Experimental Evolution of Parasites

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Serial passage experiments are a form of experimental evolution that is frequently used in applied sciences; for example, in vaccine development. During these experiments, molecular and phenotypic evolution can be monitored in real time, providing insights into the causes and consequences of parasite evolution. Within-host competition generally drives an increase in a parasite's virulence in a new host, whereas the parasite becomes avirulent to its former host, indicating a trade-off between parasite fitnesses on different hosts. Understanding why parasite virulence seldom escalates similarly in natural populations could help us to manage virulence and deal with emerging diseases.

Human health, animal welfare, and modern agriculture continue to be challenged by rapidly evolving pests, parasites, and pathogens. Adaptation of these antagonists to new hosts is often observed on time scales as short as days. Although many details about specific host-parasite systems are available, our understanding of virulence evolution, which is critical for dealing with newly emerging infectious diseases, remains rudimentary (*1–3*). Here I summarize what is known about parasite adaptation to their hosts, with particular reference to serial passage experiments (SPEs), a form of experimental evolution and a powerful tool for studying adaptation.

In SPEs, parasites (broadly defined to include pathogens, protozoa, fungi, helminths, and small herbivores) are transferred from one host to another. During SPEs, parasites are propagated under defined conditions and their evolved characters are compared with those of the ancestral parasite. Parasite transfer is either artificial (for example, by injection) or through natural transmission in dense host cultures, relaxing the constraints on real-world infectious processes. Hosts usually have low genetic diversity and may be clonal or inbred lines. First used to develop vaccines, SPEs became a tool in many disciplines and are today among the preeminent practical applications of evolutionary biology. They have provided insight into the evolution of virulence, local adaptation, host-race formation, the accumulation of deleterious mutations, and the maintenance of genetic diversity.

Phenotypic Evolution

A rapid increase of parasite-induced reduction of host fitness is the most general result of SPEs (Tables 1 and 2 and Fig. 1) and it sometimes happens within only three passages (*4–6*). The ability of passaged parasites to grow and to outcompete ancestral parasites also increases during SPEs (Table 1), indicating parasite adaptation to the new hosts. The rate at which virulence increases is most rapid for RNA viruses, slower for DNA viruses and bacteria, and slowest for eukaryotes, which suggests that generation time and mutation rate determine the rate of change.

Parasites passaged in a new host line become attenuated—their virulence and ability to grow in the former host are diminished (Tables 1 and 2 and Fig. 1). As virulence in the new host increases, attenuation in the former host increases as well (*6–10*). Recurring exposure to the former host promotes rapid reversal of attenuation, possibly because genotypes favored in the ancestral hosts are not yet lost from the population (*11–16*). Attenuation may be so complete that a parasite evolves an altered host range. A variant of Dengue-4 virus lost the ability to infect monkeys after 30 passages in cell culture (*17*). Similarly, a nucleopolyhedrosis virus passaged eight times through one moth species lost the ability to infect three of its six former host species (*15*).

Live vaccines such as Theiler's yellow fever vaccine and Sabin's polio vaccine are attenuated parasites, which elicit an immune response without inducing disease. Because reemergence of virulence after vaccination is a risk (*4*, *18*), better insight into attenuation would be valuable.

Mechanisms of Evolution

Many SPEs are started with "cocktails" that are likely to include a variety of parasite genotypes. Evolutionary change in these experiments may be rapid because the favored genotypes were present in the initial cocktail (*9*, *16*). In contrast, SPEs started from one parasite transmission stage reported slower evolutionary responses. Only RNA viruses, well known for their high mutation rates (*19*), responded rapidly in SPEs even when started from single infective stages (*20*, *21*). Rapid response to selection during SPEs might also be explained by the evolution of mutators (*22*) and horizontal DNA transfer (*23*). However, because rapid parasite evolution is usually observed in new hosts, "new-host stress" might lead to unusually strong selection and rapid evolution as well.

Mutations and recombination events are associated with changes in virulence in many SPEs (*10*, *18*, *21*, *24–28*). For example, in yellow fever virus, attenuation is correlated with a point mutation that alters the secondary structure of the 3′-untranslated region (*29*). Attenuation of the live polio vaccine is mainly caused by two mutations with additive effects (*30*). Attenuation of bacteria resulted from the loss of plasmids (*31*) or of chromosome segments termed pathogenicity islands (*32*). These reports might, however, distort the general picture because SPE protocols have not been adequate to assess the role of accumulated genetic change or to estimate the impact of different mutations across replicates of attenuated lines.

