

UC San Diego

UC San Diego Previously Published Works

Title

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

Permalink

<https://escholarship.org/uc/item/04t90475>

Journal

Nature Ecology & Evolution, 8(6)

ISSN

2397-334X

Authors

Johnson, Marc TJ

Arif, Irtaqa

Marchetti, Francesco

et al.

Publication Date

2024-06-01

DOI

10.1038/s41559-024-02401-z

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives License, available at

<https://creativecommons.org/licenses/by-nc-nd/4.0/>

Peer reviewed

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

Marc T. J. Johnson^{1,2}, Irtaqa Arif^{1,2}, Francesco Marchetti³, Jason Munshi-South⁴, Rob W. Ness^{1,2}, Marta Szulkin⁵, Brian C. Verrelli⁶, Carole L. Yauk⁷, Daniel N. Anstett⁸, Warren Booth⁹, Aude E. Caizergues^{1,2}, Elizabeth Carlen¹⁰, Anthony Dant¹¹, Josefa González¹², César González Lagos¹³, Madeleine Oman^{1,2}, Megan Phifer-Rixey¹⁴, Diana Rennison¹⁵, Michael S. Rosenberg⁶, Kristin M. Winchell¹⁶

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

Author for correspondence: marc.johnson@utoronto.ca

28 **Abstract**

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

42

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

52 One of the most consistent differences between urban and non-urban environments that could
53 influence mutation rates is higher chemical pollution. Transportation, industry, wastewater
54 management, home heating, landfills, and pesticide application are some of the activities in urban areas
55 commonly associated with elevated air, water and soil pollution {Organization, 2016 #36;FAO, 2021
56 #37;UNEP, 2016 #38}. Although less frequent in urban areas, nuclear plants, nuclear testing, and
57 warfare can also result in highly mutagenic ionizing radiation (e.g., Fukushima, Three Mile Island,
58 Hiroshima){Filburn, 2016 #13}. Studies on the mutagenic effects of radiation also provide general
59 insight into how highly mutagenic pollutants can influence organisms in cities. While pollution is not
60 unique to urban areas, the concentration and diversity of pollutants is often highest in cities (Fig. 1),
61 exposing organisms to harmful stressors in unprecedented ways {Organization, 2016 #36;FAO, 2021
62 #37;UNEP, 2016 #38}.

63 Urban chemical pollution can cause physiological and genotoxic stress to organisms that may result
64 in mutations. Such pollution is known to result in respiratory illnesses in humans {Seaton, 1995 #14},
65 reduced photosynthesis and cell damage in plants {Seyyednejad, 2011 #18}, higher mortality in fishes
66 and amphibians {Casey, 2005 #17}, and decreased fledgling success in birds {Chatelain, 2021 #16}.
67 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

93 **Urban pollutants and damage to DNA**

94 Air, water, and soil in cities are consistently associated with a diverse mixture of pollutants (Table 1,
95 Box 1). The sources of most outdoor air pollutants in cities are combustion by-products from
96 transportation, power generation, home heating and cooking, and industry {Leung, 2015
97 #34;Programme, 2017 #289}. These by-products include pollutants such as PAH, NO_x, sulphur
98 dioxide (SO₂), CO, and various metal species (e.g., Hg, Cu, Pb, Sn). These compounds can interact
99 with or bind to PM, which can then be deposited in soil {Baensch-Baltruschat, 2020 #33;Nirmalkar,
100 2021 #32;Humans, 2015 #30;Humans, 2010 #35}. Soil can also become contaminated with
101 genotoxicants from industrial by-products, manufacturing, mining, and road salting {Programme, 2017
102 #289}. Air pollutants, soil leaching, run-off, and sewage all contribute to water pollution {Martínez-
103 Bravo, 2019 #28}, which can lead to elevated levels of pesticides {Nagy, 2014 #27;Annabi, 2019 #26},
104 polychlorinated biphenyls (PCBs) {Agudo, 2016 #25}, pharmaceutical products {Chowdhury, 2020
105 #22;Isidori, 2005 #24;Metzler, 1998 #21}, and microplastics {Tagorti, 2022 #20;Roursgaard, 2022
106 #19;Programme, 2017 #289} in urban aquatic habitats.

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

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

125 The genotoxic effects of urban pollutants include chemical interactions that form DNA adducts (i.e.,
126 chemicals that bind to DNA) and reactive oxygen species that damage DNA (Box 1). When such
127 damage is improperly repaired it can cause small-scale and large-scale mutations. Small-scale
128 mutations include single nucleotide substitutions and small insertions/deletions (indels). Large-scale
129 mutations involve large indels, duplications, translocations, inversions, and aneuploidy {Iafrate, 2004
130 #139; Sebat, 2004 #140; Zhang, 2009 #141}. DNA replication errors such as unequal crossovers that can
131 result in gene duplication and deletion are also possible. The location of DNA damage (coding vs.
132 noncoding regions), the molecular function of damaged DNA (e.g., regulatory versus structural), and
133 whether coding mutations are synonymous or nonsynonymous, influence the molecular, physiological,
134 and fitness consequences of damage. The fitness effects of mutation can in turn impact the ecology and
135 evolution of populations {Griffiths, 2000 #144; Chu, 2019 #142; Scacheri, 2015 #143}.

136 The effects of urban-induced mutations may differ between species because of variation in ploidy,
137 cellular complexity, mutation rate, reproductive system, population size, and generation time. For
138 example, many animals, higher plants, and some eukaryotic microbes live primarily as diploids or
139 polyploids, which can mask the fitness effects of recessive mutations at low frequencies {Orr, 1995
140 #343; Otto, 2008 #345}. Similarly, many multicellular organisms have differentiated germ and somatic
141 cells, such that pollution induced mutations in somatic cells will not generally be passed on to

142 subsequent generations. In contrast, organisms with no distinction between germ and soma (e.g., some
143 plants and fungi), may accumulate inherited mutations more rapidly if mutations arise in the cells that
144 ultimately form gametic tissue{Anderson, 2018 #339;Burian, 2021 #337}. Moreover, mutation rates
145 vary by orders of magnitude, with bacteria and microbial eukaryotes having the lowest rates, vascular
146 plants and animals with moderate rates, and viruses with the highest mutation rates {Lynch, 2016
147 #342;Wang, 2023 #338}. Recombination in sexual organisms can allow efficient purging of harmful
148 mutations compared to asexual populations{Otto, 2009 #1806;Charlesworth, 2012 #2518}. Finally,
149 large populations with rapid generation times are expected to evolve to purge or fix environmentally
150 induced mutations that affect fitness more rapidly than small long-lived populations{Charlesworth,
151 2009 #2532}. In the sections that follow we expand on how such variation among species may lead to
152 different ecological and evolutionary consequences of urban induced mutations.

