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Author for correspondence:
Carolin C. Wendling
e-mail: cwendling@geomar.de

†Present address: Marine Ecology/Evolutionary Ecology of Marine Fish, GEOMAR – Helmholtz Centre for Ocean Research, Duesternbrookerweg 20, Kiel 24105, Germany.

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Adaptation to enemy shifts: rapid resistance evolution to local *Vibrio* spp. in invasive Pacific oysters

Carolin C. Wendling† and K. Mathias Wegner

Coastal Ecology, Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Wadden Sea Station Sylt, Hafenstraße 43, List 25992, Germany

One hypothesis for the success of invasive species is reduced pathogen burden, resulting from a release from infections or high immunological fitness of invaders. Despite strong selection exerted on the host, the evolutionary response of invaders to newly acquired pathogens has rarely been considered. The two independent and genetically distinct invasions of the Pacific oyster *Crassostrea gigas* into the North Sea represent an ideal model system to study fast evolutionary responses of invasive populations. By exposing both invasion sources to ubiquitous and phylogenetically diverse pathogens (*Vibrio* spp.), we demonstrate that within a few generations hosts adapted to newly encountered pathogen communities. However, local adaptation only became apparent in selective environments, i.e. at elevated temperatures reflecting patterns of disease outbreaks in natural populations. Resistance against sympatric and allopatric *Vibrio* spp. strains was dominantly inherited in crosses between both invasion sources, resulting in an overall higher resistance of admixed individuals than pure lines. Therefore, we suggest that a simple genetic resistance mechanism of the host is matched to a common virulence mechanism shared by local *Vibrio* strains. This combination might have facilitated a fast evolutionary response that can explain another dimension of why invasive species can be so successful in newly invaded ranges.

1. Introduction

Species invasions can be considered as evolutionary 'experiments in nature' [1] that generate new phenotypes in action [2,3]. Surprisingly, the evolutionary potential of invaders to adapt to altered abiotic and biotic selection pressures in the transition from colonization to expansion has rarely been considered [3,4]. Only recently it has been shown that the adaptation to novel climatic conditions can outweigh or at least match the majority of factors promoting invasion success [5].

One important factor for invasion success is reduced pathogen burden that can result from release from parasite infections i.e. the enemy release hypothesis [6] or immunological superiority [7]. Owing to rapid coevolutionary dynamics host–parasite interactions should also lead to adaptive clines and at least for parasites the rapid adaptation to invasive hosts has been demonstrated [8]. For the host itself an invasion of a new habitat often involves exposure to novel pathogens or parasites [9]. Instead of a release from infection pressure a shift to newly acquired pathogens can be observed. However, the importance of adaptation to new pathogen communities has so far not been investigated.

Here, we present a unique study system, where two independent invasions of Pacific oysters *Crassostrea gigas* (Thunberg, 1793) led to genetically divergent populations [10,11]. Both invasions of the Pacific oyster into the European Wadden Sea occurred throughout the 1990s. While the southern invasion wave can be traced back to the Oosterschelde, the northern invasion stems from British hatchery produced spat farmed on the island of Sylt [12]. Using independent invasion events has a clear-cut advantage over the comparison of adaptive differences between source and invasive populations. For one, adaptations to local selection pressures will be independent and will have

furthermore occurred over a similar time span [2]. Yet, only few studies focused on populations with independent invasion histories [4]. Both invasive populations also differ in their selective history of disease outbreaks. In hot summer months with water temperatures exceeding 19°C, southern oysters were frequently subjected to strong selection by oyster summer mortality with mortality rates exceeding 60% and affecting all size and age classes [13].

A complex interaction of external and internal factors including high temperatures and pathogens like herpes viruses or bacteria of the genus *Vibrio* spp. (in particular, *Vibrio splendidus)* are suggested to be involved in mortality events affecting oyster larvae [14], juveniles [15–17] and adults [18,19]. Although herpes viruses (OsHV) were observed during mortality events in the Wadden Sea in 2005 [13], high mortality rates in comparison with low infection rates suggest that also other factors were involved. On top of that screening of oysters from Texel and Sylt for herpes viruses in Pacific oysters by PCR in July 2011 did not give positive results for OsHV-1 (M.Y. Engelsma 2011, personal communication). Furthermore, resistance towards disease has a genetic basis [20], making it a likely target for selection.

Since the northern population has been spared from disease so far, the aim of this study was to test whether rapid evolution along differential selection gradients within the two invasion waves led to local host adaptation to sympatric disease agents, i.e. *Vibrio* spp. To answer this question, we conducted two consecutive experiments: in a first reciprocal infection experiment, we determined disease resistance and the underlying efficiency of the cellular immune response towards sympatric and allopatric *V. splendidus* strains in adult Pacific oysters stemming from both source populations. In order to determine the influence of environmental conditions the experiments were carried out at average summer water temperature (17°C) and elevated temperature (21°C) associated with disease outbreaks. To then generalize our findings, we tested the resistance of laboratory-bred larvae against a broad range of *Vibrio* strains. Here, we also included crosses between the invasion sources and can therefore not only detect signatures of selection within each invasion wave but are also able to predict whether admixture will put new superior genotypes into action [21–23] that can decisively influence the further spread of biological invasions. We found clear evidence of rapid immunological adaptation to sympatric *Vibrio* spp. communities and demonstrate that resistance against these is dominantly inherited resulting in increased fitness of admixed populations. Our findings therefore add a new facet to the factors explaining invasion success: rapid adaptation to enemy shifts.

