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How many species of mammals are there?

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Accurate taxonomy is central to the study of biological diversity, as it provides the needed evolutionary framework for taxon sampling and interpreting results. While the number of recognized species in the class Mammalia has increased through time, tabulation of those increases has relied on the sporadic release of revisionary compendia like the *Mammal Species of the World* (MSW) series. Here, we present the Mammal Diversity Database (MDD), a digital, publically accessible, and updateable list of all mammalian species, now available online: https://mammaldiversity.org. The MDD will continue to be updated as manuscripts describing new species and higher taxonomic changes are released. Starting from the baseline of the 3rd edition of MSW (MSW3), we performed a review of taxonomic changes published since 2004 and digitally linked species names to their original descriptions and subsequent revisionary articles in an interactive, hierarchical database. We found 6,495 species of currently recognized mammals (96 recently extinct, 6,399 extant), compared to 5,416 in MSW3 (75 extinct, 5,341 extant)—an increase of 1,079 species in about 13 years, including 11 species newly described as having gone extinct in the last 500 years. We tabulate 1,251 new species recognitions, at least 172 unions, and multiple major, higher-level changes, including an additional 88 genera (1,314 now, compared to 1,226 in MSW3) and 14 newly recognized families (167 compared to 153). Analyses of the description of new species through time and across biogeographic regions show a long-term global rate of ~25 species recognized per year, with the Neotropics as the overall most species-dense biogeographic region for mammals, followed closely by the Afrotropics. The MDD provides the mammalogical community with an updateable online database of taxonomic changes, joining digital efforts already established for amphibians (AmphibiaWeb, AMNH's Amphibian Species of the World), birds (e.g., Avibase, IOC World Bird List, HBW Alive), non-avian reptiles (The Reptile Database), and fish (e.g., FishBase, Catalog of Fishes).

Una taxonomía que precisamente refleje la realidad biológica es fundamental para el estudio de la diversidad de la vida, ya que proporciona el armazón evolutivo necesario para el muestreo de taxones e interpretación de resultados del mismo. Si bien el número de especies reconocidas en la clase Mammalia ha aumentado con el tiempo, la tabulación de esos aumentos se ha basado en las esporádicas publicaciones de compendios de revisiones taxonómicas, tales como la serie *Especies de mamíferos del mundo* (MSW por sus siglas en inglés). En este trabajo presentamos la Base de Datos de Diversidad de Mamíferos (MDD por sus siglas en inglés): una lista digital de todas las especies de mamíferos, actualizable y accesible públicamente, disponible en la dirección URL https://mammaldiversity.org/. El MDD se actualizará con regularidad a medida que se publiquen artículos que describan nuevas especies o que introduzcan cambios de diferentes categorías taxonómicas. Con la tercera edición de MSW (MSW3) como punto de partida, realizamos una revisión en profundidad de los cambios taxonómicos publicados a partir del 2004. Los nombres de las especies nuevamente descriptas (o ascendidas a partir de subespecies) fueron conectadas digitalmente en una base de datos interactiva y jerárquica con sus

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descripciones originales y con artículos de revisión posteriores. Los datos indican que existen actualmente 6,495 especies de mamíferos (96 extintas, 6,399 vivientes), en comparación con las 5,416 reconocidas en MSW3 (75 extintas, 5,341 vivientes): un aumento de 1,079 especies en aproximadamente 13 años, incluyendo 11 nuevas especies consideradas extintas en los últimos 500 años. Señalamos 1,251 nuevos reconocimientos de especies, al menos 172 uniones y varios cambios a mayor nivel taxonómico, incluyendo 88 géneros adicionales (1,314 reconocidos, comparados con 1,226 en MSW3) y 14 familias recién reconocidas (167 en comparación con 153 en MSW3). Los análisis témporo-geográficos de descripciones de nuevas especies (en las principales regiones del mundo) sugieren un promedio mundial de descripciones a largo plazo de aproximadamente 25 especies reconocidas por año, siendo el Neotrópico la región con mayor densidad de especies de mamíferos en el mundo, seguida de cerca por la region Afrotrópical. El MDD proporciona a la comunidad de mastozoólogos una base de datos de cambios taxonómicos conectada y actualizable, que se suma a los esfuerzos digitales ya establecidos para anfibios (AmphibiaWeb, Amphibian Species of the World), aves (p. ej., Avibase, IOC World Bird List, HBW Alive), reptiles "no voladores" (The Reptile Database), y peces (p. ej., FishBase, Catalog of Fishes).

Key words: biodiversity, conservation, extinction, taxonomy

Species are a fundamental unit of study in mammalogy. Yet species limits are subject to change with improved understanding of geographic distributions, field behaviors, and genetic relationships, among other advances. These changes are recorded in a vast taxonomic literature of monographs, books, and periodicals, many of which are difficult to access. As a consequence, a unified tabulation of changes to species and higher taxa has become essential to mammalogical research and conservation efforts in mammalogy. Wilson and Reeder's 3rd edition of *Mammal Species of the World* (MSW3), published in November 2005, represents the most comprehensive and up-to-date list of mammalian species, with 5,416 species (75 recently extinct, 5,341 extant), 1,229 genera, 153 families, and 29 orders. That edition relied on expertise solicited from 21 authors to deliver the most comprehensive list of extant mammals then available. However, the episodic release of these massive anthologies (MSW1—Honacki et al. 1982; MSW2—Wilson and Reeder 1993; MSW3—Wilson and Reeder 2005) means that taxonomic changes occurring during or soon after the release of a new edition may not be easily accessible for over a decade. For example, MSW3, compared to MSW2, resulted in the addition of 787 species, 94 genera, and 17 families compared to MSW2 (Solari and Baker 2007). Since the publication of MSW3, there has been a steady flow of taxonomic changes proposed in peer-reviewed journals and books; however, changes proposed more than a decade ago (e.g., Carleton et al. 2006; Woodman et al. 2006) have yet to be incorporated into a Mammalia-wide reference taxonomy. This lag between the publication of taxonomic changes and their integration into the larger field of mammalogy inhibits taxonomic consistency and accuracy in mammalogical research, and—at worst—it can impede the effective conservation of mammals in instances where management decisions depend upon the species-level designation of distinctive evolutionary units.

The genetic era has catalyzed the discovery of morphologically cryptic species and led to myriad intra- and interspecific revisions, either dividing species (splits) or uniting them (lumps). Many groups of mammals are taxonomically complex and in need of further revision, especially those that have received relatively little systematic attention or are morphologically or behaviorally cryptic (e.g., shrews, burrowing mammals). For example, the phylogenetic placement of tenrecs and golden moles (families: Tenrecidae and Chrysochloridae) has long been a point of taxonomic contention, having variously been included within Insectivora, Eulipotyphla, and Lipotyphla. Taxonomic assignment of this group was only conclusively resolved when genetic data (Madsen et al. 2001; Murphy et al. 2001), as corroborated by morphology (Asher et al. 2003), aligned Tenrecidae and Chrysochloridae in the order Afrosoricida and found it allied to other African radiations in the superorder Afrotheria (Macroscelidea, Tubulidentata, Hyracoidea, Proboscidea, Sirenia). As analytical methods evolve and techniques become more refined, mammalian taxonomy will continue to change, making it desirable to create an adjustable list of accepted species-level designations and their hierarchical placement that can be updated on a regular basis. Such a list is needed to promote consistency and accuracy of communication among mammalogists and other researchers.

Here, using MSW3 as a foundation, we provide an up-to-date list of mammal species and introduce access to this species list as an amendable digital archive: the Mammal Diversity Database (MDD), available online at http://mammaldiversity.org. We compare our list to that of MSW3 to quantify changes in mammalian taxonomy that have occurred over the last 13 years and evaluate the distribution of species diversity and new species descriptions across both geography and time. We intend the MDD as a community resource for compiling and disseminating published changes to mammalian taxonomy in real time, rather than as a subjective arbiter for the relative strength of revisionary evidence, and hence defer to the peer-reviewed literature for such debates.

MATERIALS AND METHODS

Starting from those species recognized in MSW3, we reviewed > 1,200 additional taxonomic publications appearing after MSW3's end-2003 cutoff date in order to compile a list of every recognized mammal species. In addition to evaluating peer-reviewed manuscripts, other major references included the *Handbook of the Mammals of the World* volumes 1–6 (Wilson and Mittermeier 2009, 2011, 2014, 2015; Mittermeier et al. 2013; Wilson et al. 2016), *Mammals of South America* volumes 1 and 2 (Gardner 2007; Patton et al. 2015), *Mammals of Africa* volumes 1–6 (Kingdon et al. 2013), *Rodents of Sub-Saharan Africa* (Monadjem et al. 2015), *Taxonomy of Australian Mammals* (Jackson and Groves 2015), and *Ungulate Taxonomy* (Groves and Grubb 2011). We linked each species to its primary, descriptive publication and if a species was taxonomically revised since 2004, the associated revisionary publications also were linked. The list was curated for spelling errors and compared to the species recognized in MSW3 to determine the total change in the number of recognized species over the interval 1 January 2004 to 15 August 2017; the latter date was our cutoff for reviewing literature. As with MSW3 and the IUCN (2017) RedList, species totals for the MDD include mammalian species that have gone extinct during the last 500 years, an arbitrary period of time used to delimit species "recently extinct". The IUCN taxonomy was downloaded on 28 June 2017.

