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Santangelo, James

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URBAN EVOLUTION

Global urban environmental change drives adaptation in white clover

James S. Santangelo^{1,2}, Rob W. Ness^{1,2}, Beata Cohan¹, Connor R. Fitzpatrick³, Simon G. Innes^{4,1}, Sophie Koch¹, Lindsay S. Miles^{1,2}, Samreen Mumim^{5,1}, Pedro R. Peres-Neto⁶, Cindy Prashad¹, Alex T. Tong¹, Windsor E. Aguirre⁷, Philips O. Akinwole⁸, Marina Alberti⁹, Jackie Álvarez¹⁰, Jill T. Anderson¹¹, Joseph J. Anderson¹², Yoshino Ando¹³, Nigel R. Andrew¹⁴, Fabio Angeoletto¹⁵, Daniel N. Anstett¹⁶, Julia Anstett^{17,18}, Felipe Aoki-Gonçalves¹⁹, A. Z. Andis Arietta²⁰, Mary T. K. Arroyo^{21,22}, Emily J. Austen²³, Fernanda Baena-Díaz²⁴, Cory A. Barker²⁵, Howard A. Baylis²⁶, Julia M. Beliz^{27,28}, Alfonso Benitez-Mora²⁹, David Bickford³⁰, Gabriela Biedebach³⁰, Gwylim S. Blackburn³¹, Manfred M. A. Boehm¹⁶, Stephen P. Bonser³², Dries Bonte³³, Jesse R. Braggar³⁴, Cristina Branquinho³⁵, Kristien I. Brans³⁶, Jorge C. Bresciano³⁷, Peta D. Brom³⁸, Anna Bucharova³⁹, Briana Burt⁴⁰, James F. Cahill⁴¹, Katelyn D. Campbell²⁵, Elizabeth J. Carlen⁴², Diego Carmona⁴³, Maria Clara Castellanos⁴⁴, Giada Centenaro⁴⁵, Izan Chalen^{10,46}, Jaime A. Chaves^{10,47}, Mariana Chávez-Pesqueira⁴⁸, Xiao-Yong Chen^{49,50}, Angela M. Chilton⁵¹, Kristina M. Chomiak⁴⁰, Diego F. Cisneros-Heredia^{10,46}, Ibrahim K. Cisse⁴⁰, Aimée D. Classen⁵², Mattheau S. Comerford⁵³, Camila Cordoba Fradinger⁵⁴, Hannah Corney⁵⁵, Andrew J. Crawford⁵⁶, Kerri M. Crawford⁵⁷, Maxime Dahirel⁵⁸, Santiago David⁵⁹, Robert De Haan⁶⁰, Nicholas J. Deacon⁶¹, Clare Dean⁶², Ek del-Val⁶³, Eleftherios K. Deligiannis⁶⁴, Derek Denney¹¹, Margarete A. Dettlaff⁴¹, Michelle F. DiLeo⁶⁵, Yuan-Yuan Ding⁴⁹, Moisés E. Domínguez-López^{66,67}, Davide M. Dominoni⁶⁸, Savannah L. Draud⁶⁹, Karen Dyson⁹, Jacintha Eilers⁷⁰, Carlos I. Espinosa⁷¹, Liliana Essi⁷², Mohsen Falahati-Anbaran^{73,74}, Jéssica C. F. Falcão⁷⁵, Hayden T. Fargo¹, Mark D. E. Fellowes⁷⁶, Raina M. Fitzpatrick⁷⁷, Leah E. Flaherty⁷⁸, Pádraic J. Flood⁷⁹, María F. Flores²², Juan Fornoni⁸⁰, Amy G. Foster⁸¹, Christopher J. Frost⁸², Tracy L. Fuentes⁹, Justin R. Fulkerson⁸³, Edeline Gagnon^{84,85}, Frauke Garbsch⁸¹, Colin J. Garroway⁸⁶, Aleeza C. Gerstein⁸⁷, Mischa M. Giasson⁸⁸, E. Binney Girdler⁸⁹, Spyros Gkelis⁶⁴, William Godsoe⁹⁰, Anneke M. Golemic⁵, Mireille Golemic¹, César González-Lagos^{29,91}, Amanda J. Gorton⁹², Kiyoko M. Gotanda^{93,26}, Gustaf Granath¹², Stephan Greiner⁸¹, Joanna S. Griffiths⁹⁴, Filipa Grilo³⁵, Pedro E. Gundel^{95,54}, Benjamin Hamilton⁴⁰, Joyce M. Hardin⁶⁹, Tianhua He^{96,97}, Stephen B. Heard⁸⁸, André F. Henriques³⁵, Melissa Hernández-Poveda⁹⁶, Molly C. Hetherington-Rauth¹, Sarah J. Hill¹⁴, Dieter F. Hochuli⁹⁸, Kathryn A. Hodgins⁹⁹, Glen R. Hood¹⁰⁰, Gareth R. Hopkins¹⁰¹, Katherine A. Hovanes¹⁰², Ava R. Howard¹⁰¹, Sierra C. Hubbard⁶⁹, Carlos N. Ibarra-Cerdeña¹⁰³, Carlos Iñiguez-Armijos⁷¹, Paola Jara-Arancio^{104,105}, Benjamin J. M. Jarrett^{106,26}, Manon Jeannot¹⁰⁷, Vania Jiménez-Lobato¹⁰⁸, Mae Johnson¹⁰⁹, Oscar Johnson¹¹⁰, Philip P. Johnson¹¹¹, Reagan Johnson¹¹², Matthew P. Josephson¹¹³, Meen Chel Jung⁹, Michael G. Just¹¹⁴, Aapo Kahilainen⁶⁵, Otto S. Kailing¹¹⁵, Eunice Kariñho-Betancourt¹¹⁶, Regina Karousou⁶⁴, Lauren A. Kirm⁹⁹, Anna Kirschbaum¹¹⁷, Anna-Liisa Laine^{118,65}, Jalene M. LaMontagne^{7,119}, Christian Lampe³⁹, Carlos Lara¹²⁰, Erica L. Larson¹²¹, Adrián Lázaro-Lobo¹²², Jennifer H. Le¹²³, Deleon S. Leandro¹²⁴, Christopher Lee⁹⁹, Yunting Lei¹²⁵, Carolina A. León²⁹, Manuel E. Lequerica Tamara⁹⁸, Danica C. Levesque¹²⁶, Wan-Jin Liao¹²⁷, Megan Ljubotina⁴¹, Hannah Locke⁵⁷, Martin T. Lockett¹²⁸, Tiffany C. Longo³⁴, Jeremy T. Lundholm⁵⁵, Thomas MacGillivray⁶⁸, Christopher R. Mackin⁴⁴, Alex R. Mahmoud²⁷, Isaac A. Manju¹⁰¹, Janine Mariën⁷⁰, D. Nayeli Martínez^{63,129}, Marina Martínez-Bartolomé^{130,122}, Emily K. Meineke¹³¹, Wendy Mendoza-Arroyo¹¹⁶, Thomas J. S. Merritt¹²⁶, Lila Elizabeth L. Merritt¹²⁶, Giuditta Migiani⁶⁸, Emily S. Minor¹¹¹, Nora Mitchell^{132,133}, Mitra Mohammadi Bazargani¹³⁴, Angela T. Moles³², Julia D. Monk²⁰, Christopher M. Moore¹³⁵, Paula A. Morales-Morales¹³⁶, Brook T. Moyers^{137,138}, Miriam Muñoz-Rojas^{51,139}, Jason Munshi-South⁴², Shannon M. Murphy¹²¹, Maureen M. Murúa¹⁴⁰, Melisa Neila²⁹, Ourania Nikolaidis¹²³, Iva Njunji¹⁴¹, Peter Nosko¹⁴², Juan Núñez-Farfán⁸⁰, Takayuki Ohgushi¹⁴³, Kenneth M. Olsen²⁷, Øystein H. Opedal¹⁰⁶, Cristina Ornelas¹⁴⁴, Amy L. Parachnowitsch^{88,12}, Aaron S. Paratore⁴⁰, Angela M. Parody-Merino³⁷, Juraj Paule¹⁴⁵, Octávio S. Paulo³⁵, João Carlos Pena¹⁴⁶, Vera W. Pfeiffer¹⁴⁷, Pedro Pinho³⁵, Anthony Piot³¹, Ilga M. Porth³¹, Nicholas Poulos¹⁴⁸, Adriana Puentes¹⁴⁹, Jiao Qu³³, Estela Quintero-Vallejo¹⁵⁰, Steve M. Raciti¹⁵¹, Joost A. M. Raeymaekers¹⁵², Krista M. Raveala⁶⁵, Diana J. Rennison¹⁵³, Milton C. Ribeiro¹⁴⁶, Jonathan L. Richardson¹⁵⁴, Gonzalo Rivas-Torres^{10,155}, Benjamin J. Rivera⁸⁹, Adam B. Roddy¹⁵⁶, Erika Rodriguez-Muñoz⁵⁶, José Raúl Román¹⁵⁷, Laura S. Rossi¹⁴², Jennifer K. Rowntree⁶², Travis J. Ryan¹⁵⁸, Santiago Salinas⁸⁹, Nathan J. Sanders⁵², Luis V. Santiago-Rosario¹⁵⁹, Amy M. Savage¹²³, J.F. Scheepens^{160,117}, Menno Schilthuizen¹⁶¹, Adam C. Schneider^{69,1}, Tiffany Scholier^{149,162}, Jared L. Scott¹⁶³, Sumner A. Shaheed³⁴, Richard P. Shefferson¹⁶⁴, Caralee A. Shepard⁶⁹, Jacqui A. Shykoff¹⁶⁵, Georgianna Silveira¹⁶⁶, Alexis D. Smith¹¹¹, Lizet Solis-Gabriel⁶³, Antonella Soro¹⁶⁷, Katie V. Spellman^{168,144}, Kaitlin Stack Whitney¹⁶⁹, Indra Starke-Ottich¹⁴⁵, Jörg G. Stephan^{170,149}, Jessica D. Stephens¹⁷¹, Justyna Szulc¹⁷², Marta Szulkin¹⁷², Ayco J. M. Tack⁴⁵, Ítalo Tamburrino²², Tayler D. Tate¹⁰¹, Emmanuel Tergemina⁷⁹, Panagiotis Theodorou¹⁶⁷, Ken A. Thompson^{59,173}, Caragh G. Threlfall⁹⁸, Robin M. Tinghitella¹²¹, Lilibeth Toledo-Chelala⁶³, Xin Tong⁴⁹, Léa Uroy^{58,174}, Shunsuke Utsumi¹³, Martijn L. Vandegehuchte^{107,33}, Acer VanWallendael¹⁷⁵, Paula M. Vidal²², Susana M. Wadgyar¹⁷⁶, Ai-Ying Wang¹²⁷, Nian Wang¹⁷⁷, Montana L. Warbrick¹⁴², Kenneth D. Whitney¹³², Miriam Wiesmeier¹⁷⁸, J. Tristian Wiles⁶⁹, Jianqiang Wu¹²⁵, Zoe A. Xirocostas³², Zhaogui Yan¹⁷⁷, Jiahe Yao¹⁷⁹, Jeremy B. Yoder¹⁴⁸, Owen Yoshida⁵⁵, Jingxiang Zhang¹²⁵, Zhigang Zhao¹⁷⁹, Carly D. Ziter⁶, Matthew P. Zuellig¹⁸⁰, Rebecca A. Zufall⁵⁷, Juan E. Zurita¹⁰, Sharon E. Zytynska^{178,181}, Marc T. J. Johnson^{1,2,*†}

