

Warming alters life-history traits and competition in a phage community

Samuel T.E Greenrod^{1#}, Daniel Cazares Lopez¹, Serena Johnson^{1,2}, Tobias E Hector¹, Emily J Stevens¹, R Craig MacLean¹, and Kayla C King^{1,2,3#}

¹ Department of Biology, University of Oxford, Oxford, UK

² Department of Zoology, University of British Columbia, Vancouver, Canada

³ Department of Microbiology & Immunology, University of British Columbia, Vancouver, Canada

#Correspondence: samuel.greenrod@magd.ox.ac.uk (Samuel Greenrod); kayla.king@ubc.ca (Kayla King)

Abstract

Host-parasite interactions are highly susceptible to changes in temperature due to mismatches in species thermal responses. In nature, parasites often exist in communities, and responses to temperature are expected to vary between host-parasite pairs. Temperature change thus has consequences for both host-parasite dynamics and parasite-parasite interactions. Here, we investigate the impact of warming (37°C, 40°C, 42°C) on parasite life-history traits and competition using the opportunistic bacterial pathogen *Pseudomonas aeruginosa* (host) and a panel of three genetically diverse lytic bacteriophages (parasites). We show that phages vary in their responses to temperature; while 37°C and 40°C did not have a major effect on phage infectivity, infection by two phage was restricted at 42°C. This outcome was attributed to disruption of different phage life-history traits including host attachment and replication inside hosts. Further, we show that temperature mediates competition between phages by altering their competitiveness. These results highlight phage trait variation across thermal regimes with the potential to drive community dynamics. Our results have important implications for eukaryotic viromes and the design of phage cocktail therapies.

Keywords: parasite, temperature, competition, phage, life-history

Importance

Mammalian hosts often elevate their body temperatures through fevers to restrict the growth of bacterial infections. However, the extent to which fever temperatures affects the communities of phages with the ability to parasitise those bacteria remains unclear. In this study, we investigate the impact of warming across a fever temperature range (37°C, 40°C, 42°C) on phage life-history traits

and competition using a bacterium (host) and bacteriophage (parasite) system. We show that phages vary in their responses to temperature due to disruption of different phage life-history traits. Further, we show that temperature can alter phage competitiveness and shape phage-phage competition outcomes. These results suggest that fever temperatures have the potential to restrict phage infectivity and drive phage community dynamics. We discuss implications for the role of temperature in shaping host-parasite interactions more widely.

Introduction

Organism performance typically increases with temperature up to an optimum after which performance rapidly decreases (1). Host-parasite interactions are particularly susceptible to changes in temperature due to mismatches in thermal performance between hosts and parasites (2). While parasites and their hosts often share thermal optima, parasites are thought to acclimate rapidly to changing environmental conditions resulting in higher performance either side of the thermal optimum than their hosts during interactions (2–4). Warming beyond the thermal optimum may increase parasite performance relative to host performance and exacerbate parasite burden. Increased parasite burden with warming may also feed back into host thermal responses by reducing host thermal maxima (5,6). However, in some systems, primarily those containing obligate intracellular parasites, parasites have lower thermal maxima than hosts (7,8) creating a thermal niche where hosts can grow in the absence of parasites.

Temperature generally alters parasite infectivity by disrupting parasite life-history traits (9–11). Studies in mosquito populations have shown that higher temperatures can lead to shorter development times, smaller body sizes, and higher intrinsic mortality rates (12–14). Further studies have revealed temperature-dependence of other parasite traits including attachment to hosts (15), reproductive capacity (population growth rates) (7), and virulence (16). In line with the thermal mismatch hypothesis, the impact of temperature on life-history traits often varies between hosts and parasites. *Plasmodium falciparum*, a parasite of mosquitoes and vertebrates, exhibits more rapid development and population growth with warming despite a decrease in the survival of their mosquito hosts (14,17). Similar results are shown with bacteria and their viral parasites, bacteriophages (hereafter referred to as “phages”), albeit in the opposite direction. While warming generally increases bacterial growth rates (18), phages often experience concurrent reductions in host attachment (15), reproductive capacity (7), and stability (19).

64 Hosts are often attacked by a diversity of parasite genotypes (20–23) and species (24–26). Given
65 parasite genotypes and species often vary in their upper thermal limits (27) and thermal optima (28),
66 the effects of temperature may vary between host-parasite pairs thus shaping the host-parasite
67 community. Temperature change may also alter the outcomes of inter-parasite competition in
68 communities (29) as different parasites become more or less competitive. This phenomenon is not
69 unique to parasites and has also been observed in free-living communities (30–32). Outcomes of
70 parasite competition are additionally affected by variation in specific life-history traits, such as
71 virulence, which can change with temperature (8). Parasite virulence – here defined as the rate at
72 which parasite epidemics reduce host population densities – can play a key role in parasite
73 competition (33,34). Highly virulent parasites, which rapidly reduce host population densities,
74 restrict the availability of host resources for parasite competitors thereby reducing their
75 reproductive capacities (35). By altering parasite virulence, warming may further affect competition
76 outcomes.

77 Bacteria and phages are the most abundant host-parasite system on Earth (36,37). Phages play key
78 roles in animal microbiomes and plant rhizospheres by maintaining microbiome diversity and
79 providing protection from invasive pathogens (38–41). They are also increasingly viewed as potential
80 therapeutic alternatives to antibiotics given growing levels of antimicrobial resistance (42–45).
81 Bacteria and phages experience broad temperature variation as part of the microbiomes of
82 eukaryotic hosts (39). Body temperatures vary between species (46–50) and individuals (51).
83 Temperatures also vary within individuals across space (48) and over time (52) especially following
84 the onset of fevers during pathogen infection (53). Additionally, phages typically exist in
85 communities either as complex, often host-associated phageomes (39) or through therapeutic
86 deployment in multi-phage cocktails (54). The effects of temperature on bacteria-phage and phage-
87 phage interactions therefore have acute relevance for animal and plant health.

88 Here, we investigated parasite life-history traits and competition across three temperatures (37°C,
89 40°C, and 42°C) using the opportunistic bacterial pathogen *Pseudomonas aeruginosa* and a panel of
90 three genetically diverse lytic bacteriophages targeting different bacterial surface receptors
91 (lipopolysaccharides (LPS) and type IV pili). These temperatures were intended to represent a
92 gradient of warming during a human fever (53) or a diversity of animal resting body temperatures
93 (48–50). Phage thermal responses were determined by measuring different life-history traits
94 (stability, host attachment, reproductive capacity, and virulence) across temperatures. Additionally,
95 the impact of temperature on phage-phage interactions was assessed by measuring population
96 growth in the presence or absence of phage competitors.

We hypothesised the impact of warming on phage population growth would vary across phage types given differential effects on attachment to hosts (15,55), viral replication (56), and lytic ability (57). We further hypothesised that warming would alter phage competition outcomes by increasing the competitiveness of more thermo-tolerant phages (58). Our findings provide insight into how temperature variation may affect parasite life-history traits and interactions in communities. Further, they improve our understanding of how temperature affects parasite community dynamics with important implications for the functioning of eukaryotic host viromes and the design of phage therapies.

Methods and Materials

Strains, storage, and culture conditions

Pseudomonas aeruginosa PAO1 was used with a collection of three lytic bacteriophages: ϕ PEV2, ϕ LUZ19, and ϕ 14-1. These phages, which have previously been used in ecological and evolutionary biology studies of *P. aeruginosa* at 37°C (59–62), were selected based on their genetic diversity (63) and the different bacterial receptors they target. ϕ PEV2 (N4-like phage) (64) and ϕ 14-1 (PB1-like phage) (65) are both thought to target bacterial LPS surface receptors (66,67) although N4 phage infection has also been linked to the outer-membrane protein NrfA (68). ϕ LUZ19, a T7-like phage, adsorbs to type IV pili (55,69). The three phages also vary in core phage replicative traits. While ϕ PEV2 and ϕ LUZ19 have latent periods (time from infection to burst) of ~18 minutes (67) and ~24 minutes (69) respectively, PB1-like phages (such as ϕ 14-1) have latent periods up to ~50 minutes (66). ϕ PEV2 and ϕ LUZ19 also have similar burst sizes (number of progeny) (~100-130 virions) (67,70) whereas PB1-like phages can have burst sizes up to ~180 virions (66).

Bacteria were streaked for single colonies from -80°C frozen glycerol stocks (25% glycerol) onto LB (Lennox) agar. Single colonies were inoculated into 15ml LB broth in 50ml falcons and incubated at 37°C, 40°C, or 42°C in static incubators for 16h. After incubation, cultures were centrifuged at 4000 x g for 5 mins to pellet bacterial cells and the pellet was re-suspended in 15ml fresh LB to remove potential secondary metabolites.

