

at sufficient density on the surface of tumor vascular endothelium but absent from normal vascular endothelium (15). Promising candidate molecules for humans include endoglin (16), endosialin (17), an endoglin-like molecule (18), a fibronectin isoform (19), an osteosarcoma-related antigen (20), CD34 (21), collagen type VIII (22), the vascular endothelial cell growth factor (VEGF) receptors (23), and VEGF itself (24). The induction of tumor infarction by targeting a thrombogen to these or other tumor endothelial cell markers represents an intriguing approach to the eradication of primary solid tumors and vascularized metastases.

REFERENCES AND NOTES

- J. Denekamp, *Cancer Metastasis Rev.* **9**, 267 (1990).
- R. K. Jain, *Sci. Am.* **271**, 58 (July 1994).
- E. W. Davie, K. Fujikawa, W. Kiesel, *Biochemistry* **30**, 10363 (1991).
- Human tTF (residues 1 to 219) was prepared as described [M. J. Stone, W. Ruf, D. J. Miles, T. S. Edgington, P. E. Wright, *Biochem. J.* **310**, 605 (1995)].
- W. Ruf, A. Rehemtulla, T. S. Edgington, *J. Biol. Chem.* **266**, 2158 (1991).
- L. R. Paborsky, I. W. Caras, K. L. Fisher, C. M. Gorman, *ibid.*, p. 21911; R. Bach, R. Gentry, Y. Nemerson, *Biochemistry* **25**, 4007 (1986); S. Krishnaswamy, K. A. Field, T. S. Edgington, *J. Biol. Chem.* **267**, 26110 (1992).
- The C1300(Mu γ) tumor model (14) was modified as follows: (i) we used antibody B21-2 to target I-A d ; (ii) we used C1300(Mu γ) tumor cells, a subline of C1300(Mu γ)12 tumor cells, that grew continuously in BALB/c *nu/nu* mice; and (iii) we did not add tetracycline to the mice's drinking water to prevent gut bacteria from inducing I-A d on the gastrointestinal epithelium. Unlike immunotoxins, coagulgands do not damage I-A d -expressing intestinal epithelium.
- The B21-2 (T1B-229) hybridoma, secreting a rat immunoglobulin G2b (IgG2b) antibody to the I-A d antigen, was purchased from the American Type Culture Collection. The CAMPATH-2 antibody is a rat IgG2b antibody to human CD7. The TF9-10H10 antibody (herein referred to as 10H10) is a mouse IgG1 nonneutralizing antibody to human TF [J. H. Morrissey, D. S. Fair, T. S. Edgington, *Thromb. Res.* **52**, 247 (1988)]. The MRC OX7 hybridoma (herein referred to as OX7) secretes a mouse IgG1 antibody that recognizes the Thy 1.1 antigen. The bispecific antibodies B21-2/10H10, CAMPATH-2/10H10, OX7/10H10, and B21-2/OX7 were synthesized as described [M. Brennan, P. F. Davison, H. Paulus, *Science* **229**, 81 (1985)].
- To establish solid tumors, we injected 1.5×10^7 C1300(Mu γ) cells subcutaneously into the right anterior flank of BALB/c *nu/nu* mice (Charles River Labs, Wilmington, MA). When the tumors had grown to ~ 0.8 cm in diameter, mice were randomly assigned to different experimental groups, each containing four to nine mice. Coagulgands were prepared by mixing bispecific antibodies (150 μ g) and tTF (125 μ g) in a total volume of 2.5 ml of 0.9% NaCl and incubating at 4°C for 1 hour. Mice received intravenous injections of 0.25 ml of this mixture