #20;Roursgaard, 2022 #19;Programme, 2017 #289} in urban aquatic habitats.

Pollution in urban settings varies in both time and space in complex ways. The levels and types of urban pollution have changed throughout the history of industrial and urban growth. For example, during the past 20 years, the level of $PM_{2.5}$ (particulate matter with diameters <2.5μm) in Shanghai, China, has increased by over 200%, yet it decreased by nearly 30% in New York, USA, and remained consistently low in Melbourne, Australia (Fig. 1). These changes through time are often influenced by changes in governmental policies (e.g., US's Clean Air Act, EU's Ambient Air Quality Directive) and technological change (e.g., conversion from leaded to unleaded fuels). Urban pollutants also vary spatially in their concentrations and composition (Fig. 1 insets). For example, industrial steel production often leads to some of the highest concentrations of polycyclic aromatic hydrocarbons (PAHs){Yang, 2002 #290}, whereas high vehicle traffic is typically associated with higher particulate

matter (PM), ozone, carbon monoxide (CO), and nitrous oxides (NOx) (Table 1). Socioeconomic variation among neighbourhoods often covaries with pollution levels, whereby poorer neighbourhoods are frequently in the most polluted areas, causing disparity in exposure to potentially harmful genotoxicants{Hajat, 2015 #137;Kim, 2023 #138}. Non-urban areas also frequently experience pollution due to anthropogenic activities (e.g. resource extraction, agriculture, forestry, nuclear radiation), but we focus on urban areas because they are the fastest growing ecosystem on Earth, they are consistently associated with elevated pollution of diverse mixtures of chemicals, which potentially have harmful effects on organisms including damage to DNA (i.e., genotoxicants) (Box 1).

The genotoxic effects of urban pollutants include chemical interactions that form DNA adducts (i.e., chemicals that bind to DNA) and reactive oxygen species that damage DNA (Box 1). When such damage is improperly repaired it can cause small-scale and large-scale mutations. Small-scale mutations include single nucleotide substitutions and small insertions/deletions (indels). Large-scale mutations involve large indels, duplications, translocations, inversions, and aneuploidy{Iafrate, 2004 #139;Sebat, 2004 #140;Zhang, 2009 #141}. DNA replication errors such as unequal crossovers that can result in gene duplication and deletion are also possible. The location of DNA damage (coding vs. noncoding regions), the molecular function of damaged DNA (e.g., regulatory versus structural), and whether coding mutations are synonymous or nonsynonymous, influence the molecular, physiological, and fitness consequences of damage. The fitness effects of mutation can in turn impact the ecology and evolution of populations{Griffiths, 2000 #144;Chu, 2019 #142;Scacheri, 2015 #143}.

The effects of urban-induced mutations may differ between species because of variation in ploidy, cellular complexity, mutation rate, reproductive system, population size, and generation time. For example, many animals, higher plants, and some eukaryotic microbes live primarily as diploids or polyploids, which can mask the fitness effects of recessive mutations at low frequencies{Orr, 1995 #343;Otto, 2008 #345}. Similarly, many multicellular organisms have differentiated germ and somatic cells, such that pollution induced mutations in somatic cells will not generally be passed on to

subsequent generations. In contrast, organisms with no distinction between germ and soma (e.g., some plants and fungi), may accumulate inherited mutations more rapidly if mutations arise in the cells that ultimately form gametic tissue{Anderson, 2018 #339;Burian, 2021 #337}. Moreover, mutation rates vary by orders of magnitude, with bacteria and microbial eukaryotes having the lowest rates, vascular plants and animals with moderate rates, and viruses with the highest mutation rates {Lynch, 2016 #342;Wang, 2023 #338}. Recombination in sexual organisms can allow efficient purging of harmful mutations compared to asexual populations{Otto, 2009 #1806;Charlesworth, 2012 #2518}. Finally, large populations with rapid generation times are expected to evolve to purge or fix environmentally induced mutations that affect fitness more rapidly than small long-lived populations{Charlesworth, 2009 #2532}. In the sections that follow we expand on how such variation among species may lead to different ecological and evolutionary consequences of urban induced mutations.

Somatic mutations

The primary consequence of genotoxic exposure is the induction of somatic mutations that can adversely affect molecular, cellular and tissue function. Somatic mutations are not transmitted to the next generation unless they occur in germ cell progenitors (e.g., plant apical meristems){Lanfear, 2018 #263}, and so they typically affect only the exposed individual's health and fitness. The causal role of chemically induced mutations in cancer development is well known in certain cases (e.g., lung cancer due to tobacco smoke){Hecht, 1999 #291} (Table 2). These examples show that exposure to genotoxicants can cause mutations in tumour suppressor oncogenes that function as cancer drivers that cause cellular proliferation and tumour development, or affect genes involved in DNA repair leading to genetic instability{Foo, 2014 #292}. Moreover, exposure to mutagens during key life stages (e.g., embryogenesis, organogenesis) may increase the probability of clonal expansion of mutation-bearing cells{Godschalk, 2020 #43;Whitham, 1981 #294;White, 2004 #56}. Data supporting the association between environmentally induced mutations and non-cancerous diseases are almost entirely lacking,

166 despite knowledge of mutations across the genome caused by genotoxicant exposure, and a growing
167 understanding of the role of somatic cell mutagenicity in disease more generally (e.g., ageing,
168 neurological and cardiac diseases){Schumacher, 2021 #41;Li, 2013 #293}. Thus, there is currently no
169 knowledge on the rates and functional consequences of pollution induced somatic mutations for
170 individuals, populations, and species beyond the established association with cancer.

