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Climate warming reduces gut microbiota diversity in a vertebrate ectotherm

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Climate change is now considered to be the greatest threat to biodiversity and ecological networks, but its impacts on the bacterial communities associated with plants and animals remain largely unknown. Here, we studied the consequences of climate warming on the gut bacterial communities of an ectotherm, the common lizard (*Zootoca vivipara*), using a semi-natural experimental approach. We found that 2–3 °C warmer climates cause a 34% loss of populations' microbiota diversity, with possible negative consequences for host survival.

Climate change is a major threat to biodiversity and ecosystem functioning[1,2]. It leads to phenological and range shifts[2], and population and species extinctions[2,3], and should therefore alter many interspecific interactions. In particular, species with strong, obligate interactions, such as those involving specialist predators, parasites or symbionts should be impacted by changes in their interaction networks[4].

One of the most intricate symbiotic relationships is probably that between animal hosts and the bacterial community inhabiting their guts[5]. The gut microbiota is shaped by host traits (for example, immunity[6] and metabolism[7]) and environment[8] (for example, population density and diet[9–11]) and, in turn, plays multiple essential functions for hosts, including digestion, immunity and life history[5,12]. Consequently, changes in the gut microbiota could lead to potential dysbioses with strong consequences for hosts and ecosystems[13]. These changes may be caused by rising temperatures that lead to an increase in disease prevalence, as suggested for humans[14]. Understanding the factors affecting host–microbiota interactions would improve predictions on biodiversity responses to climate warming[15]. While studying such interactions *in situ* remains a complex challenge, a promising alternative lies in warming experiments in settings that mimic natural environments.

Here, we experimentally tested the impact of climate warming on the gut bacterial microbiota of the widespread lizard species *Zootoca vivipara*. We used a system composed of large semi-natural enclosures in which climate can be manipulated[3]. In the first experiment, nine lizard populations were allocated to three climatic treatments throughout the summer: 'present climate' (current local climate), 'intermediate climate' (+2 °C) and 'warm climate' (+3 °C), which are consistent with the Intergovernmental Panel on Climate Change projections[16] and critical for the life history and population dynamics of lizards[3]. After summer climatic treatments (mid-June to mid-September; see Methods), individuals were all maintained in present climatic conditions and their cloacal bacterial communities were sampled as a proxy of hindgut the next spring.

We found warmer climates to have a strong negative effect on the total bacterial richness of individual hosts, independent of bacterial abundance (Fig. 1a and Supplementary Tables 1–4), with a particular impact on the most diverse bacterial phyla (Fig. 1b and Supplementary Tables 5–7). This effect remained after controlling for variation in the number of sequences (Supplementary Tables 1–3 and Supplementary Figs 1–4) and removing individuals with low numbers of sequences (Supplementary Table 3). However, bacterial community composition was only weakly affected by climatic conditions (Fig. 1c), with no impact on community evenness (Supplementary Table 8). Still, the relative abundance in major bacterial phyla differed between climates (Fig. 1d and Supplementary Table 9). The relative abundance was higher in the 'present climate' than in the 'warm climate' for Bacteroidetes and Firmicutes. In contrast, the relative abundance of Proteobacteria tended to be higher in the 'warm climate' (Fig. 1d and Supplementary Table 9). We further identified 24 operational taxonomic units (OTUs)—members of Bacteroidetes (Bacteroidales) and Firmicutes (Lachnospiraceae)—whose relative abundance significantly decreased with warming (Supplementary Table 10), while the abundance of eight OTUs (mainly Proteobacteria) increased in the 'warm climate' (see Supplementary Results).

In addition, individual microbiota diversity loss had consequences at the population level. There was a 34% decrease of total bacterial diversity with warming (warm: $2{,}451 \pm 1{,}194$ standard error (s.e.); present: $3{,}708 \pm 938$ s.e.). Warming also caused greater variability in microbial community composition within host populations (the mean $\pm$ s.e. Jaccard index within a population for the 'present climate' was 0.446 ± 0.002, while for the 'warm climate' it was 0.458 ± 0.004; chi-square statistic: 3.86; $P = 0.0495$), as was also found in a laboratory warming experiment on tadpoles[17]. A three-month increase in temperature thus had long-lasting (eight month) deleterious effects on gut bacterial diversity. This effect was also visible in our second short-term experiment, for which we sampled microbiota before and after two-month-long summer climatic treatments. While richness overall increased during the summer, this increase was lower in the 'warm climate' due to a higher bacteria extinction rate in warmer climates (Supplementary Tables 11 and 12). Bacterial richness was therefore lower in warmer climates in the short term, which is congruent with the long-term effects (Supplementary Table 11).

