

Evidence for parasite-mediated selection during short-lasting toxic algal blooms

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Electronic Supplementary Material

Sampling strategy

Tidal range inside the Penzé estuary fluctuates from 1.8 to 9.2 m. Because this estuary is relatively shallow (depth lower than 4 m mostly), the sea penetrates deeply (about 6 km) into the flooded valley. The Penzé river flow is rather modest, with a mean annual flow of $4 \text{ m}^3 \cdot \text{s}^{-1}$. The Penzé estuary is well-mixed, meaning that similar salinity is detected from the surface to the bottom and a continuous salinity gradient, ranging from freshwater to marine waters, is detected from upstream to downstream. Plankton samples were collected at 1 m depth using a Niskin bottle, two hours before the high tide at salinities 27 and 29 (psu), reported to display the maximal abundances of *A. minutum* (Maguer *et al.* 2004). In 2011, maximal *A. minutum* densities were detected at salinity 29 (in 80% of samplings).

The Rance estuary is larger (16 km long and 2.5 km wide) but its flow is significantly altered downstream by a tidal power station and upstream by the Châtelier lock which limits the southward progression of seawater. Ten fixed stations were defined along the Rance estuary (Sup. Table 1). Six on ten stations have been sampled every day. Their locations were daily readjusted according to the maximal *A. minutum* density measured the day before in order to maximize the chance to sample the maximal density of the microalga (in 80% of cases, maximal *A. minutum* densities have been detected at stations 3 and 4). Samples were collected two hours before gate aperture of the tidal power station. Plankton samples were collected using a Niskin bottle at the deep chlorophyll maximum (from 0.5 to 4 m depth), determined on board using a portable CTD (CastAway, ISY).

For microscopic analyses, 50 mL of the sample were fixed on board by 2% Lugol's solution. For molecular studies, seawater samples (1-2 L) were fractionated in the laboratory by sequential filtration through 47 mm diameter polycarbonate filters with 10 and 3 μm (Whatman) pore sizes and a

0.2- μ m-pore-size Sterivex unit (Millipore). Filters were submerged in lysis buffer (0.75 M sucrose, 50 mM Tris-HCl, pH 8) and immediately frozen in liquid nitrogen and stored at -80°C . The rest of the fresh sample (3-5 L) was immediately processed for strain isolation.

Details of the DNA extraction and PCR reactions

1. Identification of host and parasite species

DNA extraction was performed on strains collected during their exponential growth stage. The dinoflagellate host was directly centrifuged, while the parasite zoospores were filtered through a 5- μ m cellulose acetate filter (Minisart, Sartorius, Germany) to remove all remains of the initial host, then centrifuged.

DNA of the dinoflagellate host and the parasite strains was extracted using either the CTAB method (Lebret *et al.* 2012) or the Nucleospin 96 Plant kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions, using 5–10 mg of dried tissue resuspended in 100 μ L elution buffer. PCR reactions (14 μ L in total) included 20 ng of DNA, 1X GoTaq Flexi Green buffer (Promega, Madison, WI, USA), 150 μ M of each dNTP (Thermo Fisher Scientific Inc., Waltham, MA, USA), 20 pmol of the forward primer 329-F (5'-GTG AAC CTG CRG AAG GAT CA-3', complementary reverse of the eukaryote reverse primer 329-R (Moon-van der Staay *et al.* 2001), 20 pmol of the reverse primer DIR-R (5'- TAT GCT TAA AAT TCA GCA GGT-3' (Scholin & Anderson 1994) and 1.25 U GoTaq® Polymerase (Promega). Amplification was carried out in a DNA Engine Peltier Thermal Cycler (Bio-Rad, Hercules, CA, USA) with the following cycling conditions: an initial denaturation at 95 $^{\circ}\text{C}$ for 5 min, followed by 35 PCR cycles (denaturation at 95 $^{\circ}\text{C}$ for 45 s, annealing at 53 $^{\circ}\text{C}$ for 45 s and extension at 72 $^{\circ}\text{C}$ for 45 s), followed by a final extension at 72 $^{\circ}\text{C}$ for 10 min. The PCR products were purified using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) for subsequent bidirectional sequencing using a capillary sequencer type ABI 3130XL (Applied Biosystems).