Urbanization transforms environments in ways that alter biological evolution. We examined whether urban environmental change drives parallel evolution by sampling 110,019 white clover plants from 6169 populations in 160 cities globally. Plants were assayed for a Mendelian antiherbivore defense that also affects tolerance to abiotic stressors. Urban-rural gradients were associated with the evolution of clines in defense in 47% of cities throughout the world. Variation in the strength of clines was explained by environmental changes in drought stress and vegetation cover that varied among cities. Sequencing 2074 genomes from 26 cities revealed that the evolution of urban-rural clines was best explained by adaptive evolution, but the degree of parallel adaptation varied among cities. Our results demonstrate that urbanization leads to adaptation at a global scale.

Urbanization is a driver of both environmental and evolutionary change. Towns and cities are rapidly expanding throughout the world to accommodate human population growth. These urban areas represent novel ecosystems, in which urban development alters multiple environmental factors (1). Recent research

shows that urban environmental change can influence four evolutionary processes: mutation, genetic drift, gene flow, and adaptation due to natural selection (2, 3). Despite numerous examples of how urbanization affects genetic drift and gene flow (4, 5), the effects of urbanization on adaptive evolution have received less attention (6–8). Adaptation to

urban environments can affect species' conservation (9), the spread of pests and disease (2), and eco-evolutionary feedbacks (10), as well as urban planning and human society (11). However, the few examples of adaptation to urban environments focus on just one or a small number of cities in a single region (2). It is therefore unclear whether populations can adapt to urban habitats in similar ways across cities throughout the world.

Parallel adaptive evolution is most likely when populations experience similar environmental selective pressures on the same genes or phenotypes (12, 13). For urbanization to drive parallel evolution, urban areas must converge in environmental features that affect

*Corresponding author. Email: marc.johnson@utoronto.ca
†Affiliations are listed at the end of this paper.

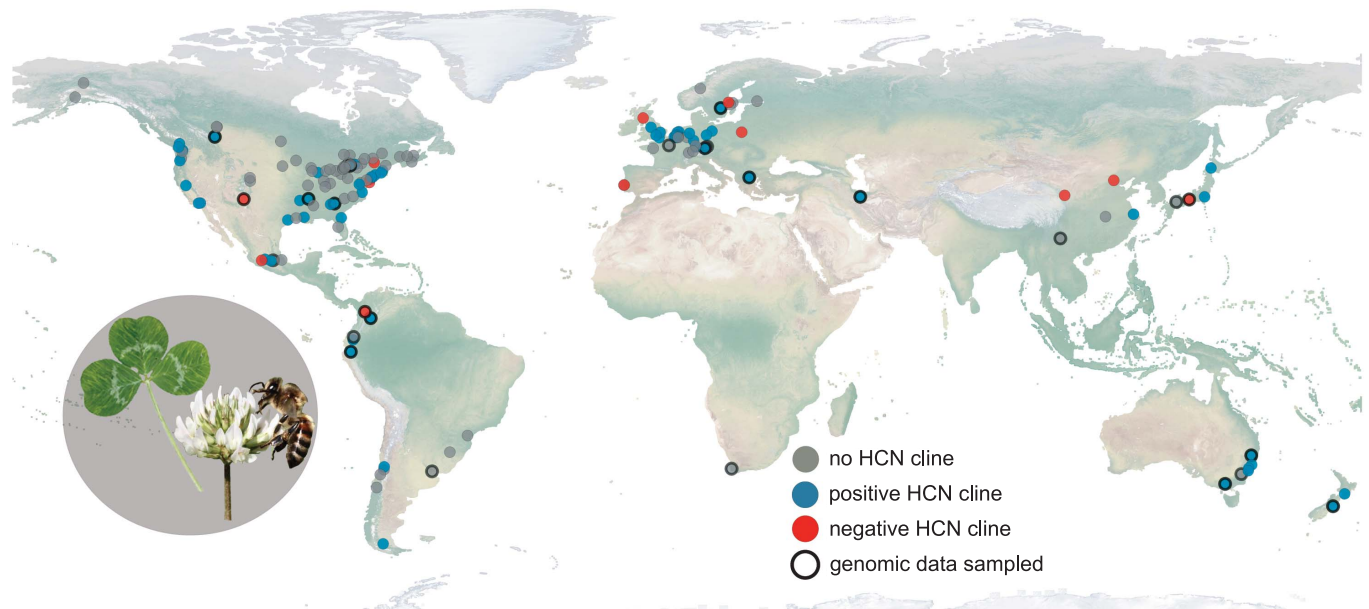


Fig. 1. Cities sampled for urban environmental and evolutionary change. Blue dots indicate cities with positive clines for hydrogen cyanide (HCN) production along urban-rural gradients ($\text{HCN}_{\text{urban}} < \text{HCN}_{\text{rural}}$). Red dots show negative clines ($\text{HCN}_{\text{urban}} > \text{HCN}_{\text{rural}}$). Gray dots indicate cities without a cline. Plants from the 26 cities outlined in black underwent whole-genome sequencing. Inset: White clover and a honey bee.

an organism's fitness. Urbanization can lead to similar environmental changes across cities (14), but whether urban environmental convergence causes parallel evolution has never been examined at a global scale.