During most SPEs, many infective stages (>100) are transferred during each passage (usually <60), which excludes genetic drift as the chief explanation for virulence evolution and attenuation. However, if fewer parasites are transferred, repeated population bottlenecks occur, increasing the rate at which deleterious mutations become fixed and decreasing the rate at which beneficial mutations become fixed. When only one or a few parasites are transferred at each passage, genetic drift can result in a failure to adapt to new hosts (*33*) and in declining fitness (*34*). Repeated transmission bottlenecks are thought to have played a role in the evolution of the endosymbiontic bacteria of aphids (*35*) and of the human immunodeficiency virus (*36*). A second mechanism that might fix deleterious mutations is hitchhiking, in which weakly deleterious mutations are carried along with a selected beneficial mutation.

Within-Host Competition Drives the Evolution of Virulence

In SPEs, the parasite strain with the highest numerical representation in the transferred inoculum has a selective advantage. The driving force may be within-host competition and selection for higher parasite growth rate (*37*, *38*). SPEs support this hypothesis, as exemplified by studies of an avian reovirus (*25*). A slowly growing strain, which resulted in infectious titers 100 times lower than other

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strains, was repeatedly outcompeted by these other strains. During coinfection SPEs, recombinant viruses were selected that grew more rapidly than did the two "parent" strains when tested individually. More rapidly growing genotypes also evolved in other systems (*21*, *39*).

Correlated Evolution of Parasite Traits

Increasing virulence during selection for higher growth rate is likely to be the consequence of the diversion of resources from the host by the reproducing parasite, and as such might be a functional constraint common to all parasites. Further, both within-host growth and virulence correlate positively with between-host transmission (*24*, *40*–*42*), but this correlation does not seem to be common to all parasites. Production rates of different parasite life stages can trade off with each other, so that an increase in the production of one stage leads to a decrease in that of another stage. For example, some parasites possess between-host transmission stages with costly protective structures, such as the polyhedrin capsules of nuclear polyhedrosis viruses. Loss of these protective capsules during SPEs accompanies increased within-host growth rate (*26*). The malaria agent *Plasmodium* propagates clonally within its vertebrate host but must produce specialized stages for transmission to its vector. During serial passage in mice, clonal growth forms became dominant and strains evolved that could no longer be transmitted to the vector (*43*). A trade-off between different parasite stages was also found for the Chagas disease agent *Trypanosoma cruzi* (*44*). Its virulence in mice was high when passaged in mice alone and lower when passages included the insect vector (*45*).

A higher within-host growth rate in the host used in the SPE accompanies reduced growth and virulence in the previous host (Table 2 and Fig. 2). The antagonistic pleiotropy hypothesis explains this negative correlation in the fitnesses of a parasite in different hosts by stating that a gene that enhances fitness on one host decreases fitness on the other host (*27*, *46*). For example, when the Sindbis RNA virus was propagated in cell cultures, mutants appeared that penetrated cells faster than the wild type but were attenuated in mice. One mutation was responsible for both changes (*27*). Antagonistic pleiotropy was also reported for changes in Venezuelan equine encephalitis virus (*47*) and poliomyelitis virus (*30*). Linkage disequilibrium can be ruled out as a general explanation for attenuation, at least for SPEs that have been started from single parasite individuals and that show consistency across replicates (*20*, *21*, *48*).

Why Does Virulence Escalate in SPEs?

Three not mutually exclusive hypotheses have been proposed to answer this question