per 25 g of body weight (that is, 0.6 mg/kg of bispecific antibody plus 0.5 mg/kg of tTF). Other mice received equivalent doses of bispecific antibodies or tTF alone. The injections were performed over ~ 45 s into one of the tail veins, followed by 200 μ l of saline. In the tumor growth-inhibition experiments, the infusions were repeated 6 days later. Perpendicular tumor diameters were measured at regular intervals and tumor volumes were calculated. Differences in tumor volume were tested for statistical significance with the Mann-Whitney rank sum test for two independent samples. For histopathologic analyses, mice were anesthetized with metopane at various intervals after treatment and were exsanguinated by perfusion with heparinized saline. Tumors and normal tissues were excised and immediately fixed in 3% (v/v) formalin. Paraffin sections were cut and stained with hematoxylin and eosin or with Martius Scarlet Blue trichrome for the detection of fibrin. Animal care in all experiments was in accordance with Institutional guidelines.
- L. B. Zacharski *et al.*, *J. Natl. Cancer Inst.* **85**, 1225 (1993); J. C. Murray, M. Clauss, G. Thurston, D. Stern, *Int. J. Radiat. Biol.* **60**, 273 (1991).
- At the treatment dose of 0.6 mg/kg B21-2/10H10 plus 0.5 mg/kg tTF, toxicity was observed in only 2 of 40 mice (thrombosis of the tail vein). The tTF itself was not toxic at 1.28 mg/kg when given intravenously.
- S. King and P. E. Thorpe, unpublished data.
- F. J. Burrows and P. E. Thorpe, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8996 (1993); F. J. Burrows and P. E. Thorpe, *Pharmacol. Ther.* **64**, 155 (1994).
- F. J. Burrows, Y. Watanabe, P. E. Thorpe, *Cancer Res.* **52**, 595 (1992).
- J. Folkman, *Semin. Cancer Biol.* **3**, 65 (1992).
- F. J. Burrows *et al.*, *Clin. Cancer Res.* **1**, 1623 (1995).
- W. J. Rettig *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10832 (1992).
- J. M. Wang *et al.*, *Int. J. Cancer* **54**, 363 (1993).
- B. Carnemolla *et al.*, *J. Cell Biol.* **108**, 1139 (1989).
- O. S. Bruland, O. Fodstad, A. E. Stenwig, A. Pihl, *Cancer Res.* **48**, 5302 (1988).
- R. O. Schlingemann *et al.*, *Lab. Invest.* **62**, 690 (1990).
- W. Paulus, E. H. Sage, U. Lischka, M. L. Iruela-Arispe, K. Jellinger, *Br. J. Cancer* **63**, 367 (1991).
- K. H. Plate, G. Breier, B. Millauer, A. Ullrich, W. Risau, *Cancer Res.* **53**, 5822 (1993); L. F. Brown *et al.*, *ibid.*, p. 4727.
- H. F. Dvorak *et al.*, *J. Exp. Med.* **174**, 1275 (1991).
- M. Trucco and S. dePetris, in *Immunological Methods*, I. Lefkovits and B. Pernis, Eds. (Academic Press, New York, 1981), vol. 2, p. 1.
- We thank G. Hale for CAMPATH-2, A. F. Williams for the OX7 hybridoma, A. Gilman for comments on the manuscript and for support, E. Derbyshire and C. Gottstein for discussions, J. Overholser for technical assistance, W. Ruf for tTF, and K. Schiller for help in manuscript preparation. Supported in part by grants from the Pardee Foundation and NIH (RO1-CA59569, RO1-CA54168, and PO1-HL16411).