The endoparasite *Amoebophrya* spp., which commonly infects dinoflagellates, was not observed infecting *A. minutum* during this study. We screened for its presence using fresh samples by

epifluorescence microscopy (BX51, Olympus) equipped with the U-MWB2 cube (450- to 480-nm excitation, 500-nm emission, (Coats & Bockstahler 1994). However *Amoebophrya* spp. infected the other dinoflagellates *Scrippsiella* spp. and *H. triquetra*.

2. Quantification of parasite species by qPCR

For environmental DNA extraction, filters were thawed at 37 °C and incubated for 20 min in presence of 70 µL of lysozyme (50 mg mL⁻¹). Then 50 µL of proteinase K (10 mg mL⁻¹) and 350 µL of sodium dodecyl sulfate (SDS) 10% were added. The mixture was incubated 20 min at 37 °C and the reaction was stopped by 10 min at 55 °C. Total gDNA was extracted using a vol/vol mixture of phenol:chloroform:isoamyl alcohol (25:24:1) and the tubes were gently agitated and incubated for 15 min at 55 °C. A 15-min centrifugation step at 4,500 g at 12°C allowed the recuperation of the aqueous phase. This step was repeated twice. Genomic material was purified and concentrated using Amicon Ultra 4 (Millipore Corporation, USA) following the manufacturer's recommendations. DNA concentration was estimated by using the Quant-it Picogreen dsDNA Assay kit (Invitrogen, Eugene, OR). Environmental DNA extracts were then stored at -80 °C until used. All DNA templates were readily amplified by PCR using general eukaryotic primers (forward primer= GTGCCAGCASCYGC GG TAA, reverse primer= TTTAAGTTTCAGHCTTGCG), so no noticeable inhibition was detected. *Parvilucifera* species abundance was monitored using two sets of specific primers (for *P. rostrata* Par1qF= CACTCTATGTTCCACCGTCGT; Par2qR= GTTCGCTGCGTCCTTCAT and for *P. infectans* Par7qF= AATACGATGAAGGACGTGGC; Par8qR= CCGACCCCTATGAAGAGACA). The presence of these species in DNA extracts was first evaluated by PCR using the following thermal cycling conditions: 95°C for 15 min, 35 cycles of denaturation at 95°C for 1 min, annealing temperature at 57°C for 75 s, and polymerase extension at 72°C for 75 s. qPCR were performed using the kit Absolute qPCR SYBR Green/ROX Master Mix (Thermo Scientific). Plasmid clones corresponding to the regions amplified by both primer sets were used as positive controls and for standard curves (10-fold dilution series) in the qPCR assays. Plasmid DNA was linearized with *HindIII* and quantified using the Picogreen dsDNA assay (Invitrogen, Eugene, OR). Extracted DNAs were normalized to 0.5 and 1 ng µL⁻¹. qPCR reactions were performed

in a total reaction volume of 10 μ l using the SYBR Green/ROX qPCR Master Mix kit (Applied Biosystems) using 96-well plates. qPCR assays were performed in triplicates using a Chromo4 Real-Time PCR Detection System and the Opticon Monitor software package (Bio-Rad) with the following thermal cycling conditions: 95°C for 15 min, 45 cycles of denaturation at 95°C for 45 s, annealing temperature at 63°C for 45 s with fluorescence measurements after this step, and polymerase extension at 72°C for 15 s. Melting curves were generated after each assay to check the specificity of the amplification by heating from 65 to 95°C and taking fluorescence measurements every 0.5°C. Only single peaks were observed in the dissociation curves for both the standards and samples, indicating specific amplification with each set of primers. Negative controls consisted in three blanks, using the same PCR mix but without DNA template. Average amplification efficiencies were as follows: *P. rostrata* primer set = 100% and *P. infectans* primer set = 98%.

Genotyping and determination of genetic clusters

Genetic differentiation among host strains was analyzed with STRUCTURE version 2.2 (Pritchard *et al.* 2000), allowing classification into three genetic clusters.

