Critically, the mean fluid flow provides the fixed-direction bias field that breaks the time-reversal invariance of the system. The two resonant modes of the system are unequally excited by an input, despite the symmetric port configuration. Careful design, by tuning of the mean fluid flow, enables the interference of these two modes to create a sound null at one of the ports, resulting in complete transmission to the other port.

In this case, the three-port device that was fabricated functions as a circulator, in which an input signal on one port at the design frequency transmits all of its acoustic energy to its neighbor port in the direction of the mean flow, and none to its neighbor port opposite the flow. The resulting nonreciprocity is easily seen: An input signal on port 1 is transmitted fully to port 2, whereas the same input signal on port 2 is fully transmitted to port 3 (not port 1, as would happen in a reciprocal device). A circulator like this can easily be converted into a two-port isolator with a matched termination on one port. The measured sample exhibits an impressive 30 dB of sound isolation in a device built from simple, off-the-shelf components.

A one-way device for sound and vibrations has broad implications. Unidirectional acoustic wave propagation has obvious uses in noise control, acoustic sensors, and manipulation of acoustic scattering. At smaller spatial scales, mechanical vibrations (called phonons in their quantum-mechanical form) are responsible for heat transport in solid materials as well. The work of Fleury *et al*. adds to the bank of ideas that can be applied to manipulating heat flow in a nonreciprocal fashion (although still subject to the laws of thermodynamics) in what is called a thermal diode (*7*, *8*). Challenges in device scaling and bandwidth control remain in applying the mean-flow–based concept demonstrated here to more specific scenarios, but that all-important first step toward general-purpose linear acoustic nonreciprocity has now been taken.

References

1. R. Fleury *et al*., *Science* **343**, 516 (2014).
2. H. B. G. Casimir, *Rev. Mod. Phys.* **17**, 343 (1945).
3. A. A. Maznev *et al*., *Wave Motion* **50**, 776 (2013).
4. D. Jalas *et al*., *Nat. Photonics* **7**, 579 (2013).
5. K. G. Budden, *Radio Waves in the Ionosphere* (Cambridge Univ. Press, Cambridge, UK, 1961).
6. D. M. Pozar, *Microwave Engineering* (Wiley, Hoboken, NJ, ed. 4, 2012).
7. N. A. Roberts, D. G. Walker, *Int. J. Therm. Sci.* **50**, 648 (2011).
8. N. Li *et al*., *Rev. Mod. Phys.* **84**, 1045 (2012).

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BOTANY

Pathogen Specialization

The ability to infect new hosts can drive the evolution and specialization of secreted pathogen proteins.

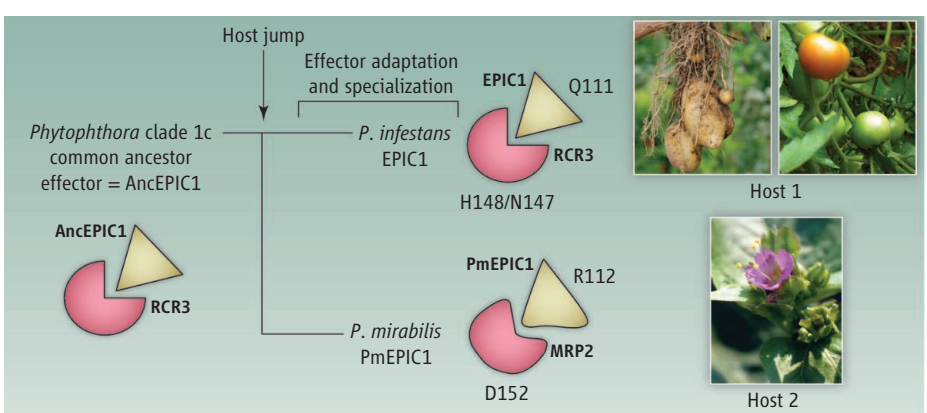
Gitta Coaker

Plants can be attacked by a vast range of pathogen classes, causing substantial agricultural losses. The *Phytophthora* (meaning "plant killer") genus is a particularly destructive pathogen that causes root and stem base decay in a wide range of plants. *Phytophthora infestans*, which precipitated the Irish potato famine, originated in Central Mexico and is closely related to other *Phytophthora* species with distinct host ranges (*1*, *2*). Pathogen effectors that are secreted during infection play a key role in disease biology, but effector-induced adaptation to new hosts is an understudied topic. On page 552 of this issue, Dong *et al.* investigate how *Phytophthora* effector proteins evolve the ability to specialize on new hosts (see the figure) (*3*).