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Geographic Distribution of Endangered Species in the United States

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Geographic distribution data for endangered species in the United States were used to locate "hot spots" of threatened biodiversity. The hot spots for different species groups rarely overlap, except where anthropogenic activities reduce natural habitat in centers of endemism. Conserving endangered plant species maximizes the incidental protection of all other species groups. The presence of endangered birds and herptiles, however, provides a more sensitive indication of overall endangered biodiversity within any region. The amount of land that needs to be managed to protect currently endangered and threatened species in the United States is a relatively small proportion of the land mass.

Previous studies have shown that, on a continental scale, the distributions of well-studied taxa can act as surrogates or indicators for the distribution of poorly studied taxa (1–4). In contrast, studies of the distribution of "hot spots" of diversity for various taxa within the British Isles suggest that there is very little correlation between the distributions of different taxonomic groups (5, 6). To date, however, no such analysis has been done on a continental or national scale for those species most likely to vanish in the foreseeable future, that is, endangered species. If significant correlations occur in the geographic distributions of different groups of endangered species, it may be possible to use a few well-studied groups as indicators for the purposes of delineating protected areas for other poorly

known taxa. The extent to which endangered species are concentrated in hot spots of potential extinctions and the extent to which hot spots for different groups overlap will influence the strategies we adopt to avert species extinctions and the impact of those strategies on other human activities (7, 8). If endangered species are highly concentrated, then fewer areas are likely to experience conflicts between species protection and other activities.

In this study, we used a database of threatened and endangered species in the United States to examine patterns in the geographic distribution of imperiled species (9). The database lists the counties of occurrence of all plants and animals protected under the federal Endangered Species Act in the 50 states, plus all species, subspecies, and populations proposed for protection under that statute as of August 1995 (a total of 924 species in 2858 counties). We grouped the species by state, county, and species group (amphibians, arachnids, birds, clams, crustacea, fish, insects, mammals, plants, reptiles, and snails) and then generated dis-

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tribution maps using a geographic information system (10). These maps were designed to identify areas with unusually large numbers of endangered species.

A sorting algorithm based on the principle of complementary subsets was used to evaluate the extent to which endangered species are clustered into hot spots (11–13). The algorithm first selected the county with the greatest number of listed species; all species found in that county were then excluded from further consideration while the algorithm searched for the county with the greatest number of species that were not already selected. Ties for number of species were broken by assignment of top rank to the county with the smallest area (or secondarily, the county with the smallest human population). This process was continued iteratively until all listed species were included. The algorithm maximizes the number of species sampled while minimizing the area required to do so. It is clearly erroneous to assume, however, that because a particular species occurs in a county, a viable population can be maintained in that county. In this respect, our analysis underestimates the amount of land necessary to preserve species with large area require-

ments (such as grizzly bears, *Ursus arctos horribilis*). On the other hand, it is equally inaccurate to assume that the entire land area of a county is occupied by its endangered species. Thus, our analysis should not be taken as a measurement of how much land must be protected to conserve endangered species but rather as an approximate indication of the extent to which endangered species are concentrated geographically. We then subdivided the data and repeated the analysis for each species group to determine whether any particular group could be used as an overall indicator for others.

The greatest numbers of endangered species occur in Hawaii, southern California, the southeastern coastal states, and southern Appalachia (Fig. 1). When counties are selected on the basis of complementarity, the algorithm first selects counties in these regions (Fig. 2). The complementary ordering of counties generates accumulation curves that can be used to examine the extent to which endangered species are clustered in hot spots. The accumulation curves represent the total area required to sample all the endangered species in each taxonomic group when the counties are

ranked from those with the most endangered species to those with the least (Fig. 3, A and B). For each group, more than 50% of endangered species are represented within 0.14 to 2.04% of the land area (14). For endangered birds, reptiles, and mammals, the sequential selection of counties on the basis of the unique species they contain leads to a steady increase in the number of populations of each endangered species already included in the counties sampled (Fig. 3C). The number of populations of most endangered plant and invertebrate species does not increase because many of these species are restricted to single counties. The data show that 48% of plants and 40% of arthropods are restricted to single counties. The average number of counties in which a listed plant or arthropod species is found is 3.9 and 4.4 counties, respectively. In contrast, only 36% of listed bird species are confined to single counties, whereas the average number of counties in which a listed bird is found is 62.7 (15). Comparable figures on the percentage of single-county species within other groups and the average number of counties in which a listed species is found are as follows: mammals, 26%, 32.9 counties per species; fish, 31%, 8.0 counties

