ulates pancreatic islet mass. Hepatic ERK activation is likely to play an important role in compensatory islet hyperplasia, although it is not yet clear how ERK signaling affects the neuronal pathway. The therapeutic effects we observed in two mouse models of insulin-deficient diabetes are especially noteworthy. Type 1 diabetes mellitus is characterized by progressive loss of pancreatic β cells, leading to a life-long insulin dependency. Recently, it was reported that β cell mass is also decreased in type 2 diabetes (*25*). Although substantial progress has been made with therapies that are based on transplantation of pancreatic islets (*26*), immune rejection and donor supply are still major challenges. In this context, therapeutic manipulation of the interorgan signaling mechanism described here may merit investigation as a potential strategy for regeneration of a patient's own β cells. Our results may open a new paradigm for regenerative medicine: regeneration of damaged tissues by targeting of interorgan communication systems, especially neural pathways.

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Control of Toxic Marine Dinoflagellate Blooms by Serial Parasitic Killers

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The marine dinoflagellates commonly responsible for toxic red tides are parasitized by other dinoflagellate species. Using culture-independent environmental ribosomal RNA sequences and fluorescence markers, we identified host-specific infections among several species. Each parasitoid produces 60 to 400 offspring, leading to extraordinarily rapid control of the host's population. During 3 consecutive years of observation in a natural estuary, all dinoflagellates observed were chronically infected, and a given host species was infected by a single genetically distinct parasite year after year. Our observations in natural ecosystems suggest that although bloom-forming dinoflagellates may escape control by grazing organisms, they eventually succumb to parasite attack.

Although photosynthetic dinoflagellates are important primary producers in marine ecosystems, some bloom-forming species produce toxins that can cause illness and even death in humans (*1*). These harmful algal blooming (HAB) species are particularly prevalent in warm, stratified, and nutrient-enriched coastal waters (*2*, *3*). Documented HAB events have increased substantially during recent decades as a result of extensive coastal eutrophication and, possibly, global climate change (*4*).

In 1968, Taylor proposed using specific dinoflagellate parasites, such as the Syndiniales *Amoebophrya* spp. (*5*), as biological control agents for HAB organisms. This idea was rejected because of the apparent lack of specificity of the parasites; however, the homogeneous morphology of these parasites masks extensive genetic diversity (*6*). Recently, the widespread existence of *Amoebophrya* spp. was "rediscovered" by culture-independent methods, and they were renamed "novel alveolate group II" (*7*–*9*). This eukaryotic lineage frequently forms 10 to 50% of sequences retrieved within coastal environmental clone libraries (*10*, *11*). Indeed, up to 44 distinct clusters have been detected, with extensive intraclade genetic diversity (*12*); the genetic diversity of the parasites appears to be comparable to the species richness of their hosts.

We sampled a marine coastal estuary (the Penzé River, northern Brittany, France) for 3 consecutive years (2004 to 2006), using catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH; tables S1 and S2) with probes specifically designed to detect group II alveolates. Our aim was to examine how the abundance and diversity of the parasites influenced their host populations in natural environments. In May and June of each year, we observed a rapid succession of four major species of photosynthetic

Table 1. Specificity of Syndiniales group II in the Penzé estuary in 2005 and 2006. Prevalences (percentage of infected cells) when a general oligonucleotide probe (ALV01) and clade-specific probes were used are shown (results for clades 1, 2, and 14; for description of clades see Fig. 3). Observations of a mature trophont inside the host cell are indicated by an asterisk. ND, not done. Numbers in parentheses show the percentage of the signal obtained when the general probe was used, explained by the clade-specific probes.

