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Author for correspondence:
Felix Moerman
e-mail: felix.moerman@ieu.uzh.ch

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Evolutionary biology

Gene swamping alters evolution during range expansions in the protist *Tetrahymena thermophila*

Felix Moerman[1,2,3,4], Emanuel A. Fronhofer[4], Andreas Wagner[1,3,5] and Florian Altermatt[1,2]

[1]Department of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, Zürich CH-8057, Switzerland
[2]Department of Aquatic Ecology, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, Dübendorf CH-8600, Switzerland
[3]Swiss Institute of Bioinformatics, Quartier Sorge—Bâtiment Génopode, Lausanne 1015, Switzerland
[4]ISEM, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France
[5]The Santa Fe Institute, Santa Fe, New Mexico 87501, USA

FM, 0000-0002-5164-0978; EAF, 0000-0002-2219-784X; AW, 0000-0003-4299-3840; FA, 0000-0002-4831-6958

At species' range edges, individuals often face novel environmental conditions that may limit range expansion until populations adapt. The potential to adapt depends on genetic variation upon which selection can act. However, populations at species' range edges are often genetically depauperate. One mechanism increasing genetic variation is reshuffling existing variation through sex. Sex, however, can potentially limit adaptation by breaking up existing beneficial allele combinations (recombination load). The gene swamping hypothesis predicts this is specifically the case when populations expand along an abiotic gradient and asymmetric dispersal leads to numerous maladapted dispersers from the range core swamping the range edge. We used the ciliate *Tetrahymena thermophila* as a model for testing the gene swamping hypothesis. We performed replicated range expansions in landscapes with or without a pH-gradient, while simultaneously manipulating the occurrence of gene flow and sexual versus asexual reproduction. We show that sex accelerated evolution of local adaptation in the absence of gene flow, but hindered it in the presence of gene flow. However, sex affected adaptation independently of the pH-gradient, indicating that both abiotic gradients and the biotic gradient in population density lead to gene swamping. Overall, our results show that gene swamping alters adaptation in life-history strategies.

1. Introduction

Individuals living at the edge of a species' range face different conditions compared to those in the core region. Selection pressures differ, and often the individuals at the edge represent only a small subset of a species' genetic variation [1]. The potential of a population to spread depends on its capacity to disperse and its ability to grow in the local abiotic environment [2]. Consequently, when populations expand their range, they experience strong selection owing to the range expansion itself, and are also affected by concurrently changing environmental conditions.

During range expansions, populations can undergo rapid evolution, as demonstrated by recent comparative and experimental work [1], showing evolution of increased dispersal [3–6], r-selected life-history strategies [7,8] and adaptation to abiotic conditions [9,10]. Expanding into previously uninhabited space allows populations to escape intraspecific competition. Consequently,

evolving in response to multiple selective pressures can potentially lead to substantial benefits, despite the challenges involved [8,11].

A major modulator of evolution is sex. Sex allows populations to reshuffle existing genetic variation [12–15]. Theoretical work suggests that sex would typically lead to offspring with lower fitness, by breaking up advantageous allele combinations (recombination load), and hence an advantage for asexual reproduction [16]. However, populations during range expansion experience strong stochasticity owing to repeated founder events, leading to maladaptive mutations becoming fixed and surfing along at the range edge (expansion load) [17,18]. Sex can strongly reduce these negative effects of expansion load, thus making it advantageous [18–20].

If populations face strong abiotic stressors or heterogeneous environments, sex may also facilitate adaptation [21–23]. Given that some experimental work found stronger benefits of sex if genetic variation is sufficiently high [24], we expect that sex is only favoured at the range edge when genetic variation is bolstered through gene flow from the high diversity core, because populations at a range edge are genetically depauperated owing to repeated founder events [1,25]. However, theory on gene swamping predicts the opposite [26–28]. As individuals bolstering the gene pool will be maladapted to the abiotic conditions at the range edge, sex may hinder adaptation when there is too much gene flow from the range core to the range edge [26–30]. Under such conditions, reproducing sexually would swamp the gene pool at the range edge with maladapted genes. This could prevent the population from adapting to the abiotic environment at the range edge, and hence slow down and even halt range expansion, leading to stable range borders [27,28]. By contrast, when drift strongly reduces adaptive variation, gene flow may positively affect adaptation by counteracting the effects of drift [29,30]. Despite extensive theory on gene swamping, surprisingly little empirical and experimental work exists (reviewed in [31–34]).