Phytophthora is a genus of oomycetes that exhibit filamentous growth on plants. Oomycetes share some phenotypes with fungi, but are phylogenetically related to photosynthetic brown algae and are thought to have initially emerged from marine environments. The *Phytophthora* genus comprises 10 main lineages designated as clades 1 to 10. Clade 1c, a subdivision of clade 1, includes *P. infestans* (infecting potato and tomato) and *P. mirabilis* (infecting 4 o'clock weeds), indicating that these species share a recent common ancestor (*2*). Genome comparisons between *P. infestans* and *P. mirabilis* highlight alterations and patterns of selection in repetitive DNA containing rapidly evolving families of virulence genes (such as effectors) (*4*).

The 82 effectors undergoing positive selection between *P. infestans* and *P. mirabilis* are promising candidates shaping host specialization (*4*). Dong *et al*. focused their efforts on the EPIC1 effector, which is abundantly secreted during infection of tomato and inhibits extracellular papain-like proteases (including RCR3) that are involved in plant immune perception (*5*).

Dong *et al*. now report that the *P. mirabilis epiC1* ortholog (*PmepiC1*) shows signatures of positive selection, suggesting that this effector has evolved to function in *Mirabilis jalapa*, the 4 o'clock plant, following the split between *P. mirabilis* and *P. infestans*. Using activity-based profiling with a probe that targets papain proteases, the authors demonstrate that recombinant EPIC1 from *P. infestans* effectively inhibits tomato and wild potato RCR3 proteases, whereas PmEPIC1 does not. The authors identify two PmEPIC1 *M. jalapa* targets with homology to RCR3 (MRP1 and MRP2). Subsequent experi-



Route to specialization. A host jump within *Phytophthora* 1c led to the emergence of *P. mirabilis*, which can infect *M. jalapa.* Dong *et al*. show that effector specialization following the host jump is associated with the R112 mutation in PmEPIC1, enabling effective inhibition of the *M. jalapa* MRP2 protease. Polymorphic residues controlling specificity for the host and pathogen are highlighted. Adapted from fig. S18 in (*3*).

PHOTO CREDITS: (POTATO) FER11/ISTOCKPHOTO; (TOMATO) WIKIMEDIA COMMONS; (4 O'CLOCK WEED) THEODORE WEBSTER/USDA AGRICULTURAL RESEARCH SERVICE

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ments confirmed that MRP2 is an active protease and can be effectively inhibited only by PmEPIC1. Thus, EPIC1 effectors function more effectively on their respective hosts, supporting the hypothesis of effector specialization after a host jump.

To define the biochemical basis of EPIC1 host specialization, Dong *et al*. inferred the ancestral allele of *epiC1* using maximum likelihood methods. Ancestral EPIC1 had similar specificity as *P. infestans* EPIC1 for RCR3. Thus, the ability of PmEPIC1 to function more effectively on MRP2 was likely acquired after host jumping.

Surprisingly, both EPIC1 and PmEPIC1 can bind MRP2 using co-immunoprecipitation. Competition experiments show that binding likely occurs at the active site of MRP2 for both EPIC1 and PmEPIC1. How can two similar EPIC1 effectors exhibit different target specificity while still binding to both effective and ineffective targets? Analyses of variant amino acid residues superimposed on an existing structure of tarocystatin in complex with papain protease (*6*) indicate that EPIC1 effectors have similar folds and likely bind proteases at three sites, two of which are polymorphic. A single amino acid polymorphism in PmEPIC1 and its corresponding protease, MRP2, determine specificity.