Host species	Dates (day/month/year)	Syndiniales group II, all clades	Syndiniales group II, clade 1	Syndiniales group II, clade 2	Syndiniales group II, clade 14
H. rotundata	03/06/2005	26*	26* (100%)	ND	ND
	29/05/2006	29*	23* (79%)	0	2 (<1%)
S. trochoidea	14/06/2005	23*	ND	11* (48%)	ND
	16/06/2006	33*	0	18* (55%)	3 (<1%)
	18/06/2006	26*	0	29* (>100%)	9 (3%)
A. minutum	14/06/2005	40*	0	0	0
	22/06/2006	19*	6 (3%)	0	0
H. triquetra	20/06/2005	10*	ND	0	11* (>100%)
	22/06/2006	14*	0	0	14* (100%)

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dinoflagellates (the dominant species changed every week; Fig. 1), namely *Heterocapsa rotundata* at the end of May, followed by *Scrippsiella trochoidea*, *Alexandrium minutum*, and *H. triquetra*. These species all have a worldwide distribution in marine coastal ecosystems, and one of them, *A. minutum*, produces paralytic shellfish poison.

No significant correlation was observed between the decline of the individual dinoflagellate species and the physicochemical environmental variables (nitrate, nitrite, phosphate, silicate, salinity, temperature, or tidal amplitude), which remained relatively constant during the 3-year study period (figs. S1 and S2). There was pervasive chronic infection of all the observed dinoflagellate species by Syndiniales; the infections occurred every year, even when host species abundance was low (Fig. 2). The prevalence of parasitized dinoflagellate cells reached 46%, with a mean value of 21% (Fig. 1) during summer. Similar values were obtained for all the species observed, including the toxic algae *A. minutum*, and are consistent with previously published data from a variety of coastal settings and several dinoflagellate host taxa (*13*–*18*). A similar average prevalence (28%) was observed for *Dinophysis norvegica* from the North Sea when a specific FISH detection approach was used (*19*). The sensitivity of the CARD-FISH analysis permitted the detection of early-stage infections as well as all the life-cycle stages of the parasite, including the very small free-living stage (3 to 5 µm in diameter). In all cases, the parasites closely corresponded to the description of *Amoebophrya* spp. provided by Cachon in 1964 (*20*).

Infections were initiated by the invasion of host cells by one or several very small infective cells called dinospores (*20*) (Fig. 2A). After several rounds of active nuclear replication, a large multinucleated trophont (the endoparasitic stage, Fig. 2, B to D) characteristic of the genus *Amoebophrya* is produced. Cultures and field observations show that this trophont matures in 2 days (*13*, *21*). The pressure of the trophont during the final stages of maturation distorts and enlarges the dinoflagellate cells (compare Fig. 2, B and C). Ultimately, the trophont ruptures the host cell wall and is elongated by a final evagination to form a swimming structure, the vermiform stage (*20*) (Fig. 2, D and E). Within a few hours, the vermiform structure fragments into 60 to 400 dinospores (*13*, *20*, *21*), each of which is able to reinfect a new host.

The amplification of newly infective parasite cells has the capacity to rapidly counter the growth rate of the host. Accordingly, the decline of the dinoflagellate populations correlated with the release of the free-living form of the parasite in the ecosystem; the dinospores were detectable in the ecosystem within 10 days of their release (a phenomenon illustrated by *H. rotundata* at the beginning of June 2005 and by *A. minutum* in 2004, Fig. 1). Although large numbers of dinospores were produced during the population decline of one dinoflagellate species, they did not prevent the growth of any other species, strongly suggesting that the parasites are host-specific. Each year, four main clades of group II Syndiniales were always detected (Fig. 1): Clade 1 was associated with the decline of *H. rotundata* in early June and clades 2, 3, and 14 appeared in late June [for clade nomenclature, see (*12*)]. Minor clades, including 32 and 42, were also detected. The parasite clades are