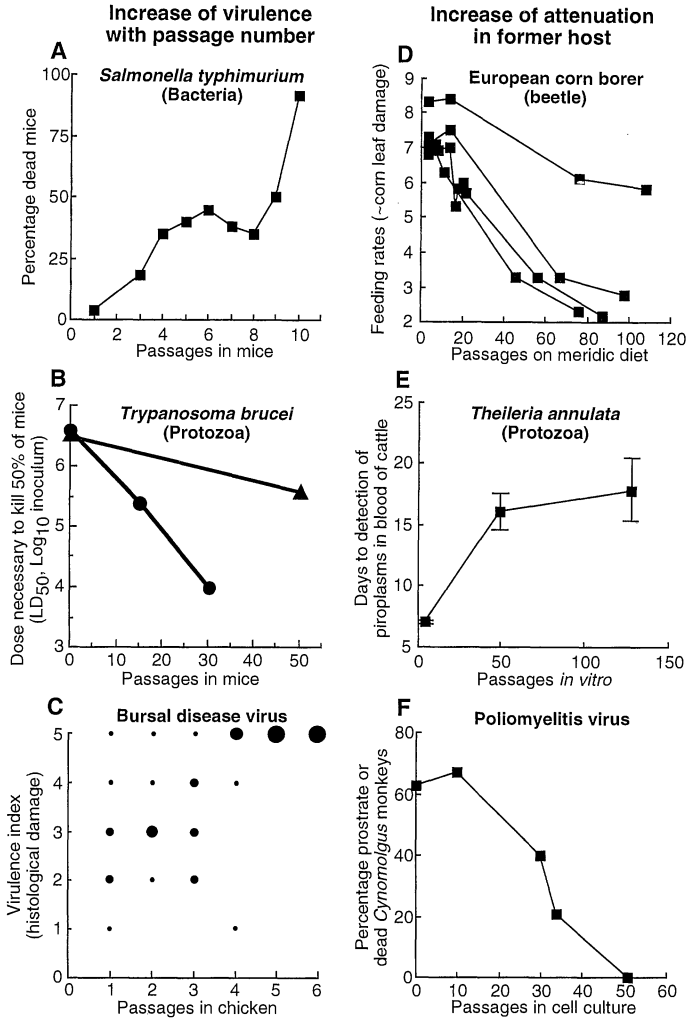

Fig. 1. Examples of the change in virulence [(A) through (C)] and attenuation [(D) through (F)] during SPEs. (**A**) *Salmonella typhimurium* in mice. (**B**) *Trypanosoma brucei* in mice. Data from two passage series are shown. (**C**) Infectious bursal disease virus in chicken. The size of the dots indicates the number of replicates (smallest dot, $n = 1$ to 4; largest dot, $n = 15$ to 20). (**D**) European corn borer (*Ostrinia nubilalis*) beetles passaged on a meridic diet became attenuated on corn. Several series are shown. (**E**) *Theileria annulata* passaged in vitro became attenuated in cattle. (**F**) Poliomyelitis virus passaged in cell culture became attenuated in *Cynomolgus* monkeys. Data for (A) through (F) are from (*10*, *18*, *33*, *39*, *48*, *77*).

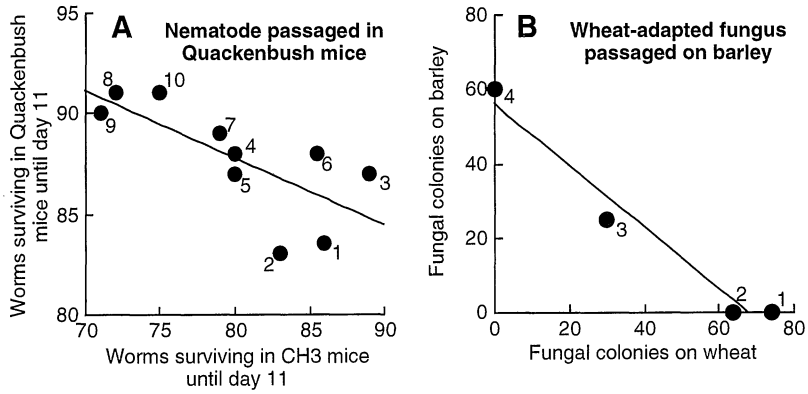

Fig. 2. Two SPEs in which a parasite fitness component was quantified in every passage in the former and the current host. Numbers indicate the passage number. Diagonal lines indicate least-square regressions [in (A), $r^2 = 0.52$; in (B), $r^2 = 0.96$]. (**A**) The nematode *Nematospiroides dubius* was passaged in the Quackenbush mouse line. Data are from figure 1 in (*8*). (**B**) Change in colony growth of a wheat-adapted isolate of the fungus *Septoria nodorum* during serial passage on barley. Data are from (*9*).