171 The study of mutagenesis in wild organisms is challenging because mutations are rare events at a
172 genomic scale. This difficulty is compounded in the case of somatic mutations because the occurrence
173 of mutations varies among tissues within a single individual. However, a variety of studies provide
174 empirical evidence supporting an association between specific urban pollutants and elevated somatic
175 cell mutation rates. The invention of the *Salmonella* mutation assay (i.e., the "Ames assay") has been
176 the single most transformative tool in the study of environmental mutagenesis{Claxton, 2010
177 #153;Claxton, 2004 #208}. Briefly, the assay assesses how frequently *Salmonella* strains lacking the
178 ability to metabolize histidine – due to engineered base pair substitutions or frameshift mutations –
179 exhibit revertant mutations to restore histidine metabolism when challenged by a potential toxin[17,64].
180 This simple bacterial assay has revealed that the air, soil and water in urban environments is replete
181 with mutagens{Claxton, 2010 #153}. Beyond *Salmonella*, observational and experimental cytogenetic
182 studies show that numerous chemical pollutants cause chromosomal abnormalities (e.g., chromosomal
183 structural aberrations, aneuploidy) in diverse organisms{Claxton, 2007 #209;White, 2004 #56;Chen,
184 2004 #57}. Additional lines of evidence are based on the types and distribution of mutations (i.e.,
185 mutation spectrum) observed in human cancers used to infer mutagenic exposures{e.g., \Olivier, 2004
186 #45}, and the COSMIC database{Alexandrov, 2020 #46}. Overall, laboratory models (e.g.,
187 *Salmonella*, mice, plants) exposed to environmental media or extracts demonstrate the widespread
188 mutagenicity of many chemical pollutants in urban areas{Olivier, 2004 #45}.

189 The most extensive evidence of pollution-induced somatic cell mutagenicity is from studies on
190 combustion-related by-products found in urban air pollution, contaminated soils, and sediments. The

weight of evidence for the mutagenicity of outdoor air pollution is high, with many specific agents declared 'carcinogenic to humans' by the International Agency for Research on Cancer (IARC){Humans, 2015 #30}. IARC monographs thoroughly describe how these urban pollutants cause mutagenicity in laboratory organisms as diverse as bacteria, plants and rodents{Ferreira, 2007 #48;Humans, 2015 #30}. For example, the mutation spectrum observed in lung tumours of non-smokers associated with air pollution is broadly consistent with exposure to bulky DNA adduct-forming chemicals (e.g., benzo[a]pyrene){DeMarini, 2001 #50;Yu, 2015 #51}. Additional evidence for the mutagenicity of air pollution comes from humans exposed to high levels of combustion by-products in residential and occupational settings, whereby individuals exhibit cytogenetic damage to various cell types{Acito, 2022 #54;León-Mejía, 2019 #55}, and the urine from such individuals is mutagenic to bacterial cells{e.g., \Hansen, 2004 #52;Wong, 2021 #53}. Moreover, soil and sediments that contain combustion-related contaminants are mutagenic to organisms that frequently come into contact with these substrates (e.g., bacteria and plants){White, 2004 #56;Chen, 2004 #57}. Undoubtedly, inhabitants of any urban ecosystem are exposed to mutagenic particulate pollutants associated with combustion emissions.

There are many other examples of mutagenic contaminants found in urban settings, from metals, to pesticides, organochlorines, and benzene (Table 1). These genotoxicants have the potential to impact somatic cell mutation burden contributing to the decreased health of individuals and populations {Humans, 2015 #30;Organization, 2020 #288}. The vast majority of mutagenicity testing is conducted in the laboratory on individual chemicals at high doses{Marchetti, 2020 #68}, leading to a major gap in understanding how lifelong, low-dose exposures of mixtures of mutagens affect mutation rates and disease outcomes. Moreover, the complex interactions between socio-demographic factors and mutagenic environmental mixtures inherent to cities have yet to be explored.

The study of environmentally induced somatic cell mutations has been considerably hampered by the lack of tools available outside of the laboratory. Although single-cell deep-sequencing{Eberwine,

2014 #317} and error-corrected sequencing{Kennedy, 2014 #315;Cho, 2023 #316} methodologies exist, these have mostly been applied in clinical settings and have yet to be extended to studies on environmental exposures in natural populations. The high levels of pollution in urban areas offer an opportunity to address these obstacles using field experiments, in addition to laboratory experiments, that apply genomic technologies to directly quantify mutation frequency and spectrum in a diverse array of organisms (see *Future Directions*).

Germline mutations

Unlike somatic mutations, germline mutations are inherited between generations. For this reason, it is primarily germline mutations that can influence the evolution of populations, with the exception of somatic mutations that are then incorporated into reproductive tissue, which is most common in fungi and plants{Lanfear, 2018 #263}. Although germline mutations are rare at the individual level, even the smallest increase in mutation rate can have significant consequences for populations{Shendure, 2015 #67}.