153 **Somatic mutations**

154 The primary consequence of genotoxic exposure is the induction of somatic mutations that can
155 adversely affect molecular, cellular and tissue function. Somatic mutations are not transmitted to the
156 next generation unless they occur in germ cell progenitors (e.g., plant apical meristems){Lanfear, 2018
157 #263}, and so they typically affect only the exposed individual's health and fitness. The causal role of
158 chemically induced mutations in cancer development is well known in certain cases (e.g., lung cancer
159 due to tobacco smoke){Hecht, 1999 #291} (Table 2). These examples show that exposure to
160 genotoxicants can cause mutations in tumour suppressor oncogenes that function as cancer drivers that
161 cause cellular proliferation and tumour development, or affect genes involved in DNA repair leading to
162 genetic instability{Foo, 2014 #292}. Moreover, exposure to mutagens during key life stages (e.g.,
163 embryogenesis, organogenesis) may increase the probability of clonal expansion of mutation-bearing
164 cells{Godschalk, 2020 #43;Whitham, 1981 #294;White, 2004 #56}. Data supporting the association
165 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

191 weight of evidence for the mutagenicity of outdoor air pollution is high, with many specific agents
192 declared ‘carcinogenic to humans’ by the International Agency for Research on Cancer
193 (IARC){Humans, 2015 #30}. IARC monographs thoroughly describe how these urban pollutants cause
194 mutagenicity in laboratory organisms as diverse as bacteria, plants and rodents{Ferreira, 2007
195 #48;Humans, 2015 #30}. For example, the mutation spectrum observed in lung tumours of non-
196 smokers associated with air pollution is broadly consistent with exposure to bulky DNA adduct-
197 forming chemicals (e.g., benzo[a]pyrene){DeMarini, 2001 #50;Yu, 2015 #51}. Additional evidence for
198 the mutagenicity of air pollution comes from humans exposed to high levels of combustion by-products
199 in residential and occupational settings, whereby individuals exhibit cytogenetic damage to various cell
200 types{Acito, 2022 #54;León-Mejía, 2019 #55}, and the urine from such individuals is mutagenic to
201 bacterial cells{e.g., Hansen, 2004 #52;Wong, 2021 #53}. Moreover, soil and sediments that contain
202 combustion-related contaminants are mutagenic to organisms that frequently come into contact with
203 these substrates (e.g., bacteria and plants){White, 2004 #56;Chen, 2004 #57}. Undoubtedly, inhabitants
204 of any urban ecosystem are exposed to mutagenic particulate pollutants associated with combustion
205 emissions.

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

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

216 2014 #317} and error-corrected sequencing{Kennedy, 2014 #315;Cho, 2023 #316} methodologies
217 exist, these have mostly been applied in clinical settings and have yet to be extended to studies on
218 environmental exposures in natural populations. The high levels of pollution in urban areas offer an
219 opportunity to address these obstacles using field experiments, in addition to laboratory experiments,
220 that apply genomic technologies to directly quantify mutation frequency and spectrum in a diverse
221 array of organisms (see *Future Directions*).

222

223 **Germline mutations**

224 Unlike somatic mutations, germline mutations are inherited between generations. For this reason, it is
225 primarily germline mutations that can influence the evolution of populations, with the exception of
226 somatic mutations that are then incorporated into reproductive tissue, which is most common in fungi
227 and plants{Lanfear, 2018 #263}. Although germline mutations are rare at the individual level, even the
228 smallest increase in mutation rate can have significant consequences for populations{Shendure, 2015
229 #67}.

230 Laboratory and field studies suggest that exposure to many common urban pollutants can induce
231 germline mutations. For example, over 80 chemical agents have been identified as germline mutagens
232 in lab mice{Marchetti, 2020 #68}. In humans, the best evidence of the impact of pollutants on germ
233 cell mutagenesis comes from studies demonstrating an increased incidence of chromosomal
234 abnormalities in human sperm{Marchetti, 2020 #68}. Such abnormalities may explain the significant
235 correlation between paternal blood dioxin levels due to occupational exposure and increased mutation
236 rates in their offspring{Ton, 2018 #77}. When considering exposure to radiation as an example of
237 extreme exposure to a mutagen, children of parents exposed to ionizing radiation following the
238 Chernobyl nuclear plant accident exhibited increased rates of tandem repeat mutations{Dubrova, 1996
239 #73}. Similar inherited mutations have been observed in plants{Kovalchuk, 1998 #74} and barn

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

248 In addition to pollution, urban and rural human populations diverge in their demographic patterns in
249 ways that are expected to influence germline mutation rates. In recent decades, there has been a trend
250 for delayed childbearing in many countries. In both developed and developing nations, this delay is
251 more pronounced in urban settings than in rural settings{Ely, 2018 #83;Lerch, 2019 #295}. Over the
252 course of the last decade, studies of human parents and offspring have consistently demonstrated an
253 age-related increase in mutation rates, especially in fathers{Goldmann, 2016 #82}. It is estimated that
254 fathers transmit ~1.2 additional mutations for each year of age, versus ~0.4 new mutations per year of
255 age in the mother. The higher paternal contribution is ascribed to the continuous production of sperm as
256 men age, while no new oocytes are generated once a female is born. The consistency of this divergence
257 between developed and developing nations requires further investigation, as a major source of
258 increased mutation rates could also result from differences in socio-cultural practices, economic
259 disparities, and racial demographics between urban and rural areas in cities throughout the world. There
260 is also evidence that non-human organisms exhibit demographic shifts in urban habitats{Merckx, 2018
261 #354}, but whether this is associated with changes in mutation rates requires investigation.

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

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

267

268 **Ecological and evolutionary consequences**

269 Alterations to the rate and spectrum of both somatic and germline mutations due to urban pollution
270 could have important ecological and evolutionary repercussions. Theoretical and empirical studies
271 show that the majority of new functionally significant mutations are deleterious and removed by
272 purifying selection {Eyre-Walker, 2007 #84}. If deleterious mutations are elevated in urban settings,
273 either due to a higher rate or as a larger fraction of deleterious mutations, we expect an increased
274 mutation load (i.e., reduced fitness due to the burden of deleterious mutations relative to an unmutated
275 individual) that will decrease population mean fitness {Schultz, 1997 #298; Sprouffske, 2018 #86}.

276 Whether urban species in fact suffer a demographic decline depends on several factors including the
277 strength of selection, N_e , and generation time (Fig. 2). Keightley {, 2012 #87} estimated that the decline
278 in human fitness due to mutation could reach 0.01% per generation, and the decline would change
279 linearly with changes in mutation rate. This estimate does not include the countering force of purifying
280 selection. It is therefore likely that organisms with long generation times will experience little effect on
281 population mean fitness in the short term. Conversely, organisms with short generation times (e.g.,
282 microbes), may experience changes in fitness over contemporary time-scales.

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

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

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

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

314 #86} (i.e., an increase in population growth rate of small populations due to adaptation) of populations
315 subject to environmental challenges in urban environments (Fig. 2). For example, pathogens whose
316 fitness in a new host is so low as to preclude persistence may benefit from higher mutation rates, where
317 the higher the mutation rate the larger the probability of evolutionary rescue {Metzgar, 2000 #324}.
318 However, this situation is highly context dependent, because once a population approaches its fitness
319 optimum, the benefit of new mutations disappears, and any new mutations are likely to be deleterious.
320 It is reasonable to speculate that urban environments will pose such strong selective pressures that
321 some populations will benefit from elevated mutational input during initial establishment (Fig. 2). The
322 extent to which mutation will provide variation to tackle new selective challenges will depend on how
323 elevated the mutation rate is in urban areas, how close a population is to a fitness optimum (i.e.,
324 selection strength), N_e , and generation time (Fig. 2). If elevated mutation rates have beneficial
325 implications for species colonizing urban environments, it may also mean that cities could facilitate
326 rapid adaptation to pesticides, herbicides, and antibiotics, or provide the raw variation needed for
327 pathogens to switch hosts.