Phage lysates were prepared by inoculating a 15ml *P. aeruginosa* PAO1 culture (at a density of ~10⁸ CFU/ml) with a sample of -80°C frozen phage glycerol stocks. Samples were taken from frozen stocks with an inoculation loop. Phage-inoculated PAO1 cultures were incubated at 37°C for ~6 hours.

127 Cultures were then centrifuged at 3,095 x g for 5 mins to pellet cell debris and the supernatants
128 (phage lysates) were sterilised using 0.22µM filters.

129 Phage stocks were quantified using the standard double-layer overlay plaque method (71). Bottom-
130 layer agar was prepared in sterile 12cm/12cm square-petri dishes with LB-agar and top-layer agar
131 was prepared with LB-agarose (0.4% agarose) supplemented with MgSO₄ and CaCl₂ (10 mM). Briefly,
132 bacterial lawns were prepared by mixing 10ml of melted top agar (~40°C) with 300µL of a *P.*
133 *aeruginosa* PAO1 overnight culture (approximately 3 x 10⁸ CFU/ml). Phage stocks were serially
134 diluted and 10µL were spotted onto the bacterial lawns. After an incubation period of the plates of
135 6-8 h at 37°C, spots with the highest number of discernible plaques were counted and reported as an
136 average of three technical replicates. Phage stocks were quantified one day before use in
137 experiments and were stored at 4°C.

138 ***Experimental design of bacterial and phage growth across temperatures***

139 Bacterial cultures grown at 37°C, 40°C, and 42°C were diluted with LB to a starting concentration of
140 ~3 x 10⁷ CFU/ml. Isogenic ϕPEV2, ϕLUZ19, and ϕ14-1 phage stocks were diluted to a concentration
141 of ~3 x 10⁷ PFU/ml. Pairwise and 3-phage combinations were prepared by mixing single-phage stocks
142 in equal volumes. Single-phage (10µL), pairwise (20µL), and 3-phage (30µL) stocks were added to
143 loose-lid 5ml falcon tubes and LB was added up to a final volume of 30µL. Diluted bacterial cultures
144 (970µL) were then added to a final volume of 1ml. A no-phage control was included containing 30µL
145 LB and 970µL bacterial culture. Starting concentrations of each phage in single and multi-phage
146 combinations were ~3 x 10⁵ PFU/ml resulting in a phage/host ratio (multiplicity of infection - MOI) =
147 0.01. Cultures were incubated statically at 37°C, 40°C, or 42°C for five hours to allow bacterial and
148 phage growth. Each hour, tubes were destructively sampled for bacterial and phage quantification.
149 Six biological replicates were carried out for each treatment at each temperature over three days
150 with two replicates performed per day.

151 ***Measuring bacterial and phage population growth***

152 Bacterial growth was measured using optical density and colony-forming units (CFU). Optical density
153 (595nm) of bacterial cultures was measured over five hours in 96-well plates using a Synergy 2 plate
154 reader (BioTek, Vermont, USA), normalised against LB blanks. Due to the high number of samples,
155 two plates were measured per hour and all phage treatments and temperatures were represented
156 on each plate. Bacterial CFUs in the no-phage control were measured at hours 0 and 5 using serial
157 dilutions in LB, of which 20µL was immediately spotted onto LB-agar. Spots with the highest number
158 of distinct colonies were counted and data represents the average of three technical replicates.

159 To measure bacterial CFUs in the presence of phage, 100µL aliquots were taken from single-phage
160 treatments at hours 0, 2, and 5, and no-phage treatments at hours 0 and 5. Aliquots were ten-fold
161 serially diluted in a solution of sodium citrate (50 mM) and phosphate-buffered saline (PBS) to
162 reduce phage binding and killing after sampling (72,73). Dilutions were spotted (20uL) onto LB-agar
163 supplemented with sodium citrate (5 mM). Plates were incubated at 37°C for ~16 hours and spots
164 with the highest number of distinct colonies were counted. Data shown represents an average of
165 three technical replicates.

166 Phage growth in single-phage samples was determined based on plaque-forming units (PFU) using
167 plaque assays as previously described. Aliquots of each sample (100µL) were transferred to 1.5ml
168 Eppendorf tubes and centrifuged at 10,000 x g for five minutes. Phage supernatants were then
169 extracted and ten-fold serially diluted in SM buffer (NaCl 100 mM, MgSO₄ 10mM, Tris-HCl (pH 8)
170 50mM). Serial dilutions were spotted (10uL) onto *P. aeruginosa* PAO1-inoculated bacterial lawns.
171 Plates were incubated at 37°C until visible plaques formed (6-8 hours), and spots with the highest
172 number of distinct plaques were counted. Data shown represents an average of three technical
173 replicates.

174 Phage growth in multi-phage samples was determined using quantitative PCR (qPCR). qPCR primers
175 were designed based on φPEV2, φLUZ19, and φ14-1 genomes downloaded from NCBI (Table S1).
176 Phage genomes were aligned using progressiveMauve (74) with Geneious (v. 2022.1.1)
177 (<https://www.geneious.com>). Phage-specific regions with no observable sequence overlap were
178 extracted from each genome and used to create qPCR primers with IDT PrimerQuest
179 (<https://eu.idtdna.com/pages/tools/primerquest>). Primers were designed to have an annealing
180 temperature of ~60°C with products ~80 bp in length. qPCR melt curves showed single peaks for
181 each primer pair indicating high specificity.

182 Phage densities were determined based on standard curves. Phage DNA for standard curves was
183 extracted from phage lysates (~10⁹ PFU/ml) using the Norgen Biotek Phage DNA Isolation Kit
184 (Norgen Biotek) with added DNase I to remove lysed bacterial DNA. Purified DNA was quantified
185 using NanoDrop 2000c (Thermo Scientific). Duplicate standard curves were generated from purified
186 DNA dilutions with Luna Universal qPCR Master Mix (New England BioLabs) using a StepOnePlus
187 Real-Time PCR System (Applied Biosystems).

188 Aliquots of phage lysates (100µL) were taken hourly for qPCR quantification. Phage aliquots were
189 boiled at 100°C for 10 mins to denature bacterial cells and phage particles. Samples were then
190 stored at -20°C. qPCR was carried out on boiled samples diluted in Ambion Nuclease-Free Water
191 (Invitrogen) using phage-specific primers with Luna Universal qPCR Master Mix. Forward and reverse

primers (10 μ M) were added to final concentration of 0.25 μ M. Amplification conditions included initial denaturation (95°C for 60 seconds) followed by 40 cycles of denaturation (95°C for 15 seconds) and annealing/extension (60°C for 30 seconds). One qPCR plate was used per primer pair. Due to space constraints, no technical replicates were performed, but all biological replicates were analysed. Phage DNA concentrations were determined through comparison with standard curves and fold change in DNA copies was calculated by dividing by the average phage DNA concentration at hour 0.

Phage decay and adsorption rates

To measure decay rate, phage stocks were diluted in LB to $\sim 10^5$ PFU/ml. Diluted phage stocks (1ml) were added to 1.5ml Eppendorf tubes and incubated statically at 37°C, 40°C, and 42°C for 24h. Samples were taken at 2.5h, 5h, 7.5h, and 24h, serially diluted in SM buffer, and spotted (10 μ L) onto PAO1-inoculated bacterial lawns. Three independent biological replicates were conducted per temperature for each phage. Plaque numbers for each biological replicate are averages of three technical replicates.

To measure phage adsorption rates, bacterial cultures were grown for ~ 16 hours at 37°C, 40°C, and 42°C, diluted into 15ml fresh LB in loose-lid 50ml falcon tubes, and grown to mid-exponential phase ($\sim 10^8$ CFU/ml). Mid-exponential phase bacterial cultures (1.485ml) were aliquoted into 1.5ml Eppendorf tubes and placed into heat blocks set at 37°C, 40°C, and 42°C. Phage stocks were diluted to $\sim 10^7$ PFU/ml and 15 μ L were added to the Eppendorf tubes to a final phage concentration of $\sim 10^5$ PFU/ml (MOI = ~ 0.001) and then mixed by inversion. At five-minute intervals, 100 μ L samples were taken and centrifuged at 17,000 x g for 1 min. To measure unadsorbed phage, supernatant was sampled, serially diluted in SM buffer, and spotted (10 μ L) onto PAO1-inoculated bacterial lawns. Three biological replicates were carried out per temperature for each phage. PFUs for each biological replicate are averages of three technical replicates.