We considered "de novo" species descriptions to be those species recognized since MSW3 and named with novel species epithets (post-MSW3 proposal date), whereas "splits" are species established by resurrecting an existing name (i.e., elevated subspecies or synonym, and pre-MSW3 proposal). We based these 2 bins of new species on the epithet authority year to enable downstream analyses of species discovery trends. However, we acknowledge that this categorization is not precise regarding the more complex (and biologically interesting) issue of how many species were derived from new field discoveries of distinctive populations versus the recognition of multiple species within named forms (Patterson 1996). Nevertheless, we expected the de novo category to encompass those field discoveries along with other types of species descriptions, and the splits category to encompass instances where existing names are elevated or validated, both of which are categories warranting future investigation.

In addition to taxonomic ranks (order, family, genus, species) and primary data links, MDD species information includes the year of description, scientific authority, and geographic occurrence by biogeographic region. Here, we approximate the biogeographic realms defined by the World Wildlife Fund (Olson and Dinerstein 1998; Olson et al. 2001), with the exception that we classified countries split across multiple biogeographic realms as belonging exclusively to the realm covering the majority of that country. We defined the Nearctic realm as all of North America, including Florida, Bermuda, and all of Mexico. The Neotropical realm included all of South America, Central America, and the insular Caribbean. The Palearctic realm included all of Europe, northern Asia (including all of China), Japan, and northern Africa (Egypt, Algeria, Tunisia, Morocco, Western Sahara, Canary Islands, and the Azores). The Indomalayan realm included southern and southeastern Asia (Pakistan, India, Nepal, Bhutan, Vietnam, Laos, Myanmar) and all islands west of Sulawesi including the Greater Sundas and Philippines. The Afrotropical realm included all of sub-Saharan Africa and the Arabian Peninsula, plus Madagascar and the nearby Indian Ocean islands (e.g., Comoros, Mauritius, Seychelles). We grouped the Australasian and Oceanian realms to include a single category for Australia, New Zealand, Sulawesi, and the islands east of Sulawesi, including Melanesia, Polynesia, Micronesia, Hawaii, and Easter Island, but excluding the Palearctic Japanese Bonin Islands. There are no terrestrial mammal species native to Antarctica. Open-water and coastal marine species, including the few Antarctic breeding species (e.g., leopard seals, *Hydrurga*), were grouped separately. Freshwater species (e.g., river dolphins, river otters) were sorted by their resident landmass.

Based on our newly curated list, we calculated the number of new species described each decade since the origin of binomial nomenclature (Linnaeus 1758) to determine the major eras of species discovery and taxonomic description. The year 1758 includes all the species described by Linnaeus that are still currently recognized. For each biogeographic realm, we calculated the total number of mammalian species recognized and the number of new species recognized since 2004. Note that the recognition of new species in a particular region can reflect greater research efforts per region or taxon and thus cannot be extrapolated to the expected number of undiscovered species in that region. We scaled the number of species by regional land area (km^2—World Atlas 2017) to determine the most species-dense region.

RESULTS

The MDD currently lists 6,495 valid species of mammals (6,399 extant, 96 recently extinct), which is 1,079 more species than were recognized in MSW3 (1,058 extant and 21 extinct) and a 19.9% increase in species during about 13 years (Table 1). The MDD recognizes 1,251 new species described since MSW3 in categories of splits (720 species; 58%) and de novo species descriptions (531 species; 42%), indicating that at least 172 species were lumped together since the release of MSW3. The MDD documents a total of 1,314 genera (increasing by 88 from MSW3), 167 families (increasing by 14), and 27 orders (decreasing by 2). The MDD also includes 17 domesticated species in the listing to facilitate the association of

Table 1.—Comparison of Mammal Diversity Database (MDD) taxonomic totals and those of *Mammal Species of the World* (MSW) editions 1–3 and the International Union of Conservation of Nature (IUCN) RedList, version 2017-1.

Taxa	MSW1	MSW2	MSW3	IUCN	MDD
	1982	1993	2005	2017	This study
Species					
Total	4,170	4,631[a]	5,416	5,560	6,495
Extinct	NA	NA	75	85[b]	96
Living	NA	NA	5,341	5,475	6,399
Living wild	NA	NA	5,338	5,475	6,382
Genera	1,033	1,135	1,230	1,267	1,314
Families	135	132	153	159	167
Orders	20	26	29	27	27

[a] Corrected total per Solari and Baker (2007).

[b] Extinct IUCN mammals include both "EX" (extinct) and "EW" (extinct in the wild).

these derivatives of wild populations with their often abundant trait data (e.g., DNA sequences, reproductive data). Details of the full MDD version 1 taxonomy, including associated citations and geographic region assignments, are provided in Supplementary Data S1.

The largest mammalian families are in the order Rodentia—Muridae (834 species versus 730 in MSW3) and Cricetidae (792 species versus 681 in MSW3)—followed by the chiropteran family Vespertilionidae (493 species versus 407 in MSW3) and the eulipotyphlan family Soricidae (440 species versus 376 in MSW3). Unsurprisingly, the 2 most speciose orders (Rodentia and Chiroptera) witnessed the most species additions: 371 and 304 species, respectively. The most speciose rodent family besides Muridae and Cricetidae is Sciuridae (298 species) and 6 rodent families are monotypic: Aplodontiidae, Diatomyidae, Dinomyidae, Heterocephalidae, Petromuridae, and Zenkerellidae. The most speciose chiropteran families along with Vespertilionidae are Phyllostomidae (214 species) and Pteropodidae (197 species), whereas there is only 1 monotypic bat family: Craseonycteridae.

The increased number of recognized genera to 1,314 (from 1,230 in MSW3) results from the demonstrated paraphyly of several speciose and widely distributed former genera. This includes *Spermophilus*, which was split into 8 distinct genera (*Spermophilus*, *Urocitellus*, *Callospermophilus*, *Otospermophilus*, *Xerospermophilus*, *Ictidomys*, *Poliocitellus*, and *Notocitellus*—Helgen et al. 2009) and *Oryzomys*, which was split into 11 genera (*Oryzomys*, *Aegialomys*, *Cerradomys*, *Eremoryzomys*, *Euryoryzomys*, *Hylaeamys*, *Mindomys*, *Nephelomys*, *Oreoryzomys*, *Sooretamys*, and *Transandinomys*—Weksler et al. 2006). Many smaller generic splits broke 1 genus into 2 or more genera and often involved the naming of a new genus, such as with *Castoria* (formerly *Akodon*—Pardiñas et al. 2016), *Paynomys* (formerly *Chelemys*—Teta et al. 2016), and *Petrosaltator* (formerly *Elephantulus*—Dumbacher 2016). Other genera were described on the basis of newly discovered taxa, such as *Laonastes* (Jenkins et al. 2005), *Xeronycteris* (Gregorin and Ditchfield 2005), *Rungwecebus* (Davenport et al. 2006), *Drymoreomys* (Percequillo et al. 2011), and *Paucidentomys* (Esselstyn et al. 2012). The most speciose currently recognized genera are *Crocidura* (197 species), *Myotis* (126 species), and *Rhinolophus* (102 species). These also are the only genera of mammals that currently exceed 100 recognized and living species, with *Rhinolophus* reaching this level only recently.

Higher-level taxonomy also was significantly altered since 2004, with the recognition of 14 additional families and 2 fewer orders than MSW3. In the MDD, we included 3 families (†Megaladapidae, †Palaeopropithecidae, †Archaeolemuridae) that were not in MSW3 but that may have gone extinct in the last 500 years (McKenna and Bell 1997; Montagnon et al. 2001; Gaudin 2004; Muldoon 2010). The net addition of 11 other families in the MDD are the result of taxonomic splits and new taxon discoveries, as well as families lumped since MSW3. For example, Dipodidae was split into 3 families (Dipodidae, Zapodidae, Sminthidae—Lebedev et al. 2013), Hipposideridae into 2 (Hipposideridae, Rhinonycteridae—Foley et al. 2015), and Bathyergidae into 2 (Bathyergidae, Heterocephalidae—Patterson and Upham 2014). One family, Diatomyidae, was added based on a species discovery (*Laonastes aenigmamus*—Jenkins et al. 2005), although it was already known as a prehistorically extinct family (Dawson et al. 2006). Additional newly recognized families are Chlamyphoridae, Cistugidae, Kogiidae, Lipotidae, Miniopteridae, Pontoporiidae, Potamogalidae, Prionodontidae, and Zenkerellidae. Three families recognized in MSW3 have since been subsumed: Myocastoridae and Heptaxodontidae inside Echimyidae (Emmons et al. 2015), and Aotidae inside Cebidae (Schneider and Sampaio 2015; Dumas and Mazzoleni 2017). Note that Capromyidae is still recognized at the family level (Fabre et al. 2017). The order Cetacea also experienced major revisions, and is now included within the order Artiodactyla based on genetic and morphological data (Gatesy et al. 1999; Adams 2001; Asher and Helgen 2010). Soricomorpha and Erinaceomorpha also are grouped together in the order Eulipotyphla, given their shared evolutionary history demonstrated by genetic analyses (Douady et al. 2002; Meredith et al. 2011).