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

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

346

347 **Applied impacts**

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

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

363 induced heritable mutations causing cancer, even for ionizing radiation {Marchetti, 2020 #68;Yeager,
364 2021 #4388;Mulvihill, 2012 #299}. However, observational studies of birds {Yauk, 1996 #3746}, and
365 laboratory studies of rodents {Somers, 2004 #3699;Somers, 2002 #3700}, confirm that air pollution
366 from steel mills can induce heritable germline mutations in repetitive DNA regions, which suggests
367 that urban induced cancers could be inherited. Understanding how, when, and where urban pollution
368 leads to inherited mutations that influence cancer risk is an important goal for future research (see
369 *Future Directions*).

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

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

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

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

402

403 **Future Directions**

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

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

410 While it is important to refine how somatic mutations rates are influenced by urban pollution, the
411 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

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

449

450 **Conclusions**

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

458 **Acknowledgements**

459 The ideas for this Perspective were developed over several workshops and meetings, including the
460 Urban Eco-Evo Research Coordination Network (NSF DEB-184063), and the “Satellite Workshop on
461 Urban Evolutionary and Ecological ‘Omics’” funded by the Society of Molecular Biology and
462 Evolution, and the Center for Biological Data Science at Virginia Commonwealth University. M.

463 Johnson was supported by an NSERC Steacie Fellowship, a Canada Research Chair (CRC) and an
464 NSERC Discovery Grant. C. Yauk was supported by CRC, and along with F. Marchetti a Burroughs
465 Wellcome Fund Innovations in Regulatory Sciences Award. D. Anstett was supported by a Plant
466 Resilience Institute (PRI) Fellowship from Michigan State University. E. Carlen was funded by NSF
467 DBI-2109587 and the Living Earth Collaborative at Washington University in St. Louis. M. Phifer-
468 Rixey (DEB-2332998) and W. Booth (DEB-1754394) received funding from the National Science
469 Foundation. C. González-Lagos was funded by ANID PIA/BASAL FB0002. J. González by grant
470 PID2020-115874GB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by grant 2021 SGR
471 00417 funded by Departament de Recerca i Universitats, Generalitat de Catalunya. M. Szulkin was
472 supported by NCN Opus grant 2021/41/B/NZ8/04472.
473

474 **References**

- 475 1 MacLean, R. C., Torres-Barceló, C. & Moxon, R. Evaluating evolutionary models of stress-
476 induced mutagenesis in bacteria. *Nature Reviews Genetics* **14**, 221-227 (2013).
- 477 2 Fitzgerald, D. M., Hastings, P. & Rosenberg, S. M. Stress-induced mutagenesis: implications in
478 cancer and drug resistance. *Annual Review of Cancer Biology* **1**, 119-140 (2017).
- 479 3 Lynch, M. *et al.* Genetic drift, selection and the evolution of the mutation rate. *Nature Reviews*
480 *Genetics* **17**, 704-714 (2016).
- 481 4 Bergeron, L. A. *et al.* Evolution of the germline mutation rate across vertebrates. *Nature* **615**, 285-
482 291 (2023).
- 483 5 Fenster, C. B. & Murren, C. J. *Evolutionary Ecology* **34**, 311-314 (2020).
- 484 6 Somers, C. M., McCarry, B. E., Malek, F. & Quinn, J. S. Reduction of particulate air pollution
485 lowers the risk of heritable mutations in mice. *Science* **304**, 1008-1010 (2004).
- 486 7 Yauk, C. L. & Quinn, J. S. Multilocus DNA fingerprinting reveals high rate of heritable genetic
487 mutation in herring gulls nesting in an industrialized urban site. *Proc. Natl. Acad. Sci. U.S.A.* **93**,
488 12137-12141 (1996).
- 489 8 World Health Organization. *Ambient Air Pollution: A Global Assessment of Exposure and Burden*
490 *of Disease*. <https://iris.who.int/handle/10665/250141> (World Health Organization, 2016).
- 491 9 FAO and UNEP. *Global Assessment of Soil Pollution – Summary for Policy Makers*.
492 <https://doi.org/10.4060/cb4827en> (2021).
- 493 10 UNEP. *A Snapshot of the World's Water Quality: Towards a Global Assessment*.
494 <https://www.unep.org/resources/publication>. (United Nations Environment Programme, 2016).
- 495 11 Filburn, T., Bullard, S. & Bullard, S. G. *Three Mile Island, Chernobyl and Fukushima*. (Springer,
496 2016).
- 497 12 Seaton, A., Godden, D., MacNee, W. & Donaldson, K. Particulate air pollution and acute health
498 effects. *The Lancet* **345**, 176-178 (1995).
- 499 13 Seyyednejad, S., Niknejad, M. & Koochak, H. A review of some different effects of air pollution
500 on plants. *Research Journal of Environmental Sciences* **5**, 302 (2011).
- 501 14 Casey, R., Shaw, A., Massal, L. & Snodgrass, J. Stormwater retention ponds in suburban
502 Maryland, USA. *Bull. Environ. Contam. Toxicol* **74**, 273-280 (2005).
- 503 15 Chatelain, M. *et al.* Urban metal pollution explains variation in reproductive outputs in great tits
504 and blue tits. *Science of the Total Environment* **776**, 145966 (2021).
- 505 16 Claxton, L. D. & Woodall Jr, G. M. A review of the mutagenicity and rodent carcinogenicity of
506 ambient air. *Mutation Research/Reviews in Mutation Research* **636**, 36-94 (2007).
- 507 17 White, P. A. & Claxton, L. D. Mutagens in contaminated soil: a review. *Mutation*
508 *Research/Reviews in Mutation Research* **567**, 227-345 (2004).
- 509 18 IARC. Outdoor Air Pollution. *IARC Monographs on the Evaluation of Carcinogenic Risks to*
510 *Humans* **109**, 1-454 (WHO Press, 2016).