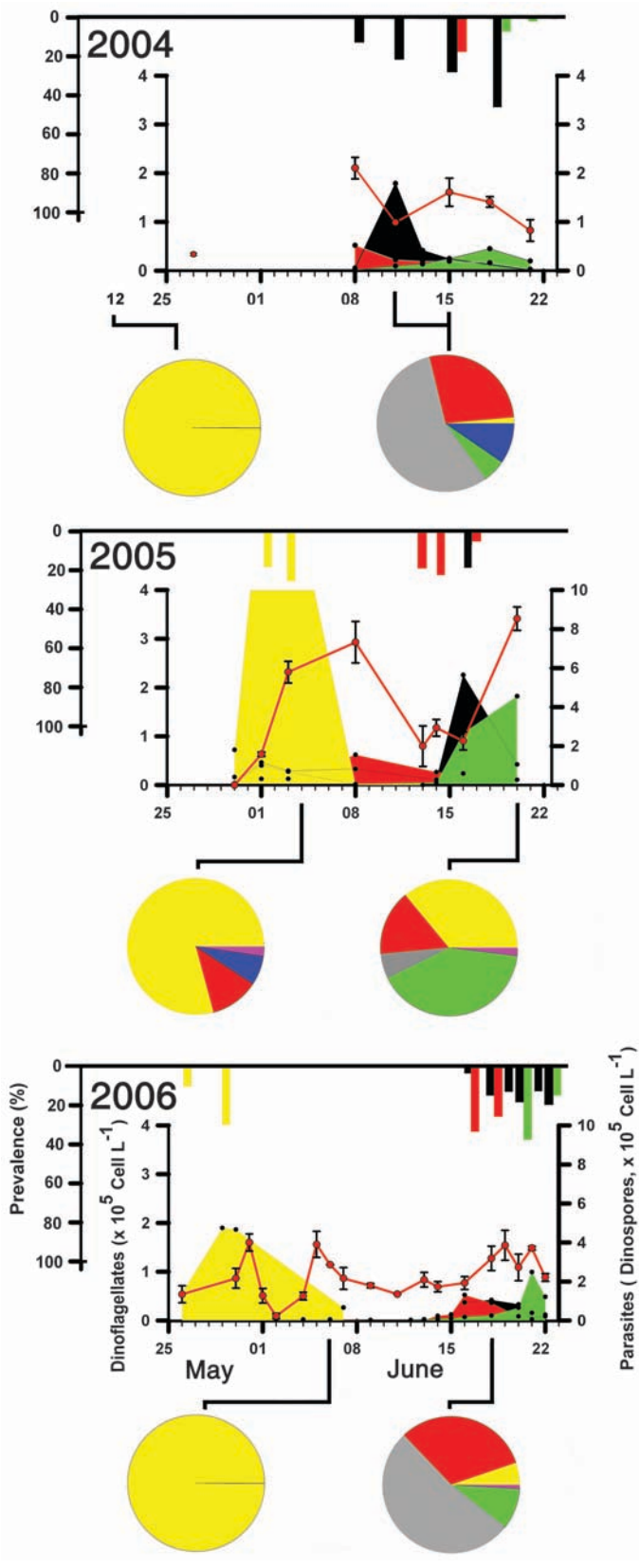

Fig. 1. Dinoflagellate-parasitoid successions and abundances in the Penzé estuary (northern Brittany, France) and the genetic clades of parasites occurring during May and June during 3 consecutive years (2004, 2005, and 2006). Dinoflagellate species are shown in the colored solid curves and by the left axis. Yellow, *H. rotundata*; red, *S. trochoidea*; black, *A. minutum*; green, *H. triquetra*. The free-living stage (dinospore) of group II Syndiniales is shown by the red line and the right axis. Prevalences (percentage of infected host) are shown in the inverted histogram at the top of each panel with the same color code as used for the host. Dinoflagellates were counted by microscopy, and dinospores and prevalence were detected with the general probe ALV01 by FISH. Error bars indicate SDs between triplicates. The pie charts represent the relative contribution, in percentages, of clones belonging to group II Syndiniales obtained from biased polymerase chain reaction amplifications made at two different dates during the monitored period. Yellow, clade 1; red, clade 2; gray, clade 3; green, clade 14; blue, clade 32; pink, clade 42.

separated from each other by at least 44 point mutations (Fig. 3).