The pathways of such climatic impacts are unclear. These impacts could arise through modification of a hosts' environment (for example, climate-induced shifts in prey communities or social/sexual context affecting the pool of environmentally acquired bacteria that can

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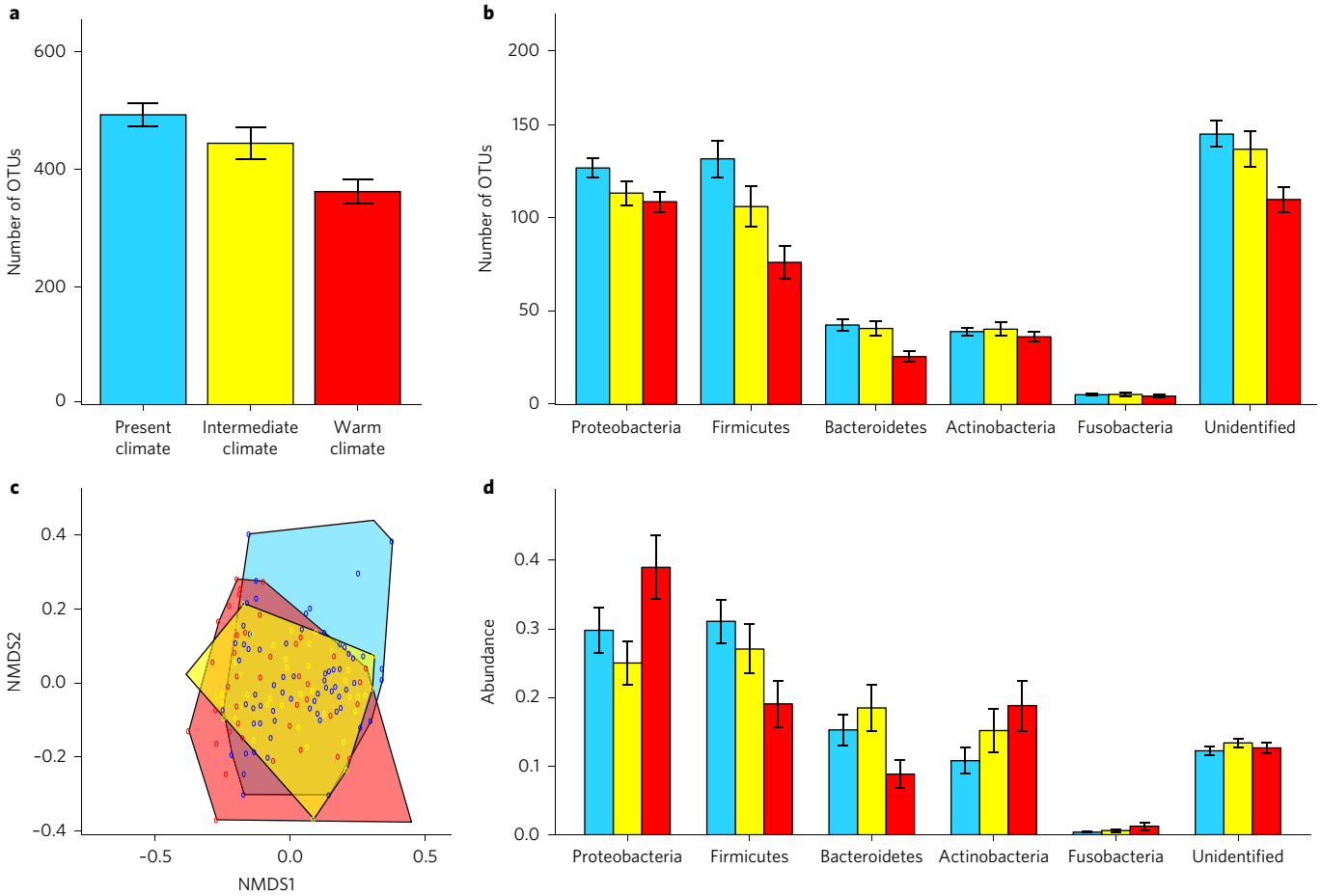