Here we test how global urbanization affects environmental change and evolution in a cosmopolitan plant species, white clover (*Trifolium repens* L., Fabaceae). White clover populations are polymorphic for the production of hydrogen cyanide (HCN), an antiherbivore chemical defense controlled by two genes (15). At least one functional allele at each of two unlinked loci (*Ac* and *Li*) is required to produce HCN following tissue damage, whereas plants that are homozygous for gene deletions (*ac* and *li* alleles) at either locus lack HCN (16, 17). Notably, these deletions occur throughout the world, resulting in standing genetic variation on which selection can act (18). Previous work showed that herbivores select for the production of HCN, and abiotic stressors (e.g., freezing and drought) influence the costs and benefits of the metabolic components underlying the defense (19, 20). Variation in these environmental factors is credited with driving the evolution of clines in HCN production at continental and regional scales (21, 22), including in response to urban environments (23–25). Thus, HCN production could evolve in response to urbanization if there are urban-rural gradients in herbivory, winter temperature, or drought.

We examined global urban environmental and evolutionary change across the diverse climates that white clover inhabits. To this end, we created the Global Urban Evolution Project

to test for parallel evolution and urban adaptation in natural populations across white clover's worldwide range. The present study builds on our previous work on white clover (23–25) by sampling cities globally across diverse climates in both the native (Europe and western Asia) and introduced ranges, by quantifying many environmental factors from each population and by integrating evolutionary genomic analyses using whole-genome sequence data. This project spanned 160 cities across 26 countries (Fig. 1) (15) in white clover's native and introduced ranges (Fig. 1 and fig. S1). From these cities, we phenotyped 110,019 plants from 6169 sampling sites (hereafter “populations,” table S1). Populations within each city were sampled along an urban-rural transect, with half of each transect in urban and suburban areas (i.e., areas with high building density) and the other half in rural areas (Fig. 2, E to G) (15).

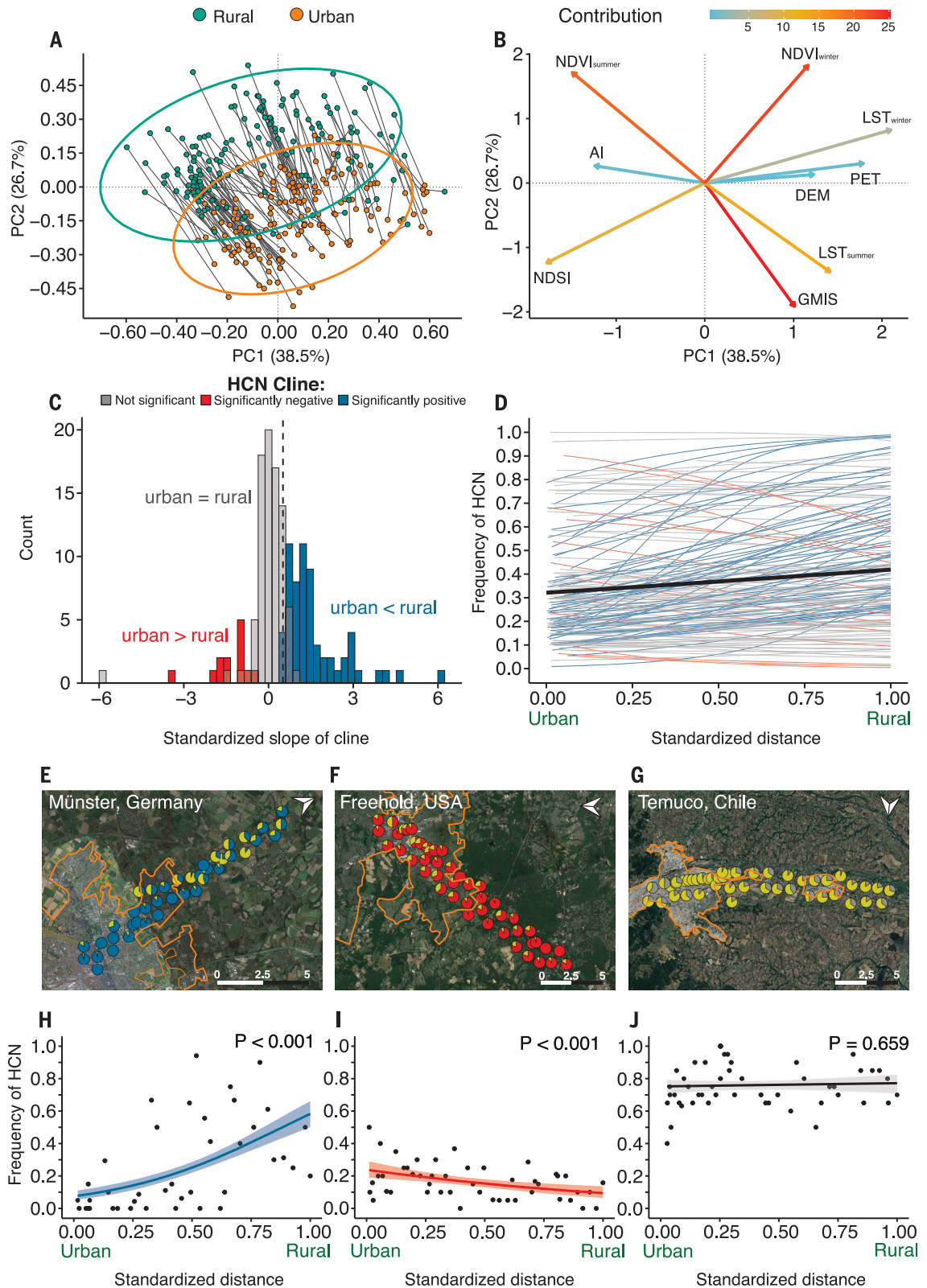
Across 160 cities, we tested whether urban white clover habitats converged to be more similar among cities and less variable within cities in their environmental characteristics compared to rural habitats (15). Urban and rural habitats significantly diverged (MANOVA H_0 : $\text{urban}_{\text{mean}} = \text{rural}_{\text{mean}}$, $P_{\text{bootstrapped}} < 0.01$, Fig. 2A) along two principal-component axes that accounted for 65% of the variation in the multivariate environments between the two habitats across cities. Urban locations consistently had more impervious surface, higher summer temperatures, and less vegetation than rural populations (Fig. 2B and fig. S2). The remaining environmental variables changed along urban-rural gradients in many cities, but

these changes were less consistent in direction among cities (fig. S2 and table S2). Although urban and rural environments diverged on average, urban-rural changes in the environment were not always parallel (MANOVA H_0 : parallel urban-rural changes among cities, $P_{\text{bootstrapped}} < 0.01$, Fig. 2A). Additionally, environmental variance among urban populations within a city was lower than the environmental variance among rural populations ($F_{9,1570} = 31.76$, $P < 0.001$, fig. S3). Together these results show that on average, urbanization leads to similar and less-variable environmental conditions in some factors (e.g., impervious surface, summer temperature, summer vegetation) but not in others (e.g., potential evapotranspiration, snow cover, winter vegetation), which could lead to variation in the degree of parallel evolution.