- 511 19 Marchetti, F., Douglas, G. R. & Yauk, C. L. A return to the origin of the EMGS: rejuvenating the
512 quest for human germ cell mutagens and determining the risk to future generations. *Environmental*
513 *and Molecular Mutagenesis* **61**, 42-54 (2020).
- 514 20 Bromham, L., Hua, X., Lanfear, R. & Cowman, P. F. Exploring the relationships between mutation
515 rates, life history, genome size, environment, and species richness in flowering plants. *American*
516 *Naturalist* **185**, 507-524 (2015).
- 517 21 Diamond, S. E. & Martin, R. A. Evolution in cities. *Annual Review of Ecology, Evolution, and*
518 *Systematics* **52**, 519-540 (2021).
- 519 22 Johnson, M. T. J. & Munshi-South, J. Evolution of life in urban environments. *Science* **358**,
520 aam8327 (2017).
- 521 23 Szulkin, M., Munshi-South, J. & Charmantier, A. (Oxford University Press, 2020).
- 522 24 Verrelli, B. C. *et al.* A global horizon scan for urban evolutionary ecology. *Trends in Ecology &*
523 *Evolution* **37**, 1006-1019 (2022).
- 524 25 Yang, H.-H., Lai, S.-O., Hsieh, L.-T., Hsueh, H.-J. & Chi, T.-W. Profiles of PAH emission from
525 steel and iron industries. *Chemosphere* **48**, 1061-1074 (2002).
- 526 26 Hajat, A., Hsia, C. & O'Neill, M. S. Socioeconomic disparities and air pollution exposure: a global
527 review. *Current Environmental Health Reports* **2**, 440-450 (2015).
- 528 27 Kim, K. *et al.* Inequalities in urban greenness and epigenetic aging: different associations by race
529 and neighborhood socioeconomic status. *Science Advances* **9**, eadf8140 (2023).
- 530 28 Leung, D. Y. Outdoor-indoor air pollution in urban environment: challenges and opportunity.
531 *Frontiers in Environmental Science* **2**, 69 (2015).
- 532 29 UNEP. *Towards a Pollution-Free Planet: Background Report*.
533 <https://www.unep.org/resources/publication> (United Nations Environment Programme, 2017).
- 534 30 Baensch-Baltruschat, B., Kocher, B., Stock, F. & Reifferscheid, G. Tyre and road wear particles
535 (TRWP)-A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in
536 the environment. *Science of the Total Environment* **733**, 137823 (2020).
- 537 31 Nirmalkar, J., Haswani, D., Singh, A., Kumar, S. & Raman, R. S. Concentrations, transport
538 characteristics, and health risks of PM2.5-bound trace elements over a national park in central
539 India. *Journal of Environmental Management* **293**, 112904 (2021).
- 540 32 IARC. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures.
541 *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* **92**, 1-868 (WHO Press,
542 2010).
- 543 33 Martínez-Bravo, M. & Martínez-del-Río, J. Urban pollution and emission reduction. *Sustainable*
544 *Cities and Communities. Encyclopedia of the UN Sustainable Development Goals*, 1-11 (2019).
- 545 34 Nagy, K., Rácz, G., Matsumoto, T., Ádány, R. & Ádám, B. Evaluation of the genotoxicity of the
546 pyrethroid insecticide phenothrin. *Mutation Research/Genetic Toxicology and Environmental*
547 *Mutagenesis* **770**, 1-5 (2014).
- 548 35 Annabi, E., Ben Salem, I. & Abid-Essefi, S. Acetamiprid, a neonicotinoid insecticide, induced
549 cytotoxicity and genotoxicity in PC12 cells. *Toxicology Mechanisms and Methods* **29**, 580-586
550 (2019).
- 551 36 Agudo, A. *et al.* *Polychlorinated Biphenyls and Polybrominated Biphenyls*. (WHO Press, 2016).
- 552 37 Chowdhury, J., Mandal, T. K. & Mondal, S. Genotoxic impact of emerging contaminant
553 amoxicillin residue on zebra fish (*Danio rerio*) embryos. *Heliyon* **6** (2020).
- 554 38 Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, L. & Parrella, A. Toxic and genotoxic
555 evaluation of six antibiotics on non-target organisms. *Science of the Total Environment* **346**, 87-98
556 (2005).
- 557 39 Metzler, M., Kulling, S. E., Pfeiffer, E. & Jacobs, E. Genotoxicity of estrogens. *Zeitschrift für*
558 *Lebensmitteluntersuchung und-Forschung A* **206**, 367-373 (1998).

- 559 40 Tagorti, G. & Kaya, B. Genotoxic effect of microplastics and COVID-19: The hidden threat.
560 *Chemosphere* **286**, 131898 (2022).
- 561 41 Roursgaard, M. *et al.* Genotoxicity of particles from grinded plastic items in Caco-2 and HepG2
562 cells. *Frontiers in Public Health* **10**, 906430 (2022).
- 563 42 Iafrate, A. J. *et al.* Detection of large-scale variation in the human genome. *Nature Genetics* **36**,
564 949-951 (2004).
- 565 43 Sebat, J. *et al.* Large-scale copy number polymorphism in the human genome. *Science* **305**, 525-
566 528 (2004).
- 567 44 Zhang, F., Gu, W., Hurles, M. E. & Lupski, J. R. Copy number variation in human health, disease,
568 and evolution. *Annual Review of Genomics and Human Genetics* **10**, 451-481 (2009).
- 569 45 Griffiths, W., Miller, A., Suzuki, J., Lewontin, D. & Gelbart, R. Chapter 14—Mutation, repair, and
570 recombination. *An Introduction to Genetic Analysis* (WH Freeman and Company, 2000).
- 571 46 Chu, D. & Wei, L. Nonsynonymous, synonymous and nonsense mutations in human cancer-related
572 genes undergo stronger purifying selections than expectation. *BMC Cancer* **19**, 1-12 (2019).
- 573 47 Scacheri, C. A. & Scacheri, P. C. Mutations in the non-coding genome. *Current Opinion in*
574 *Pediatrics* **27**, 659 (2015).
- 575 48 Orr, H. A. Somatic mutation favors the evolution of diploidy. *Genetics* **139**, 1441-1447 (1995).
- 576 49 Otto, S. P. & Gerstein, A. C. The evolution of haploidy and diploidy. *Curr. Biol.* **18**, R1121-1124
577 (2008).
- 578 50 Anderson, J. B. *et al.* Clonal evolution and genome stability in a 2500-year-old fungal individual.
579 *Proc. R. Soc. B* **285**, 20182233 (2018).
- 580 51 Burian, A. Does shoot apical meristem function as the germline in safeguarding against excess of
581 mutations? *Front. Plant Sci.* **12**, 707740 (2021).
- 582 52 Lynch, M. *et al.* Genetic drift, selection and the evolution of the mutation rate. *Nat. Rev. Genet.* **17**,
583 704-714 (2016).
- 584 53 Wang, Y. & Obbard, D. J. Experimental estimates of germline mutation rate in eukaryotes: a
585 phylogenetic meta-analysis. *Evol Lett* **7**, 216-226 (2023).
- 586 54 Otto, S. P. The evolutionary enigma of sex. *American Naturalist* **174**, S1-S14, (2009).
- 587 55 Charlesworth, B. The effects of deleterious mutations on evolution at linked sites. *Genetics* **190**, 5-
588 22, (2012).
- 589 56 Charlesworth, B. Effective population size and patterns of molecular evolution and variation.
590 *Nature Reviews Genetics* **10**, 195-205 (2009).
- 591 57 Lanfear, R. Do plants have a segregated germline? *PLoS Biology* **16**, e2005439 (2018).
- 592 58 Hecht, S. S. Tobacco smoke carcinogens and lung cancer. *Journal of the National Cancer Institute*
593 **91**, 1194-1210 (1999).
- 594 59 Foo, J. & Michor, F. Evolution of acquired resistance to anti-cancer therapy. *Journal of*
595 *Theoretical Biology* **355**, 10-20 (2014).
- 596 60 Godschalk, R. W., Yauk, C. L., van Benthem, J., Douglas, G. R. & Marchetti, F. In utero exposure
597 to genotoxicants leading to genetic mosaicism: An overlooked window of susceptibility in genetic
598 toxicology testing? *Environmental and Molecular Mutagenesis* **61**, 55-65 (2020).
- 599 61 Whitham, T. G. & Slobodchikoff, C. Evolution by individuals, plant-herbivore interactions, and
600 mosaics of genetic variability: the adaptive significance of somatic mutations in plants. *Oecologia*
601 **49**, 287-292 (1981).
- 602 62 Schumacher, B., Pothof, J., Vijg, J. & Hoeijmakers, J. H. The central role of DNA damage in the
603 ageing process. *Nature* **592**, 695-703 (2021).
- 604 63 Li, C. & Williams, S. M. Human somatic variation: it's not just for cancer anymore. *Current*
605 *Genetic Medicine Reports* **1**, 212-218 (2013).