Laboratory and field studies suggest that exposure to many common urban pollutants can induce germline mutations. For example, over 80 chemical agents have been identified as germline mutagens in lab mice{Marchetti, 2020 #68}. In humans, the best evidence of the impact of pollutants on germ cell mutagenesis comes from studies demonstrating an increased incidence of chromosomal abnormalities in human sperm{Marchetti, 2020 #68}. Such abnormalities may explain the significant correlation between paternal blood dioxin levels due to occupational exposure and increased mutation rates in their offspring{Ton, 2018 #77}. When considering exposure to radiation as an example of extreme exposure to a mutagen, children of parents exposed to ionizing radiation following the Chernobyl nuclear plant accident exhibited increased rates of tandem repeat mutations{Dubrova, 1996 #73}. Similar inherited mutations have been observed in plants{Kovalchuk, 1998 #74} and barn

swallows{Ellegren, 1997 #75}. However, increases in inherited single nucleotide variants have yet to be conclusively demonstrated for humans exposed to radiation{Yeager, 2021 #4388}. When we look to non-polluted areas, a recent study reported a reduced mutation rate in an Amish population, which has been interpreted as traditional rural lifestyles leading to low mutations rates because of reduced exposure to chemical mutagens{Kessler, 2020 #80}. Only a few studies have examined non-human populations outside of laboratory conditions, and they show that birds and rodents exhibit increased heritable mutation rates in repetitive DNA regions when exposed to ambient industrial air pollution{King, 2014 #70;Yauk, 1996 #3746;Somers, 2004 #3699;Somers, 2002 #3700}.

In addition to pollution, urban and rural human populations diverge in their demographic patterns in ways that are expected to influence germline mutation rates. In recent decades, there has been a trend for delayed childbearing in many countries. In both developed and developing nations, this delay is more pronounced in urban settings than in rural settings{Ely, 2018 #83;Lerch, 2019 #295}. Over the course of the last decade, studies of human parents and offspring have consistently demonstrated an age-related increase in mutation rates, especially in fathers{Goldmann, 2016 #82}. It is estimated that fathers transmit ~1.2 additional mutations for each year of age, versus ~0.4 new mutations per year of age in the mother. The higher paternal contribution is ascribed to the continuous production of sperm as men age, while no new oocytes are generated once a female is born. The consistency of this divergence between developed and developing nations requires further investigation, as a major source of increased mutation rates could also result from differences in socio-cultural practices, economic disparities, and racial demographics between urban and rural areas in cities throughout the world. There is also evidence that non-human organisms exhibit demographic shifts in urban habitats{Merckx, 2018 #354}, but whether this is associated with changes in mutation rates requires investigation.

Despite the circumstantial evidence mentioned above for an effect of urban pollution and demographics on increased germline mutation rates in cities, a direct link between urban pollution and mutations has yet to be directly demonstrated using modern genome sequencing techniques. Thus, we

lack information on how and when urban pollution increases rates of germline mutation, the targets of mutation, and especially their phenotypic and fitness effects.

Ecological and evolutionary consequences

Alterations to the rate and spectrum of both somatic and germline mutations due to urban pollution could have important ecological and evolutionary repercussions. Theoretical and empirical studies show that the majority of new functionally significant mutations are deleterious and removed by purifying selection{Eyre-Walker, 2007 #84}. If deleterious mutations are elevated in urban settings, either due to a higher rate or as a larger fraction of deleterious mutations, we expect an increased mutation load (i.e., reduced fitness due to the burden of deleterious mutations relative to an unmutated individual) that will decrease population mean fitness{Schultz, 1997 #298;Sprouffske, 2018 #86}. Whether urban species in fact suffer a demographic decline depends on several factors including the strength of selection, N_e, and generation time (Fig. 2). Keightley{, 2012 #87} estimated that the decline in human fitness due to mutation could reach 0.01% per generation, and the decline would change linearly with changes in mutation rate. This estimate does not include the countering force of purifying selection. It is therefore likely that organisms with long generation times will experience little effect on population mean fitness in the short term. Conversely, organisms with short generation times (e.g., microbes), may experience changes in fitness over contemporary time-scales.

Although evolutionary responses depend on inherited germline mutations, somatic mutations also have important consequences for the health and fitness of individuals that contribute to long-term population viability. In multicellular organisms, somatic mutations can create a mosaic of cells with slightly different genotypes{Pineda-Krch, 2004 #323}. These mutations can lead to developmental instability, which is particularly detrimental in organisms with strict body plans like animals (Table 2){Doonan, 2010 #322}. The genomic diversity within an individual can also produce competition

among cell lineages that can be harmful, as in the case with cancers. There is also clear evidence for intra-organismal selection for healthy cell lineages that can reduce the overall impact of deleterious mutation, including in marine tunicates, and long lived perennial plants{Doonan, 2010 #322;Pineda-Krch, 2004 #323}. These different phenomena hint at complex interactions between development, life history, and genetic systems when determining the relative impact of elevated somatic mutation rates in urban settings. Given the evidence that urban habitats experience elevated concentrations of numerous mutagens (Table 1), the impact of somatic mutation may become very important to predicting the sustainability of some urban populations (see *Applied Impacts*).

Theory generally predicts an advantage for reduced mutation rates because most non-neutral mutations will be deleterious{Jiang, 2010 #88;Sniegowski, 2000 #325}. Therefore, we might expect that urban populations will be under selection to reduce mutation rates in the presence of mutagens. The ability and time it takes for selection to reduce mutation rates will depend on numerous factors such as the mating system, N_e, and target size (i.e., amount of nucleotide sequence that can reduce mutation rate) for mutation modifiers{Wei, 2022 #89}. The drift-barrier hypothesis{Lynch, 2010 #90} predicts that directional selection will reduce mutation rates until a point at which the strength of genetic drift ($1/N_e$) overcomes the selective advantage (s) of smaller improvements in mutation rate (when $N_e s < 1$). This hypothesis is supported by recent comparative genomic analyses that show that species with higher long-term N_e, and shorter generation times, tend to have lower mutation rates per generation{Bergeron, 2023 #91}. There is an equilibrium point beyond which if mutation rates are sufficiently high, selection to reduce the mutation rate should overcome drift. Nevertheless, if urban environments reduce an organism's N_e, resulting in a loss of genetic diversity{Johnson, 2017 #3562}, we may expect a higher equilibrium mutation rate.

