Emerging Disease or Diagnosis?

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Science **338**, 750 (2012);
DOI: 10.1126/science.1225893

Sexual selection in pronghorns. Byers and Dunn show that male mating success in a pronghorn population only becomes male reproductive success in years with low coyote predation on pronghorns.

ness and trait over long periods of time is necessary to allow adaptations to be exquisitely refined.

However, in evolutionary biology, most field studies of the strength of natural and sexual selection are snapshots, constructed from a portion of the life span of the species under study. Thus, few measurements of the consistency of selection exist, and for those few, it has proven difficult to separate chance variations in fitness from variations attributable to phenotypic differences between individuals (*11*). Indeed, without data across several generations or from several different populations, it is difficult to even identify the environmental factor or factors responsible for selection.

Byers and Dunn's long-term study of a closed pronghorn population (see the second figure) addresses these shortfalls in the measurement of selection. They report a positive Bateman slope in each of the 10 years of the study and convincingly rule out random variation in male mating success as its cause. Variance in mate numbers among males accounts for three-fourths of the male variance in the numbers of weaned offspring. The authors' plot of the annual distribution of individual male mating success [figure 2 in (*1*)] conforms strikingly to the very highly skewed distributions expected on theoretical grounds (*6*, *7*) to arise from strong sexual selection. Lifetime male mating success can be very high because the males that successfully mate in one season enjoy repeated mating success in subsequent breeding seasons.

The authors' repeated measurements of the Bateman slope afford the unique opportunity to use the slope itself as the dependent variable in a generalized linear model and attempt to account for its yearly variation in terms of population density, sex ratio, age structure, and fawn mortality. Byers and Dunn find a strong negative effect of fawn mortality on the Bateman slope, indicating that male mating success becomes male reproductive success only in those years when coyote predation on antelopes is low. This is an important finding because many studies of the strength of sexual selection begin and end with mating success, whereas total fitness (measured as weaned offspring) is difficult or impossible to observe in most field systems.

Furthermore, because many research programs are necessarily focused only on a portion of the life span, we rarely gain insight into the action of multiple, sequential episodes of selection. Most long-term studies have found that the strength of selection and the direction of selection vary with changes in environmental conditions. In this respect, the study of Byers and Dunn is not novel. However, unlike most studies, they discovered an interesting and opposing interaction between sexual selection and subsequent fawn viability that mitigates the former.

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10.1126/science.1230395

EPIDEMIOLOGY

Emerging Disease or Diagnosis?

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Some viral infections may not represent emerging diseases, but improved detection and diagnosis of common diseases.

Outbreaks this year of the deadly and highly contagious Ebola and Marburg viruses in the Democratic Republic of Congo and Uganda and Lassa virus in Nigeria raised concerns about possible epidemic spread of these hemorrhagic fevers. These pathogens seemed to appear out of nowhere around the middle of the 20th century: Marburg virus in 1967, Lassa virus in 1969, and Ebola virus in 1976. By the early 1990s, public health concerns were crystallized in a landmark report (*1*) that was the first to popularize the concept of "emerging pathogens" (fig. S1). But could "emerging diagnosis" explain the rise in appearance of hemorrhagic fevers caused by these pathogens? Recent epidemiologic and genetic studies of Lassa and Ebola fevers suggest that these diseases may have widespread prevalence and ancient origins. They raise the possibility that some viral infections may reflect "emerging diagnoses" of diseases that are circulating more widely than thought, with an emerging character primarily a matter of improved detection of the culprit pathogens.

Emerging pathogens generally fall into two categories: microbes newly introduced to humans from other species, and existing but previously rare human pathogens that rise rapidly in prevalence or pathogenicity (*2*). Their appearance is often attributed to human encroachment on animal habitats, changing socioeconomic conditions, increased connectivity of the world, and genetic changes in the microorganisms. The concept of emerging pathogens arising through better diagnoses of disease is not commonly thought to apply to deadly viruses such as those causing Lassa and Ebola hemorrhagic fevers.

It may be that the causative pathogens of viral hemorrhagic fevers are characterized

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as rare because we lack the tools in the right places to routinely detect them. Contrary to popular perception, viral hemorrhagic fevers often have nonspecific symptoms such as fever, headache, and nausea, which make clinical diagnosis difficult. Without proper laboratory diagnostics, health care workers cannot accurately determine the source of fever; malaria, typhoid, or shigella is often assumed to be the culprit. Such misdiagnosis costs lives, as in a 1989 outbreak of Lassa virus in two Nigerian hospitals where 22 people died (*3*). In addition, most patients rarely present with symptoms in the hospital, and death from febrile illness is common in many developing regions of the world.