On average, since 1758, 24.95 species have been described per decade, including 3 major spikes in species recognition in the 1820–1840s, 1890–1920s, and 2000–2010s (Fig. 1). These bursts of systematic and taxonomic development were followed by 2 major troughs from about 1850–1880 and 1930–1990 (Fig. 1). Currently, we detect an accelerating rate of species description per decade, increasing from the 1990s (207 species), 2000s (341 species), and 2010s so far (298 species). A linear regression on these data suggests that if trends in mammalian species discovery continue, 120.46 species are yet to be discovered this decade, potentially resulting in a total of 418 new species to be recognized between 2010 and 2020 ($R^2 = 0.97$, $P < 0.000$; Fig. 1).

Across biogeographic regions, the Neotropics harbors the greatest number of currently recognized mammalian species (1,617 species), followed by the Afrotropics (1,572 species), and the Palearctic (1,162 species), whereas Australasia-Oceania has the least (527 species) (Fig. 2). The Neotropics also has the most newly recognized species (362 species—169 de novo and 193 split), again followed by the Afrotropics (357 species—158 de novo and 199 split), and with the fewest new species described from Australasia-Oceania (48 species—18 de novo and 30 split). Other categories included the marine (124 total species—4 de novo and 5 split), domesticated (17 total species—0 de novo and 2 split), and extinct (96 total species—7 de novo and 4 split; Fig. 2; Table 2) categories. When weighting the biogeographic realms by land area, we find the Neotropics and Afrotropics are also the most species-dense biogeographic regions (85.1 and 71.1 species per km^2, respectively), followed closely by Australasia-Oceania (61.4 species per km^2; Table 2). In all realms except the Indomalayan, more species were recognized via taxonomic splits than by de novo descriptions.

DISCUSSION

Mammalogists have a collective responsibility to serve the most current taxonomic information about mammalian biodiversity

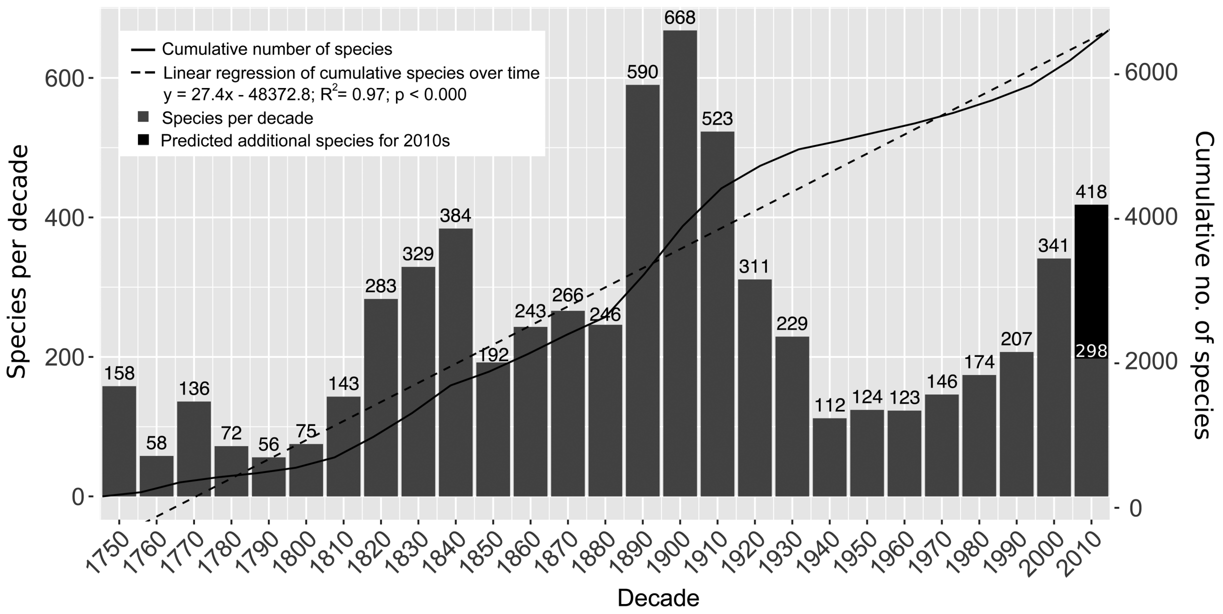

Fig. 1.—Cumulative and decadal descriptions of taxonomically valid extant mammal species from 1758 to 15 August 2017.

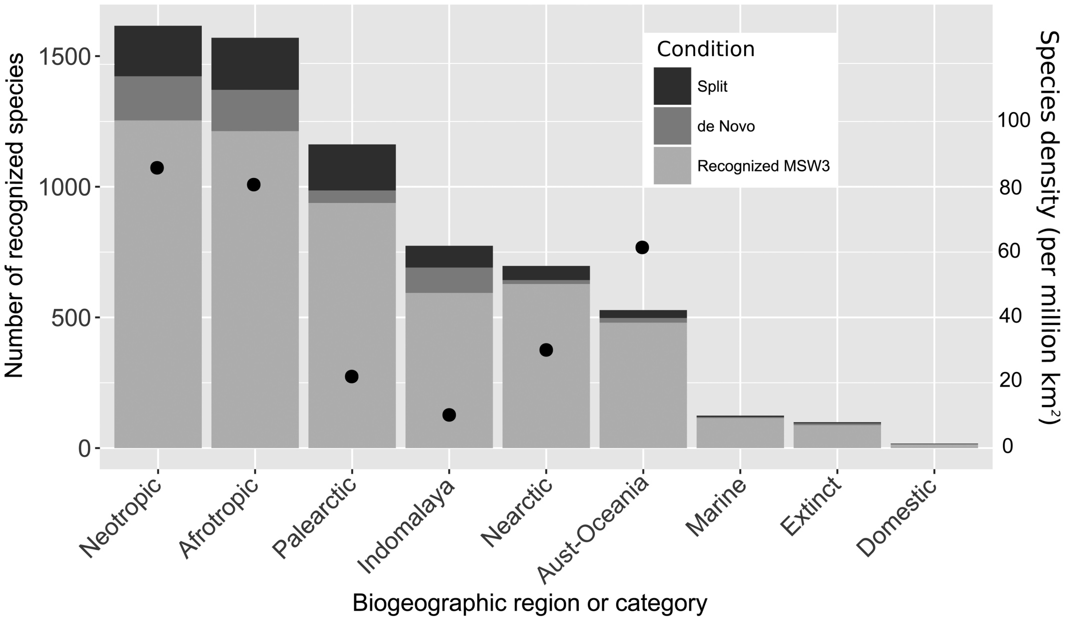

Fig. 2.—The number of mammalian species distributed in each biogeographical region: Palearctic, Afrotropic, Indomalayan, Nearctic, Neotropic, and Australasia-Oceania (i.e., Aust-Oceania), with marine, extinct, and domestic species in separate categories. Each group is divided into species recognized in both MSW3 and MDD, and new species in the MDD in categories of newly coined species epithet (de novo) versus existing species epithet (splits). The dot within each bar indicates the relative species density per km^2 land area, values are available in Table 2. MDD = Mammal Diversity Database; MSW3 = 3rd edition of *Mammal Species of the World*.

to the general public. The need for mammalian taxonomy to reflect our current understanding of species boundaries and evolutionary relationships is only expected to grow as efforts to synthesize "big data" increase in frequency, scope, and sophistication. Studies at this macroscale address major questions in evolution, ecology, and biodiversity conservation across the tree of life (e.g., Rabosky et al. 2012; Hedges et al. 2015; Hinchliff et al. 2015), yielding results relevant to global issues of sustainability that require our best data on biodiversity (Pascual et al. 2017). Mammalogists, in turn, benefit from easy access to this biodiversity data for purposes of study design, classroom teaching, analyses, and writing. The release of the MDD therefore addresses a key need in the mammalogical and global biodiversity communities alike. Whether we study the behavioral ecology of desert rodents or the macroevolution of tetrapods, biologists collectively need accurate measurements of species diversity—the most commonly assessed (but not the only) dimension of biodiversity (Jarzyna and Jetz 2016).

The MDD represents the most comprehensive taxonomic compendium of currently recognized mammals, documenting

Table 2.—The total number of mammal species in the Mammal Diversity Database (MDD) as compared to *Mammal Species of the World*, volume 3 (MSW3) that live within each biogeographic realm and those belonging to domestic and extinct categories. Numbers correspond to Fig. 2. Note that some species are found within multiple regions, so column totals do not correspond to taxonomic totals.