For this analysis, only 9 microsatellite loci were retained in order to allow a maximum of 18% of missing data per locus. STRUCTURE uses a model-based Bayesian method to delineate clusters of individuals on the basis of their genotypes at multiple loci. Hardy-Weinberg and linkage disequilibrium are minimized within clusters. The estimated log probability of data $\Pr(X | K)$ for each value of K is given, allowing the estimation of the more likely number of clusters. The analysis was run on the whole data set without any a priori population assignments and admixture was allowed. For each value of the number of cluster K (all values from 1 to 11), we ran 20 simulations using a burn-in of 100,000 iterations and a run length of 1,000,000 iterations. The best K was inferred both from the posterior probability of the data, $L(K)$, and following the ΔK choice criterion of (Evanno *et al.* 2005). For the selected K, results from the 20 replicate runs were averaged using CLUMPP version 1.1.2 (Jakobsson & Rosenberg 2007) and each strains was assigned to a genetic cluster depending on their probability of assignment (> 55%).

Local adaptation

Local adaptation could be tested only for the parasite *P. infectans*, as *P. rostrata* is present only in the Penzé estuary. To make meaningful comparisons across estuaries, we set the time of maximum *A. minutum* density to day 0 in both estuaries (i.e., day 0 is 22 June 2011 in Penzé, and 27 May 2011 in Rance). Local adaptation was defined in two ways. When two populations are assayed, it has been suggested that local adaptation is present only under the strict criterion when the reaction norms across environments cross (Kawecki & Ebert 2004). Additionally we defined local adaptation as the difference between fitness in sympatry and fitness in allopatry. However, when only two demes are sampled, this contrast could represent any form of interaction between population and habitat, and not necessarily local adaptation. Moreover, with two demes it is not possible to test for the significance of this contrast while accounting for habitat and population main effects (Blanquart *et al.* 2013). To resolve the latter problem, we tested significance of the sympatric versus allopatric (SA) contrast using a permutation test. Specifically, we generated a null distribution of the SA contrast by considering the same transfers as for the true SA contrast, but where the sympatric versus allopatric status of transfers were randomized. This test can be thought of as a test of non-parallelism of reaction norms.

Patterns of local adaptation can be more apparent when the parasites are tested with hosts time-shifted in the past (Koskella 2014). We first tested local adaptation using all transfers where the parasite and host populations were sampled after day 0. Second we measured a time-shifted local adaptation where we tested the parasite populations sampled after day 0, and the host populations sampled at or before day 0.

Because parasites from Rance were generally less infectious than parasites from Penzé, the stricter criterion (reaction norm crossing) was not fulfilled (Sup. Fig. 1). Nevertheless, our analysis revealed a pattern of non-parallelism, whereby the fraction of host strains infected by the parasite in allopatry is 0.18 greater than in sympatry ($p = 0.042$, using a permutation test with 1000 permutations). In contrast, when considering time-shifted local adaptation, using the hosts sampled at or before day 0, the parasite local adaptation was positive at 0.13 ($p = 0.068$) (Sup. Table 5). These

contrasted patterns of non-parallelism cannot be interpreted as local adaptation, but mainly emerged as the parasite in Rance, in sympatry, had quite low infectivity on the contemporaneous host (host sampled after day 0), but slightly larger infectivity on the hosts of the past (hosts sampled before day 0), as the host in Rance evolved an increased resistance (see analysis of temporal trends, Fig. 4).

Correcting the significance levels in the generalized linear model using randomized pseudo-datasets

Sampling of parasite strains was relatively limited (at several dates only 1 strain was available), which hinders detection of temporal trends if the parasite population exhibits large variability. In particular the limited number of strains sampled at each time point is not necessarily representative of the true variability within the population, such that variation across time points attributed to temporal variations could in fact be due to sampling effects. We used simulation to determine the type 1 error rate of the model (rate of false positive) for the detection of temporal trends in infectivity or resistance. Specifically we used randomized pseudo-datasets with exactly the same structure as the true dataset but where the host and parasite strains were randomized. These randomized pseudo-datasets comply to the null hypothesis (no difference in infectivity between date host and date parasite), and if the linear model behaves ideally, the type I error rate on randomized pseudo-datasets should be equal to the significance level (here, 0.05). This randomization test revealed that the limited sampling of parasite strains leads to type I error at a rate that is sometimes higher than the expected 0.05. We therefore used the distribution of p-values on 500 pseudo-datasets to adjust the significance level for the temporal effects on the true dataset, ensuring a proper estimation of p-values.