- 606 64 Claxton, L. D., de A. Umbuzeiro, G. & DeMarini, D. M. The *Salmonella* mutagenicity assay: the
607 stethoscope of genetic toxicology for the 21st century. *Environmental Health Perspectives* **118**,
608 1515-1522 (2010).
- 609 65 Claxton, L. D., Matthews, P. P. & Warren, S. H. The genotoxicity of ambient outdoor air, a
610 review: *Salmonella* mutagenicity. *Mutation Research/Reviews in Mutation Research* **567**, 347-399
611 (2004).
- 612 66 Chen, G. & White, P. A. The mutagenic hazards of aquatic sediments: a review. *Mutation
613 Research/Reviews in Mutation Research* **567**, 151-225 (2004).
- 614 67 Olivier, M., Hussain, S. P., Caron de Fromentel, C., Hainaut, P. & Harris, C. C. TP53 mutation
615 spectra and load: a tool for generating hypotheses on the etiology of cancer. *IARC Scientific
616 Publications*, 247-270 (2004).
- 617 68 Alexandrov, L. B. *et al.* The repertoire of mutational signatures in human cancer. *Nature* **578**, 94-
618 101 (2020).
- 619 69 Ferreira, M. I., Domingos, M., Gomes, H. d. A., Saldiva, P. H. & De Assuncao, J. V. Evaluation of
620 mutagenic potential of contaminated atmosphere at Ibirapuera Park, São Paulo–SP, Brazil, using
621 the *Tradescantia* stamen-hair assay. *Environmental Pollution* **145**, 219-224 (2007).
- 622 70 DeMarini, D. M. *et al.* Lung tumor KRAS and TP53 mutations in nonsmokers reflect exposure to
623 PAH-rich coal combustion emissions. *Cancer Research* **61**, 6679-6681 (2001).
- 624 71 Yu, X.-J. *et al.* Characterization of somatic mutations in air pollution-related lung cancer.
625 *EBioMedicine* **2**, 583-590 (2015).
- 626 72 Acito, M., Fatigoni, C., Villarini, M. & Moretti, M. Cytogenetic effects in children exposed to air
627 pollutants: A systematic review and meta-analysis. *International Journal of Environmental
628 Research and Public Health* **19**, 6736 (2022).
- 629 73 León-Mejía, G. *et al.* Cytotoxic and genotoxic effects in mechanics occupationally exposed to
630 diesel engine exhaust. *Ecotoxicology and Environmental Safety* **171**, 264-273 (2019).
- 631 74 Hansen, Å. M. *et al.* Urinary 1-hydroxypyrene and mutagenicity in bus drivers and mail carriers
632 exposed to urban air pollution in Denmark. *Mutation Research/Genetic Toxicology and
633 Environmental Mutagenesis* **557**, 7-17 (2004).
- 634 75 Wong, J. Y. *et al.* Elevated urinary mutagenicity among those exposed to bituminous coal
635 combustion emissions or diesel engine exhaust. *Environmental and Molecular Mutagenesis* **62**,
636 458-470 (2021).
- 637 76 Anderson, R. M. Cytogenetic biomarkers of radiation exposure. *Clinical Oncology* **31**, 311-318
638 (2019).
- 639 77 Da Cruz, A., McArthur, A., Silva, C., Curado, M. & Glickman, B. Human micronucleus counts are
640 correlated with age, smoking, and cesium-137 dose in the Goiania (Brazil) radiological accident.
641 *Mutation Research/Environmental Mutagenesis and Related Subjects* **313**, 57-68 (1994).
- 642 78 Geraskin, S., Evseeva, T. & Oudalova, A. Effects of long-term chronic exposure to radionuclides
643 in plant populations. *Journal of Environmental Radioactivity* **121**, 22-32 (2013).
- 644 79 Mousseau, T. A. & Møller, A. P. Genetic and ecological studies of animals in Chernobyl and
645 Fukushima. *Journal of Heredity* **105**, 704-709 (2014).
- 646 80 Lazutka, J. *et al.* Chromosomal aberrations and sister-chromatid exchanges in Lithuanian
647 populations: effects of occupational and environmental exposures. *Mutation Research/Genetic
648 Toxicology and Environmental Mutagenesis* **445**, 225-239 (1999).
- 649 81 Slozina, N., Neronova, E., Kharchenko, T. & Nikiforov, A. Increased level of chromosomal
650 aberrations in lymphocytes of Chernobyl liquidators 6–10 years after the accident. *Mutation
651 Research/Fundamental and Molecular Mechanisms of Mutagenesis* **379**, 121-125 (1997).
- 652 82 Fucic, A. *et al.* Genomic damage in children accidentally exposed to ionizing radiation: a review of
653 the literature. *Mutation Research/Reviews in Mutation Research* **658**, 111-123 (2008).

- 654 83 Jargin, S. Thyroid cancer after Chernobyl: re-evaluation needed. *Turk Patoloji Derg* **37**, 1-6
655 (2021).
- 656 84 Nikiforov, Y. E. Radiation-induced thyroid cancer: what we have learned from Chernobyl.
657 *Endocrine Pathology* **17**, 307-318 (2006).
- 658 85 FAO and WHO. Principles and methods for the risk assessment of chemicals in food.
659 *Environmental Health Criteria* 240. (Food and Agriculture Organization of the United Nations and
660 World Health Organization, 2020).
- 661 86 Eberwine, J., Sul, J.-Y., Bartfai, T. & Kim, J. The promise of single-cell sequencing. *Nature*
662 *Methods* **11**, 25-27 (2014).
- 663 87 Kennedy, S. R. *et al.* Detecting ultralow-frequency mutations by Duplex Sequencing. *Nature*
664 *Protocols* **9**, 2586-2606 (2014).
- 665 88 Cho, E. *et al.* Error-corrected duplex sequencing enables direct detection and quantification of
666 mutations in human TK6 cells with strong inter-laboratory consistency. *Mutation*
667 *Research/Genetic Toxicology and Environmental Mutagenesis* **889**, 503649 (2023).
- 668 89 Shendure, J. & Akey, J. M. The origins, determinants, and consequences of human mutations.
669 *Science* **349**, 1478-1483 (2015).
- 670 90 Ton, N. D. *et al.* Whole genome sequencing and mutation rate analysis of trios with paternal dioxin
671 exposure. *Human Mutation* **39**, 1384-1392 (2018).
- 672 91 Dubrova, Y. E. *et al.* Human minisatellite mutation rate after the Chernobyl accident. *Nature* **380**,
673 683-686 (1996).
- 674 92 Kovalchuk, I., Kovalchuk, O., Arkhipov, A. & Hohn, B. Transgenic plants are sensitive
675 bioindicators of nuclear pollution caused by the Chernobyl accident. *Nature Biotechnology* **16**,
676 1054-1059 (1998).
- 677 93 Ellegren, H., Lindgren, G., Primmer, C. R. & Møller, A. P. Fitness loss and germline mutations in
678 barn swallows breeding in Chernobyl. *Nature* **389**, 593-596 (1997).
- 679 94 Yeager, M. *et al.* Lack of transgenerational effects of ionizing radiation exposure from the
680 Chernobyl accident. *Science* **372**, 725-729 (2021).
- 681 95 Kessler, M. D. *et al.* De novo mutations across 1,465 diverse genomes reveal mutational insights
682 and reductions in the Amish founder population. *Proc. Natl. Acad. Sci. USA* **117**, 2560-2569
683 (2020).
- 684 96 King, L., De Solla, S., Small, J., Sverko, E. & Quinn, J. Microsatellite DNA mutations in double-
685 crested cormorants (*Phalacrocorax auritus*) associated with exposure to PAH-containing industrial
686 air pollution. *Environmental Science & Technology* **48**, 11637-11645 (2014).
- 687 97 Somers, C. M., Yauk, C. L., White, P. A., Parfett, C. L. & Quinn, J. S. Air pollution induces
688 heritable DNA mutations. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 15904-15907 (2002).
- 689 98 Ely, D. & Hamilton, B. Trends in fertility and mother's age at first birth among rural and
690 metropolitan counties: United States, 2007–2017 (NCHS Data Brief No. 323). *Hyattsville, MD:*
691 *National Center for Health Statistics, Centers for Disease Control and Prevention* (2018).
- 692 99 Lerch, M. Fertility decline in urban and rural areas of developing countries. *Population and*
693 *Development Review* **45**, 301-320 (2019).
- 694 100 Goldmann, J. M. *et al.* Parent-of-origin-specific signatures of de novo mutations. *Nature Genetics*
695 **48**, 935-939 (2016).
- 696 101 Eyre-Walker, A. & Keightley, P. D. The distribution of fitness effects of new mutations. *Nature*
697 *Reviews Genetics* **8**, 610-618 (2007).
- 698 102 Schultz, S. T. & Lynch, M. Mutation and extinction: the role of variable mutational effects,
699 synergistic epistasis, beneficial mutations, and degree of outcrossing. *Evolution* **51**, 1363-1371
700 (1997).
- 701 103 Sprouffs, K., Aguilar-Rodriguez, J., Sniegowski, P. & Wagner, A. High mutation rates limit
702 evolutionary adaptation in *Escherichia coli*. *PLoS Genetics* **14**, e1007324 (2018).