2. Material and methods

(a) *Vibrio* community structure

Pacific oysters were sampled at six sites covering the entire Wadden Sea (electronic supplementary material, figure S1) in September 2011. Haemolymph samples were taken from each oyster and 4 µl were spread on *Vibrio* selective thiosulfate citrate bile sucrose agar (TCBS) plates (Fluka Analytica, Sigma-Aldrich, Steinheim, Germany). Plates were incubated at 25°C for 24 h, before we counted the colony forming units (CFU). A random subset of 11–18 single colonies per site were resuspended in 3 ml nutrient solution 1.5% NaCl (1000 ml distilled water, 5.0 g peptone, 3.0 g meat extract) and cultured at 25°C under constant shaking for 24 h. An aliquot of each liquid culture was used for direct amplification of 16s *rRNA*, *GyrB* and *PyrH*. Amplification followed previously established protocols [24] and PCR products were purified and sequenced at the Institute for Clinical Molecular Biology (IKMB), Christian-Albrechts-University Kiel, Germany. Each remaining culture was cryopreserved in medium +50% glycerol at −80°C until further use.

(b) Infection experiments

We performed two sets of infection experiments. One using adult oysters to measure resistance and cellular immune response against two strains of *V. splendidus* and the other using laboratory-bred larvae to measure resistance against a wide variety of *Vibrio* strains.

(i) Adult infections

Adult sampling and acclimation. In May 2012, healthy adults that were showing no signs of disease were collected three weeks prior to the experiment from two sites covering the northern (Sylt Island: 55°2.33′ N, 8°26.57′ E) and southern (Texel Island: 53°08.85′ N, 4°54.53′ E) population (electronic supplementary material, figure S1). Oysters were acclimated to the experimental temperatures of 17 and 21°C in constant temperature rooms (temperature shifts during acclimation less than 0.5°C per day). We chose 17°C as it represents contemporary average summer water temperatures, and 21°C as a representative for future predicted water temperatures [25]. Oysters were kept in a flow through system and fed three times a week with 50 000 to 80 000 cells per ml^{-1} of Isochrysis 1800 Instant Algae (Varicon Aqua Solutions, Worcester, UK). One week prior to the experiment oysters were cleaned of epibionts and were notched on the dorsolateral side of the shell closest to the adductor muscle with a small hand drill.

Adult infections. For the adult infection experiment, we selected two previously described closely related isolates of *V. splendidus* from each location (O7w_July from Sylt and Tx5.1 from Texel, as described in Thieltges *et al.* [26]). Bacteria of the *Splendidus* clade have been shown to be involved in mortality events affecting oyster larvae [14], spats [15] and adult oysters [27,28]. Both strains have been successfully used in past studies [26] and did not show temporal bias in virulence [17] at 17°C. The strains will be referred to as *Vibrio* north and *Vibrio* south for geographical reference. To test for patterns of local adaptation across environments, we used a three-factorial design including origin (north, south), temperature (17 and 21°C) and infection (control, *Vibrio* north and *Vibrio* south). We used a total of 220 oysters and kept half of the oysters from each site ($n = 55$) at average summer water temperature (17°C) and the other half at elevated temperature (21°C). Experiments were carried out in one constant climate chamber where oysters were kept individually in single 1 l aerated glass jars placed in temperature controlled water baths. To avoid block effects, jars were randomly distributed over 21 water baths, each containing eight jars at 17°C and eight jars at 21°C. We exchanged the water every second day.

For each experimental group, 20 oysters were infected with *Vibrio* north or *Vibrio* south, and the remaining 15 oysters with nutrient solution 1.5% NaCl (as sham control). Treatments followed the infection protocols described in Wendling & Wegner [29]. Briefly, we injected 10^8 cells of bacterial culture or an equal volume of nutrient solution 1.5% NaCl with a syringe into the adductor muscle through the predrilled hole.

Adult cellular immune response and resistance. Cellular immunological parameters, bacterial load expressed as culturable *Vibrio* counts and survival were assayed as described in Wendling & Wegner [29]. In short, we monitored survival of all animals daily and additionally collected five random oysters from every treatment group at days 1, 3 and 7 to extract haemolymph (800 µl) from the adductor muscle. In fractions of the haemolymph, we:

(i) measured the total number of circulating haemocytes (THC) using an automated cell counter (Scepter, Merck Millipore, Darmstadt, Germany), (ii) estimated phagocytosis activity per unit of haemocyte protein using neutral red-stained, heat stabilized zymosan as described in Pipe *et al*. [30], and (iii) determined infection intensity by plating out 4 µl of haemolymph on TCBS plates to count CFU.

(ii) Larval infections

Larval crossing and rearing. Over a 2 day period in July 2012, we created 40 full-sibling families from randomly collected northern (Sylt) and southern (Texel) oysters (electronic supplementary material, figure S1). We bred four crossing groups with 10 families each: NN ($North_{female} \times North_{male}$), NS ($North_{female} \times South_{male}$), SN ($South_{female} \times North_{male}$) and SS ($South_{female} \times South_{male}$) by stripping gametes directly from the gonads. Fertilization was performed at a ratio of 200 spermatozoa per oocyte, with 4×10^5 oocytes per family. One hour after fertilization embryos were transferred to rearing tanks at a concentration of five embryos ml^{-1}. Larvae were kept at 21°C with salinity at 28 psu in 2 l rearing tanks filled with 0.45 µm filtered, ultraviolet (UV)-treated seawater (exchanged every 48 h) and fed *Isochrysis galbana* (10–150 cells μl^{-1} depending on age).