Seroprevalence surveys, which estimate the number of individuals exposed to a specific microbe (based upon the presence of antibodies for that microbe in the blood), indicate widespread exposure to Lassa and Ebola viruses in certain parts of Sub-Saharan Africa (see tables S1 and S2, and note S1). Prevalence rates for Lassa virus in surveyed regions are as high as 52% and 54.9% in parts of Sierra Leone and Guinea, respectively, with lower yet substantial rates for Nigeria (21.3%), Côte d'Ivoire (20%), Benin (9.9%), and Ghana (3.8%), suggesting that the virus is endemic in West Africa. These studies led public health officials to estimate that 100,000 to 300,000 people in West Africa become infected each year, and that Lassa infections account for as many as 15% of admissions in some regional hospitals in Sierra Leone, Guinea, and Liberia (*4*, *5*). Seroprevalence surveys of Ebola virus also indicate high exposure (table S2), with prevalence rates up to 22%, depending on the population, and 15.3% in Gabon (*6*). But the prevalence of both viruses may be higher, as assays for seroprevalence can understate actual exposure rates (because antibody concentrations in the blood wane after exposure), or lower, as they are also subject to false positives (because some assays have low specificity).

Human exposure to animal reservoirs of Lassa and Ebola viruses may underlie their widespread presence in human populations. Both viruses primarily infect nonhuman hosts, and the natural reservoirs of these hosts are abundant. Lassa virus is transmitted by the mouse *Mastomys natalensis*, which persistently maintain the infection, likely through many years of coevolution. The rodent lives in close contact with humans—in some areas, the rodents are eaten by up to 90% of the population—and a sizable fraction of the mice are infected with and shed Lassa virus. Large areas of West Africa, ranging from 10% of Ghana to 80% of Sierra Leone and Liberia, are exposed to the Lassa reservoir (*7*). The likely Ebola reservoir, the fruit bat, similarly inhabits broad swaths of Western and Central Africa (*8*).

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Viral discovery. Shown is a photo illustration (artistic rendition) of the Congo River in the Democratic Republic of Congo, near where Ebola virus was first discovered in 1976 (*1*). The image of the river was created to take the form of Ebola virus.

Although human outbreaks of Ebola are thought to quickly wane, a 2006 Ebola epidemic among nonhuman primates in Central Africa indicated that virus transmission can be wide-ranging and sustained. The epidemic wiped out ~95% of the exposed gorilla populations [almost 5000 animals (*9*)] and a large fraction of the chimpanzee population. Given the extreme fatality rates, it is possible that the virus was newly introduced to these populations of nonhuman primates or was a more pathogenic species or strain. In the case of a newly introduced virus, however, 12.9% of chimpanzees in Central Africa are seropositive, suggesting natural exposure and resistance (rather than the introduction of a new virus) (*10*). In 10 instances where human infection resulted from hunters handling nonhuman primate carcasses, the outbreaks had one point of origin with subsequent spread throughout Gabon, indicating that sustained transmission can occur (*11*).

Analyses of the evolutionary histories of both viruses hints at their long-time and widespread presence around the world, and possible long-standing potential for causing human disease. Lassa virus likely diverged around 500 years ago from other arenaviruses, some of which also cause hemorrhagic disease (*12*). Similarly, Ebola virus is estimated to have diverged from Marburg virus ~10,000 years ago (*13*), and itself occurs as at least five genetically distinct, stable subtypes that are dispersed geographically. Most cause disease in humans, suggesting that the virus has ancient origins and that its pathogenicity has been present for an extended period.

Multiple genomewide scans have uncovered potential evidence for human evolutionary adaptation to Lassa virus infection (*14*). Most notably, the gene *LARGE,* which encodes a protein that modifies α-dystroglycan, a cellular receptor for Lassa virus, is one of the strongest signals of natural selection in the Yoruba of Nigeria (table S3). The variants in *LARGE* (found to be under recent selection) are present only in certain West African and West African–derived populations and are estimated to have emerged between 3000 and 10,000 years ago. Establishing that the positively selected variants in *LARGE* and other identified genes confer resistance to Lassa fever would support long-term human exposure to Lassa virus.

Reports of subclinical infections suggest that natural human genetic resistance to Lassa and Ebola exists. Although fatality rates among hospital cases range from 12 to 78% for Lassa fever and 42 to 88% for Ebola fever (reference S1), the seroprevalence data and reports of asymptomatic individuals

during Ebola outbreaks indicate that there are many subclinical cases. Host immune responses may potentially restrict viral replication of both viruses (*15*). However, the degree and route of exposure to these viruses, as well as variable pathogenicity of viral species and strains, must also be considered.