We next tested whether convergent urban environmental change causes parallel evolution in an ecologically important trait of white clover. We examined evolution in response to urbanization by testing for a relationship between HCN production and distance to the urban center (i.e., an “HCN cline”), as well as other metrics of urbanization (15). Our model explained 28% of the variation in the frequency of HCN production within populations (table S3). Across 160 cities, distance from the city center was positively related to the frequency of HCN-producing plants (distance: $\chi^2_{df=1} = 12.35$, $P < 0.001$). The probability that a plant produced HCN increased by 44% on average from the center of an urban area to the furthest rural population (Fig. 2C, D). However, cities varied in the strength and direction

Fig. 2. Urban environmental and evolutionary change across cities. (A) Principal component analysis showing environmental differences between urban (orange dots) and rural (green dots) habitats; ovals represent 95% confidence interval (CI). Lines connect urban and rural habitats from the same city. (B) The eigenvectors for environmental variables, colored according to their contribution to PC2. The environmental variables included vegetation in winter (NDVI_{winter}) and summer (NDVI_{summer}), snow accumulation (NDSI), surface temperature in winter (LST_{winter}) and summer (LST_{summer}), aridity index (AI), potential evapotranspiration (PET), impervious surface (GMIS), and elevation (DEM).

(C) Histogram of the slopes from binomial regressions of the relationship between HCN production within populations and distance from the city center. Distance was standardized to vary between 0 (urban center) and 1 (furthest rural population) in each city, so that cities that varied in size were compared on the same scale. The dashed vertical line corresponds to the mean slope across cities, and overlap between bars showing cities with significant (blue and red) and nonsignificant (grey) is shown as muted colors. (D) The relationship between HCN production within populations and distance for each city; colors correspond to those in (C). The black line shows the positive main effect of distance across cities ($P < 0.001$). (E to G) Examples of transects, with the orange lines showing the urban boundary, and pie charts (jittered to reduce overlap) showing the proportion of HCN+ plants colored in yellow. (H to J) Frequency of HCN production versus distance for the cities shown in (E) to (G). The line shows the regression line \pm 95% CI.



of clines (distance \times city interaction: $\chi^2_{df=1} = 1001$, $P < 0.001$; Fig. 2, C and D). Overall, 47% of cities exhibited a significant ($P < 0.05$) cline (15), with 39% of cities (62 of 160) showing a

positive cline in which HCN production was less common in urban than rural populations, and 8% of cities (13 of 160) had negative clines (Fig. 2 and table S4). Positive and negative

clines occurred in both the native and introduced ranges, with the former being more prevalent among continents and across diverse climates (Fig. 1).

Given the prevalence of HCN clines at a global scale, we sought to identify the evolutionary processes driving variation in the strength and direction of clines. In addition to natural selection, nonadaptive evolution can lead to the evolution of clines (26). Notably, the epistatic genetic architecture of HCN

production makes the loss of the trait more likely with increased genetic drift (26). Therefore, the prevalence of positive clines could reflect stronger drift in urban populations (4, 5). To examine whether urban populations exhibited stronger drift, we estimated pairwise nucleotide diversity (π) of putatively neutral sites

using whole-genome sequence data from ~80 individuals per city, with samples equally split between urban and rural habitats across 26 cities ($N = 2,074$) (15). These cities were selected to capture variation in the strength and direction of clines, geography, and climate (Fig. 1) (15).

Genetic diversity was not consistently different between urban and rural habitats and did not explain variation in the slope of HCN clines along urban-rural gradients. On average, urban and rural habitats did not differ in neutral genetic diversity ($F_{1, 25} = 0.028$, $P = 0.87$; Fig. 3A). Furthermore, the difference in π between urban and rural habitats within a city was not strongly related to the slope of HCN clines ($F_{1, 24} = 0.25$, $P = 0.62$; Fig. 3B and fig. S4), and urban-rural differences in genetic diversity were similar between cities with and without clines ($F_{1, 24} = 0.017$, $P = 0.90$).

Variation in the strength of genetic differentiation and gene flow between urban and rural habitats can influence the ability of populations to adapt to urban environments (27). To test the association between genetic differentiation and the evolution of HCN clines, we estimated population genetic differentiation between urban and rural populations using both F_{ST} and principal components analysis (PCA) (fig. S5), in addition to urban-rural admixture (fig. S6) (15). Urban-rural F_{ST} was low [mean = 0.012 ± 0.002 (SE)] and did not differ significantly between cities with and without clines ($F_{1, 24} = 1.47$, $P = 0.24$; Fig. 3C and fig. S4). Neither F_{ST} ($F_{1, 24} = 1.42$, $P = 0.25$; Fig. 3D) nor urban-rural differentiation measured using PCA ($F_{1, 24} = 1.10$, $P = 0.31$, fig. S5) predicted the strength of clines in HCN production. The absence of strong differentiation was associated with extensive admixture between urban and rural populations (fig. S6). Because genetic differentiation is consistently low and gene flow appears to be high among urban and rural populations, the repeated evolution of clines suggests strong selection on HCN production along urban-rural gradients. This conclusion is further supported by direct tests of selection on the *Ac* and *Li* loci, as well as HCN production, in which differentiation (using a statistic equivalent to F_{ST}) between urban and rural populations was stronger than expected under neutral evolution in cities with HCN clines compared to cities without clines (Fig. 3, E and F) (15).

Multiple environmental stressors are known to influence the evolution of HCN production at continental scales (20–22, 28), so we asked: What environmental factors explain variation in the evolution of HCN production along urban-rural gradients? Environmental factors related to drought and vegetation cover were the strongest predictors of variation in HCN clines, accounting for 11.3% of the variation in the strength of clines (tables S5 and S6). Change in potential evapotranspiration (PET) along urban-rural gradients was one of the