Future experiments are needed to determine whether PmEPIC1 provides a fitness advantage. If it does, then this would validate the importance of effector specialization for disease biology. These experiments are possible but likely to be difficult to interpret. Oomycete genomes contain large effector arsenals, with ~563 cell-entering effectors in *P. infestans* (*7*). Collectively, effectors are critical virulence factors, but each individual effector typically provides a minor fitness advantage for the pathogen (*8*). It is likely that numerous effectors together rather than individual effectors are crucial for fitness.

By exploring both evolutionary pressures and biochemical adaptation of EPIC1 and corresponding host proteases, Dong *et al*. reveal that selection for single amino acid polymorphisms can significantly affect effector specialization. These findings open an exciting new door to understanding the role effectors play in shaping pathogen adaptation. Conserved core effectors undergoing positive selection could be promising targets for disease control. Nature's ability to select for biochemical specialization has implications for engineering enzymes and their corresponding targets for enhanced specificity. Undoubtedly, many important secrets remain to be discovered.

References and Notes

1. N. J. Grünwald, W. G. Flier, *Annu. Rev. Phytopathol.* **43**, 171 (2005).
2. J. E. Blai *et al*., *PLOS ONE* **7**, e37003 (2012).
3. S. Dong *et al*., *Science* **343**, 552 (2014).
4. S. Raffaele, S. Kamoun, *Nat. Rev. Microbiol.* **10**, 417 (2012).
5. J. Song *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **106**, 1654 (2009).
6. M. H. Chu *et al*., *Planta* **234**, 243 (2011).
7. B. J. Haas *et al*., *Nature* **461**, 393 (2009).
8. G. Fabro *et al*., *PLOS Pathog.* **7**, e1002348 (2011).

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GENETICS

A Unified Process for Neurological Disease

The discovery of mutations associated with disease pathogenesis does not have to rest solely on genetic evidence.

Andrew B. Singleton

The perennial promise of human disease genetics is the delivery of etiology-based therapies. This rests on the notion that identifying disease-causing mutations will provide a basis for determining the molecular networks that constitute the disease process—an understanding that is critical for the development of such therapies. On page 506 of this issue, Novarino *et al*. (*1*) perform what is perhaps the most complete genetic analysis of the neurological disorder hereditary spastic paraplegia (HSP), and deliver on part of this promise by creating an "HSPome," a plausible network of proteins involved in this disease.

In many fields, the hard-won discoveries linking mutations to diseases are the foundation for investigations into molecular pathogenesis. In rare instances, mutation detection has provided immediate insight into the disease process. Usually, however, moving from gene to pathogenesis has been exceptionally difficult. Functional and mechanistic work on the molecular etiology of disease remains one of the major challenges in modern biology; this is quite understandable given the inherent limitations of traditional reductionist functional work. There have been successes, however, and these have helped form the dominant theories of pathogenesis for many diseases, including Alzheimer's disease (*2*). An increasingly popular intermediate step between genetics and function is the use of pathways-based analysis. Such an approach attempts not only to produce a refined list of potential functional interactions to investigate at the bench, but also to provide a global snapshot of the landscape of a particular disease's etiology.

Novarino *et al*. have executed this approach with compelling results. The underlying strategy of their study involved the identification of disease-causing mutations using the power of small, inbred families. HSPs represent a class of inherited progressive neurodegenerative disorders that manifest with stiffness and contraction in the lower limbs—a feature believed to be the result of corticospinal tract dysfunction. These diseases are extremely genetically heterogeneous. Novarino *et al*. examined the autosomal recessive form (AR-HSP), which was already linked to mutation of more than 20 genes (*3*). The authors expand this list by means of an iterative set of analyses on an initial set of 55 AR-HSP families. Using a straightforward genetic strategy of segregation, they show that disease in one-third of the families is linked to genes previously implicated in AR-HSP, but they also identify new candidate gene mutations in more than half of the remaining families. Additional mutations were identified in one-third of these new genes, providing substantive genetic support for causality. Functional analysis showed that candidate gene mutations cause a locomotor deficit in an animal model (zebrafish) of HSP, consistent with what has been described in previous HSP modeling efforts, thus further supporting pathogenicity.

Beyond identifying new genes associated with HSP, Novarino *et al*. constructed a pro-

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