Table 1. Examples of SPEs. Care was taken to represent all major groups of horizontally transmitted parasites. Priority was given to studies with more replicates and to studies with controls. Studies with small transfer numbers were omitted (see text). Fitness refers to competitive ability relative to the ancestral strain. Growth refers to within-host growth. Attenuation indicates reduced survival, reproduction, growth, or virulence in the former host. Dashes indicate no information available; yes/no indicates that some replicates or combinations within a study showed attenuation, whereas others did not.

Parasites/hosts	Increase in passaged host			Attenuation (former host)	Reference
	Fitness	Virulence	Growth		
RNA viruses					
Poliomyelitis virus/cell culture	–	yes	–	yes (monkeys)	(33)
Influenza A seal virus/chicken eggs	–	yes	yes	–	(61)
Neurovirulence influenza virus/cell cultures	–	yes	yes	yes/no (mouse)	(62)
Sindbis virus/cell culture	–	–	–	yes (mouse)	(27)
Venezuelan equine encephalitis virus/cell cultures	–	–	–	yes (mouse)	(12)
Infectious bursal disease virus/chicken	–	yes	–	–	(18)
Chicken influenza virus/chicken	–	yes	–	–	(5)
Yellow fever virus/rhesus, mouse	–	yes	yes	yes (man)	(63)
Borna disease virus/mouse, rat	–	yes	yes	yes/no (rat, mouse)	(64)
Vesicular stomatitis virus/cell cultures	yes	yes	yes	yes (cell culture)	(21)
Foot-and-mouth disease virus/cell culture	–	yes	–	yes (mice, cattle)	(20)
Tobacco mosaic virus/legumes	yes	yes	–	yes (other legumes)	(14)
Avian reovirus/cell culture	yes	–	yes	–	(25)
Hepatitis A/cell culture	yes	yes	yes	yes (primates)	(65)
Dengue-4 virus/cell culture	–	–	–	yes (monkey)	(17)
Japanese encephalitis virus/cell culture	–	–	–	yes (mouse)	(66)
Equine infectious anemia virus/cell culture	–	yes	–	yes (ponies)	(7)
DNA viruses					
Nucleopolyhedrosis virus/*Pseudoplusia includens*	–	yes	–	yes (other moths)	(15)
Duck hepatitis virus/ducklings	–	yes	–	–	(4)
Alcelaphine gammaherpes virus/cell culture	–	yes	–	yes (rabbit)	(67)
Influenza B virus/chicken eggs	–	–	–	yes (man)	(68)
Bacteria					
Salmonella typhimurium/mice	yes	yes	–	–	(48)
Rhodococcus equi/axenic medium	yes	–	yes	yes (mouse)	(31)
Borrelia anserina/axenic medium	–	–	–	yes (chicken)	(69)
Bacillus anthracis/in vitro	–	–	–	yes (livestock, human)	(70)
Legionella pneumophila/in vitro	–	–	–	yes (guinea pig)	(71)
Fungi					
Septoria nodorum/wheat, barley	–	yes	yes	yes (wheat, barley)	(9)
Phytophthora infestans/potato	yes	yes	yes	yes (other potatoes)	(6)
Paracoccidiodes brasiliensis/in vitro	–	–	–	yes (mouse)	(72)
Coccidioides immitis/in vitro	–	yes	–	yes (mouse)	(73)
Protozoa					
Eimeria tenella and *E. necatrix*/turkey	–	no	yes	–	(74)
Trypanosoma brucei/mouse	–	yes	yes	–	(39)
T. cruzi/axenic culture	yes	yes	–	yes (mouse)	(45)
Plasmodium knowlesi/man	–	yes	–	–	(75)
P. berghei/mouse	–	–	yes	–	(43)
Naegleria fowleri/cell culture	–	–	–	yes (mouse)	(11)
Theileria annulata/cell culture	–	–	–	yes (cattle)	(10)
Toxoplasma gondii/rat	–	–	–	yes (mouse)	(76)
Babesia bovis/splenectomized cattle	–	–	–	yes (cattle)	(13)
Helminth					
Nematospiroides dubius/Quackenbush mice	–	–	–	yes (CH3 mice)	(8)
Arthropoda					
Tetranychus urticae (mite)/bean	–	yes	yes	yes (tomato, cucumber)	(46)
Ostrinia nubilalis (moth)/corn	–	–	–	yes (corn)	(77)
Nilaparvata lugens (planthopper)/rice	yes	–	yes	–	(78)

(*49*). First, parasite-induced host mortality truncates parasite transmission from one living host to another. Therefore, a parasite is expected to balance this cost with the benefits of killing the host, so that its fitness is maximized. The fitness optimum depends on the system, the ecological conditions, and the frequency of multiple infections (*2*, *37*, *38*, *50*). SPEs support the presence of genetic correlations between virulence and other parasite fitness components, a key assumption of these models. Passaged parasites pay no costs for killing the host because transmission relies on the experimenter. Therefore, an increase in virulence during SPEs can be beneficial for the parasite.