Category	Total species	Shared with MSW3	De novo	Split	Area (million km^2)	Density (species/km^2)
Neotropic	1,617	1,255	169	193	19.0	85.1
Afrotropic	1,572	1,215	158	199	22.1	71.1
Palearctic	1,162	938	48	176	54.1	21.5
Indomalaya	954	774	97	83	7.5	12.7
Nearctic	697	628	15	54	22.9	30.4
Aust-Oceania	527	479	18	30	8.6	61.4
Marine	124	115	4	5		
Domestic	17	15		2		
Extinct	96	85	7	4		

6,399 extant species (Tables 1 and 3) as well as 96 recently extinct species for a total of 6,495 species. This database is updateable and digitally searchable, tracking primary sources of species descriptions and phylogenetic studies of higher-level (genus or family) taxonomic changes and compiling them into a single listing. The MDD thus closes the gap between proposed taxonomic changes and integration into a broader understanding of mammalian diversity, and it then distributes this information to the scientific community and lay public as it is published in scientific literature. We aim for the MDD to build on this capacity as a record keeper to be a resource for hosting histories of taxonomic change. For example, the MDD records both the description of *Tapirus kabomani* (Cozzuol et al. 2013) and the later synonymy of this taxon under *T. terrestris* (Voss et al. 2014). Likewise, the revision of *Spermophilus* ground squirrels into 8 genera (Helgen et al. 2009) altered the binomial names of 28 species, a rearrangement that usefully established generic monophyly, but one that has not been readily summarized for workers without easy access to libraries. The MDD compiles data on genus transfers published since 2004 across all of Mammalia, helping to release researchers from undertaking piecemeal taxonomic updates for their projects.

Preliminary findings from the MDD compilation indicate that Primates has been a nexus of new species discovery, which is unexpected given their large body sizes. An incredible 148 primate species have been recognized since the publication of MSW3, including 67 de novo and 81 splits (Tables 1 and 3), a taxonomic outcome that is striking for our closest human relatives. Taxonomic revisions have centered around New World monkey families (Cebidae—Boubli et al. 2012; Pitheciidae—Marsh 2014) and many de novo species descriptions also occurred among Malagasy lemurs (Cheirogaleidae—Lei et al. 2014; Lepilemuridae—Louis et al. 2006). However, persistent taxonomic uncertainty within the family Cercopithecidae (Groves 2007a, 2007b; Mittermeier et al. 2013) suggests that the species-level diversity of Primates is not yet stable and will continue to fluctuate.

Among other taxonomic changes, the MDD documents the addition of 371 species of Rodentia, 304 species of Chiroptera, 86 species of Eulipotyphla, and 227 species of Artiodactyla, including many species from historically well-studied geographic regions (Table 2; Rausch et al. 2007; Castiglia et al. 2017). While the addition of > 300 species each of rodents and bats is unsurprising given their existing diversity, these clades may reasonably contain disproportionally high levels of cryptic diversity (e.g., Ruedi and Mayer 2001; Belfiore et al. 2008), and thus the application of genetic sequence data may continue to yield greater insights. Within Eulipotyphla (most particularly in shrews), we expect that the discovery of new species will continue given their rate of recent discoveries and frequency of morphological crypsis (Esselstyn et al. 2013). The species richness in *Sorex* (86 species) and *Crocidura* (197 species) suggests that genus-level revisions are needed and, when conducted, are likely to yield further taxonomic rearrangements (Castiglia et al. 2017; Matson and Ordóñez-Garza 2017).

The MDD includes a total of 465 species of non-cetacean Artiodactyla and Perissodactyla recognized by Groves and Grubb (2011) with select modifications based on taxonomic refinements published after the release of the latter (e.g., 4 species of *Giraffa* [Bercovitch et al. 2017] versus 8 [Groves and Grubb 2011]). This total compares to 240 species in these orders recognized in MSW3 (> 93% increase). Although some researchers have argued that the changes proposed by Groves and Grubb (2011) exemplify an extreme form of taxonomic inflation (Lorenzen et al. 2012; Zachos et al. 2013; Harley et al. 2016), the increase in species richness is comparable to concurrent rates of increase in the richness of Rodentia, Chiroptera, Eulipotyphla, and Primates. For now, inclusion of the taxonomy of Groves and Grubb (2011) in the MDD ensures that these taxa are vetted by the greater mammalogical community using multiple tiers of evidence (de Queiroz et al. 2007; Voss et al. 2014).

Following the publication of Linnaeus's 10th edition of *Systema Naturae* in 1758, the number of described species of mammals has increased at various rates, punctuated by factors including the efforts of prolific systematists and world events (Fig. 1). For example, Oldfield Thomas (1858–1929) of the British Museum (now the Natural History Museum, London), considered one of the "greatest taxonomists […] who ever lived" (Flannery 2012), was responsible for nearly 3,000 new names for genera, species, and subspecies (Hill 1990). In turn, reduced rates of species descriptions in the mid-20th century may be linked to periods of political instability and limited scientific activity during World War I (1914–1918) and II (1939–1945). Methodological innovations such as polymerase chain reaction (PCR—Mullis et al. 1989) may have driven

Table 3.—Totals of the genera and species per families and orders currently listed in the Mammal Diversity Database (MDD) online compilation, along with new species described since *Mammal Species of the World* volume 3 (MSW3) in categories of split or de novo, based on whether the specific epithet already existed or was newly coined, respectively.

	Genera	Species	New species since MSW3	
			Splits	De novo
Class Mammalia	1,314	6,495	720	531
Subclass Prototheria	3	5		
Order Monotremata	3	5		
Family Ornithorhynchidae	1	1		
Family Tachyglossidae	2	4		
Subclass Theria	1,311	6,490	720	531
Infraclass Marsupialia	91	379	32	29
Order Didelphimorphia	18	111	15	18
Family Didelphidae	18	111	15	18
Order Paucituberculata	3	7		1
Family Caenolestidae	3	7		1
Order Microbiotheria	1	3		2
Family Microbiotheriidae	1	3		2
Order Notoryctemorphia	1	2		
Family Notoryctidae	1	2		
Order Dasyuromorpha	19	78	5	5
Family Dasyuridae	17	76	5	5
Family Myrmecobiidae	1	1		
Family †Thylacinidae	1	1		
Order Peramelemorphia	8	23	1	1
Family †Chaeropodidae	1	1		
Family Peramelidae	6	20	1	1
Family Thylacomyidae	1	2		
Order Diprotodontia	41	155	11	2
Family Acrobatidae	2	3	1	
Family Burramyidae	2	5		
Family Hypsiprymnodontidae	1	1		
Family Macropodidae	13	67	3	
Family Petauridae	3	12		1
Family Phalangeridae	6	30	3	1
Family Phascolarctidae	1	1		
Family Potoroidae	4	12	1	
Family Pseudocheiridae	6	20	3	
Family Tarsipedidae	1	1		
Family Vombatidae	2	3		
Infraclass Placentalia	1,220	6,111	684	502
Superorder Afrotheria	34	89	8	6
Order Tubulidentata	1	1		
Family Orycteropodidae	1	1		
Order Afrosoricida	20	55	1	3
Family Chrysochloridae	10	21		
Family Potamogalidae[a]	2	3		
Family Tenrecidae	8	31	1	3
Order Macroscelidea	5	20	2	3
Family Macroscelididae	5	20	2	3
Order Hyracoidea	3	5	1	
Family Procaviidae	3	5	1	
Order Proboscidea	2	3		
Family Elephantidae	2	3		
Order Sirenia	3	5		
Family Dugongidae	2	2		
Family Trichechidae	1	3		
Superorder Xenarthra	14	30		
Order Cingulata	9	20		
Family Chlamyphoridae[b]	8	13		
Family Dasypodidae	1	7		
Order Pilosa	5	10		
Family Bradypodidae	1	4		
Family Cyclopedidae	1	1		
Family Megalonychidae	1	2		
Family Myrmecophagidae	2	3		

Table 3.—*Continued*

	Genera	Species	New species since MSW3	
			Splits	De novo
Superorder Euarchontoglires	616	3,194	285	249
Order Scandentia	4	24	4	
Family Ptilocercidae	1	1		
Family Tupaiidae	3	23	4	
Order Dermoptera	2	2		
Family Cynocephalidae	2	2		
Order Primates	84	518	81	67
Family †Archaeolemuridae[c]	1	2		
Family Atelidae	4	25	3	
Family Cebidae[d]	11	89	27	2
Family Cercopithecidae	23	160	24	5
Family Cheirogaleidae	5	40	1	20
Family Daubentoniidae	1	1		
Family Galagidae	6	20	2	2
Family Hominidae	4	7		
Family Hylobatidae	4	20	3	2
Family Indriidae[e]	3	19	2	6
Family Lemuridae	5	21	2	
Family Lepilemuridae	1	26		16
Family Lorisidae	4	15	6	1
Family †Megaladapidae[c]	1	1		
Family †Palaeopropithecidae[c]	1	1		
Family Pitheciidae	7	58	9	9
Family Tarsiidae	3	13	2	4
Order Lagomorpha	13	98	10	1
Family Leporidae	11	67	5	1
Family Ochotonidae	1	30	5	
Family †Prolagidae	1	1		
Order Rodentia	513	2,552	190	181
Family Abrocomidae	2	10		
Family Anomaluridae	2	6		
Family Aplodontiidae	1	1		
Family Bathyergidae	5	21	3	4
Family Calomyscidae	1	8		
Family Capromyidae	7	17		
Family Castoridae	1	2		
Family Caviidae	6	21	3	
Family Chinchillidae	3	7		1
Family Cricetidae	145	792	75	61
Family Ctenodactylidae	4	5		
Family Ctenomyidae	1	69	5	6
Family Cuniculidae	1	2		
Family Dasyproctidae	2	15	2	1
Family Diatomyidae[f]	1	1		1
Family Dinomyidae	1	1		
Family Dipodidae	13	37	3	
Family Echimyidae[g]	25	93	6	3
Family Erethizontidae	3	17	1	2
Family Geomyidae	7	41	8	1
Family Gliridae	9	29		1
Family Heterocephalidae[h]	1	1		
Family Heteromyidae	5	66	6	2
Family Hystricidae	3	11		
Family Muridae	157	834	41	84
Family Nesomyidae	21	68	1	6
Family Octodontidae	7	14		1
Family Pedetidae	1	2		
Family Petromuridae	1	1		
Family Platacanthomyidae	2	5	2	1
Family Sciuridae	62	298	18	5
Family Sminthidae[i]	1	14	2	