Supplementary Table 1. Coordinates of fixed stations sampled in the Rance estuary.

| Stations | Latitude | Longitude |
|----------|--------------|-------------|
| 1 | 48°30'00.15" | 1°58'49.56" |
| 2 | 48°30'11.00" | 1°58'50.94" |
| 3 | 48°30'30.48" | 1°58'59.71" |
| 4 | 48°31'24.69" | 1°58'52.58" |
| 5 | 48°32'07.51" | 1°58'19.33" |
| 6 | 48°32'45.32" | 1°58'17.30" |
| 7 | 48°32'03.58" | 1°58'11.00" |
| 8 | 48°30'52.14" | 1°58'35.62" |
| 9 | 48°29'51.67" | 1°59'11.64" |
| 10 | 48°29'49.29" | 1°59'36.86" |

Supplementary Table 2. List of host and parasite strains used in this study. RCC: Roscoff Culture Collection.

| Species | Host genotype clusters | Use in this study | RCC number | In (Dia <i>et al.</i> 2014) | Synonymous | Ecosystems | Date of isolation |
|----------------------------|------------------------|-------------------|------------|-----------------------------|-------------|------------|-------------------|
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3250 | * | PARALEX 575 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3251 | * | PARALEX 577 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3253 | * | PARALEX 579 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3255 | * | PARALEX 581 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3256 | * | PARALEX 582 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3257 | * | PARALEX 583 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3258 | * | PARALEX 584 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3259 | * | PARALEX 586 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3260 | * | PARALEX 587 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3261 | * | PARALEX 589 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3262 | * | PARALEX 590 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3263 | * | PARALEX 591 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3264 | * | PARALEX 593 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3265 | * | PARALEX 594 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3267 | * | PARALEX 597 | Penzé | 01/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | | | PARALEX 619 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | | | PARALEX 622 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 623 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 624 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | | | PARALEX 626 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 627 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | | | PARALEX 628 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 630 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 633 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | | | PARALEX 635 | Penzé | 09/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3326 | * | PARALEX 753 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3327 | * | PARALEX 754 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3331 | * | PARALEX 761 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3333 | * | PARALEX 764 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 765 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3334 | * | PARALEX 766 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3335 | * | PARALEX 767 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3336 | * | PARALEX 770 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | RCC 3337 | * | PARALEX 771 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 774 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3339 | * | PARALEX 775 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3342 | * | PARALEX 780 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3343 | * | PARALEX 781 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | RCC 3348 | * | PARALEX 788 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3349 | * | PARALEX 789 | Penzé | 22/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | | | PARALEX 821 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | | | PARALEX 822 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | | | PARALEX 825 | Penzé | 30/06/2011 |

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|----------------------------|----|------------------|----------|---|--------------|-------|------------|
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 828 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 830 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 831 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 832 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 833 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 838 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 840 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 841 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 843 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 844 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 1091 | Penzé | 30/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3352 | * | PARALEX 640 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3353 | * | PARALEX 641 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3354 | * | PARALEX 643 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3356 | * | PARALEX 645 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3357 | * | PARALEX 646 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3358 | * | PARALEX 647 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3359 | * | PARALEX 648 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3360 | * | PARALEX 655 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3361 | * | PARALEX 658 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3362 | * | PARALEX 660 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3363 | * | PARALEX 661 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 664 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3364 | * | PARALEX 665 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 676 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 678 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3365 | * | PARALEX 679 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3366 | * | PARALEX 682 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3367 | * | PARALEX 683 | Penzé | 11/07/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3186 | | PARALEX 919 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3188 | | PARALEX 922 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 924 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3189 | * | PARALEX 925 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3190 | * | PARALEX 926 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3191 | * | PARALEX 927 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3192 | * | PARALEX 930 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3193 | * | PARALEX 931 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3194 | * | PARALEX 932 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3195 | * | PARALEX 934 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | RCC 3196 | * | PARALEX 937 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3197 | * | PARALEX 938 | Rance | 09/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3224 | * | PARALEX 1019 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3227 | * | PARALEX 1022 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3228 | * | PARALEX 1024 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 1025 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3230 | * | PARALEX 1028 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3233 | * | PARALEX 1035 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 1037 | Rance | 27/05/2011 |