The second hypothesis states that under natural conditions, genetic diversity among host individuals prevents parasites from adapting to particular genotypes (*51*). Because hosts in SPEs are usually of low genetic diversity or even clonal, parasites may achieve high virulence because they adapt to specific genotypes. The notorious sensitivity of agricultural monocultures to parasites (*52*) is consistent with this "Red Queen" hypothesis, which states that genetic variation is beneficial because it hinders parasite adaptation and fuels host evolution for resistance (*51*, *53*). The benefits of genetic diversity in fighting natural enemies has been demonstrated for weeds with different breeding systems (*54*) and for multiple versus single mated queens of social insects (*55*).

A third hypothesis applies only to parasites with different stages for within-host growth and between-host transmission. If there are trade-offs in the production of these stages, evolution should favor a balance that maximizes overall parasite fitness. Because the costly between-host transmission stages are not needed during SPEs, they will be lost and within-host growth rates will increase. However, declining production of the between-host transmission stages may also be

Table 2. Summary of observed evolutionary changes (relative to the ancestral parasite) during SPEs with large transfer sizes in the host in which the passages were done and in the former host.

Trait	Direction of change
Trait measured in passaged host	
Virulence	Increase
Within-host growth rate	Increase
Competitive ability relative to ancestral host	Increase
Transmissibility	Increase/decrease*
Trait measured in former host	
Virulence	Decrease
Within-host growth rate	Decrease
Infectivity	Decrease
Clearance rate in vertebrate hosts	Increase

*Depends on the system studied.

due to the accumulation of deleterious mutations. For example, growth of *Plasmodium falciparum* in cell culture frequently leads to deletions in the parasite genome (*56*), and pathogenicity islands are often lost during microbial growth in culture (*32*).

The Evolution of Host-Race Formation

Results from SPEs support the generalist-specialist trade-off hypothesis (*46*, *57*) (Fig. 2), which is often discussed in relation to host-race formation (*58*). Other experimental tests of this hypothesis gave ambiguous results (*59*). Fitness boundaries might explain this discrepancy. Correlations between parasite fitness components are only meaningful if they are close to the fitness boundary of the parasite (*2*). At the fitness boundary, genes that code for higher fitness on both hosts or that affect only one host (being neutral on the other) are fixed by past selection. The remaining genetic variance is explained by genes with negative pleiotropic effects. The boundary can be reached when selection acts simultaneously to increase fitness on both hosts, a condition that might not hold in many natural systems.

Conclusions

The evolution of vertically transmitted parasites (*37*, *42*, *60*) is better understood than the evolution of horizontally transmitted parasites. Observations of horizontally transmitted parasites made during SPEs are concordant with evolutionary theory and help us to understand host-parasite interactions. SPEs indicate that within-host competition drives parasite adaptation and the evolution of virulence, justifying the conclusion that any factor that increases the frequency of multiple infections will lead to an increase in virulence (*37*, *38*). SPEs do not reflect epidemiological aspects of parasite evolution, and thus what prevents virulence from increasing to high levels in natural populations remains unknown. The answer is likely to be found in the relationship between within-host growth and between-host transmission. To better understand the evolution of virulence, we have to connect within-host to between-host evolutionary dynamics. Antagonistic pleiotropy and host genetic diversity are likely to play important roles in this relationship.