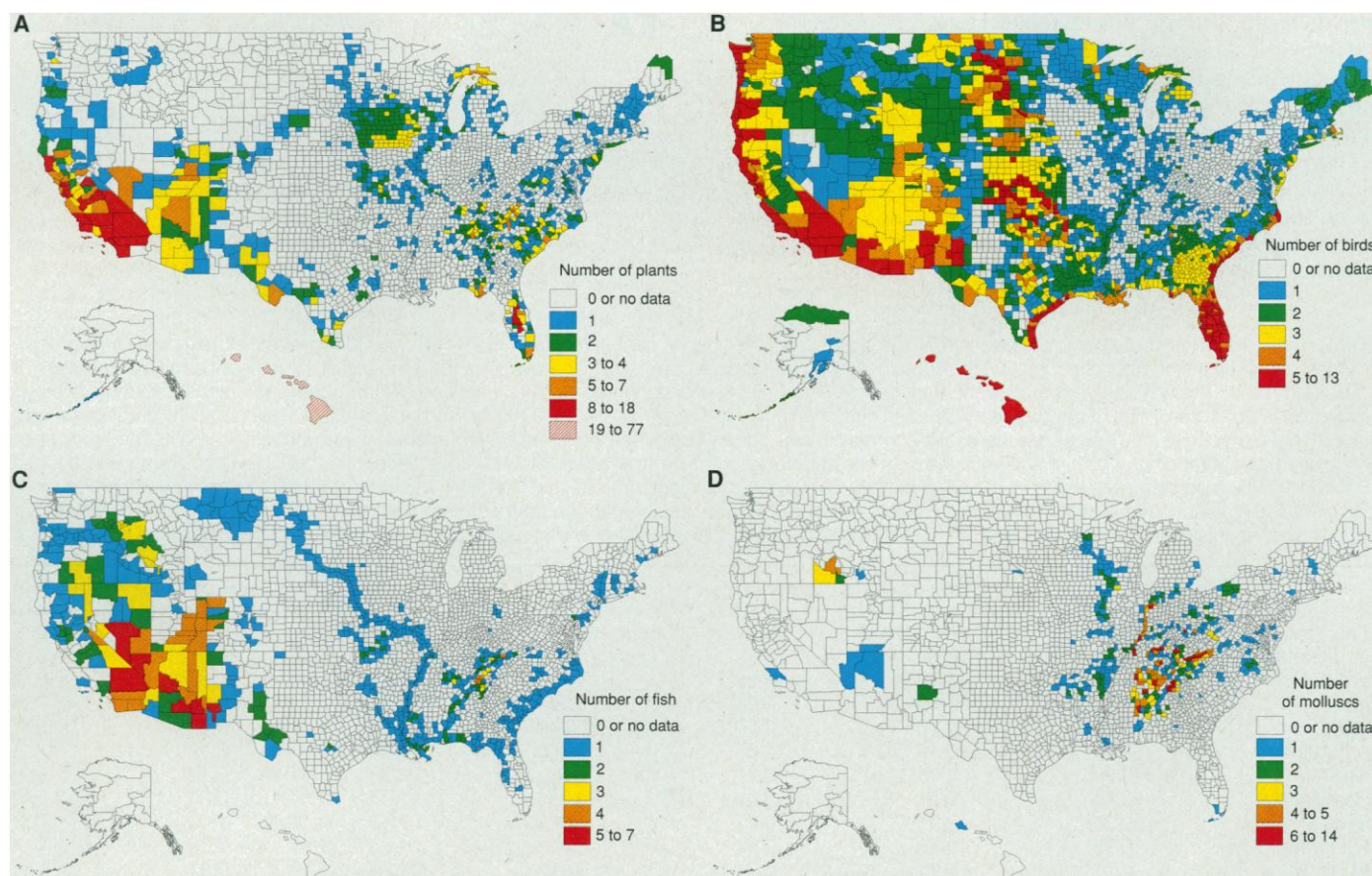


Fig. 1. The geographic distribution of four groups of endangered species in the United States. (A) Plants, (B) birds, (C) fish, and (D) molluscs. The maps illustrate the number of listed species in each county. Alaska and Hawaii are shown in the bottom left-hand corner of the maps (not to scale).

per species; herptiles (reptiles and amphibians), 14%, 18.8 counties per species; snails, 57%, 2.1 counties per species; and clams, 3%, 12.1 counties per species.