Statistical analysis

Statistical analyses and data visualisation were carried out using Microsoft Excel (v.2102) (75), R (v.4.0.3) (76) and RStudio (v1.4.1103) (77). Line graphs and boxplots were generated using the “ggplot2” R package (78). Radar charts were generated using the “ggradar” R package.

The impact of phage on bacterial population densities was determined by comparing optical densities between phage and no-phage treatments at the end of the experiment between temperatures. This was conducted using separate linear mixed effects models for each temperature with the “nlme” R package where optical density was the response variable and phage was a fixed

explanatory variable. Experimental batch (three batches as experiment performed across three days) was included as a random effect. Unequal variances between groups were accounted for using the “varIdent” function (79). Similar mixed effect models were used to compare phage PFU/ml and \log_2 (DNA concentrations) at the start and end of the experiment at each temperature. Additionally, a similar model was used to determine the impact of temperature on bacterial densities where optical density was the response variable and explanatory variables included temperature, time, and a temperature-time interaction term. Post-hoc analyses were carried out using the R “emmeans” package. The relationship between optical density and colony-forming units were determined using a linear model. A square-root transformation was used on CFUs to meet model assumptions.

The impact of temperature on phage decay and host attachment was tested using a Poisson generalised linear model with PFU/ml as response variable and time, temperature, and the interaction between time and temperature as explanatory variables. As more than ~90% attachment occurred before the first measurement for ϕ 14-1, an ANOVA between 0 and 5min was performed rather than GLM. The time required for 50% phage attachment/decay was determined by generating predicted PFU/ml over time with 95% confidence intervals from the GLM. A time range where PFU/ml confidence intervals overlapped with 50% starting densities was recorded.

The impact of temperature on bacterial doublings in the presence of phage (CFU) and phage doublings (PFU) was tested by calculating mean \log_2 ratios of T5 / T0 or T2 / T0 values. Standard

errors of log ratios were calculated using the formula: $SE \left[\log_2 \left(\frac{T_n}{T_0} \right) \right] = \sqrt{\frac{SE[\overline{T_0}]^2}{\overline{T_0}^2} + \frac{SE[\overline{T_n}]^2}{\overline{T_n}^2}}$ where

SE = standard error of mean, T_n = values at time point n (T2 or T5), T_0 = values at initial time point (T0) (80). Statistical significance between ratios at each temperature was determined by calculating 95% confidence intervals of the difference between ratio means using the formula:

$CI_{95} = \bar{x}_1 - \bar{x}_2 \pm 1.96 \sqrt{SE_{\bar{x}_1} + SE_{\bar{x}_2}}$. Comparisons were considered significant when confidence intervals did not overlap with 0.

The impact of competitors on phage growth was tested using linear mixed-effect models as previously described. We included phage DNA concentration of the focal phage as the response variable, and phage competitor, temperature, and their interaction as fixed explanatory variables.

Phage competitiveness across temperatures was determined by comparing phage resistance to competitors and phage suppression of competitors. For resistance to competitors, the ratio of phage densities (DNA concentrations) at hour 5 in the presence and absence of each competitor was

calculated. An average of ratios for all competitors was used to determine the average impact of competitors on phage growth. For suppression of competitors, the ratio of competitor densities (DNA concentrations) at hour 5 in the presence and absence of each phage was calculated. An average of ratios was determined to give the relative competitor growth compared to a single-phage control. The average of ratios was taken away from 1 to determine the average impact of focal phage on competitor growth.

R script and data files used to generate figures and carry out statistical tests are publicly available at: https://github.com/SamuelGreenrod/Warming_parasite_paper

Results

Temperature shapes infectivity across phages

We initially investigated the impact of temperature on bacteria-phage interactions by measuring bacterial population growth in the presence of three different phages at 37°C, 40°C, and 42°C (Figure 1A). At the end of the experiment, the presence of phage had significantly reduced bacterial densities relative to the no-phage control at 37°C and 40°C (ANOVA – 37°C: $F_{2,17} = 115.4$, $p < 0.001$; 40°C: $F_{2,17} = 41.7$, $p < 0.001$). All phage had a significant effect on bacterial growth (post-hoc contrasts, $p < 0.05$). While phage presence also significantly reduced bacterial densities at 42°C (ANOVA - $F_{2,17} = 578.7$, $p < 0.001$), this trend was significantly driven by ϕ LUZ19 (post-hoc - $p < 0.001$). Neither ϕ 14-1 nor ϕ PEV2 had a significant impact on bacterial growth at 42°C (post-hoc - ϕ 14-1: $p = 0.33$; ϕ PEV2: $p = 0.15$).

An important assumption of this assay is that optical density (OD) is correlated with bacterial cell density. To test this assumption, we directly measured viable cell density by counting bacterial colony-forming units (CFUs) in a sub-set of our treatments. There was a significant relationship between OD and CFU (ANOVA: $F = 121$; d.f = 1, 96; $p < 0.001$) (Figure S1). Accordingly, the results of CFU counts recapitulated the two key features of our OD data (Figure S2). First, bacterial CFUs decreased over time at 37°C and 40°C for all phages. Second, bacterial densities decreased at 42°C with ϕ LUZ19, but not ϕ PEV2 or ϕ 14-1. Notably, the relationship between OD and CFU was less clear at OD < 0.05 indicating bacterial densities reached a lower detection OD threshold (Figure S1). Therefore, measurement of CFU counts may provide greater resolution than OD as bacterial densities start to collapse.

To better understand the decrease in phage lytic activity with temperature, we also measured phage densities using both plaque-forming units (PFUs) to quantify infectious phage particles and qPCR to quantify phage DNA concentrations. All phage particle densities increased over time at 37°C and 40°C (Figure 1B). While ϕ PEV2 and ϕ LUZ19 densities started to plateau after T2, ϕ 14-1 densities plateaued at T3 (Figure 1B-C). At 42°C, ϕ LUZ19 densities increased significantly ($F_{2,5} = 129.1$, $p < 0.05$) but ϕ PEV2 densities decreased ($F_{2,5} = 270.4$, $p < 0.05$) possibly due to ϕ PEV2 particles attaching to the bacterial surface but not replicating. ϕ 14-1 densities did not change significantly ($F_{2,5} = 1.41$, $p = 0.36$). However, ϕ 14-1 densities were not static, initially decreasing and then increasing indicative of attachment followed by replication. These results were supported by measures of phage DNA concentrations (Figure 1C) which showed significant increases for all phage at 37°C and 40°C (37°C - ϕ PEV2: $F_{2,5} = 1219$; ϕ LUZ19: $F_{2,5} = 2257$; ϕ 14-1: $F_{2,5} = 299.8$; 40°C - PEV2: $F_{2,5} = 1260$; ϕ LUZ19: $F_{2,5} = 1355$; ϕ 14-1: $F_{2,5} = 1936$; $p < 0.001$ for all phage). At 42°C, significant increases in DNA concentrations were observed with ϕ LUZ19 ($F_{2,5} = 391.4$, $p < 0.001$) and ϕ 14-1 ($F_{2,5} = 44.2$, $p = 0.001$), but not with ϕ PEV2 ($F_{2,5} = 0.078$, $p = 0.79$).

Phage replication rates are intrinsically linked to bacterial growth rates (81) which typically vary with temperature (18). We assessed the potential impact of bacterial growth rates on phage activity by measuring bacterial growth in the absence of phage using both optical densities and CFUs. There was no significant impact of temperature on bacterial densities ($F_{2,79} = 2.73$, $p = 0.0713$), or interaction between temperature and time point ($F_{5,79} = 253.7$, $p = 0.465$), in the no-phage control measured with optical density (Figure S3A). Further, no significant differences were observed between temperatures at the end of the experiment based on CFU counts ($F_{2,17} = 0.43$, $p = 0.52$) (Figure S3B), despite bacteria being in mid-log phase. Temperature appears to have had a minimal impact on bacterial growth rates within the 5-hour period. However, a lack of temperature effects may reflect restricted overall bacterial growth in our experimental system; bacteria spent the start of the 5-hour period in lag-phase and only underwent a total of 3.26 (37°C), 3.07 (40°C), and 2.84 (42°C) doublings.

Temperature alters phage infectivity via life-history traits

Phage population growth is dependent on different parasite life-history traits including stability (free-phage decay rates), host attachment (adsorption to bacterial cells), and virulence (impact on bacterial densities). We investigated whether disruption of these traits may explain reduced phage infectivity at high temperatures (Figure 2A).