References and Notes

1. G. C. Williams and R. M. Nesse, *Q. Rev. Biol.* **66**, 1 (1991).
2. J. J. Bull, *Evolution* **48**, 1423 (1994).
3. P. W. Ewald, *The Evolution of Infectious Disease* (Oxford Univ. Press, Oxford, 1994).
4. P. R. Woolcock and G. W. Crighton, *Vet. Rec.* **105**, 30 (1979).
5. M. Brugh and M. L. Perdue, *Avian Dis.* **35**, 824 (1991).
6. J. L. Jinks and M. Grindle, *Heredity* **18**, 245 (1963).
7. D. E. Gutekunst and C. S. Becvar, *Am. J. Vet. Res.* **40**, 974 (1979).
8. C. Dobson and M. E. Owen, *Int. J. Parasitol.* **7**, 463 (1977).
9. B. M. Cunfer, *Ann. Appl. Biol.* **104**, 61 (1984).
10. I. A. Sutherland *et al.*, *Exp. Parasitol.* **83**, 125 (1996).
11. M. M. Wong, S. L. Karr, C. K. Chow, *J. Parasitol.* **63**, 872 (1977).
12. T. O. Berge, I. S. Banks, W. D. Tigertt, *Am. J. Hyg.* **73**, 209 (1961).
13. L. L. Callow, L. T. Mellors, W. McGregor, *Int. J. Parasitol.* **9**, 333 (1979).
14. F. C. Bawden, *J. Gen. Microbiol.* **18**, 751 (1958).
15. O. H. Pavan, D. G. Boucias, J. C. Pendland, *Entomophaga* **26**, 99 (1981).
16. C. A. Carson, P. Timms, A. F. Cowman, N. P. Stewart, *Exp. Parasitol.* **70**, 404 (1990).
17. N. J. Marchette *et al.*, *Am. J. Trop. Med. Hyg.* **43**, 212 (1990).
18. J. C. Muskett, N. E. Reed, D. H. Thornton, *Vaccine* **3**, 309 (1985).
19. L. M. Mansky, *J. Gen. Virol.* **79**, 1337 (1998).
20. J. Diez, M. Hofner, E. Domingo, A. I. Donaldson, *Virus Res.* **18**, 3 (1990).
21. I. S. Novella *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5841 (1995).
22. J. E. LeClerc, B. Li, W. L. Payne, T. A. Cebula, *Science* **274**, 1208 (1996).
23. S. F. Mel and J. J. Mekalanos, *Cell* **87**, 795 (1996).
24. J. J. Bull and I. J. Molineux, *Evolution* **46**, 882 (1992).
25. Y. Ni and M. C. Kemp, *J. Gen. Virol.* **73**, 3107 (1992).
26. S. Kumar and L. K. Miller, *Virus Res.* **7**, 335 (1987).
27. R. A. Olmsted, R. S. Baric, B. A. Sawyer, R. E. Johnston, *Science* **225**, 424 (1984).
28. G. Kurath and J. A. Dodds, *RNA* **1**, 491 (1995).
29. V. Proutski, M. W. Gaunt, E. A. Gould, E. C. Holmes, *J. Gen. Virol.* **78**, 1543 (1997).
30. G. D. Westrop *et al.*, *J. Virol.* **63**, 1338 (1989).
31. S. Takai *et al.*, *Vet. Microbiol.* **39**, 187 (1994).
32. E. A. Groisman and H. Ochman, *Cell* **87**, 791 (1996).
33. A. Sabin, W. A. Hennessen, J. Winser, *J. Exp. Med.* **99**, 551 (1954).
34. L. Chao, *Nature* **348**, 454 (1990); D. K. Clarke *et al.*, *J. Virol.* **67**, 222 (1993).
35. P. Baumann, N. Moran, L. Baumann, *BioScience* **47**, 12 (1997); N. Moran, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2873 (1996).
36. A. J. Leigh Brown, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1862 (1997).
37. E. A. Herre, *Parasitology* **111**, S179 (1995).
38. M. A. Nowak and R. M. May, *Proc. R. Soc. London Ser. B* **255**, 81 (1994).
39. P. Diffley, J. O. Scott, K. Mama, T. N. R. Tsen, *Am. J. Trop. Med. Hyg.* **36**, 533 (1987).
40. D. Ebert and K. L. Mangin, *Evolution* **51**, 1828 (1997).
41. M. Lipsitch and E. R. Moxon, *Trends Microbiol.* **5**, 31 (1997).