- 703 104 Keightley, P. D. Rates and fitness consequences of new mutations in humans. *Genetics* **190**, 295-
704 304 (2012).
- 705 105 Pineda-Krch, M. & Lehtilä, K. Costs and benefits of genetic heterogeneity within organisms.
706 *Journal of Evolutionary Biology* **17**, 1167-1177 (2004).
- 707 106 Doonan, J. H. & Sablowski, R. Walls around tumours—why plants do not develop cancer. *Nature*
708 *Reviews Cancer* **10**, 794-802 (2010).
- 709 107 Jiang, X. *et al.* Impacts of mutation effects and population size on mutation rate in asexual
710 populations: a simulation study. *BMC Evolutionary Biology* **10**, 1-13 (2010).
- 711 108 Sniegowski, P. D., Gerrish, P. J., Johnson, T. & Shaver, A. The evolution of mutation rates:
712 separating causes from consequences. *Bioessays* **22**, 1057-1066 (2000).
- 713 109 Wei, W. *et al.* Rapid evolution of mutation rate and spectrum in response to environmental and
714 population-genetic challenges. *Nature Communications* **13**, 4752 (2022).
- 715 110 Lynch, M. Evolution of the mutation rate. *Trends in Genetics* **26**, 345-352 (2010).
- 716 111 Carlson, S. M., Cunningham, C. J. & Westley, P. A. Evolutionary rescue in a changing world.
717 *Trends in Ecology & Evolution* **29**, 521-530 (2014).
- 718 112 Metzgar, D. & Wills, C. Evidence for the adaptive evolution of mutation rates. *Cell* **101**, 581-584
719 (2000).
- 720 113 Thompson, K. A., Rieseberg, L. H. & Schluter, D. Speciation and the city. *Trends in Ecology &*
721 *Evolution* **33**, 815-826 (2018).
- 722 114 Orr, H. A. & Turelli, M. The evolution of postzygotic isolation: accumulating Dobzhansky-Muller
723 incompatibilities. *Evolution* **55**, 1085-1094 (2001).
- 724 115 Van Drunen, W. E. & Johnson, M. T. J. Polyploidy in urban environments. *Trends in Ecology &*
725 *Evolution* **37**, 507-516.
- 726 116 Guo, H., Chang, Z., Wu, J. & Li, W. Air pollution and lung cancer incidence in China: Who are
727 faced with a greater effect? *Environment international* **132**, 105077 (2019).
- 728 117 Dey, S. *et al.* Urban–rural differences in breast cancer incidence in Egypt (1999–2006). *The Breast*
729 **19**, 417-423 (2010).
- 730 118 Ayuso-Álvarez, A. *et al.* Association between proximity to industrial chemical installations and
731 cancer mortality in Spain. *Environmental Pollution* **260**, 113869 (2020).
- 732 119 Giraudeau, M., Sepp, T., Ujvari, B., Ewald, P. W. & Thomas, F. Human activities might influence
733 oncogenic processes in wild animal populations. *Nature Ecology & Evolution* **2**, 1065-1070
734 (2018).
- 735 120 Sepp, T., Ujvari, B., Ewald, P. W., Thomas, F. & Giraudeau, M. Urban environment and cancer in
736 wildlife: available evidence and future research avenues. *Proc. R. Soc. B* **286**, 20182434 (2019).
- 737 121 Mulvihill, J. J. Preconception exposure to mutagens: medical and other exposures to radiation and
738 chemicals. *Journal of Community Genetics* **3**, 205-211 (2012).
- 739 122 Wang, L. *et al.* Association of ultra-processed food consumption with colorectal cancer risk among
740 men and women: results from three prospective US cohort studies. *The BMJ* **378**, e068921 (2022).
- 741 123 Kliemann, N. *et al.* Ultra-processed foods and cancer risk: from global food systems to individual
742 exposures and mechanisms. *British Journal of Cancer* **127**, 14-20 (2022).
- 743 124 Winglee, K. *et al.* Recent urbanization in China is correlated with a Westernized microbiome
744 encoding increased virulence and antibiotic resistance genes. *Microbiome* **5**, 1-13 (2017).
- 745 125 Schell, C. J. *et al.* The ecological and evolutionary consequences of systemic racism in urban
746 environments. *Science* **369**, eaay4497 (2020).
- 747 126 Des Roches, S. *et al.* Socio-eco-evolutionary dynamics in cities. *Evolutionary Applications* **14**,
748 248-267 (2020).
- 749 127 Valentine III, C. C. *et al.* Direct quantification of in vivo mutagenesis and carcinogenesis using
750 duplex sequencing. *Proceedings of the National Academy of Sciences USA* **117**, 33414-33425
751 (2020).