Figure 1 | Impact of climate on various outcomes. a, Bacterial richness (Supplementary Table 1). *P*-value for climate effect: 0.017. **b**, Bacterial richness of the most diverse phyla (phyla with the highest number of OTUs plus unidentified OTUs; see Supplementary Tables 5–7). **c**, Non-metric dimensional scaling (NMDS) representation of the community dissimilarities of hosts. Permutational multivariate analysis of variance: $F_{2,147}=1.42$, $P=0.01$, $R^2=0.02$). **d**, Individual abundance in the most diverse phyla per climate (see Supplementary Table 9 for significance). Blue indicates 'present climate' ($n=68$ lizards), yellow 'intermediate' ($n=41$) and red 'warm' ($n=41$). The effect of climate on bacterial richness did not rely on differences in sample sequencing depth or the occurrence of rare taxa (Supplementary Figs 1–4 and Supplementary Tables 1–4). Bars represent means ± s.e.m.

be ingested or sexually/socially transmitted[9–11]) or through a change in the hosts themselves (for example, in their individual condition, immune system, physiology or behaviour[7,18]). However, in the first experiment, neither host body condition, nor social, sexual or dietary contexts measured at the time of the microbiota sampling could explain the decrease in bacterial diversity in warmer climates (Supplementary Table 13). These preliminary analyses cannot disentangle the different pathways of climatic impacts, such as physiological (for example, metabolism[7]) and behavioural (for example, diet composition[11] or mating behaviour) mechanisms, which should be investigated further. Regardless of the exact pathway, a less diverse microbiota may affect a host's health and survival prospects in warmer climates.

A higher gut bacterial diversity is often beneficial to hosts[11], thus a climate-driven diversity reduction might be detrimental for them[19], particularly if it entails a loss of essential functions[20,21]. Conversely, a loss of pathogenic taxa might be beneficial. The 24 OTUs notably depleted in warm climates were mainly members of Bacteroidales and Lachnospiraceae, two groups particularly important to reptile microbiota[22,23]. Their depletion may be deleterious for the host through, for example, reduced nutrient assimilation, or may facilitate the colonization of the gut by pathogens, as suggested by a laboratory warming experiment on tadpoles[17]. To better understand these potential climate-driven changes in host–microbiota feed-backs, we studied the differences in bacterial functional profiles between warm and present climates in the first experiment through a phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis (Supplementary Tables 15 and 16). The results of such an analysis in non-model organisms such as the common lizard should be interpreted with caution. However, we found that functional features associated with metabolism, information processing and cellular processes were differentially abundant between climates, but in different directions for females and males (Supplementary Tables 15 and 16). For example, metabolism-associated functional features (for example, lipid and energy metabolism) increased with warming in male hosts and conversely decreased in female hosts. This difference in functional profiles was also supported by a permutational multivariate analysis of variance on functional features, which showed a significant interaction between climate and sex (permutational multivariate analysis of variance F statistic $(F)_{1,146}=9.35$; $P=0.001$). The functional profiles were impacted by climatic conditions in males ($F_{1,76}=4.37$; $P=0.018$) and females ($F_{1,70}=5.06$; $P=0.004$), but in different ways (Supplementary Fig. 4 and Supplementary Table 15). These contrasting functional changes in the gut microbiota shed light on interesting sex-specific responses to climatic change that will require further investigation. It is, however, difficult to infer the fitness consequences of such functional changes for hosts in the absence of a direct assessment of impacts on these hosts.