42. P. E. Turner, S. C. Cooper, R. E. Lenski, *Evolution* **52**, 315 (1998).
43. A. L. Dearsly, R. E. Sinden, I. A. Self, *Parasitology* **100**, 359 (1990).
44. J. Dönges, *Parasitologie* (Thieme, Stuttgart, Germany, 1988).
45. V. T. Contreras, W. Araque, V. S. Delgado, *Mem. Inst. Oswaldo Cruz* **89**, 253 (1994).
46. J. D. Fry, *Am. Nat.* **136**, 569 (1990); F. Gould, *Evolution* **33**, 791 (1979).
47. R. E. Johnston and J. F. Smith, *Virology* **162**, 437 (1988).
48. M. R. Zelle, *J. Infect. Dis.* **71**, 131 (1942).
49. D. Ebert, in *Evolution in Health and Disease*, S. C. Stearns, Ed. (Oxford Univ. Press, Oxford, 1999), pp. 161–172.
50. R. M. Anderson and R. M. May, *Parasitology* **85**, 411 (1982).
51. D. Ebert and W. D. Hamilton, *Trends Ecol. Evol.* **11**, 79 (1996).
52. M. W. Adams, A. H. Ellinghoe, E. C. Rossmann, *Bioscience* **21**, 1067 (1971).
53. J. Jaenike, *Evol. Theory* **3**, 191 (1978); W. D. Hamilton, *Oikos* **35**, 282 (1980); C. M. Lively, *Bioscience* **46**, 107 (1996).
54. N. E. Stevens, *J. Am. Soc. Agron.* **40**, 841 (1948); J. J. Burdon, *J. Appl. Ecol.* **18**, 649 (1981).
55. S. Liersch and P. Schmid-Hempel, *Proc. R. Soc. London Ser. B* **265**, 1 (1998).
56. K. P. Day *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8292 (1993).
57. K. M. Harrower, *Trans. Br. Mycol. Soc.* **68**, 101 (1977).
58. J. M. Smith, *Am. Nat.* **100**, 637 (1966); J. Felsenstein, *Evolution* **35**, 124 (1981).
59. S. Via, *Annu. Rev. Entomol.* **35**, 421 (1990); A. Mackenzie, *Evolution* **50**, 155 (1996).
60. J. J. Bull, I. J. Molineux, W. R. Rice, *Evolution* **45**, 875 (1991).
61. E. D. Kilbourne and J. S. Murphy, *J. Exp. Med.* **111**, 337 (1960).
62. Z. Janda and V. Vonka, *Arch. Gesamte Virusforsch.* **24**, 192 (1968).
63. M. Theiler and H. H. Smith, *J. Exp. Med.* **65**, 782 (1937).
64. S. A. Rubin, R. W. Waltrip, J. R. Bautista, K. M. Carbone, *J. Virol.* **67**, 548 (1993).
65. R. A. Karron *et al.*, *J. Infect. Dis.* **157**, 338 (1988).
66. J. X. Cao *et al.*, *J. Gen. Virol.* **76**, 2757 (1995).
67. W. Plowright, *Rev. Sci. Tech. Off. Int. Epizoo.* **5**, 897 (1986).
68. M. A. Zuckerman, J. C. Rebecca, J. S. Oxford, *J. Infect.* **28**, 41 (1994).
69. J. F. Levine *et al.*, *Res. Vet. Sci.* **48**, 64 (1990).
70. P. C. B. Turnbull, *Vaccine* **9**, 533 (1991).
71. E. E. Catrenich and W. Johnson, *Infect. Immun.* **56**, 3121 (1988).
72. E. Brummer, A. Restrepo, L. H. Hanson, D. A. Stevens, *Mycopathologia* **109**, 13 (1990).
73. H. B. Levin, D. Pappagianis, J. M. Cobb, *Mycopathol. Mycol. Appl.* **41**, 177 (1979).
74. M. H. Kogut, T. C. Gore, P. L. Long, *Parasitology* **86**, 199 (1983).
75. W. Chin, P. G. Contacos, W. E. Collins, M. H. Jeter, E. Alpert, *Am. J. Trop. Med. Hyg.* **17**, 355 (1968).
76. V. Lecompte, B. F. F. Chumpitazi, B. Pasquier, P. Ambroise-Thomas, F. Santoro, *Parasitol. Res.* **78**, 267 (1992).
77. W. D. Guthrie, Y. S. Rathore, D. F. Cox, G. L. Reed, *J. Econ. Entomol.* **67**, 605 (1974).
78. M. F. Claridge and J. Hollander, *Entomol. Exp. Appl.* **32**, 213 (1982).

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