The utility of using any one group of endangered species as an indicator for other groups can be quantified by calculating the proportion of each other group that occurs in the subsets of counties that contain all the species in any individual group (Table 1). An initial examination of this table suggests that the counties that contain a complete set of endangered plant species will contain the greatest numbers of other endangered species. However, more counties are required to adequately sample endangered plants than are required for any other taxa, so we would expect this larger area to contain more species from other taxa. An area-independent index of predictive power may be obtained by comparing the number of species contained in the complementary counties for each group with the number of species that would occur if a set of counties of about the same total area were selected at random. The ratio of these two values provides an indication of how accurately the presence of endangered species in one group indicates the presence of endangered species in other groups. This index suggests that birds and then herptiles provide the best indicators for any particular area. In contrast, the presence of endangered fish or plant species provides only a weak indication that other endangered species are present in a given county.

We also examined the associations between the density of endangered species in each state, the intensity of human economic and agricultural activities, and the cli-

mate, topology, and vegetative cover of the state. We collated data on a variety of economic and topographic indicators using the annual statistical survey of the United States (16). Although there are complex and subtle associations between the variables included in this analysis, our initial stepwise multiple-linear regression analysis reveals that the overall density of endangered species is correlated with one anthropogenic and one climatic variable (correlation coefficient $r^2 = 0.80$, $P < 0.01$): the value of agricultural output and either average temperature or rainfall (17). When the analysis was repeated for each major taxonomic group, slightly different results were obtained. In particular, agricultural ac-

tivity is the key variable for plants ($r^2 = 0.61$, $P < 0.01$), mammals ($r^2 = 0.68$, $P < 0.01$), birds ($r^2 = 0.64$, $P < 0.01$), and reptiles ($r^2 = 0.46$, $P < 0.05$). Water use and human population density are also significant predictors of the density of endangered reptiles ($r^2 = 0.42$, $P < 0.01$). As did previous studies of patterns of overall species richness (18–20), we found that geographic variables significantly influence the distribution of endangered species. For example, the diversity of endangered fish increases with the mean temperature and elevation of the state ($r^2 = 0.27$, $P < 0.01$). Climatic variables, such as mean temperature and rainfall, are the second or third most important independent variables

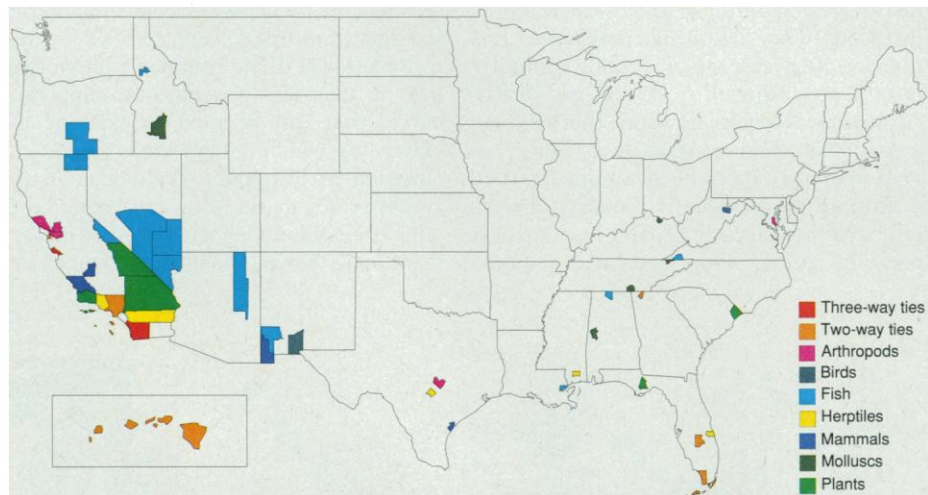


Fig. 2. Complementary set of counties that contains 50% of the listed species for each taxonomic group. The analysis identified two counties that contain large numbers of endangered species from three groups and nine counties that contain large numbers of species from two groups (Hawaii not to scale).

