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## Database Searches for Binding Sites

In their Report "Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons" (7 Apr., p. 136), G. G. Loots and colleagues identify conserved noncoding sequences (CNSs) in orthologous regions of the interleukin (IL)-4/13/5 locus of several species (interleukins are growth and differentiation factors involved in the immune response). They demonstrated that germ line deletion of one region, CNS-1 between the genes *IL-4* and *IL-13*, reduced the frequency of *IL-4* gene activation.

Referring to their sequence analysis of CNS-1, Loots and colleagues say, "Binding sites for transcription factors known to regulate the expression of *IL-4* and *IL-13* were not found in CNS-1," on the basis of searches of the Transcription Factor Database (<http://transfac.gbfraunschweig.de/TRANSFAC/index.html>). They included searches for GATA-3, c-Maf, STAT6, and NF-AT binding sites. Their statement has specific implications for the CNS-1 mechanism, excluding actions of known T helper cell type 2 (T<sub>H</sub>2)-specific factors, implying a need for unknown factors.

We searched the same database for these factor binding sites in CNS-1 and obtained one conserved consensus GATA-3 binding site and two NF-AT binding sites in the published sequence. The GATA-3 site resides 68 nucleotides upstream of the CNS-1 forward primer, within the region Loots *et al.* deleted for their experiments, and is conserved between mouse and human. The NF-AT sites are 5 nucleotides upstream and 31 nucleotides downstream of the CNS-1 forward primer and are conserved between mouse and human.

Although their biological significance requires study, these sites are within regions already described to exert GATA-3-dependent augmentation of the *IL-4* promoter (1). GATA-3 is a T<sub>H</sub>2-specific transcription factor (2) shown to exert chromatin remodeling effects on the *IL-4* and *IL-13* loci (3), and NF-AT family transcription factors regulate many T cell cytokine genes (4). These GATA-3 and NF-AT binding sites in CNS-1 may or

may not be involved in its activity, but their recognition in CNS-1 is important in consideration of this study and in future work in this field.

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### Response

Murphy points out an important issue concerning the use of transcription factor binding site databases, such as TRANSFAC (1), to characterize gene regulatory elements. Transcription factor binding sites are short (the "core sequence" is typically 4 base pairs in length) and highly degenerate; therefore, TRANSFAC searches invariably identify a greater number of false binding sites than functional binding sites. Because of this fact, we used relatively stringent search criteria to maximize the likelihood of discovering true binding sites in the conserved noncoding sequences, such as CNS-1, identified in our human-mouse sequence comparisons.

The criteria we used, as stated in reference 15 of our Report, included that the binding sites be conserved in multiple species in addition to humans and mice (rats, dogs, cows, and rabbits). This was based on the assumption that putative regulatory elements such as CNS-1 should have the same regulatory function in these mammals and, accordingly, should be a target for the same transcription factors. The second criterion used, which was not specifically outlined in our Report, was that the binding sites have a matrix similarity score (a quality rating) of  $\geq 0.9$ . The default matrix similarity threshold for MatInspector (2), the software tool that we used to search TRANSFAC, is 0.85. Neither the GATA-3 nor the two NF-AT sites pointed out by Murphy fit both of these criteria. The 3' NF-AT site, although conserved in all six species examined, had a matrix similarity score below the 0.9 cutoff value. The 5' GATA-3 and NF-AT sites, although conserved in humans and mice, were not in the region amplified and sequenced in multiple species.

Using databases such as TRANSFAC to identify putative regulatory sequences that are targets for known transcription factors is currently the only computational method available for identifying such elements. Although this is clearly a valuable approach, the results of TRANSFAC searches need to be carefully scrutinized, taking into consideration the analysis of orthologous sequences in multiple species to distinguish between real and spurious binding site matches. Although our analysis of CNS-1 did not identify binding sites that met our stringent search criteria, we do agree with Murphy that individuals studying *IL-4* and *IL-13* expression should not be deterred from examining this element for binding activity of transcription factors such as GATA-3, c-Maf, STAT6, and NF-AT.

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## Conservation Conundrum

P. Daszak, A. A. Cunningham, and A. D. Hyatt present a convincing argument in their Review "Emerging infectious diseases of wildlife—threats to biodiversity and human health" (*Science's Compass*, 21 Jan., p. 443) that emerging infectious diseases (EIDs) pose a risk to wildlife, and they suggest that EIDs most often result from a change in the ecology of the pathogen or the host (or both). A situation they did not mention is that, in some cases, the protection of threatened species can increase the risk of

an EID outbreak by allowing a close association between wildlife and domestic animals where one would not have naturally occurred. An important example is northern elephant seals (*Mirounga angustirostris*) (see figure at left), which were abundant in California and Baja California, Mexico, at the beginning of the 19th century before being nearly eliminated by hunting. During the population bottleneck that resulted, there may



have been fewer than 100 seals until sometime after 1900 (1). However, during the 20th century, this species made a remarkable recovery. In 1991, the population was estimated to be 127,000 (2).