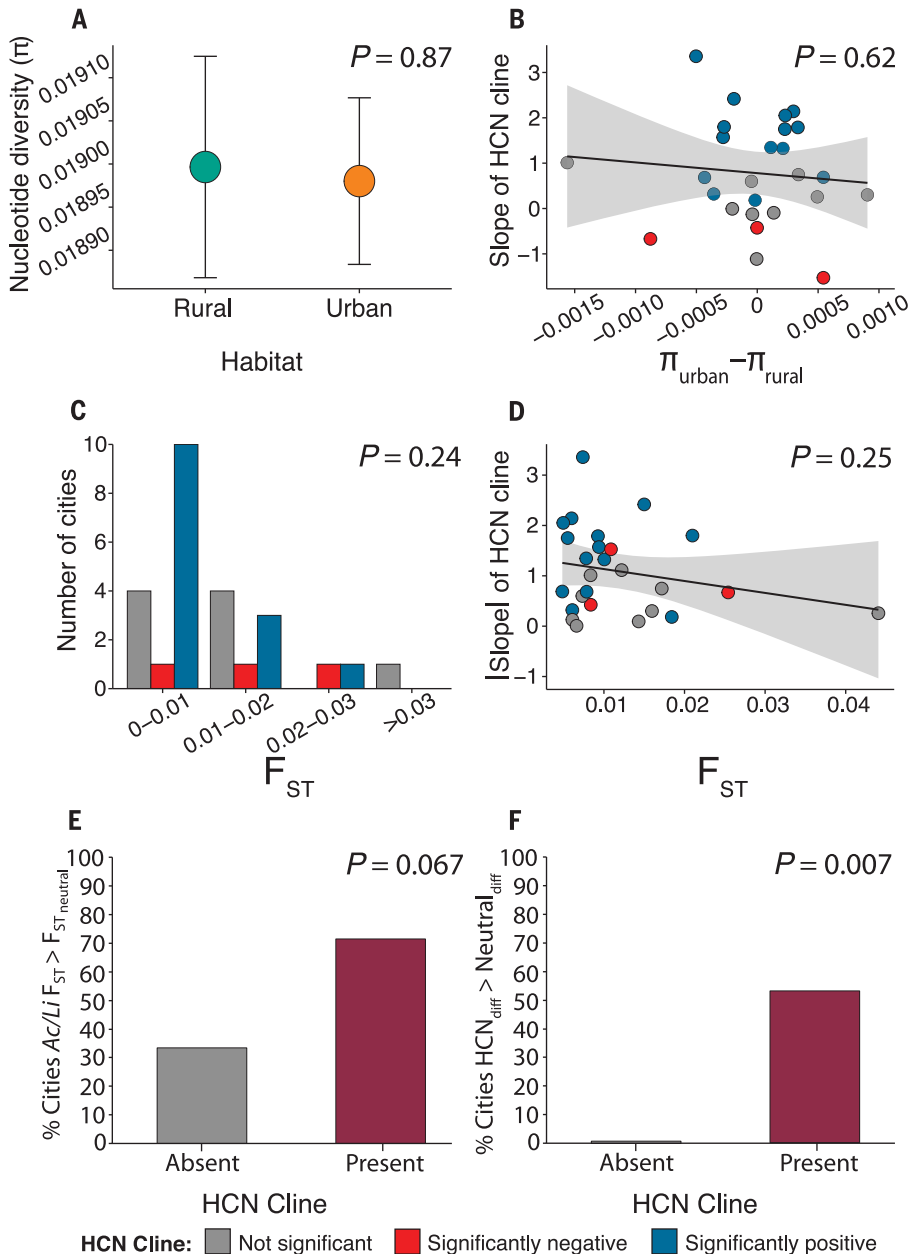


Fig. 3. Genetic diversity and differentiation within and between urban and rural habitats. (A) Mean (\pm SE) pairwise nucleotide diversity (π) for urban (orange) and rural (green) plants across cities. (B) The relationship between the slope of HCN clines versus the difference in nucleotide diversity between habitats, where each point is a city. (C) Histogram showing the distribution of genetic differentiation (F_{ST}) between urban and rural habitats for each city, colored by respect to the significance of HCN clines. (D) Relationship between the absolute value of the slope of HCN clines versus F_{ST} . (E) Percentage of cities in which differentiation between urban and rural habitats at *Ac* or *Li* exceeds neutral expectation in cities with or without significant HCN clines (15). (F) Percentage of cities with differentiation in HCN production between urban and rural habitats that exceeds neutral expectation in cities with or without significant HCN clines (15). P values in (E) and (F) correspond to χ^2 test for independence.

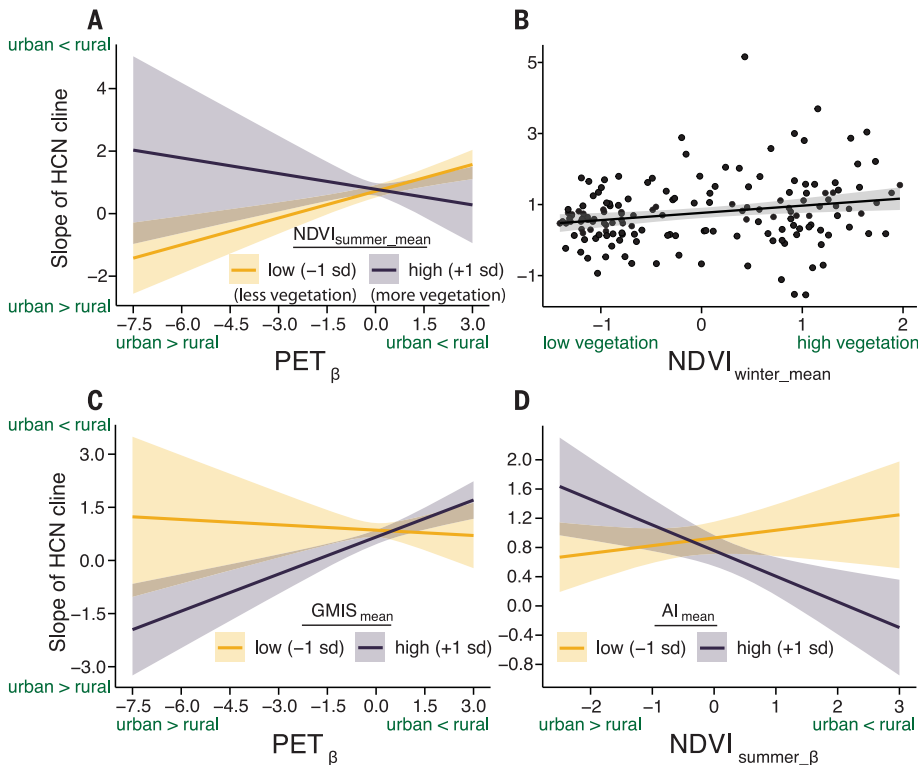


Fig. 4. Environmental predictors of urban-rural clines in HCN production. (A) Change in potential evapotranspiration along urban-rural gradients (PET_{β}) interacts with the regional amount of summer vegetation (i.e., $NDVI_{summer_mean}$) to explain variation in the slopes of HCN clines. (B) The relationship between the slopes of HCN clines and the regional amount of winter vegetation ($NDVI_{winter_mean}$). (C) PET_{β} interacts with the regional amounts of impervious surface ($GMIS_{mean}$) to predict the slope of HCN clines. (D) Change in summer vegetation along urban-rural gradients ($NDVI_{summer_beta}$) interacts with regional aridity (AI_{mean}) to explain variation in the slope of HCN clines. Acronyms as in Fig. 2.

most consistent predictors of evolution in HCN production (table S5); the frequency of HCN production tended to be higher in rural than urban populations in cities where PET was also greater in rural habitats (Fig. 4, A and C, and fig. S7). Because high PET can lead to plant water stress under low soil moisture, this result is consistent with drought selecting for higher HCN production, a pattern also observed at continental scales (27). However, the effect of PET on the evolution of HCN production only occurs when the amount of vegetation in and around cities is low (Fig. 4A). When vegetation cover is relatively high (and impervious surface is low) along the whole urban-rural transect, HCN clines tend to be positive regardless of variation in PET (Fig. 4, A to C). Notably, the amount of vegetation is positively correlated with invertebrate herbivore biomass and diversity (29), which can select for increased HCN production (20). When combined with the observation that herbivores are often less abundant in urban habitats (30), our evidence suggests that herbivores are selecting for greater HCN production in rural than urban areas. The positive association between urban-rural changes in vegetation and the positive slope of HCN clines

in some cities further supports this interpretation (Fig. 4D). Put simply, herbivory seems to select for higher HCN production in rural areas, but in the absence of strong herbivore pressure (i.e., when there is less vegetation across the whole gradient), drought is the main selective agent. Contrary to previous findings, urban-rural changes in temperature and snow cover did not explain changes in HCN production (24), suggesting that urban-rural changes in these abiotic factors are not a general explanation for the evolution of clines at a global scale.

Our results have general implications for understanding how environmental change affects adaptation in widespread species. Parallel evolution is a hallmark of natural selection because it suggests that adaptation proceeds in a repeatable way when populations face similar environments (12, 13). However, departures from parallel evolution are common, and a major goal of recent research involves quantifying how ecological and evolutionary factors interact to influence variation in adaptive responses to similar environments (12). Our results show that white clover rapidly adapts to urban environments on a global scale, but there is considerable variation in the strength

and direction of HCN clines that is driven by variation in particular biotic and abiotic factors that differ in how they change along urban-rural gradients among cities. Variation in additional unmeasured factors (e.g., gene flow from agricultural varieties, pollution, etc.) might further explain variation in the strength of clines, and future work will seek to explore such mechanisms.

Urbanization is increasingly transforming rural and natural environments into unique ecosystems that Earth's biodiversity has never experienced, and these changes are altering the evolution of life. If adaptation to urban environments is common, then this could have cascading effects on populations and ecosystems. This knowledge could help conserve some of Earth's most vulnerable species (9), mitigate the impacts of pests (2), improve human well-being (8, 11), and contribute to understanding fundamental eco-evolutionary processes (10).