Despite the genetic load created by deleterious mutations, mutation also provides the raw variation necessary for adaptation. These contrasting effects of mutations lead to the possibility that mutation-fuelled adaptation can result in a so-called "evolutionary rescue"{Carlson, 2014 #92;Sprouffske, 2018

#86} (i.e., an increase in population growth rate of small populations due to adaptation) of populations subject to environmental challenges in urban environments (Fig. 2). For example, pathogens whose fitness in a new host is so low as to preclude persistence may benefit from higher mutation rates, where the higher the mutation rate the larger the probability of evolutionary rescue{Metzgar, 2000 #324}. However, this situation is highly context dependent, because once a population approaches its fitness optimum, the benefit of new mutations disappears, and any new mutations are likely to be deleterious. It is reasonable to speculate that urban environments will pose such strong selective pressures that some populations will benefit from elevated mutational input during initial establishment (Fig. 2). The extent to which mutation will provide variation to tackle new selective challenges will depend on how elevated the mutation rate is in urban areas, how close a population is to a fitness optimum (i.e., selection strength), N_e, and generation time (Fig. 2). If elevated mutation rates have beneficial implications for species colonizing urban environments, it may also mean that cities could facilitate rapid adaptation to pesticides, herbicides, and antibiotics, or provide the raw variation needed for pathogens to switch hosts.

It is plausible that elevated patterns of mutation in cities could facilitate speciation, especially if mutations induced by urban pollution causes large-scale chromosomal abnormalities that affect mating incompatibilities. Elevated mutation rates within cities could not only lead to population divergence among urban and non-urban populations due to local adaptation, but also as a result of accelerated genetic drift due to population fragmentation{Thompson, 2018 #4191}. Under these conditions higher mutation rate in urban settings would increase the possibility of generating mutations that are compatible with population-specific local alleles at other loci, but incompatible with alleles in populations adapted to non-urban environments. Alleles that are only compatible with the genetic background they arose in are called “Bateson-Dobzhansky-Muller incompatibilities”, and are thought to form the genetic basis of speciation{Orr, 2001 #326}. Such incompatibilities may be particularly likely to occur if urban pollutants increase the frequency of large structural mutations, including

inversions, translocations, polyploidy, or elevated activity of transposable elements, since these types of large-scale structural mutations are frequently associated with genes that influence reproductive isolation{Van Drunen, #285}. Even in the absence of reproductive isolation, reduced vigour of urban and non-urban hybrids could potentially alter the fitness of nearby populations. In general, we may expect that elevated mutation in urban areas to lead to increased divergence, and potentially speciation, via both adaptive and non-adaptive processes{Thompson, 2018 #4191}. We believe cities offer unique opportunities to study the process of speciation across a myriad of taxa in real-time.

Applied impacts

Given that urbanization can increase mutation rates, we expect numerous applied consequences associated with the health and conservation of organisms inhabiting cities. The anticipated health effects of humans and nonhuman species include cancers and other diseases linked to somatic and germline mutations. The conservation consequences relate to how elevated mutation rates are expected to influence the fitness and long-term population growth of urban-dwelling species (Fig. 2).

Urban pollution causes numerous types of cancer in humans and other organisms. Contemporary urban pollution elevates lung{Guo, 2019 #106;Yu, 2015 #51}, breast{Dey, 2010 #101} and other forms of cancer{Ayuso-Álvarez, 2020 #96} by 10% to 1000% above baseline incidence rates (Table 2). The magnitude of these effects varies among cities and over time because of variation in the types and concentrations of specific pollutants (Fig. 1). Admittedly, most research on the health effects of urban pollution has been done on humans. How urban pollution affects somatic mutations and cancers in nonhumans is poorly understood outside of lab settings and represents an important gap in knowledge{Giraudeau, 2018 #327;Sepp, 2019 #328;Baines, 2021 #355} (see *Future Directions*). Although heritable germline mutations have the potential to magnify cancer risk in offspring due to pollution exposure in parents, there is currently no evidence outside of the lab of environmentally-

induced heritable mutations causing cancer, even for ionizing radiation{Marchetti, 2020 #68;Yeager, 2021 #4388;Mulvihill, 2012 #299}. However, observational studies of birds{Yauk, 1996 #3746}, and laboratory studies of rodents{Somers, 2004 #3699;Somers, 2002 #3700}, confirm that air pollution from steel mills can induce heritable germline mutations in repetitive DNA regions, which suggests that urban induced cancers could be inherited. Understanding how, when, and where urban pollution leads to inherited mutations that influence cancer risk is an important goal for future research (see *Future Directions*).

Multiple socio-ecological factors associated with urban lifestyles could interact with pollution to elevate mutation rates. The previously-mentioned shift to older parental age among people in urban compared to rural communities is the best known cause of higher germline mutations in urban populations{Goldmann, 2016 #82}. Urban mutagenic pollution likely interacts with and amplifies this demographic effect on mutation rates. Human urban populations also exhibit increased rates of obesity and associated cancers due to a large proportion of processed foods in urban diets and relatively sedentary lifestyles{Wang, 2022 #349}. Herbivorous, omnivorous and predatory wildlife species also exhibit altered diets in cities that incorporate more anthropogenic food sources such as sugar, corn and wheat. Such diet shifts have been linked to higher body mass and hyperglycemia in some species{Gámez, 2022 #356;Lyons, 2017 #357;Schulte-Hostedde, 2018 #358}. Food additives and contaminants in processed foods may influence germline mutation rates{Kliemann, 2022 #107}, as could shifts in urban gut microbiomes{Winglee, 2017 #115}. Exposure to environmental pollutants and lack of access to high-quality diets may be biased towards certain urban demographics. Thus, analyzing urban mutagenesis and other evolutionary processes is an important step to address concerns about environmental justice{Schell, 2020 #110;Des Roches, 2020 #336;Verrelli, 2022 #4482}.