Significant intraclade genetic microdiversity was found within environmental sequences (from the 238 sequences analyzed, 170 haplotypes were different). Clades 1 and 3 both have a starlike distribution, with an excess of rare haplotypes (Fig. 3). Furthermore, the results of Tajima's D test were significantly negative (table S3), suggesting a recent evolutionary origin and/or a rapid evolutionary divergence resulting from strong selection pressure. Clades 2 and 14 are more complex, probably indicating a more ancient evolutionary history and cryptic species (fig. S3).

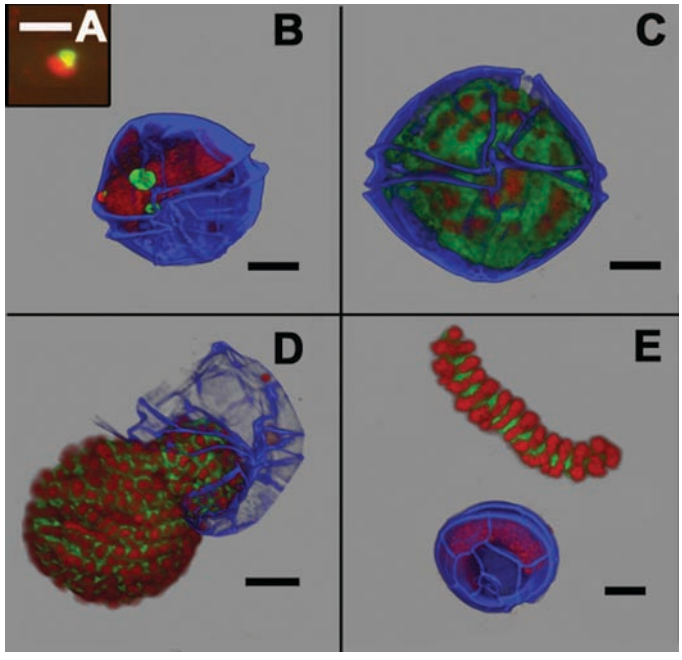

Fig. 2. Life cycle of the *A. minutum* parasitoids in the Penzé estuary. Green fluorescence shows the general oligonucleotide probe ALV01 targeting the small subunit ribosomal RNA (SSU rRNA) of the parasitoid by FISH. Red fluorescence shows nuclear genomic DNA stained by propidium iodide. Blue fluorescence shows the cellulosic theca of *A. minutum* revealed by the calcofluor. (**A**) Dinospore (free-living stage). (**B**) Early stage of *A. minutum* infection. This cell is infected by two intracellular parasites, and a third is located on the surface of the host theca. (**C**) Mature trophont inside *A. minutum*. Note the size increase of the host as compared to the previous stage. (**D**) Very late stage of trophont maturation. Note the thin ruptured host theca. (**E**) Vermiform stages, shown elongated and coiled as a ribbon. Scale bars, 3 μm in (A) and 10 μm in (B) to (E).