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|-------------------------------|----|------------------------|----------|---|--------------|----------------|------------|
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 1038 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3235 | * | PARALEX 1039 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3236 | * | PARALEX 1040 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 1041 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3237 | * | PARALEX 1042 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3239 | * | PARALEX 1044 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 1045 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 1046 | Rance | 27/05/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | RCC 3278 | * | PARALEX 873 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3279 | * | PARALEX 874 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3280 | * | PARALEX 875 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3281 | * | PARALEX 876 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3282 | * | PARALEX 877 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 2 | Cross-infections | RCC 3283 | * | PARALEX 878 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | | | PARALEX 879 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3284 | * | PARALEX 880 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3285 | * | PARALEX 881 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | RCC 3286 | * | PARALEX 882 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3287 | * | PARALEX 883 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 1 | Cross-infections | RCC 3288 | * | PARALEX 884 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | RCC 3289 | * | PARALEX 885 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3290 | * | PARALEX 886 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | 3 | Cross-infections | RCC 3291 | * | PARALEX 887 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | ND | Cross-infections | RCC 3187 | * | PARALEX 921 | Rance | 05/06/2011 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3018 | | AM89BM2 | Morlaix | 1989 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3019 | | AM99P22 | Penzé | 1999 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3020 | | PARALEX 178 | Penzé | 30/06/2007 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3022 | * | PARALEX 292 | Rance | 02/06/2010 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 2651 | | PARALEX 263 | Rance | 17/05/2010 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3023 | | PARALEX 524 | Penzé | 01/07/2010 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3024 | | PARALEX 381 | Penzé | 08/06/2010 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3026 | | PARALEX 157 | Penzé | 30/06/2007 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3027 | | PARALEX 465 | Rance | 22/06/2010 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3028 | | PARALEX 458 | Penzé | 20/06/2010 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3030 | | CBA38 | Adriatic Sea | XX/05/2008 |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3031 | | CBA42 | Adriatic Sea | XXX |
| <i>Alexandrium minutum</i> | | Isolation of parasites | RCC 3032 | | SZN30 | Gulf of Naples | 18/05/2000 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2861 | | PARALEX P163 | Penzé | 19/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2862 | | PARALEX P164 | Penzé | 21/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2829 | | PARALEX P131 | Penzé | 21/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2916 | | PARALEX P218 | Penzé | 19/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2893 | | PARALEX P195 | Penzé | 19/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2876 | | PARALEX P178 | Penzé | 21/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2885 | | PARALEX P187 | Penzé | 21/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2917 | | PARALEX P219 | Penzé | 21/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2827 | | PARALEX P129 | Penzé | 19/06/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2823 | | PARALEX P124 | Penzé | 01/07/2011 |
| <i>Parvilucifera rostrata</i> | | Cross-infections | RCC 2839 | | PARALEX P141 | Penzé | 30/06/2011 |

| | | | | | |
|--------------------------------|------------------|----------|--------------|-------|------------|
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2842 | PARALEX P144 | Penzé | 01/07/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2849 | PARALEX P151 | Penzé | 30/06/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2850 | PARALEX P152 | Penzé | 30/06/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2888 | PARALEX P190 | Penzé | 01/07/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2891 | PARALEX P193 | Penzé | 30/06/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2907 | PARALEX P209 | Penzé | 30/06/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2913 | PARALEX P215 | Penzé | 30/06/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2926 | PARALEX P228 | Penzé | 29/06/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2927 | PARALEX P229 | Penzé | 29/06/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2928 | PARALEX P230 | Penzé | 30/06/2011 |
| <i>Parvilucifera rostrata</i> | Cross-infections | RCC 2929 | PARALEX P231 | Penzé | 08/07/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2824 | PARALEX P126 | Penzé | 02/07/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2870 | PARALEX P172 | Penzé | 27/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2902 | PARALEX P204 | Penzé | 26/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2922 | PARALEX P224 | Penzé | 23/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2903 | PARALEX P205 | Penzé | 26/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2837 | PARALEX P139 | Penzé | 27/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2901 | PARALEX P203 | Penzé | 25/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2867 | PARALEX P169 | Penzé | 26/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2857 | PARALEX P159 | Rance | 08/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2853 | PARALEX P155 | Rance | 04/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2826 | PARALEX P128 | Rance | 05/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2879 | PARALEX P181 | Rance | 08/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2912 | PARALEX P214 | Rance | 15/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2819 | PARALEX P120 | Rance | 08/06/2011 |
| <i>Parvilucifera infectans</i> | Cross-infections | RCC 2852 | PARALEX P154 | Rance | 08/06/2011 |