Here, we experimentally tested the gene swamping hypothesis using the ciliate *Tetrahymena thermophila*. We assessed how reproduction (asexual or sexual) and gene flow (i.e.dispersal from the range core to the range edge) altered evolutionary adaptation during range expansions in landscapes with or without a gradient in pH. We found a distinct signal of gene swamping, where sex facilitated or hindered adaptation depending on the presence or absence of gene flow.

2. Material and methods

2.1. Study organism

Tetrahymena thermophila is a freshwater ciliate commonly used in ecological and evolutionary experiments [35–40]. We used four phenotypically divergent [41] clonal strains of *T. thermophila* obtained from the Tetrahymena Stock Center: strain B2086.2 (Research Resource Identifier TSC_SD00709), strain CU427.4 (TSC_SD00715), strain CU428.2 (TSC_SD00178) and strain SB3539 (TSC_SD00660).

2.2. Experiment

2.2.1. Microcosms

We performed all evolution experiments and all bioassays in a 20°C climate-controlled room. Following an established method [4], we experimentally emulated an expanding range front with two-patch landscapes, which consisted of two 25 ml Sarstedt tubes connected by an 8 cm long silicone tube (inner diameter 4 mm). See also electronic supplementary material, figure S1.

We prepared 40 two-patch landscapes, and filled patches of each landscape with 15 ml modified Neff-medium [42]. We complemented the medium for experimental evolution and bioassays with 10 µg ml^{-1} Fungin and 100 µg ml^{-1} Ampicillin to prevent bacterial and fungal contamination. We then inoculated one patch of each two-patch landscape with 200 µl of ancestor culture (50 µl from each of the four ancestral strains). This allowed adaptation through clonal selection and de novo mutation [43] in populations designated for asexual reproduction, as well as recombination [44] in populations designated for sexual reproduction.

2.2.2. Treatment groups

We designed a full-factorial experiment that tested the effect of (1) abiotic conditions, with two treatment levels ('uniform': pH always 6.5, 'gradient': pH starts at 6.5 and then gradually decreases), (2) reproduction, with two treatment levels ('asexual': pure asexual reproduction, 'sexual': asexual and sexual reproduction) and (3) gene flow, with two treatment levels ('absent': no gene flow; 'present': gene flow from the range core to range edge). We evolved five replicate populations per treatment, for a total of 40 evolving populations.

2.2.3. Experimental evolution

We performed a range expansion experiment that lasted 10 weeks, in which we repeated the same procedure cycle every 14 days. This cycle consisted of three dispersal events (on days 1, 3 and 5). These events were followed by a gene flow and sexual reproduction event or the appropriate controls depending on the treatment groups (on day 8), and subsequently an additional two dispersal events (on days 10 and 12).

We initiated dispersal by opening the clamps in the two-patch landscapes for one hour, which allowed cells to disperse from their original (home) patch to the target patch. After dispersal, we prepared 40 new two-patch landscapes. If population density was measurable (1 or more cell observed during video analysis; see below) in the target patch, we transferred the content of the target patch to a new two-patch landscape. If no measurable dispersal occurred, we transferred the content of the home patch to the new two-patch landscape.

In treatment groups designated for gene flow to occur, we emulated long-distance gene flow (from the range core to the edge, following theoretical predictions [27,28]) by transferring 1.5 ml of culture from the core population to the range front.

To control reproduction, we transferred all populations to a starvation medium, because *T. thermophila* only mates when starved [44]. We incubated the starvation cultures on a shaker rotating at 120 r.p.m. After 36 h, we removed the populations designated for sexual reproduction from the shaker, but kept populations designated for asexual reproduction on the shaker, because the shaking movement prevents cells from mating. We left cells to mate overnight, after which we transferred populations to new two-patch landscapes. For a more extensive technical description, see electronic supplementary material, section S1.2.

2.2.4. Common garden

After experimental evolution, we sampled 100 µl of culture from all surviving populations, and transferred this sample to 25 ml Sarstedt tubes containing 15 ml Neff-medium at pH 6.5. We maintained these populations in the common garden for 72 h before starting bioassays, to reduce epigenetic and trans-generational effects.