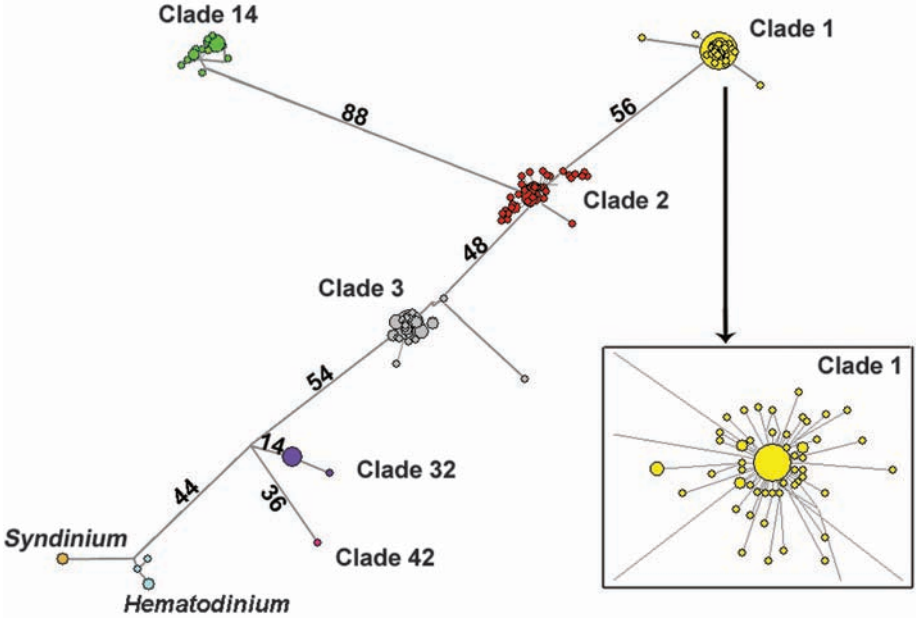

Fig. 3. Median-joining network depicting the phylogenetic relationship among environmental Syndiniales group II SSU rRNA gene sequences obtained from the Penzé estuary over 3 years (238 sequences in total). The solid colored circles follow the same color code as in Fig. 1. The size of each circle is proportional to the corresponding haplotype frequency. Each branch length is proportional to the number of mutational steps (the number of mutations between major clades is written above the branch). Four sequences of *Hematodinium* sp. and four of *Syndinium* sp. (group IV Syndiniales that are parasites of decapods and copepods, respectively) were used as outgroups.

The specificity of the four main parasite clades detected was assessed by FISH, using specific oligonucleotidic probes (tables S1 and S2). Clades 1, 2, and 14 each infected a single dinoflagellate species: *H. rotundata*, *S. trochoidea*, and *H. triquetra*, respectively (Table 1). This specificity is very stable over time; the same parasite clade infected the same species year after year. In all cases, for a given host/parasite pair, the prevalence threshold detected for a given host species when the general probe for group II Syndiniales was used was similar to the value obtained when the clade-specific probe was used (Table 1). Occasionally, parasites attacked nonoptimal host species, but mature trophonts were not observed (Table 1). Similar nonspecific and unproductive infections have been also observed in cultured strains of *Amoebophrya* (*21*, *22*). Unfortunately, we were not able to detect any cells targeted by clade 3 using the FISH technique, and none of the major clades (1, 2, 3, and 14) detected in our genetic clone libraries seemed to be specific to the toxic dinoflagellate *A. minutum*.

HABs occur when dinoflagellates escape not only predation but also parasitic infection. *A. minutum* had been suspected to be invasive after being introduced along the Atlantic coast of France, where blooms were first observed in the late 1980s (*22*). Blooms of *A. minutum* in the Penzé estuary were recorded for the first time in 1994 (fig. S4). Cell densities were reported to exceed 10×10^6 cells/liter (*22*). Over the ensuing 9 years, toxic blooms occurred with remarkable regularity in the Penzé (*22*), but although this species is still present in the ecosystem, blooms no longer occur (fig. S5). The population of *A. minutum* is now regulated by the parasitoids.

Environmental sequences belonging to Syndiniales have been detected in almost every marine ecosystem (*12*). Their host ranges are extremely diverse, extending from dinoflagellates to ciliates, radiolarians, cercozoans, chaetognaths, copepods, cnidarians, appendicularians, crabs, and even fish eggs. Thus, most marine planktonic groups are potentially affected by these parasites, which like the viruses that control bacterial populations, play a top-down control role in their host populations. However, the dinoflagellate parasites have a different impact on organic carbon transfer: In contrast to viruses, dinospores can be directly grazed by larger predators and thus are implicated in carbon transfer to higher trophic levels.

The capacity of these parasitoids to control their hosts is highly dependent on the parasitic fitness and mechanisms underlying the parasitic specificity. This also means that these natural biological controls are potentially less efficient when an exotic species is newly imported into an ecosystem (the enemy release hypothesis) (*23*) or when a rare species is promoted into abundance by substantial environmental change (such as coastal eutrophication or climate change).

Thus, the recent increase of inshore HAB events may originate in geographical and temporary disruptions between these dinoflagellates and their natural parasites.