Historically, grizzly bears (*Ursus arctos*), wolves (*Canis lupus*), mountain lions (*Puma concolor*), and humans were all potential seal predators (3), limiting distribution of seal populations. Predators are no longer present on the California mainland, and legal protection has allowed elephant seals to colonize the region. Since 1975, four breeding populations have established on the California mainland (2, 4, 5).

With expansion of their range in mainland areas, elephant seals face an increasing threat of EIDs. Seals may be especially vulnerable in both urban and agricultural areas, where they will encounter potential disease reservoirs in domestic animals and wildlife. Elephant seals also have extremely low variation in the class II major histocompatibility complex (6), which may increase the vulnerability of the species to disease (7, 8). Thus, an outbreak of morbillivirus (9) or other disease such as brucellosis (10) in elephant seals is possible. If spillover does occur, the disease could infect a large portion of the population and incur high mortality (11). In the case of northern elephant seals, an outbreak might persist and reoccur seasonally as individuals occasionally travel among breeding colonies, until surviving seals are immune to the disease. The risk of disease transmission to other marine species or terrestrial mammals and of spillback to domestic animals or even humans (10, 12) should also be considered.

Many injured or sick marine mammals haul-out or strand on mainland beaches. Stranding networks in Europe, the United States, and elsewhere should have direct lines of frequent communication with one another, and should be adequately staffed to serve as disease sentinels. Further, rehabilitation centers should restrict contact with domestic species and take precautions, including virus screening, when rereleasing animals to the wild.

It seems unwise to allow pinniped populations to establish new breeding or haul-out colonies on the mainland of California, especially in areas associated with humans and domestic animals. Without costly and risky intervention (13), this will be the best method to control the spread of EIDs to elephant seals and other pinniped populations.

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9. Seven known single-stranded RNA viruses (rinderpest virus, peste des petits ruminants virus, canine distemper virus, measles virus, phocine distemper virus, dolphin morbillivirus, and porpoise morbillivirus) make up the genus *Morbillivirus* (family Paramyxoviridae). These viruses are highly contagious and often cause epizootic events in previously unexposed host populations. At least five such epizootics have caused mass mortality in marine mammals since 1988, and numerous cases of distemper and incidence of morbillivirus infection have recently been reported in marine mammal species.
10. H. M. Ross *et al.*, *Vet. Rec.* **138**, 647 (1996).
11. H. McCallum and A. Dobson, *Trends. Ecol. Evol.* **10**, 190 (1995).
12. A. D. M. E. Osterhaus *et al.*, *Science* **288**, 1051 (2000).
13. Vaccines against morbillivirus in marine mammals are unwarranted and ill advised in most cases. For most populations, it would be unfeasible to vaccinate a significant portion of animals, and vaccination could have unforeseen genetic consequences for the population involved.

#### Response

Brownell *et al.* highlight a dilemma that is likely to increasingly challenge wildlife conservation programs: Protection measures may drive disease emergence by increasing populations to artificially high levels, particularly if there has been prior anthropogenic removal of predation or other pressures. In our Review, in which we categorized EIDs of wildlife, we commented on this irony, citing published examples such as the in situ provisioning for wild birds leading to the emergence of salmonellosis (United Kingdom) and conjunctival mycoplasmosis (United States).

Brownell *et al.* suggest that the recovery of the northern elephant seal population, subsequent to specific protection measures, has led to an expansion of this species' range and, consequently, to an unnatural association with domestic animals and their pathogens. They are correct to draw attention to such a risk. For example, the "spillover" of pathogens from domestic animals to pinnipeds and cetaceans has been previously proposed to explain the occurrence of canine distemper virus in Siberian seals (*Phoca*

*siberica*) (1) and a Caspian seal (*P. caspica*) (2), and toxoplasmosis in a spinner dolphin (*Stenella longirostris*) (3) and two Beluga whales (*Delphinapterus leucas*) (4). Spillover events underlie a large proportion of wildlife EIDs and usually result from the anthropogenic translocation of domestic animals with their pathogens (pathogen co-introduction).

Although Brownell *et al.* propose some useful measures to combat such disease threats to elephant seals, their proposal to prevent establishment of new breeding or haul-out colonies on mainland California appears to be based on a false premise, favoring an unnatural situation over the natural ecology of California. The northern elephant seal was historically abundant along the Californian coast, despite the presence of predators. The ultimate goal of most conservation programs for species that have had their population reduced by human activities is to return them to their former ranges and levels. We believe that the northern elephant seal should not be prevented from returning to former breeding sites, but other protective measures should be put in place, such as the elimination of exotic (domestic) species from these areas, or the prevention of contact between these two groups. The disease risk posed to the northern elephant seal by domestic species and their pathogens fits into our category of "pathogen pollution" described in our Review. We coined this term to firmly implicate human activity in driving wildlife disease emergence, and to raise awareness that this is a form of pollution in much the same way as chemical pollution. The former is, perhaps, even more insidious a threat to biodiversity than the latter, given the global nature of biological introductions, and the high impact of introduced diseases on naïve host populations.

The example of the northern elephant seal illustrates the complex issues involved in developing conservation policies for endangered species, and emphasizes that protective measures, including legislation, should encompass strategies to counter disease threats. These measures should be adapted as we learn more about threats to biodiversity.

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