- 752 128 Marchetti, F. *et al.* Error-corrected next-generation sequencing to advance nonclinical genotoxicity
753 and carcinogenicity testing. *Nature Reviews Drug Discovery* **22**, 165-166 (2023).
- 754 129 Salk, J. J. & Kennedy, S. R. Next-generation genotoxicology: using modern sequencing
755 technologies to assess somatic mutagenesis and cancer risk. *Environmental and Molecular*
756 *Mutagenesis* **61**, 135-151 (2020).
- 757 130 Du Four, V., Janssen, C., Brits, E. & Van Larebeke, N. Genotoxic and mutagenic activity of
758 environmental air samples from different rural, urban and industrial sites in Flanders, Belgium.
759 *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **588**, 106-117 (2005).
- 760 131 Ceretti, E. *et al.* Monitoring of volatile and non-volatile urban air genotoxins using bacteria, human
761 cells and plants. *Chemosphere* **120**, 221-229 (2015).
- 762 132 Figueroa, X. F., Lillo, M. A., Gaete, P. S., Riquelme, M. A. & Sáez, J. C. Diffusion of nitric oxide
763 across cell membranes of the vascular wall requires specific connexin-based channels.
764 *Neuropharmacology* **75**, 471-478 (2013).
- 765 133 Su, R., Jin, X., Li, H., Huang, L. & Li, Z. The mechanisms of PM_{2.5} and its main components
766 penetrate into HUVEC cells and effects on cell organelles. *Chemosphere* **241**, 125127 (2020).
- 767 134 Yauk, C., Lambert, I., Marchetti, F. & Douglas, G. *Adverse outcome pathway on alkylation of*
768 *DNA in male pre-meiotic germ cells leading to heritable mutations*. (OECD Publishing, 2016).
- 769 135 Cho, E. *et al.* AOP report: Development of an adverse outcome pathway for oxidative DNA
770 damage leading to mutations and chromosomal aberrations. *Environmental and Molecular*
771 *Mutagenesis* **63**, 118-134 (2022).
- 772 136 Lakey, P. S. *et al.* Chemical exposure-response relationship between air pollutants and reactive
773 oxygen species in the human respiratory tract. *Scientific Reports* **6**, 32916 (2016).
- 774 137 Sasaki, J. C. *et al.* Application of the adverse outcome pathway framework to genotoxic modes of
775 action. *Environmental and Molecular Mutagenesis* **61**, 114-134 (2020).
- 776 138 Chauhan, V., Sherman, S., Said, Z., Yauk, C. L. & Stainforth, R. A case example of a radiation-
777 relevant adverse outcome pathway to lung cancer. *International Journal of Radiation Biology* **97**,
778 68-84 (2021).
- 779 139 Ignatov, A. V., Bondarenko, K. & Makarova, A. Non-bulky lesions in human DNA: the ways of
780 formation, repair, and replication. *Acta Naturae* **9**, 12-26 (2017).
- 781 140 David, E. & Niculescu, V.-C. Volatile organic compounds (VOCs) as environmental pollutants:
782 occurrence and mitigation using nanomaterials. *International Journal of Environmental Research*
783 *and Public Health* **18**, 13147 (2021).
- 784 141 Jameson, C. W. Chapter 7: Polycyclic aromatic hydrocarbons and associated occupational
785 exposures. *Tumour Site Concordance and Mechanisms of Carcinogenesis* (WHO Press, 2021).
- 786 142 Ravindra, K., Sokhi, R. & Van Grieken, R. Atmospheric polycyclic aromatic hydrocarbons: source
787 attribution, emission factors and regulation. *Atmospheric Environment* **42**, 2895-2921 (2008).
- 788 143 Abdel-Shafy, H. I. & Mansour, M. S. A review on polycyclic aromatic hydrocarbons: source,
789 environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* **25**,
790 107-123 (2016).
- 791 144 Levy, R. J. Carbon monoxide pollution and neurodevelopment: a public health concern.
792 *Neurotoxicology and Teratology* **49**, 31-40 (2015).
- 793 145 Brook, J. R. *et al.* Further interpretation of the acute effect of nitrogen dioxide observed in
794 Canadian time-series studies. *Journal of Exposure Science & Environmental Epidemiology* **17**,
795 S36-S44 (2007).
- 796 146 Zhang, L. *et al.* Understanding the industrial NO_x and SO₂ pollutant emissions in China from
797 sector linkage perspective. *Science of the Total Environment* **770**, 145242 (2021).
- 798 147 Meftaul, I. M., Venkateswarlu, K., Dharmarajan, R., Annamalai, P. & Megharaj, M. Pesticides in
799 the urban environment: a potential threat that knocks at the door. *Science of the Total Environment*
800 **711**, 134612 (2020).

- 801 148 Li, Z., Liang, Y., Zhou, J. & Sun, X. Impacts of de-icing salt pollution on urban road greenspace: a
802 case study of Beijing. *Frontiers of Environmental Science & Engineering* **8**, 747-756 (2014).
- 803 149 García-Pérez, J., Gómez-Barroso, D., Tamayo-Uria, I. & Ramis, R. Methodological approaches to
804 the study of cancer risk in the vicinity of pollution sources: the experience of a population-based
805 case-control study of childhood cancer. *International Journal of Health Geographics* **18**, 1-18
806 (2019).
- 807 150 García-Pérez, J. *et al.* Childhood leukemia and residential proximity to industrial and urban sites.
808 *Environmental Research* **140**, 542-553 (2015).
- 809 151 García-Pérez, J. *et al.* Association between residential proximity to environmental pollution
810 sources and childhood renal tumors. *Environmental Research* **147**, 405-414 (2016).
- 811 152 Chen, X. *et al.* Long-term exposure to urban air pollution and lung cancer mortality: A 12-year
812 cohort study in Northern China. *Science of the Total Environment* **571**, 855-861 (2016).
- 813 153 Beeson, W. L., Abbey, D. E. & Knutsen, S. F. Long-term concentrations of ambient air pollutants
814 and incident lung cancer in California adults: results from the AHSMOG study. *Environmental*
815 *Health Perspectives* **106**, 813-823 (1998).
- 816 154 Bai, X. *et al.* Linking urbanization and the environment: conceptual and empirical advances.
817 *Annual Review of Environment and Resources* **42**, 215-240 (2017).
- 818 155 Gogna, P. *et al.* Estimates of the current and future burden of lung cancer attributable to PM2.5 in
819 Canada. *Preventive Medicine* **122**, 91-99 (2019).
- 820 156 Nyberg, F. *et al.* Urban air pollution and lung cancer in Stockholm. *Epidemiology*, 487-495 (2000).
- 821 157 Fei, X. *et al.* The association between heavy metal soil pollution and stomach cancer: a case study
822 in Hangzhou City, China. *Environmental Geochemistry and Health* **40**, 2481-2490 (2018).
- 823 158 Cheng, I. *et al.* Association between ambient air pollution and breast cancer risk: the multiethnic
824 cohort study. *International Journal of Cancer* **146**, 699-711 (2020).
- 825 159 Ebenstein, A. The consequences of industrialization: evidence from water pollution and digestive
826 cancers in China. *Review of Economics and Statistics* **94**, 186-201 (2012).
- 827 160 Wei, J. & Zhanqing, L. GlobalHighPM2.5: Big data seamless 1km global ground-level PM2.5
828 dataset over land (Version 1) [Data set]. <https://doi.org/10.5281/zenodo.6449741> (2022).
- 829 161 Center for International Earth Science Information Network - CIESIN. Annual PM2.5
830 concentrations for countries and urban areas, 1998-2016. (Columbia University, 2021).
- 831 162 Wolf, M. J. *et al.* Country trends in major air pollutants, v1 (2003-2018).
832 <https://sedac.ciesin.columbia.edu/data/set/aqdh-country-trends-major-air-pollutants-2003-2018>
833 (Socioeconomic Data and Applications Center - SEDAC, 2022).
- 834 163 Wolf, M. J. *et al.* New insights for tracking global and local trends in exposure to air pollutants.
835 *Environmental Science & Technology* **56**, 3984-3996 (2022).
- 836

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 {Figuroa, 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.

837

838

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 [SO₂], nitrous oxides [NO_x], and ozone [O₃] 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).