Larval infection. On day 10 after fertilization we conducted controlled infection experiments with 76 different *Vibrio* strains isolated from all sites throughout the entire Wadden Sea (electronic supplementary material, figure S1). We created three groups by pooling families with equal larvae contributions: NORTH (all NN families), HYBRIDS (all NS and SN families) and SOUTH (all SS families). Experimental challenges were carried out using sterile 96-well culture plates as described in Wendling *et al*. [24]. Briefly, 10–15 larvae per group were placed in one well containing 0.45 µm filtered, UV-treated seawater and bath challenged with the *Vibrio* isolates at a concentration of 10^7 cells ml^{-1}. Each combination (larval group × *Vibrio* isolate) was assayed in duplicates. Survival was observed three days post infection using an inverted microscope by counting the amount of dead larvae.

(c) Data analysis

All statistics were performed in the R v. 2.15.2 statistical environment (R Development Core Team, 2011) and only minimal adequate models of generalized linear models (GLMs) were fitted unless stated otherwise. All data were checked to meet underlying assumptions and proper model fits.

(i) *Vibrio* community structure

Phylogenetic analysis. A detailed description of our analysis procedure is given in Wendling *et al*. [24]. Briefly, after assembly and alignment of concatenated sequences, we constructed a phylogenetic tree using maximum-likelihood in PhyML v. 3.0 [31] from the 2560 positions long alignment. We applied the *general time reversible model* plus a discrete γ-distribution to account for rate heterogeneity among sites plus invariant sites (GTR + G + I) [32] as suggested by the Akaike information criterion (AIC) given by jModelTest [33]. *Allovibrio fischerii* was used as outgroup and 14 reference strains were included for species identification. A radial cladogram was drawn using the Interactive Tree of Life web service at http://itol.embl.de/ [34].

(ii) Adult infections

Survival. We performed two independent logistic regression analyses for each temperature, with survival as the dependent variable and oyster origin (north, south), *Vibrio* origin (north, south), as well as all interactions as independent variables. Oysters that survived longer than nine days were recorded as survivors, while oysters used for immunological analyses were dropped from the analysis.

Cellular immunological parameters and infection intensity. We used a multivariate analysis of variance (MANOVA) using the Pillai's trace statistics with all response variables (i.e. total haemocyte counts, phagocytosis rate (%) and amount of CFU) as dependent variable and oyster origin, temperature, *Vibrio* origin and time and all interaction terms as independent variables.

(iii) Larval infections

Resistance against all strains was assessed as survival on day 3 of the infection experiment. We used a binomial generalized linear model to identify local adaptation with numbers of surviving and dead larvae as the dependent variable and oyster origin (NN, SS, hybrids) as well as *Vibrio* group as independent variables. We grouped *Vibrio* strains to contrast effects between both larvae sources (Sylt and Texel) and other sites (Husum, Büsum, Wilhemshaven, Norden). To further identify differences in oyster survival depending on geographical origins of *Vibrio* isolates, we used a linear weighted regression with survival rate averaged over all *Vibrio* strains per site (weighted by sd^{-1}) as the dependent variable and geographical distance of to the *Vibrio* community to the northern site as the independent variable for all crossing groups. Since the relationship between survival rate and geographical distance in the hybrid group was not linear, we chose a quadratic polynomial regression to determine the relationship between survival rate and geographical distance of the *Vibrio* community.

We used a heuristic approach to identify *Vibrio* strains causing patterns of local adaptation. To do so, we calculated the deviance explained by the interaction term of a generalized linear model predicting survival by cross type and *Vibrio* strain. We repeated this analysis after removal of each *Vibrio* strain and recorded the resulting deviance change of the interaction term when compared with the model containing all strains. We then ordered all 76 strains based on their contribution to the interaction deviance term and subsequently removed strains in descending order until the crossing group × *Vibrio* strain interaction term was not significant any more. Strains that were removed from the model this way can be considered to show patterns of local adaptation. We conducted a Fisher's exact test to identify whether *Vibrio* origin and genetic affiliation were responsible for the observed pattern of local adaptation.

3. Results

(a) *Vibrio* community structure

We successfully genotyped 76 different *Vibrio* strains for all three genes (16s, *PyrH*, *GyrB*), of which we could unambiguously assign 75 strains to nine different *Vibrio* species from three distinct clades (figure 1), i.e. the *Splendidus* clade (92%), the *Vibrio* core (7%) and the *Anguillarum* clade (1%). Within the *Splendidus* clade, we found seven different species (*V. chagasii*, *V. crassostreae*, *V. cyclitrophicus*, *V. gigantis*, *V. kanaloae*, *V. splendidus*, *V. tasmaniensis*), while all members of the *Vibrio* core belonged to the species *V. alginolyticus*, and the member of the *Anguillarum* clade was identified as *V. aestuarianus*. The *Vibrio* community composition did not significantly differ between all sampling sites (Unifrac significant test, $p = 0.07$) and overall was dominated by the *Splendidus* clade (electronic supplementary material, figure S1).