How does the possibility that some "emerging diseases" are ancient and widespread affect approaches to fighting them? If exposure is indeed common, then the causative pathogens are likely already circulating—undetected—in communities and health clinics. With the right detection tools in place, we can identify where their corresponding diseases are likely to be prevalent, and begin to develop treatment, surveillance, and research capacity without waiting for the next outbreak.

Efforts to reduce the burden of Lassa fever at the Kenema Government Hospital (KGH) in Sierra Leone and the Irrua Specialist Teaching Hospital (ISTH) in Irrua, Nigeria, are informative examples. At KGH and ISTH, Lassa virus is recognized as a common cause of illness, and local and international partners work together to diagnose and treat it (*16*, *17*). These efforts have saved numerous lives and the institutions have become thriving research centers; in the process they have raised awareness nationally. In 2012, the Nigerian Federal Ministry of Health reported an increased incidence of suspected Lassa fever cases, with nearly 1000 cases reported by 41 local government agencies in 23 states, potentially due to "emergence of diagnosis."

Intriguingly, as patient outcomes for Lassa fever have improved at KGH and ISTH, the surrounding communities have become more engaged, and the sites are now referral clinics for patients with undiagnosed (or unexplained) febrile illness within hundreds of kilometers. Consequently, the sites have amassed not only numerous cases of Lassa fever but thousands of cases of unexplained fever. They provide an opportunity both to investigate Lassa virus and to identify or discover other microbes, thereby becoming sentinel sites for emerging infectious diseases.

This kind of strong and sustained partnership between local health care clinics and their communities, along with access to effective diagnostics and treatment, motivates more members of the community to seek care, resulting in a positive feedback cycle that can save lives and facilitate rapid detection of pathogens, both "emerging" ones and common pathogens whose improved detection has labeled them as emerging pathogens. Ideally, after local analysis, samples can be sent to laboratories for identification and discovery of known and new microbes through next-generation DNA sequencing. Implemented more widely, this approach could create a worldwide surveillance capacity with the ability to monitor known disease agents (including the prevalence and evolution) and to discover new disease agents. As pathogens are identified, affordable, field-deployable diagnostics could be developed to reduce the burden of disease. These efforts will thus not only have an immediate impact on affected communities, but can help detect, monitor, and characterize emerging diseases before they become global threats.

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Acknowledgments: The work is supported by by NIH 1DP2OD006514-01, BAA-NIAID-DAIT-NIHAI2009061, and BAA–NAID-DAIT-NIHAI2008031. We thank S. Hart, C. Edwards, J. Schieffelin, and the Sabeti Lab.

Supplementary Materials

www.sciencemag.org/cgi/content/full/338/6108/750/DC1
Figs. S1 to S2
Tables S1 to S3
Reference S1
Note S1

10.1126/science.1225893

CHEMISTRY

Driving the Formation of Molecular Knots

A molecule with a trefoil knot topology can form spontaneously through noncovalent interactions, avoiding deliberate anchoring and assembly steps.

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Tying knots and handwriting constitute basic motor skills associated with the development of a child's conception of spatial relationships (*1*). For synthetic chemists, molecular knot tying—so-called topological stereochemistry (*2*)—often requires the slow, deliberate steps of children learning to tie their shoes. On page 783 of this issue, Ponnuswamy *et al*. report reaction conditions that appear to drive the spontaneous formation of a knotted organic molecule (*3*). Previous studies from Sanders' group under conditions known as dynamic combinatorial equilibria revealed a preference for the formation of densely packed aggregates, including topologically complex structures, such as catenanes (linked rings) (*4*). Recently, investigating the early stages of polymerization by disulfide linkage formation, they have found an unexpected product that appears to be a bona fide molecular knot.

Early studies discussed the improbability of spontaneous knot formation—the accidental threading of a knot in a molecular string [see, for example, (*5*)]. Strand anchoring—holding the relative orientation of the entwined strands in place until the knot is tied off—greatly improves the chances for threading, and by using coordination bonds to transition metal complexes (*6*, *7*), one can synthesize knots in high yield and ever-increasing variety (see the figure, panel A). More elaborate anchoring scaffolds have been used to obtain trefoil-like structures, but generality is still sought (*8*).

Anchoring strategies toward the synthesis of topologically complex structures are inherently focused on kinetic control of product formation; that is, the pieces are

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