Table 3.—*Continued*

	Genera	Species	New species since MSW3	
			Splits	De novo
Family Spalacidae	7	28	8	
Family Thryonomyidae	1	2		
Family Zapodidae[i]	3	12	6	1
Family Zenkerellidae[j]	1	1		
Superorder Laurasiatheria	556	2,798	399	247
Order Eulipotyphla[k]	56	527	23	63
Family Erinaceidae	10	24		
Family †Nesophontidae	1	6		
Family Solenodontidae	1	3		
Family Soricidae	26	440	16	55
Family Talpidae	18	54	7	8
Order Chiroptera	227	1,386	130	174
Family Cistugidae[l]	1	2		
Family Craseonycteridae	1	1		
Family Emballonuridae	14	54		3
Family Furipteridae	2	2		
Family Hipposideridae	7	88	6	8
Family Megadermatidae	5	6		1
Family Miniopteridae[l]	1	35	7	9
Family Molossidae	19	122	12	13
Family Mormoopidae	2	17	8	
Family Mystacinidae	1	2		
Family Myzopodidae	1	2		1
Family Natalidae	3	11	3	
Family Noctilionidae	1	2		
Family Nycteridae	1	16		
Family Phyllostomidae	62	214	22	37
Family Pteropodidae	45	197	5	12
Family Rhinolophidae	1	102	10	14
Family Rhinonycteridae[m]	4	9	1	3
Family Rhinopomatidae	1	6	1	1
Family Thyropteridae	1	5		2
Family Vespertilionidae	54	493	55	70
Order Carnivora	130	305	23	2
Family Ailuridae	1	2	1	
Family Canidae	13	39	3	
Family Eupleridae	7	8		
Family Felidae	14	42	5	
Family Herpestidae	16	36	2	
Family Hyaenidae	3	4		
Family Mephitidae	4	12	1	
Family Mustelidae	23	64	5	1
Family Nandiniidae	1	1		
Family Odobenidae	1	1		
Family Otariidae	7	16		
Family Phocidae	14	19		
Family Prionodontidae[n]	1	2		
Family Procyonidae	6	14	2	1
Family Ursidae	5	8		
Family Viverridae	14	37	4	
Order Pholidota	3	8		
Family Manidae	3	8		
Order Perissodactyla	8	21	4	
Family Equidae	1	12	4	
Family Rhinocerotidae	4	5		
Family Tapiridae	3	4		
Order Artiodactyla[o]	132	551	219	8
Family Antilocapridae	1	1		
Family Balaenidae	2	4		
Family Balaenopteridae	2	8		1
Family Bovidae	54	297	152	2
Family Camelidae	2	7	1	

Table 3.—*Continued*

	Genera	Species	New species since MSW3	
			Splits	De novo
Family Cervidae	18	93	43	
Family Delphinidae	17	40	3	3
Family Eschrichtiidae	1	1		
Family Giraffidae	2	5	3	
Family Hippopotamidae	2	4		
Family Iniidae	1	3	1	1
Family Kogiidae[p]	1	2		
Family Lipotidae[q]	1	1		
Family Monodontidae	2	2		
Family Moschidae	1	7		
Family Neobalaenidae	1	1		
Family Phocoenidae	3	7	1	
Family Physeteridae	1	1		
Family Platanistidae	1	1		
Family Pontoporiidae[q]	1	1		
Family Suidae	6	28	11	
Family Tayassuidae	3	5	2	
Family Tragulidae	3	10	1	1
Family Ziphiidae	6	22	1	

[a] Split from Tenrecidae.
[b] Split from Dasypodidae.
[c] Recently extinct families not included in MSW3.
[d] Includes Aotidae and Callitrichidae.
[e] Was spelled as "Indridae" in MSW3.
[f] Recognized as extant based on *Laonastes aenigmamus*.
[g] Includes Heptaxodontidae and Myocastoridae.
[h] Split from Bathyergidae.
[i] Split from Dipodidae.
[j] Split from Anomaluridae.
[k] Includes Soricomorpha and Erinaceomorpha.
[l] Split from Vespertilionidae.
[m] Split from Hipposideridae.
[n] Split from Felidae.
[o] Includes Cetacea.
[p] Split from Physeteridae.
[q] Split from Iniidae.
†Extinct.

later bursts of species descriptions by allowing morphologically cryptic but genetically divergent evolutionary lineages to be recognized as species. For example, over one-half of the species described since 2004 appear to have stemmed from taxonomic splits (~58%), many based in part or whole on genetic data, to go with at least 172 species unions (lumps) during the same period. As we continue to progress within the genomic era, where data on millions of independent genetic loci can be readily generated for taxonomic studies, there is a growing understanding that hybridization and introgression commonly occur among mammalian species that may otherwise maintain genetic integrity (e.g., Larsen et al. 2010; Miller et al. 2012; vonHoldt et al. 2016). Characterizing species and their boundaries using multiple tiers of evidence will continue to be essential given the profound impact of species delimitation on legislative decisions (e.g., U.S. Endangered Species Act of 1973—Department of the Interior, U.S. Fish and Wildlife Service 1973).

At the current rate of taxonomic description of mammals (~25 species/year from 1750 to 2017), we predict that 7,342 mammalian species will be recognized by 2050 and 8,590 by 2100. Alternatively, if we consider the increased rate of taxonomic descriptions since the advent of PCR (~30 species/year from 1990 to 2017), our estimates increase to 7,509 species recognized by 2050 and 9,009 by 2100. These estimates surpass Reeder and Helgen's (2007) prediction of > 7,000 total mammalian species, but echo their observation that mammals contain considerably greater species diversity than is commonly recognized. Remarkably, the same estimate of ~25 species/year was derived somewhat independently from tracking 14 estimates of global diversity (1961–1999—Patterson 2001) and from species-level changes between MSW2 and MSW3 (Reeder and Helgen 2007), thereby affirming the robustness of that estimate across both data sources and eras.

Assumed in all taxonomic forecasts is the stability of global ecosystems, scientific institutions, and natural history collections. With mammals being disproportionately impacted by human-induced extinctions (Ceballos et al. 2017), especially in insular regions like the Caribbean (Cooke et al. in press), efforts to protect threatened habitats and their resident mammalian species are key to the continued persistence, existence, and discovery of mammals. The Neotropics is the most species-dense biogeographic region in the world, followed closely by the Afrotropics and Australasia-Oceania, the latter of which is one of the least explored terrestrial regions on Earth, with the second fewest de novo species descriptions (18 species; Table 2). Inventory efforts may thus be fruitfully prioritized in northern Australia, Melanesia, Sulawesi, and other oceanic islands east of Wallace's Line. However, we note that obtaining collecting permissions is a barrier to species description in any region. The continued description and discovery of mammalian species diversity hinges on investment in both natural history collecting and in the physical collections that house the specimens essential for taxonomic research. Natural history collections are repositories for the genetic and morphological vouchers used to describe every new species listed in the MDD, a fact that highlights the indispensable role of museums and universities in understanding species and the ecosystems in which they live (McLean et al. 2015). As our planet changes, the need to support geographically broad and site-intensive biological archives only grows in relevance. Collections represent time series of change in biodiversity and often harbor undiscovered species (e.g., Helgen et al. 2013), including those vulnerable or already extinct.

Acting under the supervision of the American Society of Mammalogists' Biodiversity Committee, the MDD has a 2018–2020 plan to further integrate synonym data, track Holocene-extinct taxa, and add links to outside data sources. While full synonymies are not feasible, inclusion of common synonyms will facilitate tracking taxonomic changes through time, especially within controversial groups (e.g., Artiodactyla and Perissodactyla—Groves and Grubb 2011). Controversial taxonomic assignments also will be "flagged" as tentative or pending further scientific investigation. The MDD aims to link taxon entries to a variety of relevant per-species and per-higher taxon data pages on other web platforms, including geographic range maps, trait database entries, museum records, genetic resources, and other ecological information. *Mammalian Species* accounts, published by the American Society of Mammalogists since 1969 and consisting of over 950 species-level treatments, will be linked to relevant MDD species pages, including synonym-based links. In this manner, the MDD's efforts parallel initiatives in other vertebrate taxa to digitize taxonomic resources (amphibians—AmphibiaWeb 2017; Amphibian Species of the World—Frost 2017; birds: Avibase—LePage et al. 2014; IOC World Bird List—Gill and Donsker 2017; the Handbook of the Birds of the World Alive—del Hoyo et al. 2017; non-avian reptiles, turtles, crocodiles, and tuatara—Uetz et al. 2016; and bony fish: FishBase—Froese and Pauly 2017; Catalog of Fishes—Eschmeyer et al. 2017). The new mammalian taxonomic database summarized herein aims to advance the study of mammals while bringing it to par with the digital resources available in other tetrapod clades, to the benefit of future mammalogists and non-mammalogists alike.