2.2.5. Bioassays

We quantified the population growth rate of ancestral and evolved populations, after common garden cultivation, at eight different pH values (pH 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, 3.5 and 3.0). Specifically, for every population we prepared Sarstedt tubes containing Neff-medium whose pH we had adjusted to the desired value using 1 M HCL, and inoculated this medium with 100 µl of culture from the evolved or ancestral populations. We grew the resulting cultures for 12 days, sampling populations twice on the first 2 days, and once per day on all subsequent days. Every 2 days, we replaced 1 ml of culture with fresh medium to prevent population decline.

2.2.6. Sampling and video analysis

We measured population density and cell characteristics (morphology and movement) using an established method [36,45]. We sampled 200 µl of culture from every population, and diluted samples 10–100 fold in Neff-medium to ensure densities were similar, as excessive density prevents accurate video analysis. We then took 10 s videos (250 frames, 25 fps) using a Leica M165FC stereomicroscope and top-mounted Hamamatsu Orca Flash 4.0 camera. We analysed videos using the BEMOVI R-package [45] (parameters in electronic supplementary material, section S2).

2.3. Beverton–Holt model fitting

To analyse local adaptation, we assessed growth rates by fitting a continuous-time version of the Beverton–Holt model [46], as this model is well suited for microcosm data and facilitates biological interpretation of parameters [47,48]. The Beverton–Holt model is given by the equation

$$\frac{dN}{dt} = \left(\frac{r_0 + d}{1 + \alpha N} - d\right)N, \tag{2.1}$$

where the intraspecific competitive ability (α) is equal to

$$\alpha = \frac{r_0}{\widehat{N}d}, \tag{2.2}$$

and r_0 is the intrinsic rate of increase, N the population size, α the intraspecific competitive ability, $\widehat{N}$ the equilibrium population density and d the death rate of the population. We estimated the parameters using a Bayesian approach adapted from Rosenbaum *et al*. [49]. For model code see https://zenodo.org/record/2658131.

2.4. Statistical analysis

All statistical analyses were performed with the R language for statistical computing, v. 3.5.1. We calculated local adaptation by assessing changes in the intrinsic rate of increase r_0 of evolved populations under the pH conditions they experienced during evolution, compared to the ancestor under the same pH conditions. This was done by dividing the r_0 estimates of evolved populations by the mean r_0 of the mixed ancestral populations (populations with the initial ancestral genotype mixture), and by subsequently calculating the logarithm (base 2) of this ratio (log-ratio response).

Next, we created linear models assessing the effect of reproduction, gene flow and abiotic conditions (explanatory variables) on range expansion distance (number of successful dispersal events) and local adaptation, respectively. We additionally created a linear mixed model ('nlme'-package, v. 3.1-137) to assess how population density during range expansion was influenced by the three treatments: reproduction, gene flow, abiotic conditions, as well as the covariate range expansion distance (the number of successful dispersal events). We included population ID as a random effect. We subsequently compared all possible models for these three response variables using the dredge function ('MuMin'-package, v. 1.43.6) to select the model with lowest AICc (Akaike information criterion, corrected for small sample size [50]) score for local adaptation and range expansion distance, and lowest BIC (Bayesian information criterion [51]) for population density. We report relative importance and model output. See electronic supplementary material, section S4 for additional analyses on population survival and cell movement and morphology.

3. Results

Population densities (figure 1*a*,*b*,*e*,*f* and table 1) showed strong temporal variation in all replicates. Mean density decreased marginally for populations expanding into uniform abiotic conditions ($\chi^2_{1,746} = 4.526$, $p = 0.034$), whereas population density of populations expanding into a gradient decreased strongly ($\chi^2_{1,746} = 108.258$, $p < 0.0001$). Additionally, we observed that populations faced with a gradient showed significantly slower range expansion (figure 1*c*,*d*,*h*; $F_{1,31} = 141.4$, $p < 0.0001$; table 2), and were more prone to go extinct, in the absence of gene flow (see electronic supplementary material, section S4.6).