Supplementary Table 3. Characteristics of host-parasite networks. H = number of host strains. P = number of parasite strains. I = number of interactions (total of positive infection). NA= number of none acquired data. M = size ($M=H*P-NA$). C = infectivity (or connectance, $C=I/M$). LP = average number of interactions across parasite strains ($LP=I/P$). PZ, Penzé; RC, Rance.

| | H | P | I | NA | M | C | LP |
|--|----|----|-----|----|-----|-------------|--------------|
| <i>Parvilucifera rostrata</i> PZ | | | | | | | |
| Sympatry (host from PZ) | | | | | | | |
| 01/06/2011 | 15 | 22 | 244 | 1 | 329 | 0.74 | 11.09 |
| 09/06/2011 | 10 | 14 | 125 | 0 | 140 | 0.89 | 8.93 |
| 22/06/2011 | 15 | 22 | 274 | 0 | 330 | 0.83 | 12.45 |
| 30/06/2011 | 14 | 14 | 171 | 0 | 196 | 0.87 | 12.21 |
| 11/07/2011 | 18 | 22 | 319 | 0 | 396 | 0.81 | 14.50 |
| Average Sympatry | | | | | | 0.83 | 11.84 |
| Median Sympatry | | | | | | 0.83 | 12.21 |
| Allopatry (host from RC) | | | | | | | |
| 09/05/2011 | 12 | 14 | 146 | 0 | 168 | 0.87 | 10.43 |
| 27/05/2011 | 15 | 14 | 180 | 0 | 210 | 0.86 | 12.86 |
| 05/06/2011 | 16 | 14 | 189 | 0 | 224 | 0.84 | 13.50 |
| Average Allopatry | | | | | | 0.86 | 12.26 |
| Median Allopatry | | | | | | 0.86 | 12.86 |
| Total Average | | | | | | 0.84 | 12.00 |
| Total Median | | | | | | 0.85 | 12.33 |
| <i>Parvilucifera infectans</i> PZ | | | | | | | |
| Sympatry (host from PZ) | | | | | | | |
| 01/06/2011 | 15 | 8 | 90 | 3 | 117 | 0.77 | 11.25 |
| 09/06/2011 | 10 | 8 | 61 | 0 | 80 | 0.76 | 7.63 |
| 22/06/2011 | 15 | 8 | 103 | 0 | 120 | 0.86 | 12.88 |
| 30/06/2011 | 14 | 8 | 72 | 0 | 112 | 0.64 | 9.00 |
| 11/07/2011 | 18 | 8 | 103 | 0 | 144 | 0.72 | 12.88 |
| Average Sympatry | | | | | | 0.75 | 10.73 |
| Median Sympatry | | | | | | 0.76 | 11.25 |
| Allopatry (host from RC) | | | | | | | |
| 09/05/2011 | 12 | 8 | 63 | 0 | 96 | 0.66 | 7.88 |
| 27/05/2011 | 15 | 8 | 101 | 1 | 119 | 0.85 | 12.63 |
| 05/06/2011 | 16 | 8 | 84 | 0 | 128 | 0.66 | 10.50 |
| Average Allopatry | | | | | | 0.72 | 10.33 |
| Median Allopatry | | | | | | 0.66 | 10.50 |
| Total Average | | | | | | 0.74 | 10.58 |
| Total Median | | | | | | 0.74 | 10.88 |
| <i>Parvilucifera infectans</i> RC | | | | | | | |
| Sympatry (host from RC) | | | | | | | |
| 09/05/2011 | 12 | 7 | 32 | 0 | 84 | 0.38 | 4.57 |
| 27/05/2011 | 15 | 7 | 44 | 2 | 103 | 0.43 | 6.29 |
| 05/06/2011 | 16 | 7 | 24 | 1 | 111 | 0.22 | 3.43 |
| Average Sympatry | | | | | | 0.34 | 4.76 |
| Median Sympatry | | | | | | 0.38 | 4.57 |