Table 1. Proportion of endangered species in other groups that are included in complementary county sets containing all the species in a given group. The second row gives the number of counties in the complementary set for each group; the third row gives the total area of these counties as a percentage of the U.S. land mass. The next eight rows give the total proportion of all other endangered species contained in the complementary set for any given group (columns). Power is an index of how well each species group indicates endangered species diversity in other groups; it is calculated by dividing the number of endangered species from other groups in this complementary county set by the number of such species in an equivalent area of randomly selected counties. A bootstrapping algorithm accumulated counties at random until their total area matched or just exceeded that of the complementary county set. For power_a, the algorithm selected from all U.S. counties. For power_b, the algorithm selected only from counties listed as containing endangered species. Because the area encompassed by the random county sets typically was greater than that of the complementary county sets, power underestimates the efficiency of each species group as an indicator for other groups. Power values are means ($\pm$ SE) of 200 runs of the bootstrapping algorithm.

	Plants	Molluscs	Arthropods	Fish	Herptiles	Birds	Mammals
Species (<i>n</i>)	503	84	57	107	43	72	58
Counties (<i>n</i>)	136	38	37	57	28	19	29
Area (%)	9.61	1.15	2.38	4.76	0.97	1.59	2.08
Plants	1.00	0.16	0.22	0.15	0.14	0.38	0.27
Molluscs	0.39	1.00	0.29	0.44	0.01	0.02	0.06
Arthropods	0.54	0.14	1.00	0.16	0.44	0.12	0.19
Fish	0.55	0.15	0.21	1.00	0.09	0.13	0.21
Herptiles	0.74	0.21	0.49	0.35	1.00	0.35	0.42
Birds	0.94	0.43	0.47	0.38	0.42	1.00	0.53
Mammals	0.76	0.38	0.43	0.40	0.33	0.38	1.00
All others	0.73	0.21	0.31	0.18	0.25	0.31	0.28
Power _a	1.63 (0.02)	2.92 (0.08)	2.44 (0.11)	1.24 (0.04)	3.26 (0.17)	4.00 (0.16)	2.61 (0.08)
Power _b	1.46 (0.01)	2.67 (0.06)	2.66 (0.59)	1.10 (0.02)	2.67 (0.06)	3.29 (0.09)	2.40 (0.08)

for endangered plants, reptiles, and clams.

Virtually all taxa are characterized by aggregated geographic distributions of endangered species (21). These hot spots are probably the product of two interacting factors: centers of endemism [for example, clams in southwest Appalachia (22) and plants in Florida (20)] and anthropogenic activities (for example, urbanization and agricultural development). Consequently, in a few areas of the United States, the centers of endangered richness for different groups overlap. Two counties are hot spots for three groups: San Diego, California (fish, mammals, and plants), and Santa Cruz, California (arthropods, herptiles, and plants). Nine counties are hot spots for two groups: Hawaii, Honolulu, Kauai, and Maui, Hawaii (all birds and plants); Los Angeles, California (arthropods and birds); San

Francisco, California (arthropods and plants); Highlands, Florida (herptiles and plants); Monroe, Florida (birds and mammals); and Whitfield, Georgia (fish and molluscs). Aside from these locations, the key areas for most groups overlap only weakly, which suggests that the endangered species hot spots for one group do not necessarily correspond with those for other groups. Nevertheless, the analysis confirms previous studies that suggest birds (2, 23), and perhaps arthropods (1), act as important indicators for the presence of other endangered species. Unfortunately, the data available for endangered plants and arthropods are considerably less complete than those for other taxa (24, 25). Increasing efforts to obtain information on these taxa is crucial to obtain a more complete picture of the geographic distribution of endangered species in the United States.