(b) Infection experiments

We infected two different life stages to address two different scientific questions. First, we were interested in differences of

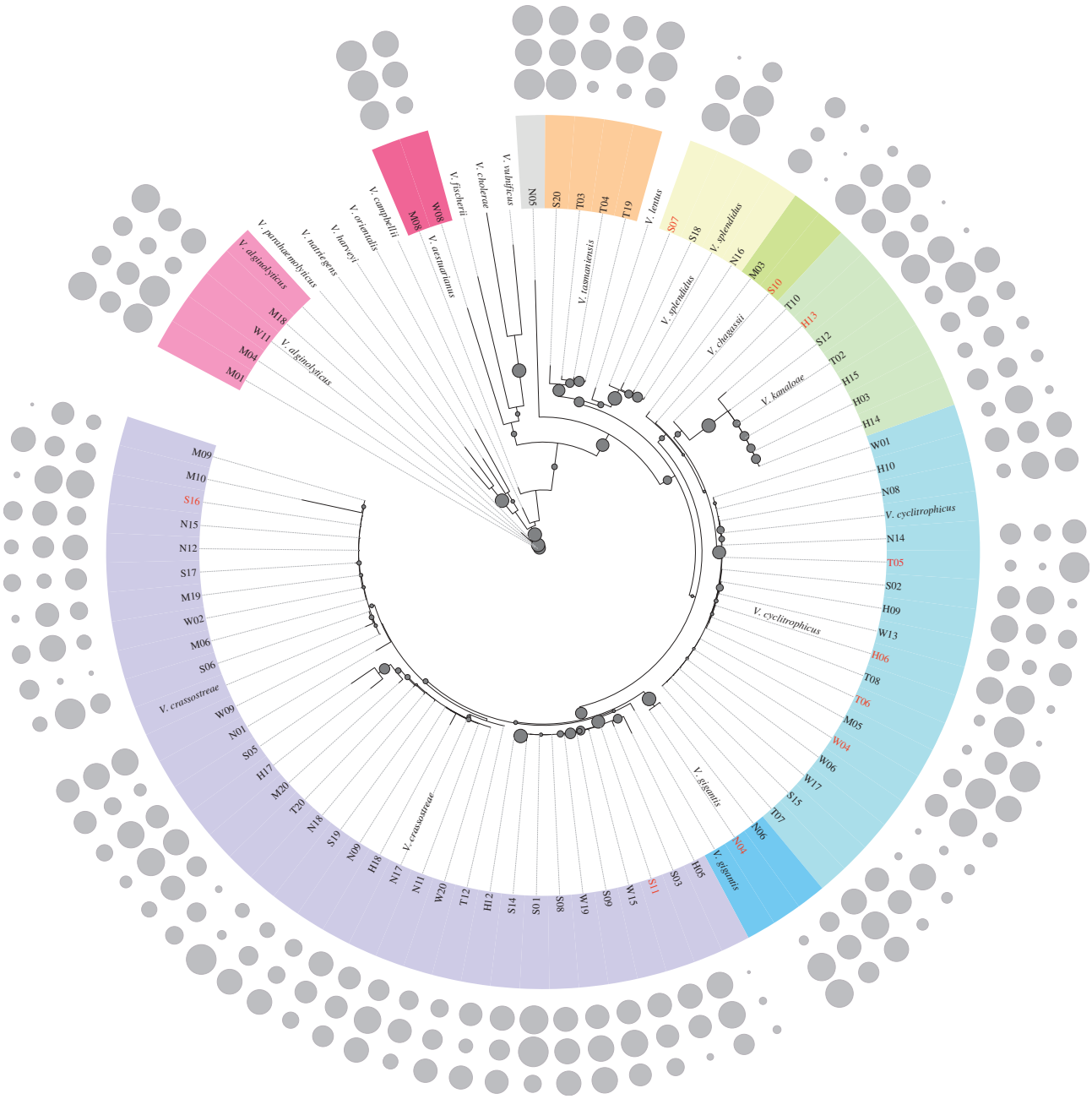

Figure 1. Phylogenetic relationship based on maximum-likelihood method (GTR + G + I substitution model) using concatenated sequences of three genes (16s *rRNA*, *GyrB* and *Pyr*) (2566 bp) of *Vibrio* isolates sampled from oyster haemolymph. Bootstrap percentages above 80% are represented by grey dots at the parent nodes. The size of grey circles on the three outer rings corresponds to resistance of the different cross types (from outside to inside: southern oysters—hybrids—northern oysters) against the selected *Vibrio* isolate. Assignment to the different clades (see legend) based on 99% sequence similarity by BLASTN analysis is depicted in colour on the inner ring. Those strains, responsible for promoting local adaptation are marked in red.

immune response between sympatric and allopatric infections, for which we had to use adults. To extrapolate our results obtained from adult infection experiments to a wider range of *Vibrio* isolates, we conducted a larvae infection experiment.

(i) Adult infections

Survival. Upon infection, mortality started at day 1 and continued until day 5 with no more deaths observed until day 7. Therefore, experiments were terminated at day 7. Infection and temperature significantly increased mortality (logistic regression: infection $\chi^2_1 = 20.82$, $p < 0.001$; temperature: $\chi^2_1 = 16.86$, $p < 0.001$). At 17°C mortality did not differ with respect to oyster and *Vibrio* origin, $\chi^2_3 = 2.37$, $p = 0.5$. However, at 21°C, oysters from north and south showed significantly lower mortality rates when infected with their sympatric strain compared with the allopatric infection: significant oyster origin × *Vibrio* origin interaction: $\chi^2_3 = 14.3$, $p = 0.003$ (figure 2).