Phage stability outside of cells can be temperature-sensitive (82). Using Poisson generalised linear models, we observed a significant interaction between temperature and time on phage stability for

all phage (Poisson GLM. ϕ PEV2: LR $\chi^2 = 30359$, $p < 0.001$; ϕ LUZ19: LR $\chi^2 = 17172$, $p < 0.001$; ϕ 14-1: LR $\chi^2 = 5986.5$, $p < 0.001$) (Figure S4). ϕ 14-1 stability was found to decrease significantly from 37°C to 40°C but no change was observed from 40°C to 42°C (post-hoc – 37-40C: $p < 0.0001$; 40-42C: $p = 0.50$). While ϕ PEV2 stability also decreased significantly with temperature (post-hoc - $p < 0.0001$), ϕ LUZ19 stability significantly increased (post-hoc – $p < 0.0001$). All phage were predicted to require a minimum of 18 hours before phage densities reduced by 50% at any temperature (Table S3). Stability thus likely played a minimal role in temperature effects on phage infectivity.

Similarly, the interaction between temperature and time on host attachment varied across phage. A temperature by time interaction was observed for ϕ PEV2 and ϕ LUZ19 attachment, but not for ϕ 14-1 (Poisson GLM. ϕ PEV2: LR $\chi^2 = 19489$, $p < 0.001$; ϕ LUZ19: LR $\chi^2 = 116.5$, $p < 0.001$; ANOVA. ϕ 14-1: $F_{2,5} = 0.029$, $p = 0.97$) (Figure S5). ϕ LUZ19 attachment was found to increase significantly with temperature (post-hoc - $p < 0.0001$). However, ϕ PEV2 attachment significantly decreased (post-hoc – $p < 0.0001$) with no attachment observed at 42°C. Following attachment, phage reproduction is dependent on the ability to replicate and lyse bacterial host cells. The impact of temperature on phage virulence and population growth was determined by calculating the mean number of bacterial doublings in the presence of phage (CFU/ml) or phage doublings (PFU/ml), respectively, between the beginning and end of the experiment (Figure S2 & 1B; Table S3). Statistical significance between temperatures ($p < 0.05$) was determined by measuring the overlap in 95% confidence intervals of the difference between bacterial or phage doublings for each temperature.

The virulence of all phage varied significantly with temperature ($p < 0.05$), decreasing with warming for ϕ PEV2 and ϕ 14-1 ($p < 0.05$) and increasing with warming for ϕ LUZ19 ($p < 0.05$). In line with this, population growth of ϕ PEV2 and ϕ 14-1 also decreased significantly with warming ($p < 0.05$) although differences were greater between 40°C and 42°C than between 37°C and 40°C. The relationship between virulence and population growth was less clear with ϕ LUZ19; while ϕ LUZ19 population growth significantly decreased with warming ($p < 0.05$), virulence significantly increased.

Variation in phage virulence and population growth across temperatures may be masked as phages reached maximum densities with all available hosts lysed. Virulence and population growth were re-analysed within the first two hours (Figure 2B). The virulence and population growth of all phage significantly varied between temperatures ($p < 0.05$). However, the magnitude of virulence changes were greater for ϕ PEV2 than for other phage, particularly between 37°C and 40°C.

Competition is temperature-dependent in phage communities

347 Parasites often exist in communities where they compete to acquire host resources (29,83). To
348 determine whether temperature variation affects competition between phages, we measured the
349 impact of competitors on phage population growth in pairwise and 3-phage combinations across
350 temperatures.

351 At 37°C, the presence of competitors significantly reduced phage population growth by hour 5 for all
352 phages (ANOVA. ϕ PEV2: $F_{2,17} = 9.16$, $p < 0.001$; ϕ LUZ19: $F_{2,17} = 18.1$, $p < 0.001$; ϕ 14-1: $F_{2,17} = 23.8$, $p <$
353 0.001) (Figure 3A). Competition was less clear at hour 2 compared to the hour 5 endpoint with no
354 significant impact of competitors on population growth observed for ϕ PEV2 or ϕ LUZ19 (ANOVA.
355 ϕ PEV2: $F_{2,17} = 0.88$, $p = 0.47$; ϕ LUZ19: $F_{2,17} = 1.11$, $p = 0.37$) (Figure S6). Competitors were found to
356 have a significant impact on ϕ 14-1 population growth after 2h ($F_{2,17} = 8.16$, $p = 0.001$), although post-
357 hoc contrasts showed this was only with the 3-phage combination ($p < 0.05$).

358 Phages varied in competitiveness at 37°C (Figure 3A). ϕ PEV2 was the most resistant to competitors
359 with only ϕ LUZ19 significantly reducing ϕ PEV2 population growth (post-hoc contrasts, $p < 0.01$).
360 ϕ PEV2 also had a significantly greater impact on competitor growth than ϕ LUZ19 or ϕ 14-1 (post-hoc
361 contrasts, PEV2 vs. ϕ LUZ19: $p < 0.001$; ϕ PEV2 vs ϕ 14-1: $p < 0.01$). Competition with ϕ LUZ19 and
362 ϕ 14-1 was weaker and more varied. While 14-1 population growth was unaffected by the presence
363 of ϕ LUZ19 (post-hoc contrasts, $p = 0.15$), ϕ 14-1 significantly reduced ϕ LUZ19 population growth.
364 ϕ LUZ19 was significantly more restrictive than ϕ 14-1 against ϕ PEV2 growth (post-hoc contrasts, $p <$
365 0.05).

366 Phage competition was found to be temperature-dependent, with significant interactions detected
367 between competitor and temperature for all phages (ANOVA. ϕ PEV2: $F_{2,47} = 4.28$, $p = 0.001$;
368 ϕ LUZ19: $F_{2,47} = 4.15$, $p = 0.001$; ϕ 14-1: $F_{2,47} = 16.5$, $p < 0.001$) (Figure 3A). Between 37°C and 40°C,
369 ϕ 14-1 restriction of ϕ PEV2 growth increased significantly (post-hoc contrasts, $p < 0.05$) and ϕ PEV2
370 and 3-phage restriction of ϕ 14-1 both significantly decreased (post-hoc contrasts, PEV2: $p < 0.001$; 3-
371 phage: $p < 0.001$). In contrast, there was no change in ϕ LUZ19 restriction of ϕ PEV2 (post-hoc
372 contrasts, $p = 1.0$) or ϕ 14-1 (post-hoc contrasts, $p = 0.93$) between 37°C and 40°C. No significant
373 difference in phage growth, with or without competitors, was detected at 42°C (Figure S7; post-hoc
374 contrasts, $p > 0.05$) indicating competition was removed.

375 The change in phage competitiveness across temperatures was analysed further by comparing
376 phage growth in the presence of competitors (Resistance to competitors) with the reduction in
377 competitor growth caused by each phage (Suppression of competitors) (see methods for more
378 detailed description) (Figure 3B). This showed that between 37°C and 40°C, ϕ PEV2 became less
379 competitive and ϕ 14-1 became more competitive. As ϕ LUZ19 competitiveness was unchanged, the

competitiveness of all phages appeared to converge. Phage competition was removed between 40°C and 42°C with all phages growing equally with and without competitors.

Discussion

The effects of temperature on host-parasite interactions depend on mismatches in host and parasite thermal responses (7,84,85). Using a bacteria-phage system, we show that thermal response mismatches are not only present between parasites and their hosts, but also between different parasites. This finding supports previous studies which identified thermal performance variation in malaria parasites and their mosquito vectors (27,86,87), midges (88,89), and honeybee parasites (90). However, our study made several additional contributions. Firstly, we found that the temperature range between phage thermal optima and phage inactivation can be as small as 2°C. Secondly, we found that phage upper thermal limits may sit within feasible human fever/animal resting temperatures. These findings indicate that even small increases in body temperature may have significant effects on the functioning of animal gut viromes. Further, they suggest that body temperature variation may limit the functional diversity, and ultimately efficacy, of phage therapy treatments. The notion that thermal regulation is used by eukaryotes to restrict the growth of parasites is not a new one (91). However, our results highlight that temperature change can affect hyperparasites, as well as parasites, possibly to the detriment of their eukaryotic hosts.