Elevated mutation rates in cities have the potential to influence the dynamics of urban populations (Fig. 2). Given that most mutations are deleterious, it is likely that urban induced mutations will frequently have negative effects on individual fitness and the growth rate of populations{Schultz, 1997

#298;Sprouffske, 2018 #86}. Whether such negative demographic effects will be sufficiently large to outweigh the influence of other factors affecting populations requires careful quantification and modelling. We expect that urban pollution induced mutational load will be one of many factors threatening the persistence of populations, and may become a conservation concern for rare or declining native species in cities. By contrast, we predict that populations of pests and other organisms that maintain large populations are less likely to be negatively affected by elevated mutation rates.

It is unlikely that urban-induced mutations will positively influence conservation through evolutionary rescue for most species. Only organisms with rapid generation times and high N_e are expected to experience positive long-term fitness effects of elevated mutation rates in cities, and even then, only when selection pressures are strong (Fig. 2). Such scenarios are most likely to apply to viruses, bacteria and some eukaryotic microbes (e.g., yeast, algae), raising the possibility that elevated mutation rates in cities could promote the spread of pathogenic organisms{Metzgar, 2000 #324}. Field and lab experiments that examine how urban induced changes in mutations rates affect known and emerging diseases and pests could have important implications for public health.

Future Directions

Our Perspective illustrates that water, soil and air pollution in urban areas increases mutation rates, but the magnitude and mutational spectrum of this increase, as well as its ecological and evolutionary consequences, remain unresolved. These gaps represent important problems requiring attention, which we outline as research questions below.

What is the magnitude of increase in somatic and germline mutation rates and what are the types of mutations caused by urban pollution?

While it is important to refine how somatic mutations rates are influenced by urban pollution, the greatest need remains establishing whether, and under what circumstances, urban pollution causes

412 germline mutations in wild populations{Marchetti, 2020 #68}. Conventional genomic technologies are
413 poorly suited for quickly surveying the mutagenic properties of changing environments like urban
414 areas. New error-corrected sequencing approaches enable the study of rare mutations within a
415 heterogenous population of cells{Valentine III, 2020 #333;Marchetti, 2023 #81}. These methods can
416 facilitate more rapid and definitive tests of how urban pollution affect mutation rates because they all
417 but eliminate the need for extensive validation via additional molecular analyses conventionally needed
418 to confirm the accuracy of mutation calls.

419 **What are the fitness effects of urban induced mutations and how do these influence the ecology**
420 **and evolution of populations?**

421 Answering this question will require a combination of laboratory and field experiments, coupled with
422 genome sequencing. Laboratory experiments could establish how mutations caused by specific urban
423 pollutants influence individual fitness, population growth, and (mal)adaptation. Field experiments
424 could follow the fitness of individuals that exhibit the presence/absence of mutations. Such experiments
425 could be expanded upon by experimentally recreating mutations via transgenic or CRISPR
426 manipulations. Finally, identification of somatic and germline mutations from human and wild urban
427 populations of diverse organisms (Fig. 3) could be used to infer fitness and health effects based on how
428 the types and locations of mutations are expected to disrupt homeostasis using deep learning models of
429 DNA sequence evolution across thousands of species{Frazer, 2021 #359}.

430 **How do urban induced mutations vary among species?**

431 There is a need to expand the investigation of mutations caused by pollution to a wider diversity of
432 organisms beyond humans given the indiscriminate threats that urban pollutants are expected to have
433 on all species. We propose a global research programme that uses a range of organisms to be used as
434 biosentinels (i.e., organisms to assay mutations induced by pollution), where the species chosen would
435 vary in their relevance to humans, prevalence in urban areas, generation time, and genomic resources
436 (Fig. 3). Such a biosentinel programme is an important strategy that can detect mutagenic effects even

when specific mutagens are difficult to identify{Salk, 2020 #329;Du Four, 2005 #103}. Bacteria, plants, and human cell lines have all been proposed as urban biosentinels{Ceretti, 2015 #98}. *Salmonella* has been the vanguard biosentinel because it responds readily to both known and unknown mutagens{Claxton, 2004 #208}, and we see it as an ideal bacterial model moving forward (Fig. 3). Existing plant (*Arabidopsis*) and animal (*Drosophila*, *C. elegans*) model organisms offer a rich genomic tool kit, although given their marginal importance to humans and/or prevalence in urban areas, non-model organisms that have been the focus of studies in urban areas should also be included (e.g., white clover, dogs, various birds). Rodents, particularly house mouse (*Mus musculus*) and Norway rat (*Rattus norvegicus*), are important pests in urban areas that are commonly used in laboratories, offering a biosentinel model that more closely resembles human physiology{Claxton, 2007 #209}. The deployment of such biosentinels could provide a rapid and accurate view of how urban induced mutations affect the biology of urban-dwelling species, including humans.

Conclusions

Our Perspective highlights the potential broad ranging mutagenic effects of urban pollution on virtually all life that inhabits cities. These mutagenic effects are expected to influence the fitness, ecology, and evolution of wild populations, but these effects are largely unstudied outside of laboratory settings, and even there, only a small subset of species have been studied. Given the many mutagens that are prevalent in urban areas, and their potentially large impacts on human and wildlife fitness, we argue that the study of urban mutagenesis is in urgent need of attention and should be prioritized in future applied research in ecology and evolution.