Local adaptation (evolution of intrinsic rate of increase r_0; figure 2 and table 2) increased only slightly for populations expanding into uniform abiotic conditions, whereas populations that expanded into a gradient greatly increased local adaptation ($F_{1,29} = 128.58$, $p < 0.0001$). Although sexual reproduction ($F_{1,29} = 3.96$, $p = 0.056$) and the presence of gene flow ($F_{1,29} = 5.55$, $p = 0.025$) individually slightly increased local adaptation, their interaction strongly decreased local adaptation ($F_{1,29} = 10.67$, $p = 0.003$), with populations evolving lower intrinsic rates of increase either when reproduction was sexual and gene flow present, or with asexual reproduction but gene flow absent.

4. Discussion

We experimentally assessed the gene swamping hypothesis using replicated range expansions of the protist *Tetrahymena thermophila*. We experimentally manipulated abiotic conditions (uniform versus gradient), reproduction (asexual versus sexual) and gene flow (absent versus present). We demonstrated how sex interacts with gene flow, affecting local adaptation of organisms at the range edge (figure 2 and table 2).

Populations undergoing range expansions face multiple selective pressures [1], and hence face a strong pressure to adapt. Theoretical predictions suggest that sex can be advantageous or disadvantageous during range expansion, depending on the context. Theory on gene swamping predicts that sex hinders adaptation during range expansions when populations undergo strong asymmetrical dispersal from a range core to a range edge [26–28]. We showed here that the effect of sex is conditional on the presence of gene flow. Despite having only four distinct events of sexual reproduction in otherwise asexually reproducing populations, we found a beneficial effect of sex on local adaptation in the absence of gene flow. However, when gene flow was present and swamped the edge population with maladapted individuals, sex hindered adaptation. Surprisingly, while the gene swamping hypothesis predicts this pattern exclusively in the presence of abiotic gradients [26–28], we observed similar effects of gene