Cellular immune parameters and infection intensity. After 7 days, we lost all southern oysters that had been infected with *Vibrio* north at 21°C and therefore excluded day 7 from the analysis. Infection with either *Vibrio* isolate significantly increased the amount of total culturable *Vibrio* counts, i.e. CFU and cellular immune parameters (figure 3). To specifically examine local adaptation between oysters and *Vibrio*, we excluded the control group from further analysis. Cellular immune parameters and infection intensity significantly increased with warmer temperatures (table 1 and figure 3). CFU was highest after 12 h and decreased

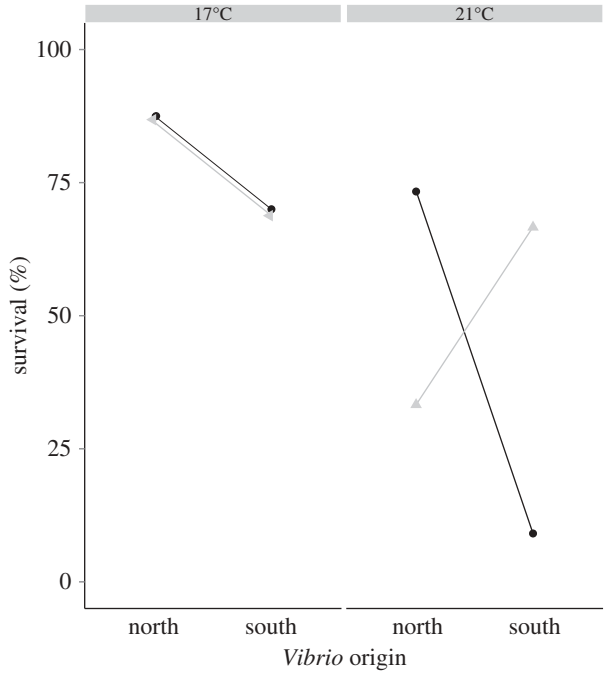

Figure 2. Endpoint survival (%) after 9 days at 17°C (left side) and 21°C (right side) for southern and northern *Vibrio* (right and left) and all three oyster origins (south, triangles and north, circles).

throughout the experiment, reaching values indistinguishable from those of controls after one week. Cellular immune parameters peaked on day 3 showing mainly a response to the immune challenge (figure 3*a*,*c*). Oyster origin and *Vibrio* strain did not show a significant effect at 17°C, but at 21°C, oysters from south and north showed significantly lower infection intensities and higher cellular immune response when infected with sympatric strains (significant origin × strain × temperature interaction, table 1).

(ii) Larval infections

Three days after artificial fertilization, we lost four families (1 NN, 1 SS and 2 SN). Resistance of cross types varied significantly between *Vibrio* strains ($F_{1,391} = 6.47$, $p = 0.01$), but did not depend on phylogenetic distance between strains within each cross type (Mantel test: northern oysters: $p = 0.91$, southern oysters: $p = 0.63$, artificial hybrids: $p = 0.87$) nor for the whole dataset (Mantel test: $p = 0.77$). On the other hand, we observed a significant main effect for oyster origin (binomial GLM: $\chi^2_2 = 55.04$, $p < 0.001$), *Vibrio* group (binomial GLM: $\chi^2_2 = 31.32$, $p < 0.001$) with the interaction between *Vibrio* group and oyster origin explaining most of the variation (binomial GLM: $\chi^2_4 = 183.64$, $p < 0.001$). For both northern and southern cross types ($R^2 = 0.84$ and $R^2 = 0.62$ respectively, figure 4*a*), we observed a linear relationship with distance to the infecting *Vibrio* community, i.e. both cross types displayed highest survival rates when infected with sympatric *Vibrio* spp. strains. By contrast, hybrids were equally resistant to northern and southern *Vibrio* spp. strains as the pure cross types (NN and SS) and showed lowest survival at intermediate distance from both origins (quadratic distance $R^2 = 0.81$; figure 4*a*) indicating that resistance to infection with sympatric *Vibrio* strains is dominantly inherited. Averaged over all *Vibrio* sampling sites, hybrids had a higher mean survival than pure lines (ANOVA: $F_{2,\ 280} = 5.38$, $p = 0.006$; figure 4*b*).