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BOX 1

Genotoxicity of urban pollutants and induction of mutations

Chemical pollutants are the primary cause of DNA damage induced by urban pollution. Ionizing radiation is less common, but a more extreme mechanism of DNA damage in and around cities. When an organism is exposed to a chemical pollutant, it can cause DNA damage and mutation through several steps:

1. The pollutant can enter the cell via diffusion{Figueroa, 2013 #267} or receptor mediated endocytosis{Su, 2020 #268}.
2. Once inside the cell:
    a. Pollutants (e.g., polycyclic aromatic hydrocarbons) can form bonds with nitrogenous DNA bases resulting in DNA adducts{Yauk, 2016 #330}
    b. Presence and interaction of pollutants with cellular processes or proteins causes increases in reactive oxygen species (ROS) that can oxidize DNA and proteins{Cho, 2022 #269;Lakey, 2016 #270}.
3. Chemically induced DNA lesions may be subject to error-prone DNA repair processes that cause mutations, or if the amount of damage exceeds the cell's capacity for DNA repair, it can result in mutations or chromosome damage{Sasaki, 2020 #331}.
4. Air pollutants can also cause oxidative stress via chronic inflammation and subsequent formation of ROS{Humans, 2015 #30}.

Ionizing radiation and radiomimetic compounds can alter DNA sequence through a different mechanism:

1. Radiation directly deposits energy in DNA causing strand breaks, or it creates free radicals that damage DNA and proteins{Fucic, 2008 #64;Chauhan, 2021 #332}.
2. Free radical DNA damage includes apurinic/apyrimidinic sites and deamination of DNA bases (among others), both of which have unique mutagenic mechanisms{Ignatov, 2017 #307}
3. Lack of repair or error-prone repair of this damage can cause chromosomal aberrations and mutations.

Table 1 | Common urban chemical mutagens and carcinogens. For each pollutant we indicate the chemical species, most common anthropogenic source, medium in which the pollutant is typically encountered (i.e., air, water, soil), and references.

Pollutant	Chemical species	Sources	Medium	References
Particulate matter (PM)	$PM_{2.5}$ PM_{10}: inorganic ionic compounds, metal oxides, organic and elemental carbon	Combustion by-products from traffic and industrial emissions, residential heating, and reactions between pollutants	Air	{Humans, 2015 #30;Organization, 2016 #36}
Volatile organic compounds (VOCs)	Aldehydes, ketones, aromatics, and alkanes	Household products, building materials and combustion sources	Air	{David, 2021 #308;Humans, 2015 #30}
Polycyclic aromatic hydrocarbons (PAHs)	Examples include: Benzo[a]pyrene, Benzo[a]anthracene, chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene	Combustion by-products from industrial, residential and transport emissions	Air/water/soil	{Jameson, 2021 #301;Ravindra, 2008 #277;Abdel-Shafy, 2016 #274;Humans, 2010 #35}
Sulphur oxides (SO_x)	Sulphur dioxide (SO_2), sulphur trioxide (SO_3)	Fossil fuel combustion, other industrial processes	Air	{Humans, 2015 #30;Organization, 2016 #36}
Carbon monoxide (CO)	--	Fossil fuel combustion, transport emissions	Air	{Levy, 2015 #309;Organization, 2016 #36}
Nitrogen Oxides (NO_x)	Nitrous oxide (NO), Nitrogen dioxide (NO_2)	Transport and industrial emissions	Air	{Brook, 2007 #310;Organization, 2016 #36;Zhang,

				2021 #311}
Pesticides	Organophosphates, pyrethroids, carbamates, polychlorinated biphenyls (PCBs), polybrominated biphenyls, persistent organic pollutants	Pesticide use in urban areas	Water/soil	{Meftaul, 2020 #275}
Heavy metals	mercury, arsenic, cadmium, chromium, and lead	Industrial processes, mining	Water/soil	{Brook, 2007 #310;FAO, 2021 #37}
High salt	Salt (NaCl)	Road salting	Soil/water	{Li, 2014 #276}

Table 2 | Cancers associated with urban induced mutations. Examples of the most common cancers associated with urban induced mutations, including changes in rates of cancer in urban and non-urban populations. For each example we indicate the region of study, pollutant studied and description of findings.

Health effect	Region of study	Pollutant	Description of findings	Reference
Childhood cancers (leukaemia, neuroblastoma, renal and bone tumours)	Spain	Air pollution	Risk of cancer increased with decreased distance from industrial or urban area studied	{García-Pérez, 2019 #119;García-Pérez, 2015 #117;García-Pérez, 2016 #118}
Lung cancer	China	Particulate matter (PM_{10}: SO_2)	Lung cancer incidence and mortality increased with increased PM_{10}; SO_2 also positively correlated with cancer	{Chen, 2016 #120}
	USA	Particulate matter (PM_{10}: SO_2, ozone)	Lung cancer was most strongly correlated with PM_{10} exposure, followed by SO_2 and ozone in males; in females lung cancer correlated with SO_2, followed by PM_{10}	{Beeson, 1998 #121}
	Canada	Air pollution ($PM_{2.5}$)	$PM_{2.5}$ associated with increased risk of lung cancer	{Bai, 2017 #124;Gogna, 2019 #123}
	Sweden	Air pollution (NO_2)	NO_2 exposure correlated to increased lung cancer	{Nyberg, 2000 #125}