The effects of temperature on parasite performance are typically attributed to changes in parasite life-history traits (9,12,15). We found that temperature change affected different life-history traits in different parasites (also found in (91)). For one phage, high temperatures reduced attachment to the bacterial cell thereby restricting the phage's ability to infect host cells. In *P. aeruginosa*, variable phage attachment has been attributed to the production of bacterial surface exopolysaccharides (EPS) which block phage adsorption (92,93). However, while *P. aeruginosa* EPS production generally increases with temperature from 25°C to 37°C, it decreases between 37°C and 42°C (94) and so cannot explain the decrease in adsorption with warming. Disruption of host attachment by temperature has previously been reported in other bacteria-phage pairs (15,91) and in more complex host-parasite systems (95,96). Host attachment therefore appears to be particularly sensitive to temperature change, perhaps due to its dependence on the parasite's binding ability (97) and host's attachment susceptibility (8,95,98). In a different phage, high temperatures restricted phage population growth while having no impact on phage attachment ability. Phage replication inside host cells, like other viruses (99), depends on multiple reproductive stages possibly disrupted by high temperatures. For example, temperature change may affect phage ability to replicate their

413 DNA (100,101), form phage particles, or lyse the bacterial cell (57). Further work is needed to
414 characterise the mechanisms underlying temperature disruption on life-history traits and would
415 improve our understanding of both parasite thermal ecology and the evolutionary hurdles parasites
416 must overcome to adapt to temperature variation.

417 Similar to other parasite systems (102–104), phages typically exist in communities and compete to
418 infect hosts. Phages are also increasingly deployed in multi-phage cocktails for the therapeutic
419 treatment of bacterial infections (43,105). We show that while phages compete in combinations,
420 they vary in their competitiveness; some phages were more restrictive of and resistant to
421 competitors than others. Parasite competitive success during epidemics has previously been linked
422 to virulence as highly virulent parasites can rapidly transmit and deplete host resources thereby
423 reducing the availability of hosts for other parasite competitors (33–35). We found that the most
424 competitive phage, ϕ PEV2, was also the most virulent and caused the earliest collapse in host
425 population densities. The least competitive phage, ϕ 14-1, was the least virulent. Phage virulence
426 depends on two correlated life-history traits: latent period (time between infection and burst) and
427 burst size (number of viral progeny). Specifically, at high bacterial densities, phage virulence and
428 competitiveness both increase when latent periods are shorter despite a reduced burst size (106).
429 Supporting this, ϕ PEV2 is known to have the shortest latent period (~18 mins) out of the phage
430 tested (67) while PB1-like phages, such as ϕ 14-1, have longer latent periods (~50 mins) (66).
431 Notably, the relationship between virulence and competitiveness depends on host availability; at
432 low bacterial densities selection favours longer latent periods with larger bursts (106). Our results
433 highlight that viromes and phage cocktails may be less functionally diverse than previously thought
434 as relative phage densities are skewed towards the most competitive phage genotypes/species (62).

435 Phage virulence varied with temperature. Therefore, we hypothesised that a change in temperature
436 may also result in a change in phage competition outcomes. We found that temperature-mediated
437 reductions in phage virulence corresponded with reductions in phage competitiveness. Temperature
438 has previously been shown to affect competition outcomes in parasites of mosquitoes (10) and fruit
439 flies (11). However, in these studies competition was tested in the absence of hosts and so changes
440 were attributed to non-host related parasite traits such as larval survivorship and development times
441 (10). Our results highlight that temperature can also affect competition for access to hosts and
442 therefore may drive parasite community dynamics during epidemics. Surprisingly, temperature
443 primarily affected interactions between two phages (ϕ PEV2 and ϕ 14-1). This could be explained by
444 the co-occurrence of competition for host resources and phage-phage synergy in specific phage pairs
445 (reviewed in (107)). However, variation between phages could reflect parasite-specific temperature
446 effects given both ϕ PEV2 and ϕ 14-1 are thought to target LPS-associated bacterial surface receptors

(59,67). Alternatively, a reduction in ϕ PEV2 virulence may have a greater impact on competition with low virulence competitors whose growth rate depends on the increase in host availability.

In conclusion, our study demonstrates that variation in temperature, particularly within a human fever range, can have a significant impact on phage population and community dynamics. Specifically, rising temperatures may alter the composition and functioning of phage communities and restrict the efficacy of phage to treat bacterial infections. Our findings also highlight the complex effects of temperature within tri-partite host-parasite-hyperparasite systems. Fevers often push both mammalian hosts and their parasites above their thermal optima, with parasites incurring greater costs. However, high temperatures may also restrict the growth of hyperparasites which target higher-level parasites thereby reducing their benefit to the mammalian host.

Acknowledgments

We thank R. Salguero-Gomez for advice on data analysis, as well as T. Richards, T. Barraclough, and K. Foster for feedback on the experimental design and results. This work was supported by the Biotechnology and Biosciences Research Council (BB/T008784/1) to S.T.E.G. as well as the Natural Environment Research Council (NE/X000540/1) and NSERC Canada Excellence Research Chair to K.C.K. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Author contributions

S.T.E.G. and K.C.K. conceived the project. S.T.E.G., C.M., T.H., E.J.S., and K.C.K. designed the experiment. S.T.E.G., D.C.L., and S.J. conducted the experiment and collected the data, with guidance from C.M. and K.C.K. S.T.E.G. analysed the data. S.T.E.G., C.M. and K.C.K. wrote the initial draft, with input from all other authors.

References

1. Schulte PM, Healy TM, Fanguie NA. Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integrative and Comparative Biology*. 2011 Nov 1;51(5):691–702.

- 477 2. Cohen JM, Venesky MD, Sauer EL, Civitello DJ, McMahon TA, Roznik EA, et al. The thermal
478 mismatch hypothesis explains host susceptibility to an emerging infectious disease. *Ecology*
479 *Letters*. 2017;20(2):184–93.
- 480 3. Mojica KDA, Brussaard CPD. Factors affecting virus dynamics and microbial host–virus
481 interactions in marine environments. *FEMS Microbiology Ecology*. 2014 Sep 1;89(3):495–515.
- 482 4. Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. Toward a Metabolic Theory of Ecology.
483 *Ecology*. 2004;85(7):1771–89.
- 484 5. Hector TE, Gehman ALM, King KC. Infection burdens and virulence under heat stress: ecological
485 and evolutionary considerations. *Philos Trans R Soc Lond B Biol Sci*. 378(1873):20220018.
- 486 6. Hector TE, Sgrò CM, Hall MD. Pathogen exposure disrupts an organism’s ability to cope with
487 thermal stress. *Global Change Biology*. 2019;25(11):3893–905.
- 488 7. Padfield D, Castledine M, Buckling A. Temperature-dependent changes to host–parasite
489 interactions alter the thermal performance of a bacterial host. *ISME J*. 2020 Feb;14(2):389–98.
- 490 8. Sillankorva S, Oliveira R, Vieira MJ, Sutherland I, Azeredo J. *Pseudomonas fluorescens* infection
491 by bacteriophage Φ S1: the influence of temperature, host growth phase and media. *FEMS*
492 *Microbiology Letters*. 2004 Dec 1;241(1):13–20.
- 493 9. Singh R, Prathibha P, Jain M. Effect of temperature on life-history traits and mating calls of a
494 field cricket, *Acanthogryllus asiaticus*. *Journal of Thermal Biology*. 2020 Oct 1;93:102740.
- 495 10. Evans MV, Drake JM, Jones L, Murdock CC. Assessing temperature-dependent competition
496 between two invasive mosquito species. *Ecological Applications*. 2021;31(5):e02334.
- 497 11. Comeault AA, Matute DR. Temperature-Dependent Competitive Outcomes between the Fruit
498 Flies *Drosophila santomea* and *Drosophila yakuba*. *The American Naturalist*. 2021
499 Mar;197(3):312–23.
- 500 12. Ciota AT, Matakchiero AC, Kilpatrick AM, Kramer LD. The effect of temperature on life history
501 traits of *Culex* mosquitoes. *J Med Entomol*. 2014 Jan;51(1):55–62.
- 502 13. Christiansen-Jucht C, Parham PE, Saddler A, Koella JC, Basáñez MG. Temperature during larval
503 development and adult maintenance influences the survival of *Anopheles gambiae* s.s.
504 *Parasites & Vectors*. 2014 Nov 5;7(1):489.
- 505 14. Shapiro LLM, Whitehead SA, Thomas MB. Quantifying the effects of temperature on mosquito
506 and parasite traits that determine the transmission potential of human malaria. *PLOS Biology*.
507 2017 Oct 16;15(10):e2003489.
- 508 15. Tokman JI, Kent DJ, Wiedmann M, Denes T. Temperature Significantly Affects the Plaquing and
509 Adsorption Efficiencies of *Listeria* Phages. *Frontiers in Microbiology* [Internet]. 2016 [cited 2022
510 Aug 25];7. Available from: <https://www.frontiersin.org/articles/10.3389/fmicb.2016.00631>
- 511 16. Kimes NE, Grim CJ, Johnson WR, Hasan NA, Tall BD, Kothary MH, et al. Temperature regulation
512 of virulence factors in the pathogen *Vibrio coralliilyticus*. *ISME J*. 2012 Apr;6(4):835–46.
- 513 17. Waite JL, Suh E, Lynch PA, Thomas MB. Exploring the lower thermal limits for development of
514 the human malaria parasite, *Plasmodium falciparum*. *Biol Lett*. 2019 Jun;15(6):20190275.