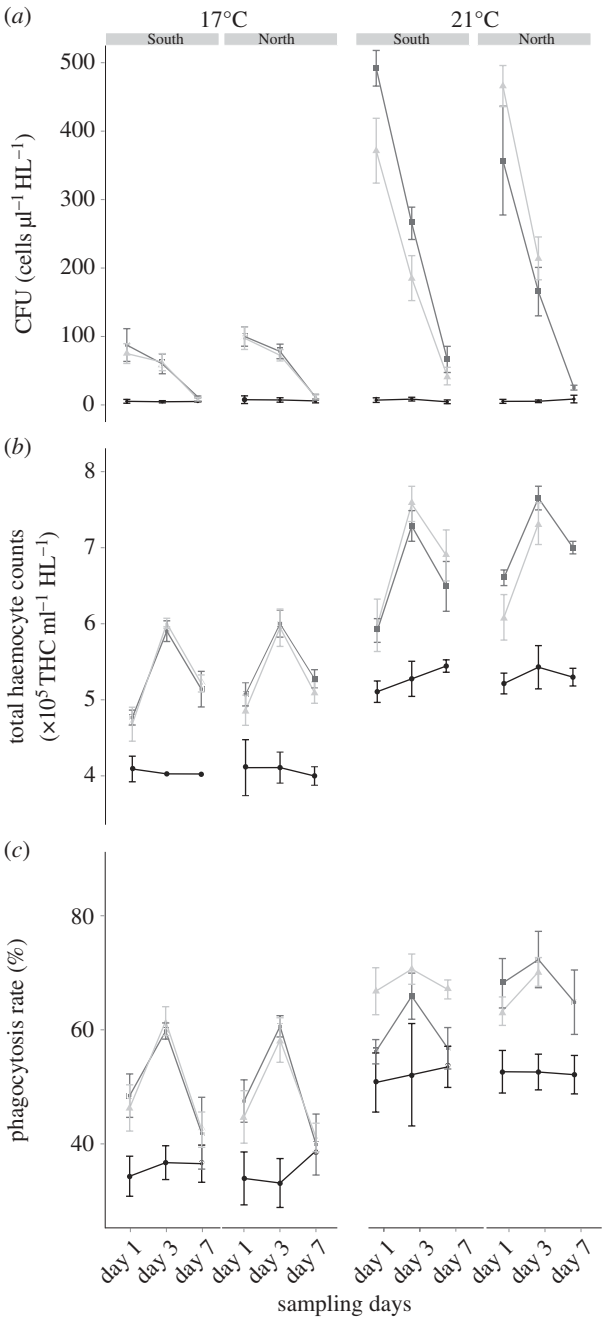

Figure 3. Infection intensity (total culturable *Vibrio* counts) and cellular immunological parameters at 17°C (left side) and 21°C (right side) measured on days 1 (i.e. 12 h), 3 and 7 after infection for each treatment (control: black circles, *Vibrio* south: light grey triangles, *Vibrio* north: dark grey squares, mean ± s.e.m., $n = 90$). (*a*) Infection intensity: CFU, (*b*) total haemocyte counts, (*c*) phagocytosis rate.

Out of the tested 76 *Vibrio* strains, our heuristic approach identified 10 strains from several groups within the *V. splendidus* clade that showed typical local adaptation patterns (figure 4*c*). Out of these 10 strains the major proportion (60%, Fisher's exact test: $p = 0.05$) was isolated from the sites where brood stocks were collected (i.e. Sylt and Texel, figure 4*c*) indicating that the evolution of specific resistance resulted from a local process over short period of time.

4. Discussion

Rapid evolution of invasive species in response to natural selection in the novel environment is a key feature in determining