Stomach cancer	China	Soil pollution (heavy metals; Cd, Cr, Pb, Hg, As)	Heavy metals in soils correlated with higher stomach cancer incidence	{Fei, 2018 #122}
Breast cancer	USA	Air pollution (NO_x)	Increased risk of breast cancer following NO_x exposure in women living near major roads	{Cheng, 2020 #126}
Digestive system cancers	China	Water pollution	Large-scale study identifying covariation between decreasing water quality and increased incidence of digestive cancers	{Ebenstein, 2012 #282}

Fig. 1 | Global concentrations and composition of mutagenic and carcinogenic pollutants. Concentrations of particulate matter that is 2.5 microns diameter or smaller ($PM_{2.5}$) across terrestrial Earth in 2019-2020, with inset panels illustrating how concentrations are frequently highest in and around cities{Wei, 2022 #305}. $PM_{2.5}$ concentrations have been changing through time (top right inset), increasing in some cities (e.g. Shanghai, China) and decreasing in others (e.g., New York, USA) {CIESIN, 2021 #304}. Pie charts show how composition of major carcinogenic pollutants (i.e., carbon monoxide [CO], volatile organic compounds [VOC], sulphur dioxide [SO_2], nitrous oxides [NOx], and ozone [O_3] in urban areas vary among countries{Wolf, 2022 #302;Wolf, 2022 #303}. High concentrations of $PM_{2.5}$ outside of urban areas are caused by a combination of anthropogenic sources such as long-distance dispersal of industrial pollution, burning of crops in agricultural regions, forest fires, and naturally occurring fine dust picked up by strong winds from bare soil, especially in arid regions (e.g., Saharan and Sub-Saharan Africa).