515 18. Ratkowsky DA, Olley J, McMeekin TA, Ball A. Relationship between temperature and growth
516 rate of bacterial cultures. *J Bacteriol.* 1982 Jan;149(1):1–5.

517 19. Blazantin M, Lam WT, Vasen E, Chan BK, Turner PE. Decay and damage of therapeutic phage
518 OMKO1 by environmental stressors. *PLOS ONE.* 2022 Feb 23;17(2):e0263887.

519 20. Orsucci M, Navajas M, Fellous S. Genotype-specific interactions between parasitic arthropods.
520 *Heredity.* 2017 Mar;118(3):260–5.

521 21. Lambrechts L, Halbert J, Durand P, Gouagna LC, Koella JC. Host genotype by parasite genotype
522 interactions underlying the resistance of anopheline mosquitoes to *Plasmodium falciparum*.
523 *Malar J.* 2005 Jan 11;4:3.

524 22. Klemme I, Louhi KR, Karvonen A. Host infection history modifies co-infection success of
525 multiple parasite genotypes. *Journal of Animal Ecology.* 2016;85(2):591–7.

526 23. Read AF, Taylor LH. The ecology of genetically diverse infections. *Science.* 2001 May
527 11;292(5519):1099–102.

528 24. Eswarappa SM, Estrela S, Brown SP. Within-Host Dynamics of Multi-Species Infections:
529 Facilitation, Competition and Virulence. *PLoS One.* 2012 Jun 21;7(6):e38730.

530 25. Tang J, Templeton TJ, Cao J, Culleton R. The Consequences of Mixed-Species Malaria Parasite
531 Co-Infections in Mice and Mosquitoes for Disease Severity, Parasite Fitness, and Transmission
532 Success. *Frontiers in Immunology [Internet].* 2020 [cited 2023 Jul 27];10. Available from:
533 <https://www.frontiersin.org/articles/10.3389/fimmu.2019.03072>

534 26. Pedersen AB, Fenton A. Emphasizing the ecology in parasite community ecology. *Trends in*
535 *Ecology & Evolution.* 2007 Mar 1;22(3):133–9.

536 27. Mozaffer F, Menon GI, Ishtiaq F. Exploring the thermal limits of malaria transmission in the
537 western Himalaya. *Ecology and Evolution.* 2022;12(9):e9278.

538 28. Mordecai EA, Caldwell JM, Grossman MK, Lippi CA, Johnson LR, Neira M, et al. Thermal biology
539 of mosquito-borne disease. *Ecol Lett.* 2019 Oct;22(10):1690–708.

540 29. Mideo N. Parasite adaptations to within-host competition. *Trends in Parasitology.* 2009 Jun
541 1;25(6):261–8.

542 30. Mei X, Gao S, Liu Y, Hu J, Razluskij V, Rudstam LG, et al. Effects of Elevated Temperature on
543 Resources Competition of Nutrient and Light Between Benthic and Planktonic Algae. *Frontiers*
544 *in Environmental Science [Internet].* 2022 [cited 2023 Jul 27];10. Available from:
545 <https://www.frontiersin.org/articles/10.3389/fenvs.2022.908088>

546 31. Kordas RL, Harley CDG, O'Connor MI. Community ecology in a warming world: The influence of
547 temperature on interspecific interactions in marine systems. *Journal of Experimental Marine*
548 *Biology and Ecology.* 2011 Apr 30;400(1):218–26.

549 32. Seth H, Gräns A, Sandblom E, Olsson C, Wiklander K, Johnsson JJ, et al. Metabolic Scope and
550 Interspecific Competition in Sculpins of Greenland Are Influenced by Increased Temperatures
551 Due to Climate Change. *PLOS ONE.* 2013 May 14;8(5):e62859.

552 33. Berngruber TW, Froissart R, Choisy M, Gandon S. Evolution of Virulence in Emerging Epidemics.
553 PLoS Pathog. 2013 Mar 14;9(3):e1003209.

554 34. Griette Q, Raoul G, Gandon S. Virulence evolution at the front line of spreading epidemics.
555 Evolution. 2015;69(11):2810–9.

556 35. Hasik AZ, King KC, Hawlena H. Interspecific host competition and parasite virulence evolution.
557 Biology Letters. 2023 May 3;19(5):20220553.

558 36. Breitbart M, Bonnain C, Malki K, Sawaya NA. Phage puppet masters of the marine microbial
559 realm. Nat Microbiol. 2018 Jul;3(7):754–66.

560 37. Williamson KE. Soil Phage Ecology: Abundance, Distribution, and Interactions with Bacterial
561 Hosts. In: Witzany G, editor. Biocommunication in Soil Microorganisms [Internet]. Berlin,
562 Heidelberg: Springer; 2011 [cited 2023 Jun 15]. p. 113–36. (Soil Biology). Available from:
563 https://doi.org/10.1007/978-3-642-14512-4_4

564 38. Marantos A, Mitarai N, Sneppen K. From kill the winner to eliminate the winner in open phage-
565 bacteria systems. PLoS Comput Biol. 2022 Aug 8;18(8):e1010400.

566 39. Manrique P, Bolduc B, Walk ST, van der Oost J, de Vos WM, Young MJ. Healthy human gut
567 phageome. Proceedings of the National Academy of Sciences. 2016 Sep 13;113(37):10400–5.

568 40. Ashy RA, Jalal RS, Sonbol HS, Alqahtani MD, Sefrji FO, Alshareef SA, et al. Functional annotation
569 of rhizospheric phageome of the wild plant species *Moringa oleifera*. Frontiers in Microbiology
570 [Internet]. 2023 [cited 2023 Jul 6];14. Available from:
571 <https://www.frontiersin.org/articles/10.3389/fmicb.2023.1166148>

572 41. Pratama AA, Terpstra J, de Oliveria ALM, Salles JF. The Role of Rhizosphere Bacteriophages in
573 Plant Health. Trends in Microbiology. 2020 Sep 1;28(9):709–18.

574 42. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. Global burden of
575 bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet. 2022 Feb
576 12;399(10325):629–55.

577 43. Van Nieuwenhuysse B, Van der Linden D, Chatzis O, Lood C, Wagemans J, Lavigne R, et al.
578 Bacteriophage-antibiotic combination therapy against extensively drug-resistant *Pseudomonas*
579 *aeruginosa* infection to allow liver transplantation in a toddler. Nat Commun. 2022 Sep
580 29;13(1):5725.

581 44. Gigante A, Atterbury RJ. Veterinary use of bacteriophage therapy in intensively-reared
582 livestock. Virology Journal. 2019 Dec 12;16(1):155.

583 45. Eskenazi A, Lood C, Wubbolts J, Hites M, Balarjishvili N, Leshkasheli L, et al. Combination of pre-
584 adapted bacteriophage therapy and antibiotics for treatment of fracture-related infection due
585 to pandrug-resistant *Klebsiella pneumoniae*. Nat Commun. 2022 Jan 18;13(1):302.

586 46. Garipey C, Amiot J, Nadai S. Ante-mortem detection of PSE and DFD by infrared thermography
587 of pigs before stunning. Meat Science. 1989 Jan 1;25(1):37–41.

588 47. Hannon JP, Bossone CA, Wade CE. Normal physiological values for conscious pigs used in
589 biomedical research. Lab Anim Sci. 1990 May;40(3):293–8.