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Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.— Details of the full Mammal Diversity Database (MDD) version 1 taxonomy, including associated citations and geographic regions.

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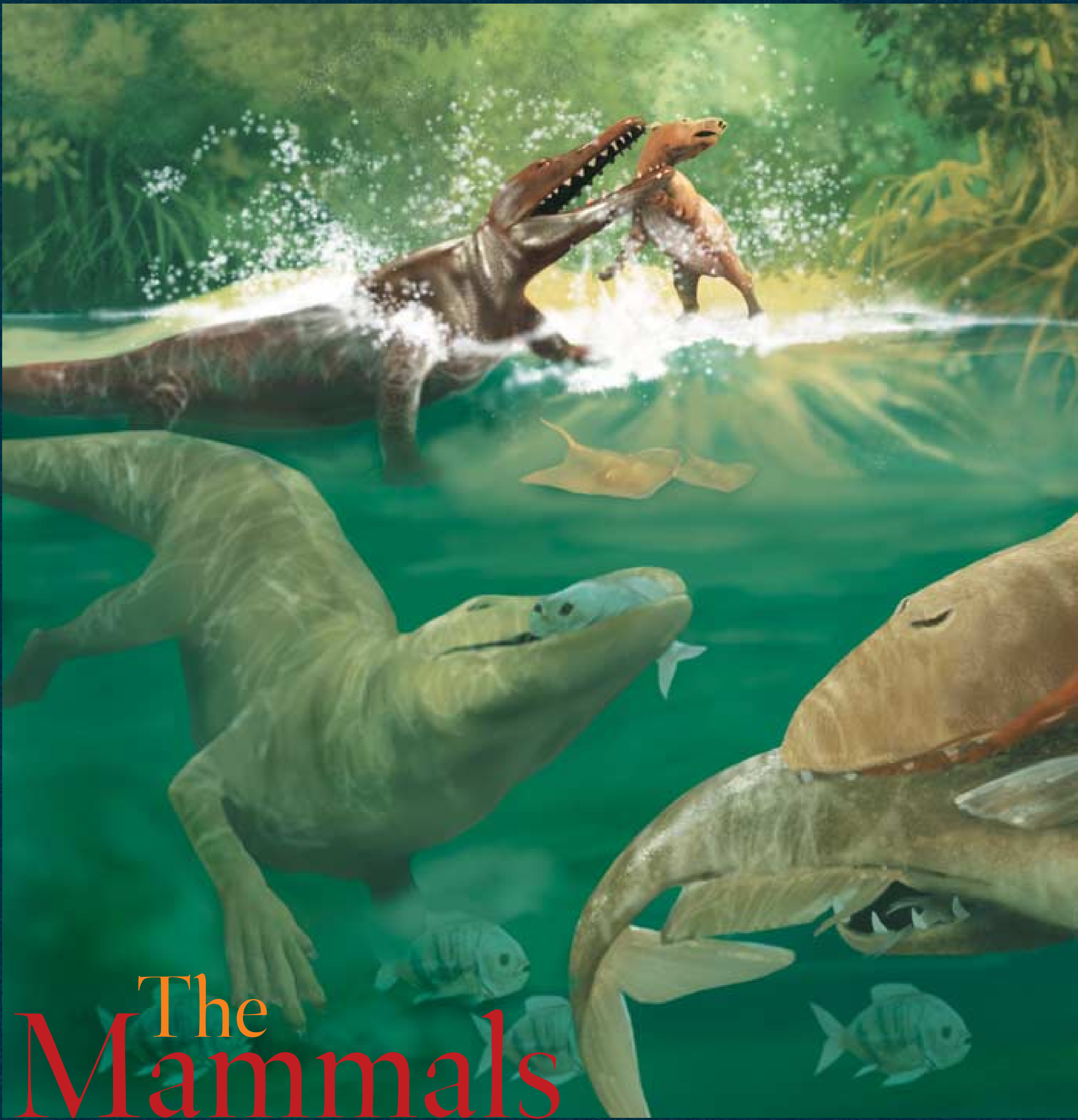


The Mammals That Conquered the

New fossils and DNA analyses elucidate the remarkable

“They say the sea is cold,
but the sea contains
the hottest blood of all,
and the wildest, the most urgent.”

—D. H. Lawrence,
“Whales Weep Not!”

ANCIENT WHALE *Rodhocetus* (*right* and *left front*) feasts on the bounty of the sea, while *Ambulocetus* (*rear*) attacks a small land mammal some 48 million years ago in what is now Pakistan.

Dawn breaks over the Tethys Sea, 48 million years ago, and the blue-green water sparkles with the day’s first light. But for one small mammal, this new day will end almost as soon as it has started.

Seas

evolutionary history of whales

By Kate Wong

Tapir-like *Eotitanops* has wandered perilously close to the water's edge, ignoring its mother's warning call. For the brute lurking motionless among the mangroves, the opportunity is simply too good to pass up. It lunges landward, propelled by powerful hind limbs, and sinks its formidable teeth into the calf, dragging it back into the surf. The victim's frantic struggling subsides as it drowns, trapped in the viselike jaws of its captor. Victorious, the beast shambles out of the water to devour its kill on terra firma. At first glance, this fearsome predator resembles a crocodile, with its squat legs, stout tail, long snout and eyes that sit high on its skull. But on closer inspection, it has not armor but fur, not claws but hooves. And the cusps on its teeth clearly identify it not as a reptile but as a mammal. In fact, this improbable creature is *Ambulocetus*, an early whale, and one of a series of intermediates linking the land-dwelling ancestors of cetaceans to the 80 or so species of whales, dolphins and porpoises that rule the oceans today.

Until recently, the emergence of whales was one of the most intractable mysteries facing evolutionary biologists. Lacking fur and hind limbs and unable to go ashore for so much as a sip of freshwater, living cetaceans represent a dramatic departure from the mammalian norm. Indeed, their piscine form led Herman Melville in 1851 to describe Moby Dick and his fellow whales as fishes. But to 19th-century naturalists such as Charles Darwin, these air-breathing, warm-blooded animals that nurse their young with milk distinctly grouped with mammals. And because ancestral mammals lived on land, it stood to reason that whales ultimately descended from a terrestrial ancestor. Exactly how that might have happened, however, eluded scholars. For his part, Darwin noted in *On the Origin of Species* that a bear swimming with its mouth agape to catch insects was a plausible evolutionary starting point for whales. But the proposition attracted so much ridicule that in later editions of the book he said just that such a bear was "almost like a whale."

Guide to *Terminology*

CETACEA is the order of mammals that comprises living whales, dolphins and porpoises and their extinct ancestors, the archaeocetes. Living members fall into two suborders: the odontocetes, or toothed whales, including sperm whales, pilot whales, belugas, and all dolphins and porpoises; and the mysticetes, or baleen whales, including blue whales and fin whales. The term "whale" is often used to refer to all cetaceans.

MESONYCHIDS are a group of primitive hoofed, wolflike mammals once widely thought to have given rise to whales.

ARTIODACTYLA is the order of even-toed, hoofed mammals that includes camels; ruminants such as cows; hippos; and, most researchers now agree, whales.

EOCENE is the epoch between 55 million and 34 million years ago, during which early whales made their transition from land to sea.

OLIGOCENE is the epoch between 34 million and 24 million years ago, during which odontocetes and mysticetes evolved from their archaeocete ancestors.

The fossil record of cetaceans did little to advance the study of whale origins. Of the few remains known, none were sufficiently complete or primitive to throw much light on the matter. And further analyses of the bizarre anatomy of living whales led only to more scientific head scratching. Thus, even a century after Darwin, these aquatic mammals remained an evolutionary enigma. In fact, in his 1945 classification of mammals, famed paleontologist George Gaylord Simpson noted that whales had evolved in the oceans for so long that nothing informative about their ancestry remained. Calling them "on the whole, the most peculiar and aberrant of mammals," he inserted cetaceans arbitrarily among the other orders. Where whales belonged in the mammalian family tree and how they took to the seas defied explanation, it seemed.