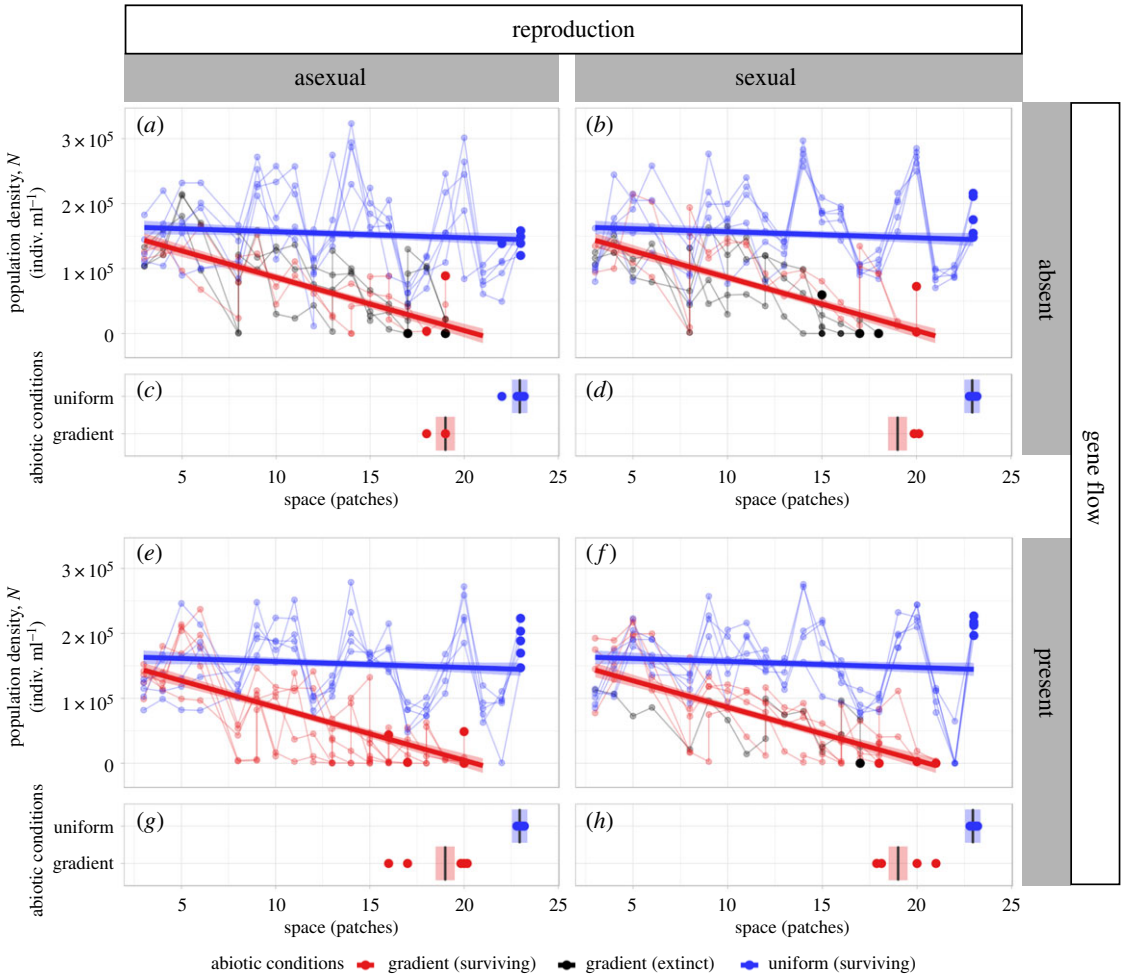

Figure 1. Population dynamics for the different treatment groups over the course of the range expansion dynamics. Faint blue lines and dots represent data for the populations expanding into uniform abiotic conditions. Faint red lines and dots show the data for populations expanding into a gradient (only given for the populations that survived until the bioassays). Faint black lines and dots show data for populations expanding into a gradient, but that went extinct before the start of the bioassays. The larger and opaque dots represent the population densities measured at the last timepoint. Thick lines and shaded areas show the mean model predictions and 95%-confidence intervals, respectively, for the best model (according to BIC/AICc comparisons through the dredge function) on population densities/range expansion distances of surviving populations expanding into a gradient (red) or uniform abiotic conditions (blue). The large panels (*a*,*b*,*e*,*f*) show population densities as a function of distance dispersed during the range expansion experiment. The small plots (*c*,*d*,*g*,*h*) show the data and model predictions on total distance expanded by the end of the range expansion experiment of the surviving populations.

Table 1. Type III ANOVA table of the best model for population density during range expansion according to BIC model comparison.

model and explanatory variables	degrees of freedom	χ^2-value	Pr (>χ^2)
abiotic conditions	1	0.044	0.833
range expansion distance	1	4.526	0.034
abiotic conditions × position	1	108.258	<0.0001

Table 2. Type III ANOVA table of the best model for local adaptation (evolution of intrinsic rate of increase r_0) and range expansion distance (total number of successful dispersal events) during range expansion according to AICc model comparison.

model and explanatory variables	degrees of freedom	*F*-value	Pr (>*F*)
local adaptation			
reproduction	1	3.96	0.056
gene flow	1	5.55	0.025
abiotic conditions	1	122.58	<0.0001
reproduction × gene flow	1	10.67	0.003
residuals	29		
range expansion distance			
abiotic conditions	1	141.4	<0.0001
residuals	31		

swamping in the presence and absence of an abiotic gradient. We argue that gene swamping in the absence of an abiotic gradient could stem from evolving life-history strategies during range expansions. Range expanding populations are thought to exhibit a gradient of decreased density towards the range front, which translates to decreased competition and selection