- 590 48. Godyń D, Herbut P, Angrecka S. Measurements of peripheral and deep body temperature in
591 cattle – A review. *Journal of Thermal Biology*. 2019 Jan 1;79:42–9.
- 592 49. Protsiv M, Ley C, Lankester J, Hastie T, Parsonnet J. Decreasing human body temperature in the
593 United States since the Industrial Revolution. Jit M, Franco E, Waalen J, Rühli F, editors. *eLife*.
594 2020 Jan 7;9:e49555.
- 595 50. Prinzinger R, Preßmar A, Schleucher E. Body temperature in birds. *Comparative Biochemistry
596 and Physiology Part A: Physiology*. 1991 Jan 1;99(4):499–506.
- 597 51. Obermeyer Z, Samra JK, Mullainathan S. Individual differences in normal body temperature:
598 longitudinal big data analysis of patient records. *BMJ*. 2017 Dec 13;359:j5468.
- 599 52. Harding C, Pompei F, Bordonaro SF, McGillicuddy DC, Burmistrov D, Sanchez LD. The daily,
600 weekly, and seasonal cycles of body temperature analyzed at large scale. *Chronobiol Int*. 2019
601 Dec;36(12):1646–57.
- 602 53. Leggett JE. Approach to fever or suspected infection in the normal host. In: *Goldman-Cecil
603 Medicine*. 26th ed. Philadelphia, PA: Elsevier; 2020. p. 1809–15.
- 604 54. Abedon ST, Danis-Wlodarczyk KM, Wozniak DJ. Phage Cocktail Development for Bacteriophage
605 Therapy: Toward Improving Spectrum of Activity Breadth and Depth. *Pharmaceuticals (Basel)*.
606 2021 Oct 3;14(10):1019.
- 607 55. Chibeu A, Ceyssens PJ, Hertveldt K, Volckaert G, Cornelis P, Matthijs S, et al. The adsorption of
608 *Pseudomonas aeruginosa* bacteriophage phiKMV is dependent on expression regulation of
609 type IV pili genes. *FEMS Microbiol Lett*. 2009 Jun;296(2):210–8.
- 610 56. Pollard E, Woodyatt S. The Effect of Temperature on the Formation of T1 and T2r
611 Bacteriophage. *Biophys J*. 1964 Sep;4(5):367–85.
- 612 57. Park J, Yun J, Lim JA, Kang DH, Ryu S. Characterization of an endolysin, LysBPS13, from a
613 *Bacillus cereus* bacteriophage. *FEMS Microbiology Letters*. 2012 Jul 1;332(1):76–83.
- 614 58. Costaz TPM, de Jong PW, Harvey JA, van Loon JJA, Dicke M, Gols R. Temperature affects the
615 outcome of competition between two sympatric endoparasitoids. *Animal Behaviour*. 2023 Sep
616 1;203:11–20.
- 617 59. Betts A, Gifford DR, MacLean RC, King KC. Parasite diversity drives rapid host dynamics and
618 evolution of resistance in a bacteria-phage system. *Evolution*. 2016;70(5):969–78.
- 619 60. Betts A, Vasse M, Kaltz O, Hochberg ME. Back to the future: evolving bacteriophages to
620 increase their effectiveness against the pathogen *Pseudomonas aeruginosa* PAO1. *Evolutionary
621 Applications*. 2013;6(7):1054–63.
- 622 61. Betts A, Kaltz O, Hochberg ME. Contrasted coevolutionary dynamics between a bacterial
623 pathogen and its bacteriophages. *Proceedings of the National Academy of Sciences*. 2014 Jul
624 29;111(30):11109–14.
- 625 62. Betts A, Gray C, Zelek M, MacLean RC, King KC. High parasite diversity accelerates host
626 adaptation and diversification. *Science*. 2018 May 25;360(6391):907–11.

627 63. De Smet J, Zimmermann M, Kogadeeva M, Ceyssens PJ, Vermaelen W, Blasdel B, et al. High
628 coverage metabolomics analysis reveals phage-specific alterations to *Pseudomonas aeruginosa*
629 physiology during infection. *ISME J*. 2016 Aug;10(8):1823–35.

630 64. Ceyssens PJ, Brabban A, Rogge L, Lewis MS, Pickard D, Goulding D, et al. Molecular and
631 physiological analysis of three *Pseudomonas aeruginosa* phages belonging to the “N4-like
632 viruses”. *Virology*. 2010 Sep 15;405(1):26–30.

633 65. Ceyssens PJ, Miroshnikov K, Mattheus W, Krylov V, Robben J, Noben JP, et al. Comparative
634 analysis of the widespread and conserved PB1-like viruses infecting *Pseudomonas aeruginosa*.
635 *Environmental Microbiology*. 2009;11(11):2874–83.

636 66. Garbe J, Wesche A, Bunk B, Kazmierczak M, Selezska K, Rohde C, et al. Characterization of
637 JG024, a *pseudomonas aeruginosa* PB1-like broad host range phage under simulated infection
638 conditions. *BMC Microbiology*. 2010 Nov 26;10(1):301.

639 67. Danis-Wlodarczyk K, Cai A, Chen A, Gittrich M, Sullivan M, Wozniak D, et al. Friends or Foes?
640 Rapid Determination of Dissimilar Colistin and Ciprofloxacin Antagonism of *Pseudomonas*
641 *aeruginosa* Phages. *Pharmaceuticals*. 2021 Nov 15;14:1162.

642 68. Kiino DR, Rothman-Denes LB. Genetic analysis of bacteriophage N4 adsorption. *J Bacteriol*.
643 1989 Sep;171(9):4595–602.

644 69. Lavigne R, Lecoutere E, Wagemans J, Cenens W, Aertsen A, Schoofs L, et al. A multifaceted
645 study of *Pseudomonas aeruginosa* shutdown by virulent podovirus LUZ19. *mBio*. 2013 Mar
646 19;4(2):e00061-00013.

647 70. Brandão A, Pires DP, Coppens L, Voet M, Lavigne R, Azeredo J. Differential transcription
648 profiling of the phage LUZ19 infection process in different growth media. *RNA Biology*. 2021
649 Nov 2;18(11):1778–90.

650 71. Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of Bacteriophages
651 by Double Agar Overlay Plaque Assay. In: Clokie MRJ, Kropinski AM, editors. *Bacteriophages:*
652 *Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions* [Internet].
653 Totowa, NJ: Humana Press; 2009 [cited 2023 Jun 2]. p. 69–76. (Methods in Molecular
654 Biology™). Available from: https://doi.org/10.1007/978-1-60327-164-6_7

655 72. Kuo TT, Chow TY, Lin YT, Yang CM, Li HW. Specific Dissociation of Phage Xp12 by Sodium
656 Citrate. *Journal of General Virology*. 1971;10(2):199–202.

657 73. Yamamoto N, Fraser D, Mahler HR. Chelating agent shock of bacteriophage T5. *J Virol*. 1968
658 Sep;2(9):944–50.

659 74. Darling AE, Mau B, Perna NT. progressiveMauve: Multiple Genome Alignment with Gene Gain,
660 Loss and Rearrangement. *PLoS One*. 2010 Jun 25;5(6):e11147.

661 75. Microsoft Corporation. Microsoft Excel [Internet]. 2018. Available from:
662 <https://office.microsoft.com/excel>

663 76. R Core Team. R: A language and environment for statistical computing. [Internet]. Foundation
664 for Statistical Computing, Vienna, Austria.; 2021. Available from: <https://www.R-project.org/>

665 77. RStudio Team. RStudio: Integrated Development for R. [Internet]. RStudio, PBC, Boston, M;
666 2020. Available from: <http://www.rstudio.com/>

667 78. Wickham H. ggplot2. WIREs Computational Statistics. 2011;3(2):180–5.

668 79. Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. Mixed effects models and extensions in
669 ecology with R [Internet]. New York, NY: Springer; 2009 [cited 2023 Sep 28]. (Statistics for
670 Biology and Health). Available from: <http://link.springer.com/10.1007/978-0-387-87458-6>

671 80. Hedges LV, Gurevitch J, Curtis PS. The Meta-Analysis of Response Ratios in Experimental
672 Ecology. Ecology. 1999;80(4):1150–6.

673 81. Nabergoj D, Modic P, Podgornik A. Effect of bacterial growth rate on bacteriophage population
674 growth rate. Microbiologyopen. 2017 Dec 1;7(2):e00558.

675 82. Mocé-Llivina L, Muniesa M, Pimenta-Vale H, Lucena F, Jofre J. Survival of Bacterial Indicator
676 Species and Bacteriophages after Thermal Treatment of Sludge and Sewage. Applied and
677 Environmental Microbiology. 2003 Mar;69(3):1452–6.

678 83. Refardt D. Within-host competition determines reproductive success of temperate
679 bacteriophages. ISME J. 2011 Sep;5(9):1451–60.

680 84. Nowakowski AJ, Whitfield SM, Eskew EA, Thompson ME, Rose JP, Caraballo BL, et al. Infection
681 risk decreases with increasing mismatch in host and pathogen environmental tolerances.
682 Ecology Letters. 2016;19(9):1051–61.