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¹Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada. ²Centre for Urban Environments, University of Toronto Mississauga, Mississauga, ON, Canada. ³Department of Biology, University of North Carolina, Chapel Hill, NC, USA. ⁴Department of Biology, University of Louisiana, Lafayette, LA, USA. ⁵Department of Biology, Queen's University, Kingston, ON, Canada. ⁶Department of Biology, Concordia University, Montreal, QC, Canada. ⁷Department of Biological Sciences, DePaul University, Chicago, IL, USA. ⁸Department of Biology, DePaul University, Greencastle, IN, USA. ⁹Department of Urban Design and Planning, University of Washington, Seattle, WA, USA. ¹⁰Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito USFQ, Quito, Ecuador. ¹¹Department of Genetics, University of Georgia, Athens, GA, USA. ¹²Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden. ¹³Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Hokkaido, Japan. ¹⁴Natural History Museum, Zoology, University of New England, Armidale, NSW, Australia. ¹⁵Programa de Pós-Graduação em Geografia da UFMT, campus de Rondonópolis, Cuiabá, Brazil. ¹⁶Department of Botany and Biodiversity Research Centre, University of British Columbia, Vancouver, BC, Canada. ¹⁷Graduate Program in Genome Sciences and Technology, Genome Sciences Centre, University of British Columbia, Vancouver, British Columbia, Canada. ¹⁸Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada. ¹⁹Red de Biología Evolutiva, Instituto de Ecología, A. C., Xalapa, Mexico. ²⁰School of the

Environment, Yale University, New Haven, CT, USA. ²¹Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago, Chile. ²²Instituto de Ecología y Biodiversidad, Universidad de Chile, Santiago, Chile. ²³Department of Biology, Mount Allison University, Sackville, NB, Canada. ²⁴Red de Ecología, Instituto de Ecología A. C., Xalapa, Mexico. ²⁵Department of Biology, University of Ottawa, Ottawa, ON, Canada. ²⁶Department of Zoology, University of Cambridge, Cambridge, UK. ²⁷Department of Biology, Washington University in St. Louis, St. Louis, MO, USA. ²⁸Department of Biology, University of Miami, Miami, FL, USA. ²⁹Centro de Investigación en Recursos Naturales y Sustentabilidad (CIRESYS), Universidad Bernardo O'Higgins, Santiago, Chile. ³⁰Department of Biology, University of La Verne, La Verne, CA, USA. ³¹Département des sciences du bois et de la forêt, Université Laval, Québec, QC, Canada. ³²Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, UNSW Sydney, Sydney, NSW, Australia. ³³Department of Biology, Ghent University, Ghent, Belgium. ³⁴Department of Biology, Monmouth University, West Long Branch, NJ, USA. ³⁵Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisboa, Portugal. ³⁶Department of Biology, KU Leuven, Leuven, Belgium. ³⁷School of Agriculture and Environment, Wildlife and Ecology group, Massey University, Palmerston North, Manawatu, New Zealand. ³⁸Department of Biological Sciences, University of Cape Town, Cape Town, South Africa. ³⁹Institute of Landscape Ecology, University of Münster, Münster, Germany. ⁴⁰Gosnell School of Life Sciences, Rochester Institute of Technology, Rochester, NY, USA. ⁴¹Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada. ⁴²Louis Calder Center and Department of Biological Sciences, Fordham University, Armonk, NY, USA. ⁴³Departamento de Ecología Tropical, Universidad Autónoma de Yucatán, Mérida, Yucatán, México. ⁴⁴School of Life Sciences, University of Sussex, Brighton, UK. ⁴⁵Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden. ⁴⁶BIOTROP Instituto de Biodiversidad Tropical, Universidad San Francisco de Quito, Quito, Ecuador. ⁴⁷Department of Biology, San Francisco State University, San Francisco, CA, USA. ⁴⁸Unidad de Recursos Naturales, Centro de Investigación Científica de Yucatán AC, Mérida, Yucatán, México. ⁴⁹School of Ecological and Environmental Sciences, East China Normal University, Shanghai, China. ⁵⁰Shanghai Engineering Research Center of Sustainable Plant Innovation, Shanghai 200231, China. ⁵¹Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, UNSW Sydney, Sydney, NSW, Australia. ⁵²Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA. ⁵³Department of Biosciences, Rice University, Houston, TX, USA. ⁵⁴FEVA, Universidad de Buenos Aires, CONICET, Facultad de Agronomía, Buenos Aires, Argentina. ⁵⁵Biology Department, Saint Mary's University, Halifax, NS, Canada. ⁵⁶Department of Biological Sciences, Universidad de los Andes, Bogotá, Colombia. ⁵⁷Department of Biology and Biochemistry, University of Houston, Houston, TX, USA. ⁵⁸ECOBIO (Ecosystèmes, biodiversité, évolution), Université de Rennes, Rennes, France. ⁵⁹Department of Zoology and Biodiversity Research Centre, University of British Columbia, Vancouver, BC, Canada. ⁶⁰Department of Environmental Studies, Dordt University, Sioux Center, IA, USA. ⁶¹Department of Biology, Minneapolis Community and Technical College, Minneapolis, MN, USA. ⁶²Department of Natural Sciences, Ecology and Environment Research Centre, Manchester Metropolitan University, Manchester, UK. ⁶³Instituto de Investigaciones en Ecosistemas y Sustentabilidad, UNAM, Morelia, Mexico. ⁶⁴Department of Botany, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece. ⁶⁵Faculty of Biological and Environmental Science, Organismal & Evolutionary Biology Research Programme, University of Helsinki, Helsinki, Finland. ⁶⁶Corporación Científica Ingeobosque, Medellín, Antioquia, Colombia. ⁶⁷GTA Colombia S.A.S. Enviado, Antioquia, Colombia. ⁶⁸Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, Scotland, UK. ⁶⁹Department of Biology, Hendrix College, Conway, AR, USA. ⁷⁰Department of Ecological Science, Vrije Universiteit Amsterdam, Amsterdam, Netherlands. ⁷¹Departamento de Ciencias Biológicas y Agropecuarias, Universidad Técnica Particular de Loja, Loja, Ecuador. ⁷²Departamento de Biología, Universidade Federal de Santa Maria (UFSM), Santa Maria, Rio Grande do Sul, Brazil. ⁷³Department of Plant Sciences, School of Biology, College of Science, University of Tehran, Tehran, Iran. ⁷⁴NTNU University Museum, Norwegian University of Science and Technology, 7491 Trondheim, Norway. ⁷⁵Red de Estudios Moleculares Avanzados, Instituto de Ecología A. C., Xalapa, Mexico. ⁷⁶School of Biological Sciences, University of Reading, Whiteknights Park, Reading, Berkshire, UK. ⁷⁷Department of Biology, Northern Arizona University, Flagstaff, AZ, USA. ⁷⁸Department of Biological Sciences, MacEwan University, Edmonton, AB, Canada. ⁷⁹Max Planck Institute for Plant Breeding Research, Cologne, Germany. ⁸⁰Departamento