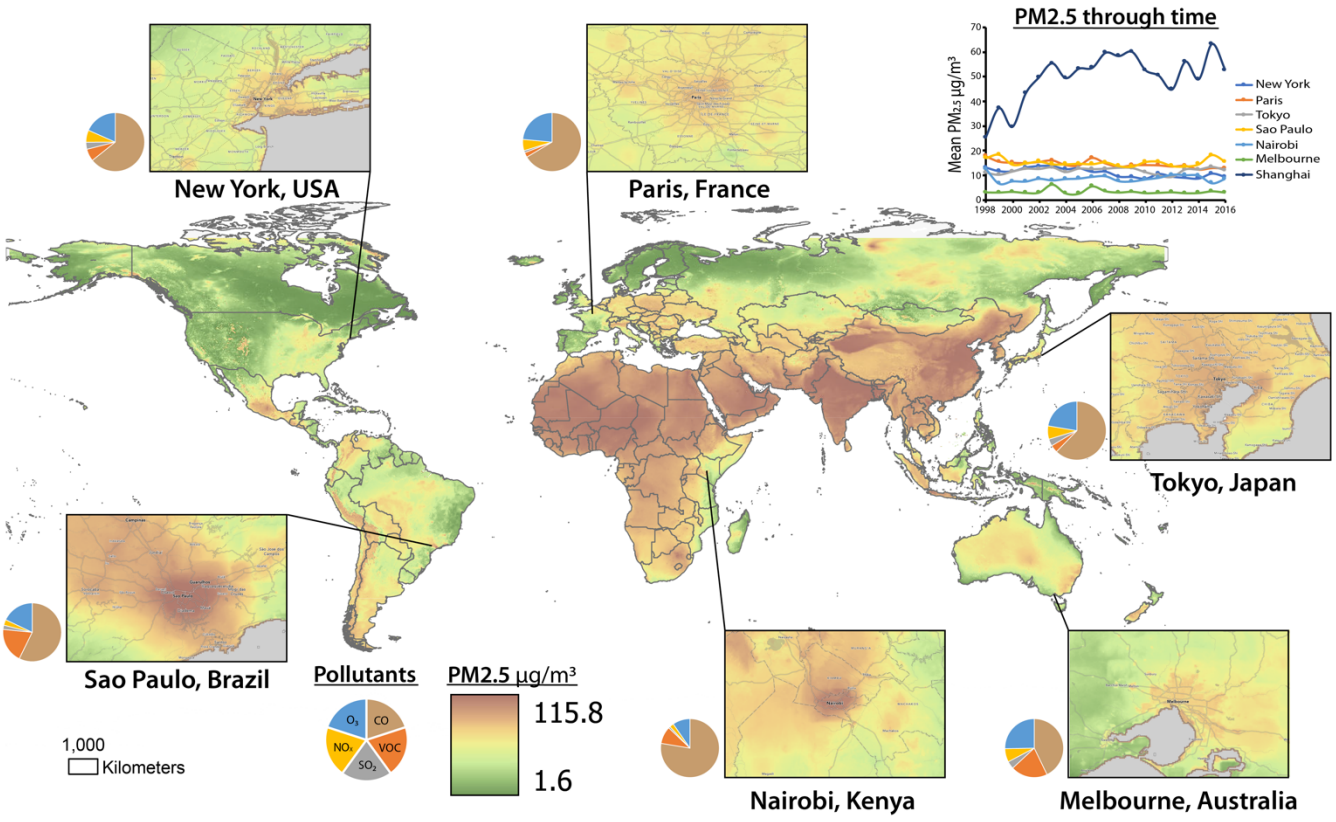

Fig. 2 | The potential for elevated mutation rates in cities to affect evolution of a population relative to a fitness optimum. When a population starts at a fitness optimum (dotted horizontal black line) in an urban environment (blue lines), any increase in mutation rate will lead to a net increase in deleterious mutations within a population, moving the population further from a fitness optimum. If urban pollution elevates mutation rates in urban areas (i.e., high Δμ - solid blue line), then we expect a population will move further from a fitness optimum through time. If Δμ is low but still >0, then this effect will be relatively small. By contrast, when a population is initially maladapted to an urban environment (red lines), such that it starts far away from a fitness optimum, then higher mutation rates in urban areas (solid red line) can lead to rapid adaptation such that the population quickly evolves towards the fitness optimum. The rate of this evolution will be slower when Δμ is lower (red dashed line). Such adaptive evolution could lead to evolutionary rescue, but such dynamics are only likely over contemporary time when *Ne* is high and generation times are fast (e.g., viruses, bacteria, eukaryotic microbes). At equilibrium, populations experiencing higher Δμ are expected to have lower fitness than those with lower Δμ because most new mutations will be deleterious when a population is close to its fitness optimum. A population may remain maladapted (scenario not shown) when *Ne* is low and there is long generation time, which could lead to extinction if population growth rates are negative.

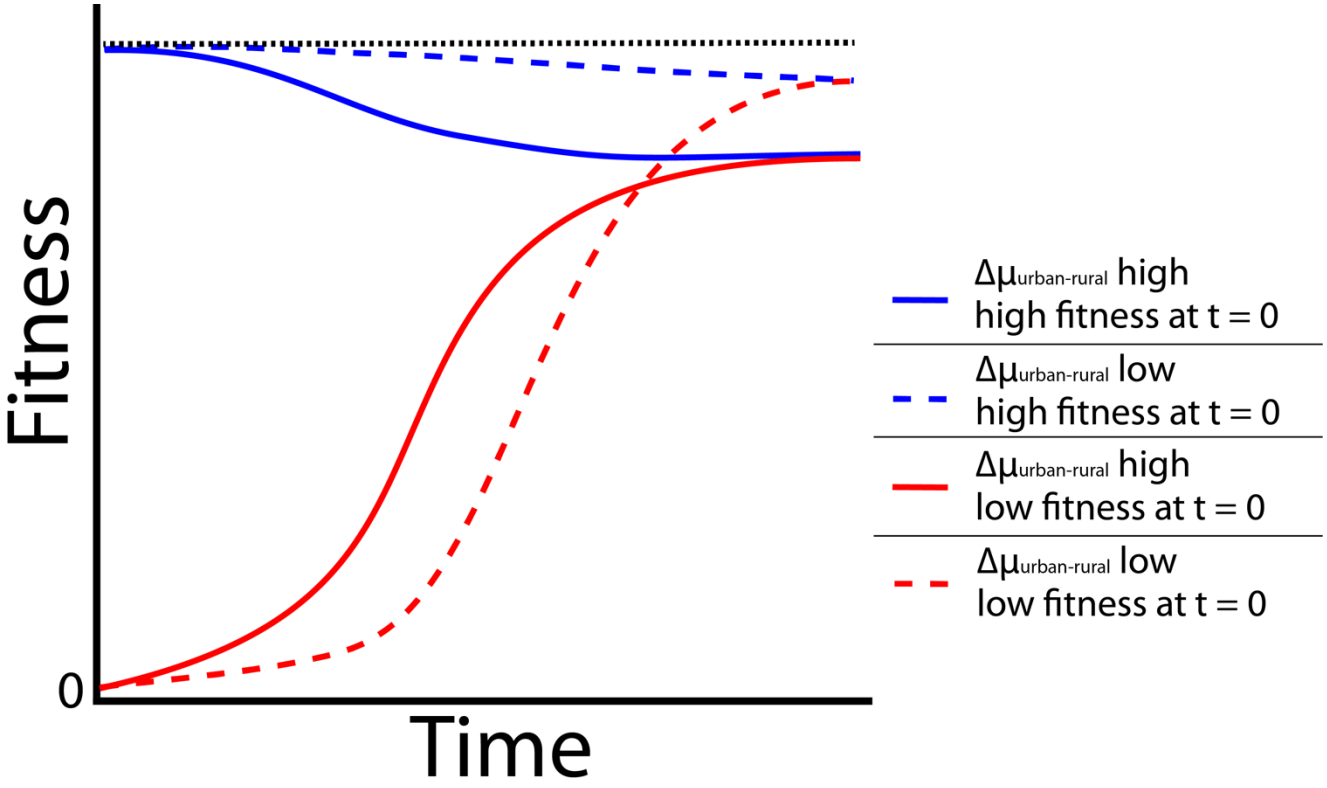

Fig. 3 | Potential biosentinel species for studying urban-associated mutations. Proposed biosentinels include: A) *Salmonella enterica*, B) *Caenorhabditis elegans*, C) *Drosophila melanogaster*, D) *Arabidopsis thaliana*, E) *Trifolium repens*, F) *Flavoparmelia caperata* (a lichen), G) *Fundulus heteroclitus*, H) *Passer domesticus*, I) *Columba livia*, J) *Mus musculus*, K) *Rattus norvegicus*, L) *Canis lupus familiaris*. An image of humans (*Homo sapiens*) is not shown but included on the schematics below. The species below represent a range of traditional laboratory model organisms used for studying genetic and evolutionary processes, as well as emerging models for studying ecological responses to pollution (e.g. lichen) or evolution in urban areas. Some species offer a combination of fast generation time and excellent genomic resources for mutagenic studies (bottom right panel), whereas others are more directly relevant to humans (i.e. with respect to health and well-being) and urbanization (i.e. there relative abundance in urban vs. nonurban habitats) given their commensal status with humans (bottom right panel).