683 85. Gehman ALM, Hall RJ, Byers JE. Host and parasite thermal ecology jointly determine the effect
684 of climate warming on epidemic dynamics. Proceedings of the National Academy of Sciences.
685 2018 Jan 23;115(4):744–9.

686 86. Buxton M, Nyamukondiwa C, Dalu T, Cuthbert RN, Wasserman RJ. Implications of increasing
687 temperature stress for predatory biocontrol of vector mosquitoes. Parasites & Vectors. 2020
688 Dec 1;13(1):604.

689 87. Villena OC, Ryan SJ, Murdock CC, Johnson LR. Temperature impacts the environmental
690 suitability for malaria transmission by *Anopheles gambiae* and *Anopheles stephensi*. Ecology.
691 2022;103(8):e3685.

692 88. Verhoef FA, Venter GJ, Weldon CW. Thermal limits of two biting midges, *Culicoides imicola*
693 Kieffer and *C. bolitinos* Meiswinkel (Diptera: Ceratopogonidae). Parasites & Vectors. 2014 Aug
694 20;7(1):384.

695 89. Tugwell LA, England ME, Gubbins S, Sanders CJ, Stokes JE, Stoner J, et al. Thermal limits for
696 flight activity of field-collected *Culicoides* in the United Kingdom defined under laboratory
697 conditions. Parasites & Vectors. 2021 Jan 18;14(1):55.

698 90. Palmer-Young EC, Raffel TR, Evans JD. Hot and sour: parasite adaptations to honeybee body
699 temperature and pH. Proceedings of the Royal Society B: Biological Sciences. 2021
700 Dec;288(1964):20211517.

701 91. Seeley ND, Primrose SB. The Effect of Temperature on the Ecology of Aquatic Bacteriophages.
702 Journal of General Virology. 1980;46(1):87–95.

703 92. Knecht LE, Veljkovic M, Fieseler L. Diversity and Function of Phage Encoded Depolymerases.
704 Frontiers in Microbiology [Internet]. 2020 [cited 2024 Feb 16];10. Available from:
705 <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2019.02949>

706 93. Chegini Z, Khoshbayan A, Taati Moghadam M, Farahani I, Jazireian P, Shariati A. Bacteriophage
707 therapy against *Pseudomonas aeruginosa* biofilms: a review. *Ann Clin Microbiol Antimicrob*.
708 2020 Sep 30;19:45.

709 94. Chug R, Mathur S, Kothari SL, Harish, Gour VS. Maximizing EPS production from *Pseudomonas*
710 *aeruginosa* and its application in Cr and Ni sequestration. *Biochemistry and Biophysics Reports*.
711 2021 Jul 1;26:100972.

712 95. Sun SJ, Dziuba MK, Jaye RN, Duffy MA. Temperature modifies trait-mediated infection
713 outcomes in a *Daphnia*–fungal parasite system. *Philosophical Transactions of the Royal Society*
714 *B: Biological Sciences*. 2023 Feb 6;378(1873):20220009.

715 96. Dalvin S, Are Hamre L, Skern-Mauritzen R, Vågseth T, Stien L, Oppedal F, et al. The effect of
716 temperature on ability of *Lepeophtheirus salmonis* to infect and persist on Atlantic salmon.
717 *Journal of Fish Diseases*. 2020;43(12):1519–29.

718 97. Conley MP, Wood WB. Bacteriophage T4 whiskers: a rudimentary environment-sensing device.
719 *Proceedings of the National Academy of Sciences*. 1975 Sep;72(9):3701–5.

720 98. Leon-Velarde CG, Happonen L, Pajunen M, Leskinen K, Kropinski AM, Mattinen L, et al. *Yersinia*
721 *enterocolitica*-Specific Infection by Bacteriophages TG1 and ϕ R1-RT Is Dependent on
722 Temperature-Regulated Expression of the Phage Host Receptor OmpF. *Appl Environ Microbiol*.
723 2016 Sep 1;82(17):5340–53.

724 99. Ryu WS. Virus Life Cycle. *Molecular Virology of Human Pathogenic Viruses*. 2017;31–45.

725 100. Bull JJ, Badgett MR, Wichman HA, Huelsenbeck JP, Hillis DM, Gulati A, et al. Exceptional
726 Convergent Evolution in a Virus. *Genetics*. 1997 Dec;147(4):1497–507.

727 101. Brown CJ, Zhao L, Evans KJ, Ally D, Stancik AD. Positive selection at high temperature reduces
728 gene transcription in the bacteriophage ϕ X174. *BMC Evolutionary Biology*. 2010 Dec
729 3;10(1):378.

730 102. Bashey F. Within-host competitive interactions as a mechanism for the maintenance of
731 parasite diversity. *Philos Trans R Soc Lond B Biol Sci*. 2015 Aug 19;370(1675):20140301.

732 103. Hudson P, Greenman J. Competition mediated by parasites: biological and theoretical progress.
733 *Trends in Ecology & Evolution*. 1998 Oct 1;13(10):387–90.

734 104. Shen SS, Qu XY, Zhang WZ, Li J, Lv ZY. Infection against infection: parasite antagonism against
735 parasites, viruses and bacteria. *Infectious Diseases of Poverty*. 2019 Jun 15;8(1):49.

736 105. Wang X, Wei Z, Yang K, Wang J, Jousset A, Xu Y, et al. Phage combination therapies for
737 bacterial wilt disease in tomato. *Nature Biotechnology*. 2019 Dec;37(12):1513–20.

738 106. Abedon ST, Hyman P, Thomas C. Experimental Examination of Bacteriophage Latent-Period
739 Evolution as a Response to Bacterial Availability. *Appl Environ Microbiol*. 2003
740 Dec;69(12):7499–506.

107. Sanjuán R. The Social Life of Viruses. Annu Rev Virol. 2021 Sep 29;8(1):183–99.

Figure legends

Figure 1. High temperatures can restrict phage infectivity. (A) Bacterial growth curves over 5h in the presence of phage at 37°C, 40°C, and 42°C. Growth curves are split by temperature and coloured by phage treatment. Results shown represent six biological replicates. (B & C) Phage growth curves over 5h at 37°C, 40°C, and 42°C measured using plaque-forming units (B) or phage DNA concentrations (C). For (C), Y-axis shows the fold change in phage DNA determined based on the change in phage DNA concentration relative to T0. Plots are split by phage and coloured by temperature. For (A) and (C), populations were destructively sampled at one-hour intervals. Results shown represent six biological replicates. For (B), populations were destructively sampled at hours 0, 2, and 5. Results shown represent three biological replicates. Error bars show standard error of the mean.

Figure 2. Temperature drives variation in phage life-history traits. (A) Radar charts showing relative change in phage life-history traits and population growth at 37°C, 40°C, and 42°C. Stability and host attachment reflect average time for 50% of phage particles to decay or adsorb to bacterial cells, respectively. Virulence and population growth reflect the number of bacterial doublings (in presence of phage) or phage doublings, respectively during the five-hour experiment. Data points show relative values across temperatures by dividing values at each temperature by 1.2 x highest value. All life-history trait values including 95% confidence interval ranges and standard errors are shown in Table S3. (B) Scatter plot shows relationship between phage virulence and population growth measured within the first two hours. Data points are shaped by phage and coloured by temperature. Error bars (inside data points) show standard errors of the $\log_2(T2/T0)$ ratios. Grey dotted line shows starting bacterial density where > 0 reflects an increase in bacterial densities and < 0 reflects a decrease in bacterial densities (due to phage lysis).

Figure 3. Phage competition is temperature-dependent. (A) Fold change in phage DNA concentration in the presence and absence of competitors between T0 and T5. Data is divided into 37°C and 40°C treatments. Asterisks show significant differences between competitor and no-competitor treatments based on a mixed effect model, with phage DNA concentration as the response variable and competitor as the explanatory variable. Significant differences within

773 temperatures are shown with a black asterisk whereas differences between temperatures are shown
774 with a red asterisk. * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.0001$). Boxes and points are coloured by
775 temperature. Results shown represent six biological replicates. **(B)** Change in phage competitiveness
776 across temperatures (see materials and methods for more information). Y-axis shows the resistance
777 of focal phage to competitors (average focal phage DNA in the presence of competitors relative to
778 no-competitor control). X-axis shows suppression of competitors by focal phage (average competitor
779 DNA in the presence of focal phage relative to no-competitor control). Black arrows show change in
780 phage competitiveness with warming. Phage are distinguished by point shape with temperatures by
781 point colour.

782