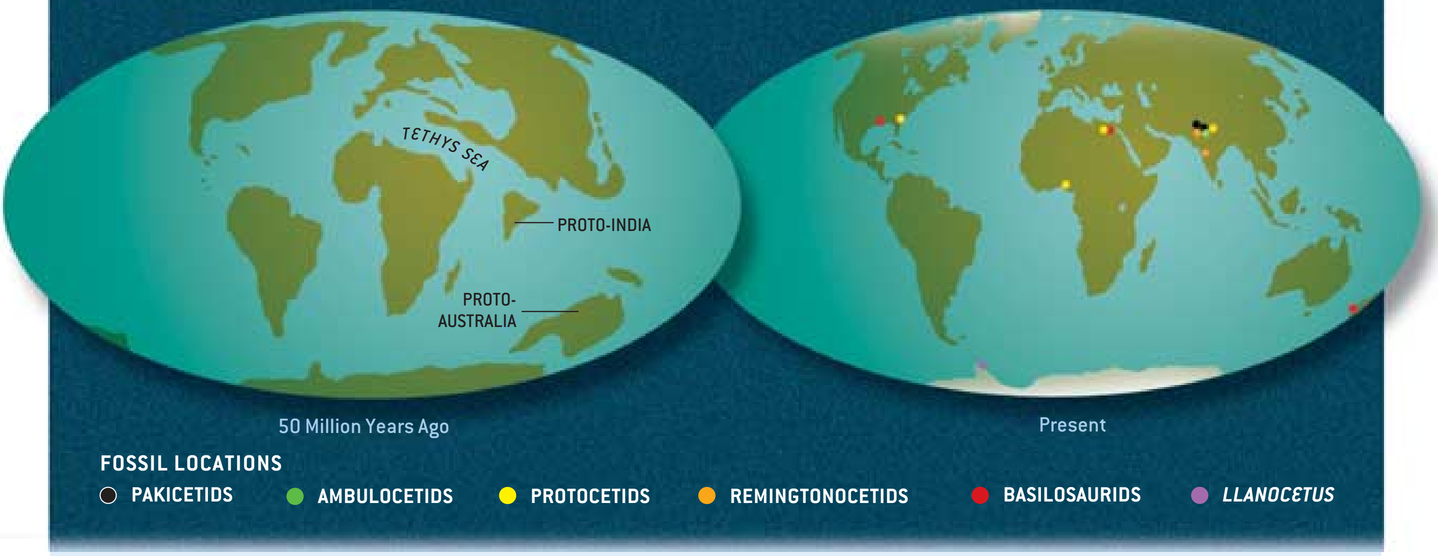
Over the past two decades, however, many of the pieces of this once imponderable puzzle have fallen into place. Paleontologists have uncovered a wealth of whale fossils spanning the Eocene epoch, the time between 55 million and 34 million years ago when archaic whales, or archaeocetes, made their transition from land to sea. They have also unearthed some clues from the ensuing Oligocene, when the modern suborders of cetaceans—the mysticetes (baleen whales) and the odontocetes (toothed whales)—arose. That fossil material, along with analyses of DNA from living animals, has enabled scientists to paint a detailed picture of when, where and how whales evolved from their terrestrial forebears. Today their transformation—from landlubbers to Leviathans—stands as one of the most profound evolutionary metamorphoses on record.

Evolving Ideas

AT AROUND THE SAME TIME that Simpson declared the relationship of whales to other mammals undecipherable on the basis of anatomy, a new comparative approach emerged, one that looked at antibody-antigen reactions in living animals. In response to Simpson's assertion, Alan Boyden of Rutgers University and a colleague applied the technique to the whale question. Their results showed convincingly that among living animals, whales are most closely related to the even-toed hoofed

KAREN CARR (*preceding pages*)

THE WHALE'S CHANGING WORLD



It might seem odd that 300 million years after vertebrates first established a toehold on land, some returned to the sea. But the setting in which early whales evolved offers hints as to what lured them back to the water. For much of the Eocene epoch (roughly between 55 million and 34 million years ago), a sea called Tethys, after a goddess of Greek mythology, stretched from Spain to Indonesia. Although the continents and ocean plates we know now had taken shape, India was still adrift, Australia hadn't yet fully separated from Antarctica, and great swaths of Africa and Eurasia lay submerged under Tethys. Those shallow, warm waters incubated abundant nutrients and teemed with fish. Furthermore, the space vacated by the plesiosaurs, mosasaurs and other large marine reptiles that perished along with the dinosaurs created room for new top predators (although sharks and crocodiles still provided a healthy dose of competition). It is difficult to imagine a more enticing invitation to aquatic life for a mammal.

During the Oligocene epoch that followed, sea levels sank and India docked with the rest of Asia, forming the crumpled interface we know as the Himalayas. More important, University of Michigan paleontologist Philip Gingerich notes, Australia and Antarctica divorced, opening up the Southern Ocean and creating a south circumpolar current that eventually transformed the balmy Eocene earth into the ice-capped planet we inhabit today. The modern current and climate systems brought about radical changes in the quantity and distribution of nutrients in the sea, generating a whole new set of ecological opportunities for the cetaceans.

As posited by paleontologist Ewan Fordyce of the University of Otago in New Zealand, that set the stage for the replacement of the archaeocetes by the odontocetes and mysticetes (toothed and baleen whales, respectively). The earliest known link between archaeocetes and the modern cetacean orders, Fordyce says, is *Llanocetus,* a 34-million-year-old protobaleen whale from Antarctica that may well have trawled for krill in the chilly Antarctic waters, just as living baleen whales do. Odontocetes arose at around the same time, he adds, specializing to become echolocators that could hunt in the deep.

Unfortunately, fossils documenting the origins of mysticetes and odontocetes are vanishingly rare. Low sea levels during the middle Oligocene exposed most potential whale-bearing sediments from the early Oligocene to erosive winds and rains, making that period largely "a fossil wasteland," says paleontologist Mark Uhen of the Cranbrook Institute of Science in Bloomfield Hills, Mich. The later fossil record clearly shows, however, that shortly after, by about 30 million years ago, the baleen and toothed whales had diversified into many of the cetacean families that reign over the oceans today. *—K.W.*

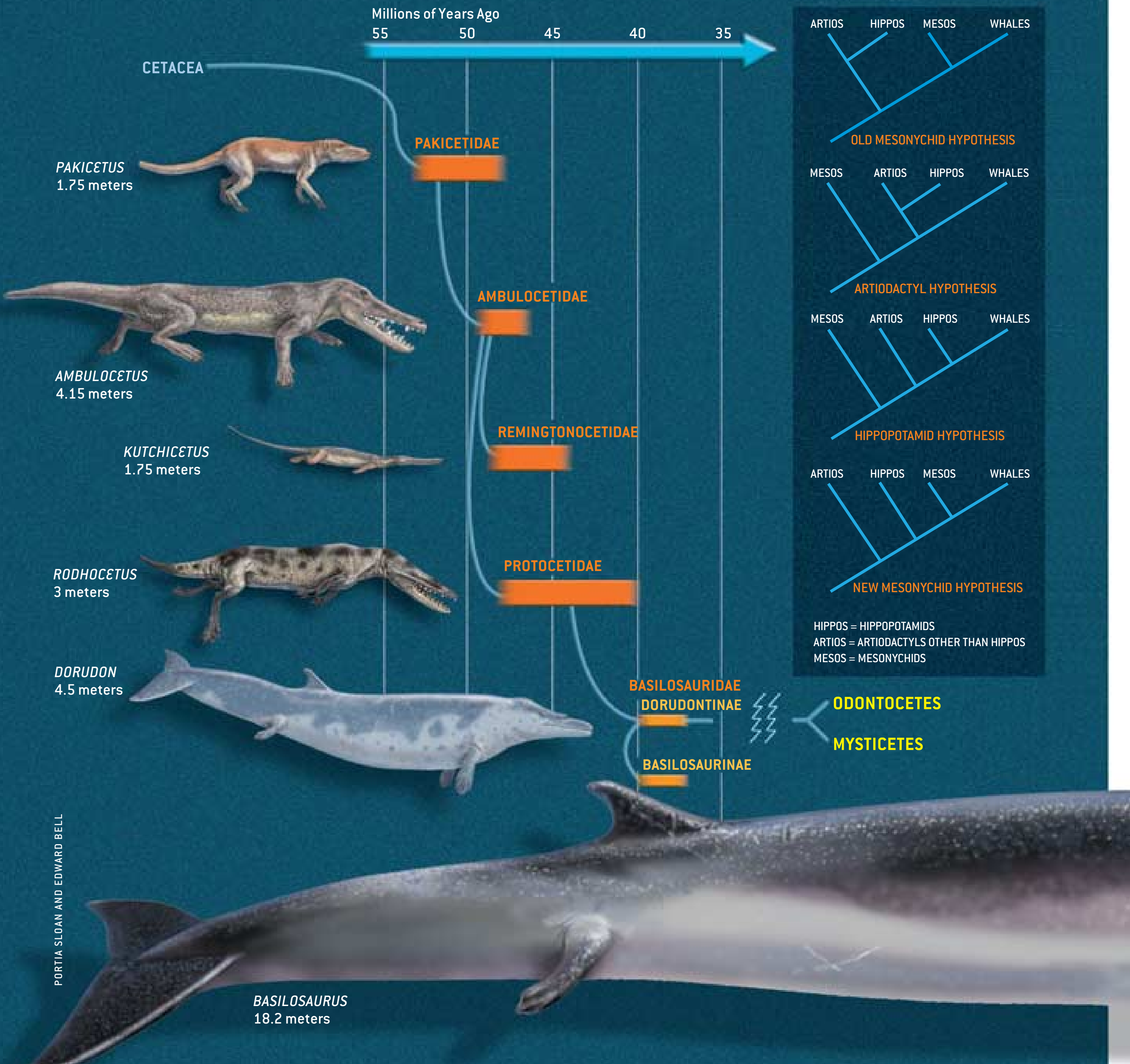
mammals, or artiodactyls, a group whose members include camels, hippopotamuses, pigs and ruminants such as cows. Still, the exact nature of that relationship remained unclear. Were whales themselves artiodactyls? Or did they occupy their own branch of the mammalian family tree, linked to the artiodactyl branch via an ancient common ancestor?