Allopatry (host from PZ)

| | | | | | | | |
|---|----|---|----|---|-------------|-------------|-------------|
| 01/06/2011 | 15 | 7 | 34 | 0 | 105 | 0.32 | 4.86 |
| 09/06/2011 | 10 | 7 | 33 | 0 | 70 | 0.47 | 4.71 |
| 22/06/2011 | 15 | 7 | 30 | 0 | 105 | 0.29 | 4.29 |
| 30/06/2011 | 14 | 7 | 35 | 0 | 98 | 0.36 | 5.00 |
| 11/07/2011 | 18 | 7 | 47 | 0 | 126 | 0.37 | 6.71 |
| Average Allopatry | | | | | | 0.36 | 5.11 |
| Median Allopatry | | | | | | 0.36 | 4.86 |
| Total Average | | | | | | 0.35 | 4.98 |
| Total Median | | | | | | 0.37 | 4.79 |
| Total number of cross-infections | | | | | 3711 | | |

Supplementary Table 4. Infectivity matrices are significantly nested. For each matrix, we calculate temperature, an indicator of nestedness, using the program BINMATNEST, implemented in R (package bipartite, (Rodríguez-Gironés & Santamaría 2006)). The p-value is the probability that the same temperature arises under the null model where average infectivity is the same, but where host strain x parasite strain combinations leading to infection are randomly chosen in the matrix. This probability was computed using 10^3 randomized matrices. PZ, Penzé; RC, Rance.

| Parasite, hosts | Number of parasite strains | Number of host strains | Average infectivity | Temperature | p-value |
|---------------------------------|-----------------------------------|-------------------------------|----------------------------|--------------------|----------------|
| <i>P. infectans</i> PZ, host PZ | 8 | 72 | 0.75 | 8.9 | 0 |
| <i>P. infectans</i> RC, host RC | 7 | 33 | 0.34 | 26.9 | 0.002 |
| <i>P. rostrata</i> PZ, host PZ | 22 | 72 | 0.81 | 16 | 0 |

Supplementary Table 5. Local adaptation tested using reciprocal experimental design on *P. infectans*. LA = mean infectivity obtained with cross-infections in sympatry (PZ*PZ + RC*RC) – mean infectivity obtained with cross-infections in allopatry (PZ*RC + RC*PZ). PZ=Penzé and RC=Rance. For the first row, all host and parasite strains sampled after time 0 were used. For the second row, we used the same parasite strains as for the first row, but used time-shifted host strains sampled before time 0 (time 0 is defined for each estuary as the date of maximum density of the host *A. minutum*).

| | LA | Permutation test |
|-------------------------|--------|------------------|
| host and parasite > 0 | -0.175 | 0.042 |
| host <= 0, parasite > 0 | 0.128 | 0.068 |

Supplementary Tables 6. Summary tables of the generalized linear mixed model explaining infection outcome (binary variable, 1 or 0) as a function of an intercept, host date, parasite date, host cluster (1, 2, 3 or unknown), the time shift effect (“far past”, “near past”, “present”, “near future”), a random effect for host strain representing host genetic variability for resistance, and a random effect for parasite strain representing parasite genetic variability for infectivity. All effects are presented on the logit scale. 95% confidence intervals were computed using a parametric bootstrap with resampling of residual errors. For host and parasite date, effects were computed relative to the earliest date; for host cluster, relative to cluster 1; for time-shift, relative to “far past”. p-values for the fixed effects (date host, date parasite, host cluster and time shift) as well as the random effects (host strain, parasite strain) were computed using a type II analysis of deviance based on chisquare tests. p-values for “date host”, “date parasite”, and “time shift” were corrected using the distribution of p-values obtained in datasets where strains were randomized with respect to date. This ensures the reported p-values are uniformly distributed on [0, 1] under the null hypothesis where there is no temporal variation in infectivity and resistance. Furthermore, for date host and date parasite we also compute p-values based on an analysis of variance on the data averaged over all parasite strains (for hosts) or all host strains (for parasites) (the second p-value reported).

| P. infectans from Rance with Rance host | | | |
|--|----------------------------|----------------|-------|
| Effect | Estimate and 95% CI | p-value | |
| Intercept | -2.29 [-3.44; -1.5] | - | - |
| Host Date 0 | 0.0981 [-1.12; 1.15] | 0.082 | 0.092 |
| Host Date 9 | -2.12 [-3.76; -0.889] | | |
| Parasite Date 9 | 1.1 [-0.017; 2.26] | 0.41 | 0.68 |
| Parasite Date 12 | 1.64 [0.944; 2.78] | | |
| Parasite Date 19 | 2.5 [1.33; 4.04] | | |
| Host Cluster 2 | -0.935 [-1.84; -0.0148] | 0.33 | |
| Host Cluster 3 | 0.445 [-0.412; 1.23] | | |
| Host Cluster NA | 0.283 [-0.555; 1.04] | | |
| Time Shift near past | 0.408 [-0.676; 1.57] | 0.62 | |
| Time Shift present | 1.09 [-0.25; 2.92] | | |
| Host Strain Variance | 2.01 [0.351; 2.39] | 9.10e-08 | |
| Parasite Strain Variance | 0.284 [1.81e-06; 0.489] | 0.021 | |