Although there are no consistent correlations in the distributions of endangered species from different taxa, the existence of hot spots for most groups indicates that a large proportion of endangered species can be protected on a small proportion of land (26). If conservation efforts and funds can be expanded in a few key areas, it should be possible to conserve endangered species with great efficiency.

REFERENCES AND NOTES

1. D. L. Pearson and F. Cassola, *Conserv. Biol.* **6**, 376 (1992).
2. C. J. Bibby *et al.*, *Putting Biodiversity on the Map: Priority Areas for Global Conservation* (International Council for Bird Preservation, Cambridge, 1992).
3. J. M. Scott, B. Csuti, J. D. Jacobi, J. E. Estes, *BioScience* **37**, 782 (1987).
4. J. M. Scott *et al.*, *Wildl. Monogr.* **123**, 1 (1993).
5. J. R. Prendergast, R. M. Quinn, J. H. Lawton, B. C. Eversham, D. W. Gibbons, *Nature* **365**, 335 (1993).
6. P. Williams *et al.*, *Conserv. Biol.* **10**, 155 (1996).
7. E. Dinerstein and E. D. Wikramanayake, *ibid.* **7**, 53 (1993).
8. R. L. Pressey, *ibid.* **8**, 662 (1994).
9. United States Environmental Protection Agency, *Endangered Species by County Database* (Office of Pesticide Programs, Washington, DC, 1995).
10. MapViewer.I. Golden Software (Golden Software, Golden, CO, 1995).
11. R. I. Vane-Wright, C. J. Humphries, P. H. Williams, *Biol. Conserv.* **55**, 235 (1991).
12. R. L. Pressey, H. P. Possingham, C. R. Margules, *ibid.* **76**, 259 (1996).
13. R. L. Pressey, C. J. Humphries, C. R. Margules, R. I. Vane-Wright, P. H. Williams, *Trends Ecol. Evol.* **8**, 124 (1993).
14. Half of the currently listed plant species are found in the 13 highest ranked counties in their complementary county subset; the total area of these counties is 1.33% of the U.S. land mass. The equivalent figures for the other groups are as follows: molluscs, 6 counties (0.14%); arthropods, 9 counties (0.46%); fish, 14 counties (2.04%); herptiles, 7 counties (0.34%); birds, 4 counties (0.28%); and mammals, 7 counties (0.40%).
15. Mean values for birds are inflated by the occurrence of peregrine falcons (*Falco peregrinus*) and bald eagles (*Haliaeetus leucocephalus*) in a large number of counties throughout the United States. If data for these two species are excluded, the mean number of counties that each endangered bird species was

located in would drop to 31.7, with 37% of endangered birds restricted to a single county.