We therefore further monitored the survival of lizards involved in the first experiment in common garden enclosures in the 'present climate' for one extra year. In this third experiment, bacterial richness positively correlated with host survival one year later, and this was independent of previous climatic treatments and body condition (Supplementary Table 17 and Supplementary Fig. 5). Host survival was further correlated with a principal component axis summarizing taxa differently abundant between climates (Supplementary Table 20). Loss of bacterial taxa might therefore have a negative impact on lizard survival. As our statistical models included climatic treatments and body condition, the effect of bacterial diversity is likely to be independent of other variables potentially modified by climatic conditions (for example, host physiology and diet). However, since these results are correlative, further studies involving direct manipulation of microbial diversity (for example, through faecal transplant experiments) would be needed to fully validate our conclusion. Nonetheless, to our knowledge, this is the first study to show that climate warming induces alterations in the diversity, structure and function of a neglected level of biological diversity in a natural setting, with potential adverse consequences for the host at both individual and population scales. Future studies should aim to investigate this overlooked effect of climate change to better predict its impact on biodiversity.

Methods

Long-term effect of climate on microbiota. In the first experiment, we used lizard populations previously captured from natural populations in the Cevennes mountains in accordance with French authority regulations and maintained using methods approved by the French authorities (licence no. 2010-189-16 Direction Régionale de l'Environnement, de l'Aménagement et du Logement). We worked in the Metatron, an ensemble of 48 semi-natural enclosures of 100 m^2 typical of lizards' habitats, in which climate could be manipulated[3] (see Supplementary Methods and http://themetatron.weebly.com/). In June 2012, nine enclosures were allocated to three treatments: present, intermediate or warm climate[3]. Two-hundred-and-forty-one adults and yearlings and 365 juveniles were then released into nine populations (adults: 6 ± 1 males, 10 ± 1 females; yearlings: 11 ± 1; juveniles: 40 ± 3).

Lizards were maintained for one year in the enclosures. Climatic treatments were effective from mid-June to mid-September 2012 (mean $\pm$ s.e. daily temperatures, present: 26.6 ± 0.4 °C; intermediate: 28.2 ± 0.5 °C; warm: 28.4 ± 0.5 °C; maximum daily temperatures: present: 29.3 ± 0.4 °C; intermediate: 31.5 ± 0.5 °C; warm: 32.1 ± 0.6 °C)[3]. In May 2013, surviving lizards (92 adults and 73 yearlings) were sampled for cloacal microbiota analyses. In reptiles, cloacal samples may encompass the breadth of bacterial diversity in the gut[23], even if they should more likely be representative of the hindgut bacterial community[22]. We used high-throughput sequencing of the bacterial 16S ribosomal ribonucleic acid gene to quantify the diversity and composition of the cloacal microbiota[24]. Molecular, bioinformatic and statistical analyses are described in the Supplementary Methods.

Short-term microbiota changes. To investigate whether climate-induced changes in bacterial communities resulted from differences in bacterial extinction rates or in the proportion of new taxa gained, we created a second experiment during summer 2013 with a set of 84 new adult individuals. After cloacal microbiota sampling in June, individuals were released into four 'present climate' and four 'warm climate' enclosures in mid-July (adults: 11 males, 10 females). Populations were maintained for two months in present or warm climates and the microbiota of the surviving ($n = 48$) lizards was resampled in mid-September.

Effect of microbiota on survival in a common garden. In July 2013, we released the lizards from the first experiment into five 'present climate' enclosures to test the impact of microbiota richness on survival one year later. In total, 78 individuals (present: 37; intermediate: 20; warm: 21) were randomly assigned to an enclosure. Populations were completed with juveniles and other adults and yearlings previously maintained in separate enclosures (adults: 12 ± 0 females, 7 ± 1 males; yearlings: 7 ± 1; juveniles: 35 ± 2). Populations were left for one year, and in May 2014, we recaptured all of the survivors.

Data availability. The data presented in this manuscript are available on Pangaea.de (https://doi.pangaea.de/10.1594/PANGAEA.874404 (Elvire Bestion, University of Exeter)).

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Author contributions

E.B., J.W. and J.C. designed the study. E.B. and J.C. performed the experiments. E.B. collected genetic data, L.D.G. and J.W. extracted microbial genetic data, and M.R. extracted and analysed lizard genetic data. S.J., J.W. and L.Z. performed molecular and bioinformatics analyses, and E.B., J.C., L.D.G. and S.J. analysed the data. E.B. wrote the first draft, and J.C., J.W., S.J. and L.Z. contributed significantly to the writing of the manuscript.

Additional information

Supplementary information is available for this paper.

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Competing interests

The authors declare no competing financial interests.