de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad de México, México. ⁸¹Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany. ⁸²BIO5 Institute, University of Arizona, Tucson, AZ, USA. ⁸³Alaska Center for Conservation Science, University of Alaska Anchorage, Anchorage, AK, USA. ⁸⁴Tropical Diversity, Royal Botanical Garden of Edinburgh, Edinburgh, UK. ⁸⁵Département de biologie, Université de Moncton, Moncton, New Brunswick, Canada. ⁸⁶Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada. ⁸⁷Departments of Microbiology & Statistics, University of Manitoba, Winnipeg, MB, Canada. ⁸⁸Department of Biology, University of New Brunswick, Fredericton, NB, Canada. ⁸⁹Department of Biology, Kalamazoo College, Kalamazoo, MI, USA. ⁹⁰BioProtection Research Centre, Lincoln University, Lincoln, Canterbury, New Zealand. ⁹¹Departamento de Ciencias, Facultad de Artes Liberales, Universidad Adolfo Ibáñez, Santiago, Chile. ⁹²Department of Ecology, Evolution, and Behaviour University of Minnesota, Minneapolis, MN, USA. ⁹³Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada. ⁹⁴Department of Environmental Toxicology, University of California, Davis, CA, USA. ⁹⁵CB - University of Talca, Chile. ⁹⁶School of Molecular and Life Science, Curtin University, Perth, Australia. ⁹⁷College of Science, Health, Engineering and Education, Murdoch University, Murdoch, WA, Australia. ⁹⁸School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia. ⁹⁹School of Biological Sciences, Monash University, Melbourne, VIC, Australia. ¹⁰⁰Department of Biological Sciences, Wayne State University, Detroit, MI, USA. ¹⁰¹Department of Biology, Western Oregon University, Monmouth, OR, USA. ¹⁰²School of Natural Resources and the Environment, University of Arizona, Tucson, AZ, USA. ¹⁰³Departamento de Ecología Humana, Cinvestav Mérida, Yucatán, México. ¹⁰⁴Departamento de Ciencias Biológicas y Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile. ¹⁰⁵Institute of Ecology and Biodiversity (IEB), Chile. ¹⁰⁶Department of Biology, Lund University, Lund, Sweden. ¹⁰⁷Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway. ¹⁰⁸Escuela Superior de Desarrollo Sustentable, Universidad Autónoma de Guerrero -CONACYT, Las Tunas, Mexico. ¹⁰⁹Clarkson Secondary School, Peel District School Board, Mississauga, ON, Canada. ¹¹⁰Homelands Sr. Public School, Peel District School Board, Mississauga, ON, Canada. ¹¹¹Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, USA. ¹¹²St. James Catholic Global Learning Centre, Dufferin-Peel Catholic District School Board, Mississauga, ON, Canada. ¹¹³Department of Biosciences, University of Calgary, Calgary, AB, Canada. ¹¹⁴Ecological Processes Branch, U.S. Army ERDC-CERL, Champaign, IL, USA. ¹¹⁵Department of Biology, Oberlin College, Oberlin, OH, USA. ¹¹⁶Escuela Nacional de Estudios Superiores Unidad Morelia, UNAM, Morelia, Mexico. ¹¹⁷Institute of Evolution and Ecology, University of Tübingen, Tübingen, Germany. ¹¹⁸Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse, Zurich, Switzerland. ¹¹⁹Urban Wildlife Institute, Department of Conservation and Science, Lincoln Park Zoo, Chicago, IL, USA. ¹²⁰Departamento de Ecología, Universidad Católica de la Santísima Concepción, Concepción, Chile. ¹²¹Department of Biological Sciences, University of Denver, Denver, CO, USA. ¹²²Department of Biological Sciences, Mississippi State University, Starkville, MS, USA. ¹²³Department of Biology, Center for Computational & Integrative Biology, Rutgers University-Camden, Camden, NJ, USA. ¹²⁴Programa de Pós-Graduação em Geografia da UFMT, campus de Rondonópolis, Brasil. ¹²⁵Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China. ¹²⁶Department of Chemistry & Biochemistry, Laurentian University, Sudbury, ON, Canada. ¹²⁷Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, College of Life Sciences, Beijing Normal University, Beijing, China. ¹²⁸School of BioSciences, University of Melbourne, Melbourne, VIC, Australia. ¹²⁹Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Coyoacán, Mexico City, 04510, Mexico. ¹³⁰Department of Biological Sciences, Auburn University, Auburn, AL, USA. ¹³¹Department of Entomology and Nematology, University of California, Davis, CA, USA. ¹³²Department of Biology, University of New Mexico, Albuquerque, NM, USA. ¹³³Department of Biology, University of Wisconsin - Eau Claire, Eau Claire, WI 54701. ¹³⁴Agriculture Institute, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. ¹³⁵Department of Biology, Colby College, Waterville, ME, USA. ¹³⁶Instituto de Biología, Universidad de Antioquia, Medellín, Colombia. ¹³⁷Department of Biology, University of Massachusetts Boston, Boston, MA, USA. ¹³⁸Agricultural Biology, Colorado State University, Fort Collins, CO, USA. ¹³⁹Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Av. Reina Mercedes s/n, 41012

Sevilla, Spain. ¹⁴⁰Facultad de Estudios Interdisciplinarios, Centro GEMA- Genómica, Ecología y Medio Ambiente, Universidad Mayor, Santiago, Chile. ¹⁴¹Evolutionary Ecology Group, Naturalis Biodiversity Center, Leiden, Netherlands. ¹⁴²Department of Biology and Chemistry, Nipissing University, North Bay, ON, Canada. ¹⁴³Center for Ecological Research, Kyoto University, Otsu, Shiga, Japan. ¹⁴⁴Bonanza Creek Long Term Ecological Research Program, University of Alaska Fairbanks, Fairbanks, AK, USA. ¹⁴⁵Department of Botany and Molecular Evolution, Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt am Main, Germany. ¹⁴⁶Departamento de Biodiversidade, Instituto de Biociências, Univ Estadual Paulista - UNESP, Rio Claro, São Paulo, Brazil. ¹⁴⁷Nelson Institute for Environmental Studies, University of Wisconsin-Madison, Madison, WI, USA. ¹⁴⁸Department of Biology, California State University, Northridge, Los Angeles, CA, USA. ¹⁴⁹Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden. ¹⁵⁰Facultad de Ciencias y Biotecnología, Universidad CES, Medellín, Colombia. ¹⁵¹Department of Biology, Hofstra University, Long Island, NY, USA. ¹⁵²Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway. ¹⁵³Division of Biological Sciences, University of California San Diego, San Diego, CA, USA. ¹⁵⁴Department of Biology, University of Richmond, Richmond, VA, USA. ¹⁵⁵Estación de Biodiversidad Tiputini, Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito USFQ, Quito, Ecuador. ¹⁵⁶Department of

Biological Sciences, Institute of Environment, Florida International University, Miami, FL, USA. ¹⁵⁷Agronomy Department, University of Almería, Almería, Spain. ¹⁵⁸Department of Biological Sciences and Center for Urban Ecology and Sustainability, Butler University, Indianapolis, IN, USA. ¹⁵⁹Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA. ¹⁶⁰Faculty of Biological Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany. ¹⁶¹Institute of Biology Leiden, Leiden University, Leiden, Netherlands. ¹⁶²Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland. ¹⁶³Department of Biology, University of Louisville, Louisville, KY, USA. ¹⁶⁴Organization for Programs on Environmental Science, University of Tokyo, Tokyo, Japan. ¹⁶⁵Université Paris-Saclay, CNRS, AgroParisTech, Ecologie Systématique et Evolution, 91405, Orsay, France. ¹⁶⁶Department of Biology, Providence College, Providence, RI, USA. ¹⁶⁷General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle, Germany. ¹⁶⁸International Arctic Research Center, University of Alaska Fairbanks, Fairbanks, AK, USA. ¹⁶⁹Science, Technology and Society Department, Rochester Institute of Technology, Rochester, NY, USA. ¹⁷⁰SLU Swedish Species Information Centre, Swedish University of Agricultural Sciences, Uppsala, Sweden. ¹⁷¹Department of Biology, Westfield State University, Westfield, MA, USA. ¹⁷²Centre of New Technologies, University of Warsaw, Warsaw, Poland. ¹⁷³Department of Biology, Stanford

University, Stanford, CA, USA. ¹⁷⁴UMR 0980 BAGAP, Agrocampus Ouest-ESA-INRA, Rennes, France. ¹⁷⁵Plant Biology Department, Michigan State University, East Lansing, MI, USA. ¹⁷⁶Biology Department, Davidson College, Davidson, NC, USA. ¹⁷⁷College of Horticulture and Forestry Sciences/ Hubei Engineering Technology Research Center for Forestry Information, Huazhong Agricultural University, Wuhan, China, Hubei, China. ¹⁷⁸School of Life Sciences, Technical University of Munich, Munich, Germany. ¹⁷⁹School of Life Sciences, Lanzhou University, Lanzhou, China. ¹⁸⁰Institute of Ecology and Evolution, University of Bern, Bern, Switzerland. ¹⁸¹Department of Evolution, Ecology and Behaviour, University of Liverpool, Liverpool, UK.