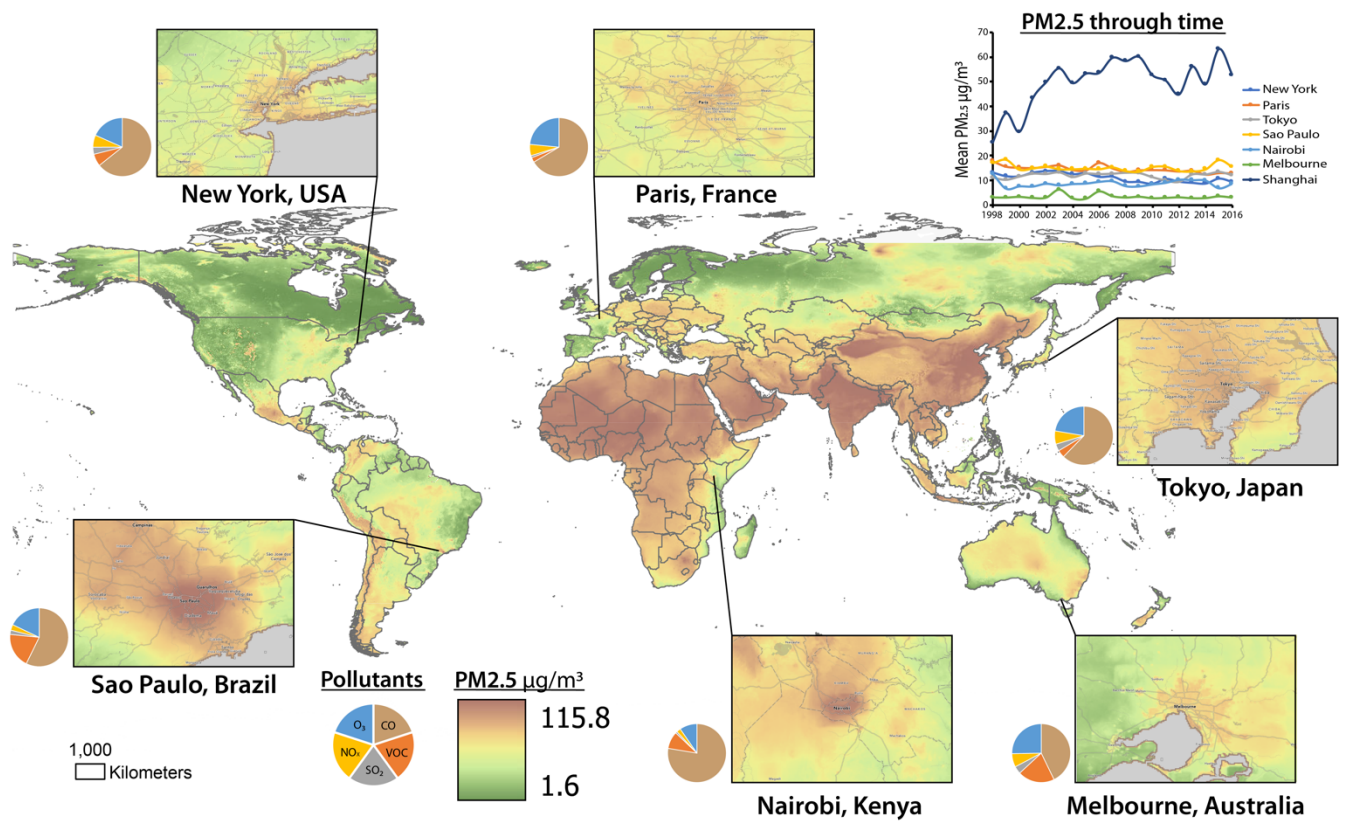


Fig. 2 | The potential for elevated mutation rates in cities to affect evolution of a population relative to a fitness optimum. When a population starts at a fitness optimum (dotted horizontal black line) in an urban environment (blue lines), any increase in mutation rate will lead to a net increase 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 $\Delta\mu$ - solid blue line), then we expect a population will move further from a fitness optimum through time. If $\Delta\mu$ 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 $\Delta\mu$ is lower (red dashed line). Such adaptive evolution could lead to evolutionary rescue, but such dynamics are only likely over contemporary time when N_e is high and generation times are fast (e.g., viruses, bacteria, eukaryotic microbes). At equilibrium, populations experiencing higher $\Delta\mu$ are expected to have lower fitness than those with lower $\Delta\mu$ 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 N_e is low and there is long generation time, which could lead to extinction if population growth rates are negative.

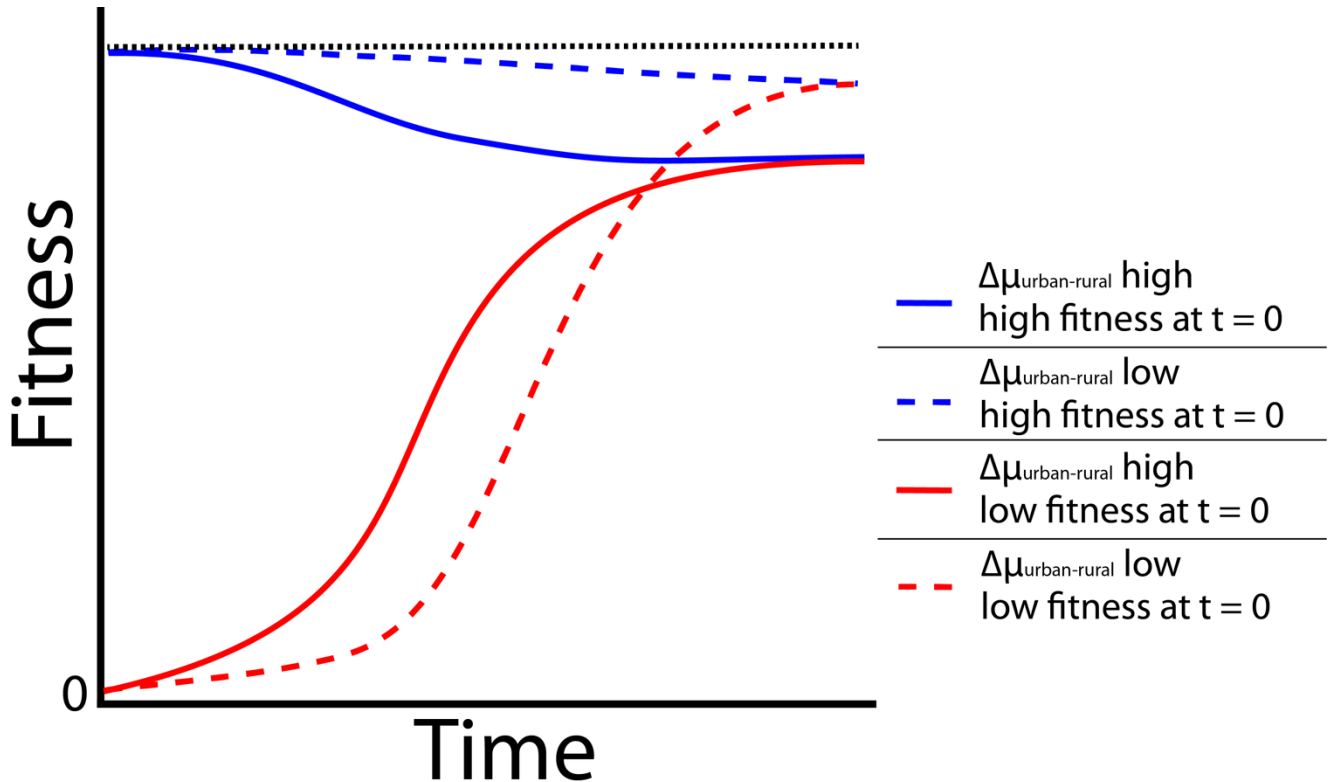


Fig. 3 | Potential biosentinel species for studying urban-associated mutations. Proposed biosentinels include: A) *Salmonella enterica*, B) *Caenorhabditis elegans*, C) *Drosophila melanogaster*, D) *Arabidopsis thaliana*, E) *Trifolium repens*, F) *Flavoparmelia caperata* (a lichen), G) *Fundulus heteroclitus*, 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. their relative abundance in urban vs. nonurban habitats) given their commensal status with humans (bottom right panel).