| P. infectans from Penzé with Penzé host | | | |
|--|----------------------------|----------------|---------|
| Effect | Estimate and 95% CI | p-value | |
| Intercept | 0.897 [-0.121; 1.92] | - | - |
| Host Date -13 | -0.104 [-0.962; 0.775] | | |
| Host Date 0 | 0.12 [-0.956; 1.16] | 0.024 | 0.00088 |
| Host Date 8 | -1.75 [-2.77; -0.454] | | |
| Host Date 19 | -0.134 [-1.28; 1.76] | | |
| Parasite Date 3 | 21 [19.2; 23.2] | | |
| Parasite Date 4 | 1.04 [0.345; 1.93] | 0.34 | 0.63 |
| Parasite Date 5 | -0.646 [-1.32; 0.221] | | |
| Parasite Date 10 | -0.584 [-1.26; 0.517] | | |
| Host Cluster 2 | 0.0955 [-0.585; 0.849] | | |
| Host Cluster 3 | 0.17 [-0.489; 0.912] | 0.97 | |
| Host Cluster NA | 0.119 [-0.528; 0.881] | | |
| Time Shift near past | 0.183 [-0.994; 1.31] | | |
| Time Shift present | 0.989 [-0.577; 2.31] | 0.06 | |
| Time Shift near future | -0.4 [-2.42; 0.773] | | |
| Host Strain Variance | 6.88e-07 [1.41e-08; 0.213] | 1 | |
| Parasite Strain Variance | 0.759 [0.359; 1.3] | 6.70e-12 | |

| P. rostrata from Penzé with Penzé host | | | |
|---|----------------------------|----------------|--------|
| Effect | Estimate and 95% CI | p-value | |
| Intercept | -1.45 [-2.25; -0.541] | - | - |
| Host Date -13 | 0.478 [-0.17; 1.29] | | |
| Host Date 0 | 1.11 [0.0971; 2.11] | 0.12 | 0.0058 |
| Host Date 8 | 0.552 [-0.459; 1.68] | | |
| Host Date 19 | 0.0754 [-0.929; 1.12] | | |
| Parasite Date -1 | 0.548 [-0.153; 1.03] | | |
| Parasite Date 7 | 0.623 [-0.256; 1.37] | | |
| Parasite Date 8 | 1.59 [0.903; 2.13] | 0.062 | 0.28 |
| Parasite Date 9 | 6.48 [5.55; 8.65] | | |
| Parasite Date 16 | 0.381 [-0.648; 1.38] | | |
| Host Cluster 2 | 0.0863 [-0.343; 0.62] | | |
| Host Cluster 3 | 0.382 [-0.0924; 0.811] | 0.73 | |
| Host Cluster NA | 0.281 [-0.211; 0.824] | | |
| Time Shift near past | 1.97 [1.05; 2.99] | | |
| Time Shift present | 1.54 [0.325; 2.6] | 0.096 | |
| Time Shift near future | 2.33 [0.894; 3.75] | | |
| Host Strain Variance | 0.63 [0.0375; 0.466*] | 1.70e-06 | |
| Parasite Strain Variance | 2.29 [1.19; 2.58] | 8.70e-40 | |

* The upper confidence limit for the genetic variance was below the maximum likelihood estimate of 0.63. This indicates the bootstrap procedure is not adequate to estimate confidence intervals for the two random effects variances (“Host Strain” and “Parasite Strain”). This is perhaps because the bootstrap uses resampling of the residual errors, which may tend to decrease the variance and thus underestimate the variances of random effects.

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Supplementary Figure 1. Local adaptation pattern for the parasite *P. infectans*, using all host and parasite populations sampled after day 0 (top row), or using the same set of parasites with time-shifted hosts sampled at or before day 0 (bottom row).