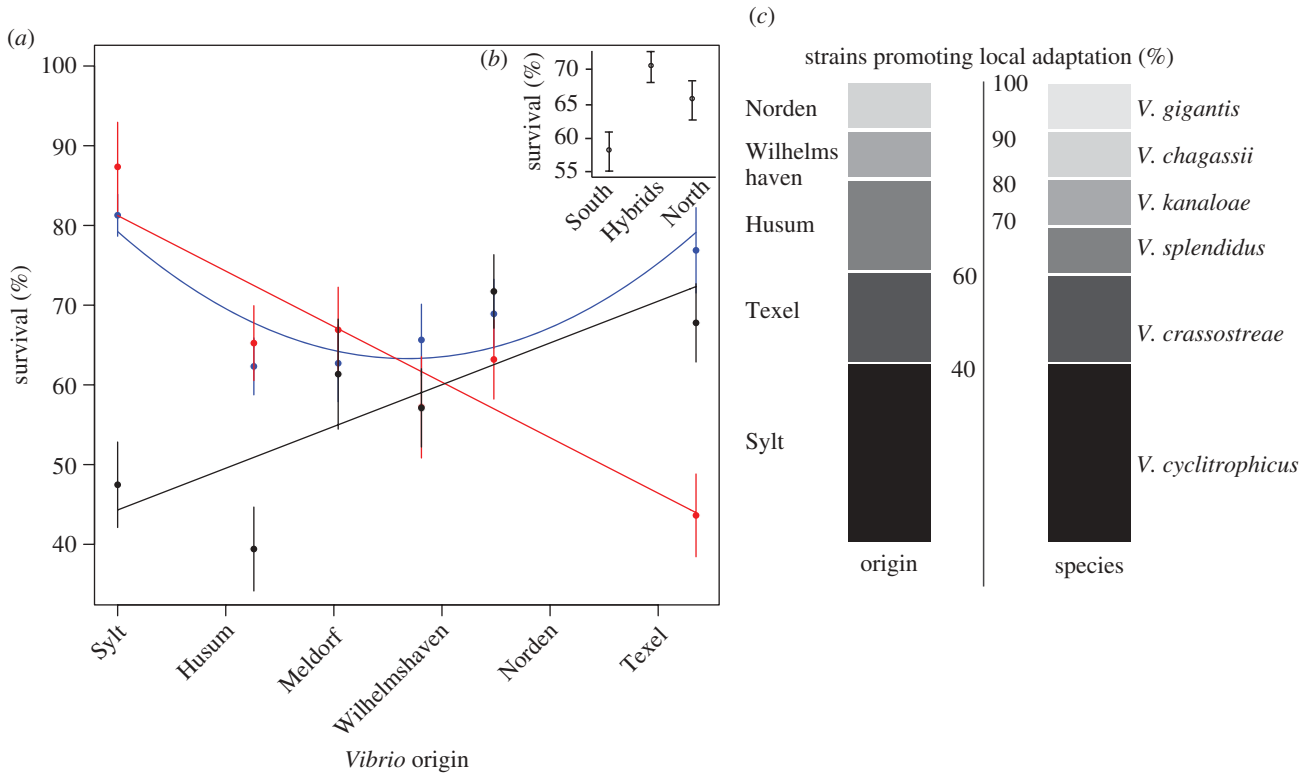

Figure 4. (*a*) Survival rate (%) of artificially bred oyster populations: NN (red), SS (black) and hybrids (blue), infected with *Vibrio* spp. from six different sampling sites along the Wadden Sea coast from north, i.e. Sylt to south, i.e. Texel. Weighted linear regression: NN (t_1, −4.69, $p = 0.009$), SS (t_1, 2.57, $p = 0.06$), polynomial regression: Hybrids (t_1, −3.59, $p = 0.04$). (*b*) Mean survival rate (mean ± s.e.m., $n = 688$) per host population. (*c*) Strains (%) identified to promote local adaptation displayed per origin (left) and *Vibrio* species (right).

Table 1. Infection intensity (i.e. total culturable *Vibrio* counts) and cellular immune parameters (MANOVA, Pillai's trace statistic). (Significant factors ($\alpha = 0.05$) are highlighted in bold.)

fixed factors	d.f.	Pilai's trace	approx. *F*	*p*
origin	1	0.005	0.1	0.96
temperature	**1**	**0.91**	**158.6**	**<0.001**
strain	1	0.012	0.2	0.88
day	**1**	**0.81**	**71.2**	**<0.001**
origin:temperature	1	0.06	1.0	0.39
origin:strain	1	0.22	4.8	0.005
temperature:strain	1	0.01	0.2	0.87
temperature:day	**1**	**0.41**	**11.5**	**<0.001**
origin:temperature:strain	**1**	**0.19**	**3.9**	**0.01**

invasion success [5]. Based on controlled infection experiments with two genetically divergent invasive populations of the Pacific oyster *C. gigas*, we could show that rapid evolution can also occur to adapt to a broad range of newly acquired opportunistic pathogens encountered in the new habitat. Oysters showed increased cellular immune efficiency giving them higher resistance when infected with sympatric *Vibrio* spp., suggesting that a common feature shared by otherwise only distantly related *Vibrio* spp. strains can serve as a target for local immunological adaptation. The dominant inheritance of resistance in hybrids between the two invasions further suggested that the genetic mechanism of resistance relies on only few loci, thus facilitating a fast and independent evolutionary response over only 20 years since the introduction of Pacific oysters into the Wadden Sea. This corresponds to six to seven generations at most when considering that Pacific oysters populations have been established in the mid-1990s (K Reise 2012, personal communication; [35]) and that they need approximately 3 years to reach maturity.

(a) Local adaptation to *Vibrio* communities is environment dependent

For all oyster origins, we detected no significant difference in disease resistance to either *Vibrio* strain at ambient water temperature (17°C). However, at higher water temperatures we detected higher resistance to sympatric *Vibrio* mediated by a stronger cellular immune response (figure 3*b*,*c*). High water

temperatures on the one side led to increased proliferation rate of the pathogen (figure 3*a*), while it also led to an increased immune response compared with 17°C (figure 3*b*,*c*). Our experimental results thus match the natural condition under which oyster mass mortalities occurred, i.e. when water temperatures exceed a critical value of 19°C [36] leading to more favourable conditions for *Vibrio* growth. This may suggest that immune surveillance may be costly [7] and is enhanced when conditions are favourable for bacterial disease. With the risk of disease being increased with rising water temperature [37], the environment becomes more selective and allowed us to detect otherwise cryptic patterns of local adaptation.

In invertebrates, phagocytosis provides an important component of cellular immunity [38]. Upon pathogen infection, haemocytes migrate actively towards the site of infection, locally increasing the concentration of immune effectors attacking the pathogen [39]. At high temperatures, a higher heartbeat rate can result in enhanced production and circulation of haemocytes [40]. Consistently, at higher temperatures, we observed increased haemocyte counts (THC) and a higher phagocytosis rate. And while we are aware that several other molecular as well as cellular immune parameters may have contributed to local immunological adaptation, it is nevertheless tempting to speculate that the increased number of THC and the enhanced phagocytosis rate are involved in limiting the pathogen load of the faster growing *Vibrio* population in the case of sympatric combinations at elevated temperatures (figure 3*b*).

By using 76 different *Vibrio* strains isolated from haemolymph of six different oyster populations covering 535 km of Wadden Sea coastline, we could further show that resistance against sympatric *Vibrio* strains is valid for a broad range of strains. Overall, *Vibrio* spp. communities associated with oyster haemolymph showed similar species distributions throughout the entire Wadden Sea (electronic supplementary material, figure S1). This indicates that the taxonomic composition of strains used here was independent of the invasion source of the host. While oyster associated microbiota can assemble according to host genotype [41], *Vibrio* spp. in oyster haemolymph are most probably taken up from the environment and vary seasonally with environmental temperature [24,42]. *Vibrio* spp. are virtually absent from oyster tissue during winter months [41], and their abundance and diversity increases from spring to summer, reaches a peak during spawning season before decreasing towards autumn [24]. Thus, Pacific oysters do not have a constant *Vibrio* community but rather take up strains from the local environment making it highly unlikely that the strains used here share a longer coevolutionary history exceeding the time since invasion into the Wadden Sea. Warm summer months also showed the highest chances of encountering highly virulent strains [24]. Therefore, our sampling in September should have captured a representative high diversity of *Vibrio* spp., including several virulent strains (figure 1).