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Material and Methods

Supplementary Text

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Global urban environmental change drives adaptation in white clover

James S. Santangelo Rob W. Ness Beata Cohan Connor R. Fitzpatrick Simon G. Innes Sophie Koch Lindsay S. Miles Samreen Munim Pedro R. Peres-Neto Cindy Prashad Alex T. Tong Windsor E. Aguirre Philips O. Akinwole Marina Alberti Jackie Álvarez Jill T. Anderson Joseph J. Anderson Yoshino Ando Nigel R. Andrew Fabio Angeoletto Daniel N. Anstett Julia Anstett Felipe Aoki-Gonçalves A. Z. Andis Arietta Mary T. K. Arroyo Emily J. Austen Fernanda Baena-Díaz Cory A. Barker Howard A. Baylis Julia M. Beliz Alfonso Benitez-Mora David Bickford Gabriela Biedebach Gwylim S. Blackburn Manfred M. A. Boehm Stephen P. Bonser Dries Bonte Jesse R. Bragger Cristina Branquinho Kristian I. Brans Jorge C. Bresciano Peta D. Brom Anna Bucharova Briana Burt James F. Cahill Katelyn D. Campbell Elizabeth J. Carlen Diego Carmona Maria Clara Castellanos Giada Centenarolzan Chalen Jaime A. Chaves Mariana Chávez-Pesqueira Xiao-Yong Chen Angela M. Chilton Kristina M. Chomiak Diego F. Cisneros-Heredia Ibrahim K. Cisse Aimée T. Classen Mattheau S. Comerford Camila Cordoba Fradinger Hannah Corney Andrew J. Crawford Kerri M. Crawford Maxime Dahirel Santiago David Robert De Haan Nicholas J. Deacon Clare Dean Ek del-Val Eleftherios K. Deligiannis Derek Denney Margaret A. Dettlaff Michelle F. Di Leo Yuan-Yuan Ding Moisés E. Dominguez-López Davide M. Dominoni Savannah L. Draud Karen Dyson Jacintha Eilers Carlos I. Espinosa Liliana Essi Mohsen Falahati-Anbaran Jéssica C. F. Falcão Hayden T. Fargo Mark D. E. Fellowes Raina M. Fitzpatrick Leah E. Flaherty Pádraic J. Flood María F. Flores Juan Fornoni Amy G. Foster Christopher J. Frost Tracy L. Fuentes Justin R. Fulkerson Edeline Gagnon Frauke Garbsch Colin J. Garroway Aleeza C. Gerstein Mischa M. Giasson E. Binney Girdler Spyros Gkelis William Godsoe Anneke M. Golemić Mireille Golemić César González-Lagos Amanda J. Gorton Kiyoko M. Gotanda Gustaf Granath Stephan Greiner Joanna S. Griffiths Filipa Grilo Pedro E. Gundel Benjamin Hamilton Joyce M. Hardin Tianhua He Stephen B. Heard André F. Henriques Melissa Hernández-Poveda Molly C. Hetherington-Rauth Sarah J. Hill Dieter F. Hochuli Kathryn A. Hodgins Glen R. Hood Gareth R. Hopkins Katherine A. Hovanes Ava R. Howard Sierra C. Hubbard Carlos N. Ibarra-Cerdeña Carlos Iñiguez-Armijos Paola Jara-Arancibia Benjamin J. M. Jarrett Manon Jeannot Vania Jiménez-Lobato Mae Johnson Oscar Johnson Philip P. Johnson Reagan Johnson Matthew P. Josephson Meen Chel Jung Michael G. Just Aapo Kahilainen Otto S. Kailing Eunice Kariño-Betancourt Regina Karousou Lauren A. Kirn Anna Kirschbaum Anna-Liisa Laine Jalene M. La Montagne Christian Lampe Carlos Lara Erica L. Larson Adrián Lázaro-Lobo Jennifer H. LeDeleon S. Leandro Christopher Lee Yunting Lei Carolina A. León Manuel E. Lequerica Tamara Danica C. Levesque Wan-Jin Liao Megan Ljubotina Hannah Locke Martin T. Lockett Tiffany C. Longo Jeremy T. Lundholm Thomas MacGillavry Christopher R. Mackin Alex R. Mahmoud Isaac A. Manju Janine Mariën D. Nayeli Martínez Marina Martínez-Bartolomé Emily K. Meineke Wendy Mendoza-Arroyo Thomas J. S. Merritt Lila Elizabeth L. Merritt Giuditta Migiani Emily S. Minor Nora Mitchell Mitra Mohammadi Bazargani Angela T. Moles Julia D. Monk Christopher M. Moore Paula A. Morales-Morales Brook T. Moyers Miriam Muñoz-Rojas Jason Munshi-South Shannon M. Murphy Maureen M. Murúa Melisa Neila Ourania Nikolaidis Iva Njunji#Peter Nosko Juan Núñez-Farfán Takayuki Ohgushi Kenneth M. Olsen Øystein H. Opedal Cristina Ornelas Amy L. Parachnowitsch Aaron S. Paratore Angela M. Parody-Merino Juraj Paule Octávio S. Paulo João Carlos Pena Vera W. Pfeiffer Pedro Pinho Anthony Pottluga M. Porth Nicholas Poulos Adriana Puentes Jiao Qu Estela Quintero-Vallejo Steve M. Raciti Joost A. M. Raeymaekers Krista M. Raveala Diana J. Rennison Milton C. Ribeiro Jonathan L. Richardson Gonzalo Rivas-Torres Benjamin J. Rivera Adam B. Roddy Erika Rodriguez-Muñoz José Raúl Román Laura S. Rossi Jennifer K. Rowntree Travis J. Ryan Santiago Salinas Nathan J. Sanders Luis Y. Santiago-Rosario Amy M. Savage J.F. Scheepens Menno Schilthuizen Adam C. Schneider Tiffany Scholier Jared L. Scott Summer A. Shaheed Richard P. Shefferson Caralee A. Shepard Jacquie A. Shykoff Georgianna Silveira Alexis D. Smith Lizet Solis-Gabriel Antonella Soro Katie V. Spellman Kaitlin Stack Whitney Indra Starke-Ottich Jörg G. Stephan Jessica D. Stephens Justyna Szulc Marta Szulkin Ayco J. M. Tackálo Tamburrino Tayler D. Tate Emmanuel Tergemina Panagiotis Theodorou Ken A. Thompson Caragh G. Threlfall Robin M. Tinghitella Lilibeth Toledo-Chelala Xin Tong Léa Uroy Shunsuke Utsumi Martijn L. Vandegehuchte Acer Van Wallendael Paula M. Vidal Susana M. Wadgyamar Ai-Ying Wang Nian Wang Montana L. Warbrick Kenneth D. Whitney Miriam Wiesmeier J. Tristian Wiles Jianqiang Wu Zoe A. Xirocostas Zhaogui Yan Jiahe Yao Jeremy B. Yoder Owen Yoshida Jingxiang Zhang Zhigang Zhao Carly D. Ziter Matthew P. Zuellig Rebecca A. Zufall Juan E. Zurita Sharon E. Zytynska Marc T. J. Johnson

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Plants adapt to city environments

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Urban development alters the local environment, potentially driving rapid evolution. Santangelo *et al.* collected data on white clover populations from 160 cities to test for consistent responses to urban environments. They found that the production of an antiherbivore chemical defense increased with greater distance from the urban center in many cities. Genomic data suggest that this trend is adaptive, likely in response to lowered drought stress and herbivory pressure in urban centers. This study from the Global Urban Evolution Project provides evidence of widespread adaptation to urbanization. —BEL

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