16. U. S. Bureau of the Census, *Statistical Abstract of the United States: 1991* (U.S. Government Printing Office, Washington, DC, 1991).
17. The stepwise multiple regression analysis was performed on the entire data set and then on each major taxonomic division. Because complete sets of economic and geographic data are only available at the state level, the analysis was performed at this coarser geographic scale. The density of endangered species was expressed as the total number of endangered species recorded in the state, divided by total area of the state for all terrestrial species. In the case of predominantly aquatic species (fish and clams), only the area of each state classified as water or wetland was used to calculate density. The variables included in the analysis were the annual value of farm products produced in the state, the year in which the state was incorporated into the United States, water use in the state, manufacturing exports, percent of the net state area that is forested, percent of the state that is urban, percent of the state classified as wetlands, percent of the state classified as agricultural land, human population density in the state, percent of the human population living in urban areas, highest point in the state, average annual temperature in the state, and average annual rainfall in the state. The analysis was undertaken twice—once including Hawaii and once for just the mainland states. In both cases there was no substantial difference in the analyses, except for birds, plants, and all species combined. A large proportion of the endangered birds and plants occur only in Hawaii. When Hawaii is included in the analysis, its high density of endangered species and extreme values for several independent variables (such as extreme topography and tropical climate) combine to yield trends that are unrepresentative of the continental United States. For this reason, we have only provided results for the 49 continental states in the main text.
18. R. H. MacArthur, *Geographical Ecology: Patterns in the Distribution of Species* (Harper and Row, New York, 1972).
19. J. M. Adams, *Plants Today* **2**, 183 (1989).
20. A. H. Gentry, in *Conservation Biology: The Science of Scarcity and Diversity*, M. E. Soule, Ed. (Sinauer, Sunderland, MA, 1986), pp. 153–181.
21. C. H. Flather, L. A. Joyce, C. A. Bloomgarden, Anonymous, *Species Endangerment Patterns in the United States* RM-241, General Technical Report, Rocky Mountain Forest and Range Experimental Station, U.S. Department of Agriculture, Fort Collins, CO, 1994.
22. P. Banarescu, *Zoogeography of Fresh Waters*, vol. 2, *Distribution and Dispersal of Freshwater Animals in North America and Eurasia* (AULA-Verlag, Wiesbaden, Germany, 1992).
23. A. R. Kiestner *et al.*, *Conserv. Biol.*, in press.
24. B. A. Stein and R. M. Chipley, *Priorities for Conservation: 1996 Annual Report Card for U.S. Plant and Animal Species* (The Nature Conservancy, Arlington, VA, 1996).
25. D. S. Wilcove, M. McMillan, K. C. Winston, *Conserv. Biol.* **7**, 87 (1993).
26. Bringing these species to the point of recovery (by increasing their populations) would involve a greater amount of land than they currently occupy. However, as the geographic distributions of many endangered species do not overlap more than a single county, this is likely to be less of a problem for species groups with restricted ranges (such as plants and arthropods) than it is for birds and mammals.
27. We thank L. Turner and M. Hood at the Environmental Protection Agency for comments on the manuscript and for providing us with the raw data for this analysis; user support services at Golden Software, CO, for providing help in producing the maps in Figs. 1 and 2; and M. Scott, M. Bean, and three anonymous referees for comments on the manuscript. The work was made possible by a grant to the Environmental Defense Fund from the Charles Stewart Mott Foundation.

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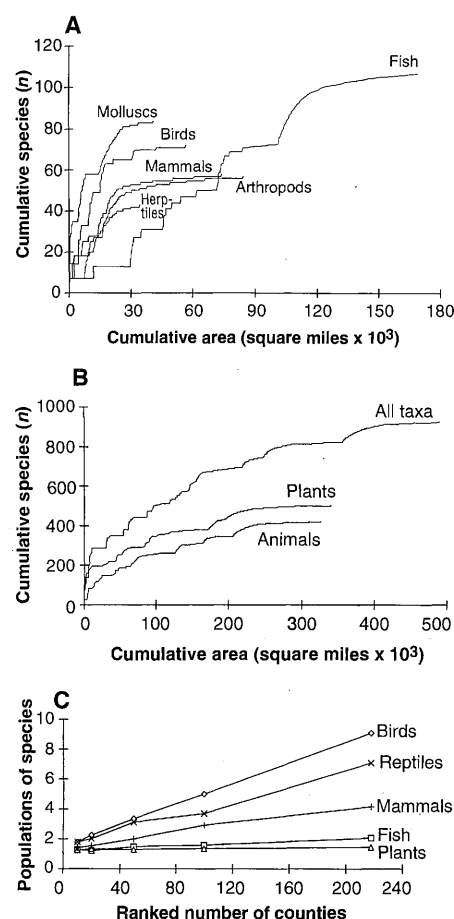


Fig. 3. (A and B) The relation between the cumulative area of land sampled and the cumulative number of listed species that are included. The sudden increases in the slopes of the curves occur when the algorithm switches to adding the next lowest integer number of species to the pool of species sampled—counties are added by picking the smallest counties that add this number of new species to the pool. (C) The average number of populations of each species in the sequentially selected counties.