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Supporting Online Material

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Antimicrobial Defense and Persistent Infection in Insects

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During 400 million years of existence, insects have rarely succumbed to the evolution of microbial resistance against their potent antimicrobial immune defenses. We found that microbial clearance after infection is extremely fast and that induced antimicrobial activity starts to increase only when most of the bacteria (99.5%) have been removed. Our experiments showed that those bacteria that survived exposure to the insect's constitutive immune response were subsequently more resistant to it. These results imply that induced antimicrobial compounds function primarily to protect the insect against the bacteria that persist within their body, rather than to clear microbial infections. These findings suggest that understanding of the management of antimicrobial peptides in natural systems might inform medical treatment strategies that avoid the risk of drug resistance.

By contrast with the clinical use of antibiotics, resistance to natural antibiotics appears to be rare (*1*, *2*). Possibly, natural antibiotics play a different role in the wild than in medical applications (*3*), and our lack of understanding of their natural role results in unforeseen problems when they are used therapeutically, such as the rapid emergence of antibiotic-resistant pathogens.

Insects rely on a suite of systemic responses to combat infection (*4*) that can be classified into two main types. "Constitutive" defenses are always present and ready to act; they rely on the response of insect immune cells (haemocytes) and several rapidly activated enzyme cascades such as phenoloxidase (*5*, *6*) to defend against pathogens. Coupled with this line of defense is the "induced" response, which consists mainly of a suite of antimicrobial peptides (*7*). This component of the antimicrobial response takes at least 1 to 3 hours to generate (*8*) and 12 to 48 hours to reach peak levels (*9*). The induced response persists for weeks in a variety of insects: for example, at least 14 days in bumble bees (*10*) and mealworm beetles (*9*), and up to 44 days in dragonflies (*11*). Because immune responses bear costs [e.g., antagonistic pleiotropy (*12*), metabolic costs (*13*), and self-harm (*14*)], these slow and long-lasting antimicrobial responses, which are

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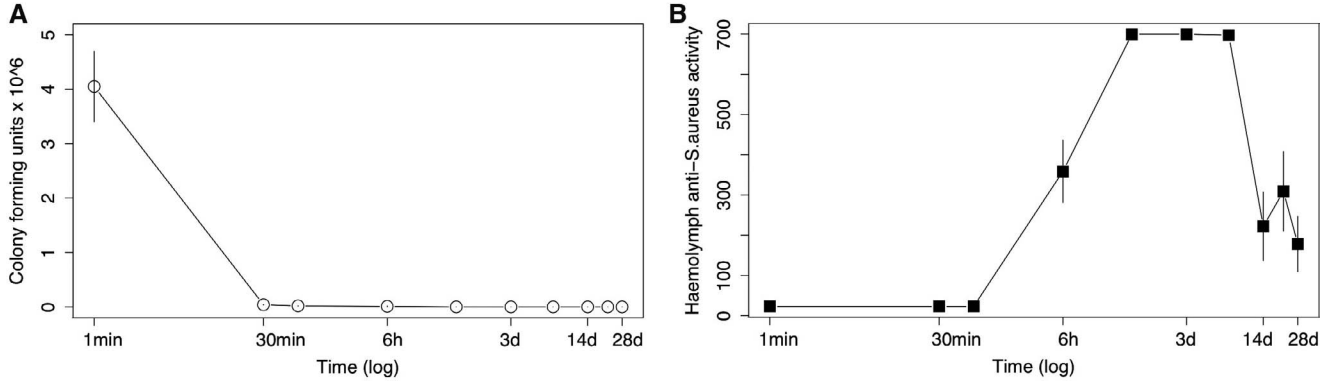

Fig. 1. The number of colony-forming units (CFU) recovered from *T. molitor* haemolymph over 28 days (**A**), and the haemolymph anti–*S. aureus* activity from the same individuals (**B**). Induced haemolymph anti–*S. aureus* activity was measured as the number of *S. aureus* CFUs killed during 2 hours of exposure to *T. molitor* haemolymph and is shown as CFU × 10^3. Each point represents the mean number of CFUs from 7 to 10 beetles (±1 SEM).