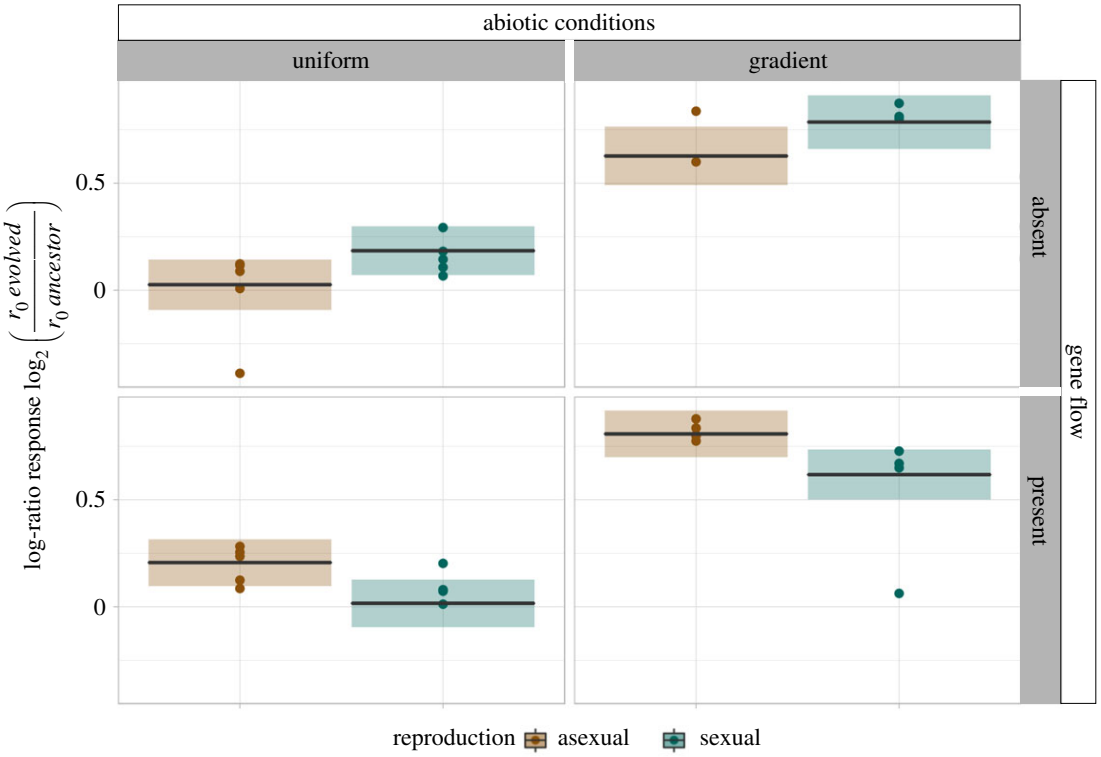

Figure 2. Local adaptation, measured as the evolution of intrinsic rate of increase r_0 in the abiotic conditions experienced during range expansion (uniform or gradient), compared to the ancestor population. The *y*-axis shows the change in r_0 compared to the ancestor (log-ratio response). Dots represent individual data points, black lines and shaded areas show the model predictions of the best model (mean and 95% CI).

for fast reproduction [52]. Hence, gene swamping may imply that individuals maladapted in life-history strategy interbreed with the population at the range edge. Consequently, gene swamping affects adaptation during range expansions even without an abiotic gradient, leading to analogous changes in adaptation as for range expansions into abiotic gradients.

Although we show that gene swamping affects adaptation during range expansions, we could not detect effects of gene swamping on range expansion rates as described by theory, despite population growth rate being a driving force behind expansion rate [2,53,54]. This discrepancy could result from our experimental set-up, where we used discrete landscapes connected through repeated dispersal events, rather than continuous dispersal. This set-up may be insufficiently sensitive to detect signals in expansion rate. Alternatively, this set-up may lead to pushed rather than pulled waves (see Pachepsky & Levine [55]), which changes predictions. Under pulled waves, dispersers from the low-density range front drive further range expansion. By contrast, further spread in pulled waves will only be possible after the population at the front has grown sufficiently large. Although it is possible that the abiotic gradient leads to a pushed wave, for example by reducing survival during the dispersal stage, determining this with absolute certainty would require extensive dispersal measurements at a temporal resolution that we lack in this experiment. Testing the interaction between pushed/pulled waves and gene swamping would, however, be interesting, as pushed waves might be less susceptible to gene swamping, because the population density gradient from the range core to the range edge is less steep compared to pulled waves.

Data accessibility. Data are available from the Dryad Digital Repository (doi:10.5061/dryad.6wwpzgmtk) [56] and analysis scripts on Github (doi:10.5281/zenodo.3560982) [57].

Authors' contributions. F.M., E.A.F., A.W. and F.A. designed the experiment. F.M. performed experimental work and statistical analyses. F.M., F.A., A.W. and E.A.F. interpreted the results. F.M. and F.A. wrote the first version of the manuscript and all authors commented on and approved of the final version. All authors are accountable for all aspects of this work.

Competing interests. We declare we have no competing interests.

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