The consistent pattern of resistance against sympatric *Vibrio* strains suggests that strains causing the pattern of local adaptation in the community of oyster haemolymph symbionts share a common factor to which oysters have adapted. Since symbiont *Vibrio* strains were only distantly related, such a factor is unlikely to be shared by descent. Horizontal gene transfer is common in the genus *Vibrio* [43] and it is tempting to speculate that the common factor was acquired in *Vibrio* communities resident in oyster tissues. Horizontal gene transfer as well as local adaptation are localized processes and with 60% of the strains showing local adaptation stemming from the source populations used for breeding, we can confirm that adaptation to local pathogen communities was such a localized process.

Vibrio are ubiquitous in the marine realm and pathogenic strains are often generalists with a broad host range [43] that encounter a vast range of resistance mechanisms from alternative host species [44]. Indeed, *Vibrio* communities associated with benthic marine invertebrates including oyster populations used here were not host specific and rather reflected a random assemblage of *Vibrio* spp. from the environment [24,45]. For generalist pathogens, adaptation to a particular host species would be disadvantageous, as it will limit the host range [46,47]. This results in asymmetric selection pressures in host–parasite coevolution favouring host adaptation over parasite adaptation. Indeed, it has previously been shown that activation of the pipefish *Syngnathus typhle* immune system is locally adapted to their phylogenetically divergent *Vibrio* communities [48].

(b) Dominant inheritance facilitates fast adaptation

Hybrid larvae (H) stemming from crosses between both genetically differentiated invasions showed similar survival rates as both parent populations (NN and SS) when infected with sympatric *Vibrio* strains (figure 4). The higher overall resistance against a wider scope of pathogens of hybrids can spread rapidly through the secondary contact zone. Such increased resistance in the admixed population might have beneficial effects during the invasion event by increasing the chance of range expansion [23]. The clear-cut resistance pattern of hybrids further suggests a simple underlying genetic architecture of resistance that is dominantly inherited. Dominance of resistance alleles over the susceptible alleles will lead to faster fixation of resistance alleles, which might explain the fast rate of local adaptation and clearly demonstrates the evolutionary importance of disease as a major selective force [49]. Indeed, extensive mortalities associated with disease in several oyster species suggests that adaptation can occur quickly within a few generations in natural populations [20,50,51]. Direct field observations and laboratory experiments of the Eastern oyster *Crassostrea virginica*, of different genetic origin and parasite exposure histories, revealed that resistance of *C. virginica* to two different protozoan parasites, i.e. *Minchinia nelsonii* (MSX disease) and *Perkinsus marinus* (Dermo disease) has evolved within a few generations [51]. Also, in *C. gigas* the genetic basis for sensitivity to summer mortality syndrome (SMS) varied with the intensity of selection with narrow-sense heritability being higher in sites where selection by SMS was stronger [20]. In addition, resistance to *V. harveyi* and *V. parahemolyticus* in the clam *Meretriix meretrix* is associated with single nucleotide polymorphisms in the I-type lysozyme gene [52]. If resistance of Pacific oysters to *Vibrio* spp. has a similar genetic basis, rapid evolution of disease resistance may result from a 'regime shift', where extensive mortalities remove susceptible individuals [53], supported by sweepstake reproductive success, where only a small fraction of adults, i.e. the most resistant ones, will reproduce successfully [53,54]. Hence abiotic and biotic environmental threats can rapidly alter the genetic composition of a single oyster population, thereby creating spatially and temporally varying populations that possess higher fitness in their native habitat.

5. Conclusion

We could show that oysters can rapidly adapt to widespread communities of pathogenic *Vibrio* spp. While invasion success has partly been attributed to a release from parasites encountered in the native habitat (i.e. enemy release [6]), we can now add a new facet for explaining invasion success: rapid adaptation to enemy shifts. Conditions supporting rapid adaptation were the likely generalism of *Vibrio* spp. in terms of host choice [43], a genetic mechanism shared by local *Vibrio* strains and the dominant inheritance of resistance. Immunological superiority in terms of reduced self-harm has been implicated in invasion success [7], but it is unclear to which extent evolutionary potential of resistance/tolerance contributes to population growth in new environments. Since any pattern of local adaptation is constrained to a given environment with all its abiotic and biotic features, evolutionary potential of any host (invader and native) seems to be an important aspect of species persistence in the light of fast changing environments like coastal oceans. For *Vibrio* spp., a group containing many widespread opportunistic pathogens, it is well known, that virulence depends on environmental parameters, such as temperature or salinity [55,56]. If temperature rises, as predicted by current climate change models, an increase in virulence has to be expected [37] that potentially disrupts patterns of local host adaptation of all potential hosts.

Invasive species can have high evolutionary potential to adapt to changing abiotic conditions [5]. If they also have a higher potential to rapidly adapt to altered virulence of generalist pathogens in general, far reaching consequences for future development of marine ecosystems can be expected. Therefore, knowledge of underlying genetic mechanisms of rapid local adaptation and their interactions with the biotic and abiotic environment will be a crucial component in predicting evolutionary change in response to increasing virulence of generalist pathogens resulting from rising temperatures.

Ethics statement. Approval for collecting oysters was given by the Nationalparkamt Schleswig-Holstein http://www.nationalpark-wattenmeer.de/.

Data accessibility. Data are available at http://doi.pangaea.de/10.1594/PANGAEA.833020. All DNA sequences are available at GenBank with Accession Numbers KM356003 through to KM356074 and KM579635 through to KM579705.

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