Support for the latter interpretation came in the 1960s, from studies of primitive hoofed mammals known as condylarths that had not yet evolved the specialized characteristics of artiodactyls or the other mammalian orders. Paleontologist Leigh Van Valen, then at the American Museum of Natural History in New York City, discovered striking resemblances between the three-cusped teeth of the few known fossil whales and those of a group of meat-eating condylarths called mesonychids. Likewise, he found shared dental characteristics between artiodactyls and another group of condylarths, the arctocyonids, close relatives of the mesonychids. Van Valen concluded that whales descended from the carnivorous, wolflike mesonychids and thus were linked to artiodactyls through the condylarths.

SARA CHEN AND EDWARD BELL

CETACEAN RELATIONS

FAMILY TREE OF CETACEANS shows the descent of the two modern suborders of whales, the odontocetes and mysticetes, from the extinct archaeocetes. Representative members of each archaeocete family or subfamily are depicted (*left*). Branching diagrams illustrate various hypotheses of the relationship of whales to other mammals (*right*). The old mesonychid hypothesis, which posits that extinct wolflike beasts known as mesonychids are the closest relatives of whales, now seems unlikely in light of new fossil whale discoveries. The anklebones of those ancient whales bear the distinctive characteristics of artiodactyl ankles, suggesting that whales are themselves artiodactyls, as envisioned by the artiodactyl hypothesis. Molecular studies indicate that whales are more closely related to hippopotamuses than to any other artiodactyl group. Whether the fossil record can support the hippopotamid hypothesis, however, remains to be seen. A fourth scenario, denoted here as the new mesonychid hypothesis, proposes that mesonychids could still be the whale's closest kin if they, too, were included in the artiodactyl order, instead of the extinct order Condylarthra, in which they currently reside. If so, they would have to have lost the ankle traits that characterize all known artiodactyls. *—K.W.*



PORTIA SLOAN AND EDWARD BELL

Walking Whales

A DECADE OR SO PASSED before paleontologists finally began unearthing fossils close enough to the evolutionary branching point of whales to address Van Valen's mesonychid hypothesis. Even then the significance of these finds took a while to sink in. It started when University of Michigan paleontologist Philip Gingerich went to Pakistan in 1977 in search of Eocene land mammals, visiting an area previously reported to shelter such remains. The expedition proved disappointing because the spot turned out to contain only marine fossils. Finding traces of ancient ocean life in Pakistan, far from the country's modern coast, is not surprising: during the Eocene, the vast Tethys Sea periodically covered great swaths of what is now the Indian subcontinent [*see box on page 73*]. Intriguingly, though, the team discovered among those ancient fish and snail remnants two pelvis fragments that appeared to have come from relatively large, walking beasts. "We joked about walking whales," Gingerich recalls with a chuckle. "It was unthinkable." Curious as the pelvis pieces were, the only fossil collected during that field season that seemed important at the time was a primitive artiodactyl jaw that had turned up in another part of the country.

Two years later, in the Himalayan foothills of northern Pakistan, Gingerich's team found another weird whale clue: a partial braincase from a wolf-size creature—found in the company of 50-million-year-old land mammal remains—that bore some distinctive cetacean characteristics. All modern whales have features in their ears that do not appear in any other vertebrates. Although the fossil skull lacked the anatomy necessary for hearing directionally in water (a critical skill for living whales), it clearly had the diagnostic cetacean ear traits. The team had discovered the oldest and most primitive whale then known—one that must have spent some, if not most, of its time on land. Gingerich christened the creature *Pakicetus* for its place of origin and, thus hooked, began hunting for ancient whales in earnest.

At around the same time, another group recovered additional remains of *Pakicetus*—a lower jaw fragment and some isolated teeth—that bolstered the link to mesonychids through strong dental similarities. With *Pakicetus* showing up around 50 million years ago and mesonychids known from around the same time in the same part of the world, it looked increasingly likely that cetaceans had indeed descended from the mesonychids or something closely related to them. Still, what the earliest whales looked like from the neck down was a mystery.

Further insights from Pakistan would have to wait, however. By 1983 Gingerich was no longer able to work there because of the Soviet Union's invasion of Afghanistan. He decided to cast his net in Egypt instead, journeying some 95 miles southwest of Cairo to the Western Desert's Zeuglodon Valley, so named for early 20th-century reports of fossils of archaic whales—or zeuglodons, as they were then known—in the area. Like Pakistan, much of Egypt once lay submerged under Tethys. Today the skeletons of creatures that swam in that ancient sea lie entombed in sandstone. After several field seasons, Gingerich and his crew hit pay dirt: tiny hind limbs belonging to a 60-foot-long sea snake of a whale known as *Basilosaurus* and the first evidence of cetacean feet.

Earlier finds of *Basilosaurus,* a fully aquatic monster that slithered through the seas between some 40 million and 37 million years ago, preserved only a partial femur, which its discoverers interpreted as vestigial. But the well-formed legs and feet revealed by this discovery hinted at functionality. Although at less than half a meter in length the diminutive limbs probably would not have assisted *Basilosaurus* in swimming and certainly would not have enabled it to walk on land, they may well have helped guide the beast's serpentine body during the difficult activity of aquatic mating. Whatever their purpose, if any, the little legs had big implications. "I immediately thought, we're *10 million* years after *Pakicetus,*" Gingerich recounts excitedly. "If these things still have feet and toes, we've got 10 million years of history to look at." Suddenly, the walking whales they had scoffed at in Pakistan seemed entirely plausible.

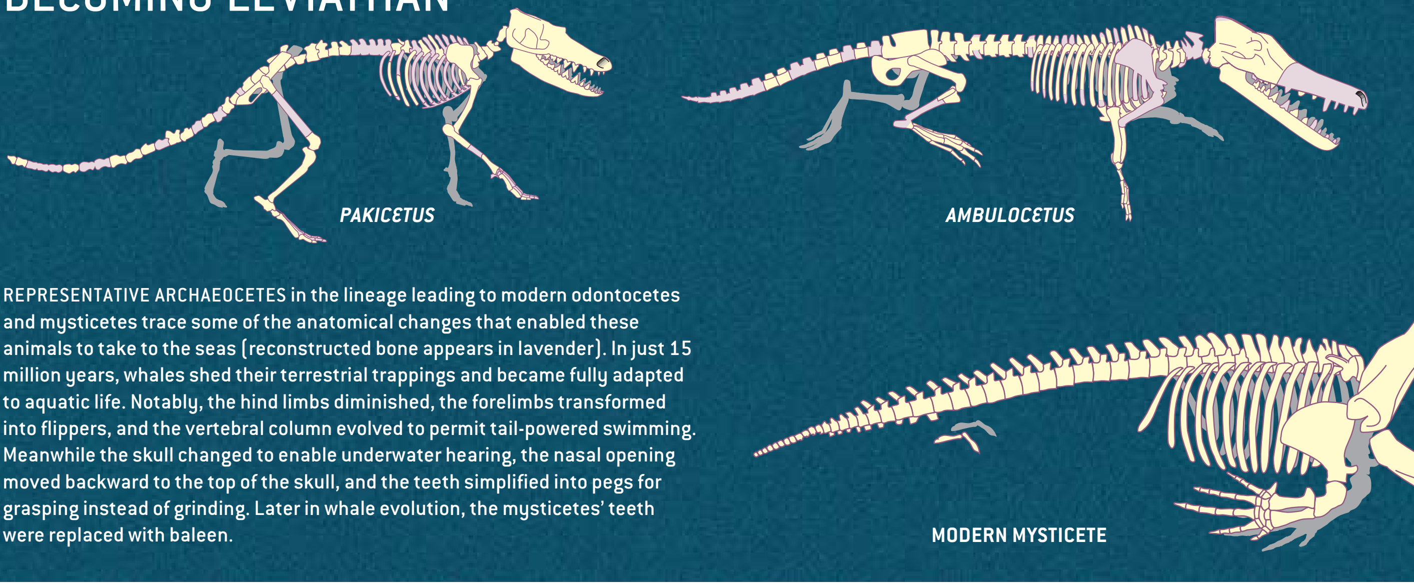
Just such a remarkable creature came to light in 1992. A team led by J.G.M. (Hans) Thewissen of the Northeastern Ohio Universities College of Medicine recovered from 48-million-year-old marine rocks in northern Pakistan a nearly complete skeleton of a perfect intermediate between modern whales and their terrestrial ancestors. Its large feet and powerful tail bespoke strong swimming skills, while its sturdy leg bones and mobile elbow and wrist joints suggested an ability to locomote on land. He dubbed the animal *Ambulocetus natans,* the walking and swimming whale.

Shape Shifters

SINCE THEN, Thewissen, Gingerich and others have unearthed a plethora of fossils documenting subsequent stages of the whale's transition from land to sea. The picture emerging from those specimens is one in which *Ambulocetus* and its kin—themselves descended from the more terrestrial pakicetids—spawned needle-nosed beasts known as remingtonocetids and the intrepid protocetids—the first whales seaworthy enough to fan out from Indo-Pakistan across the globe. From the protocetids arose the dolphinlike dorudontines, the probable progenitors of the snakelike basilosaurines and modern whales [*see box on opposite page*].

In addition to furnishing supporting branches for the whale family tree, these discoveries have enabled researchers to chart many of the spectacular anatomical and physiological changes that allowed cetaceans to establish permanent residency in the

BECOMING LEVIATHAN



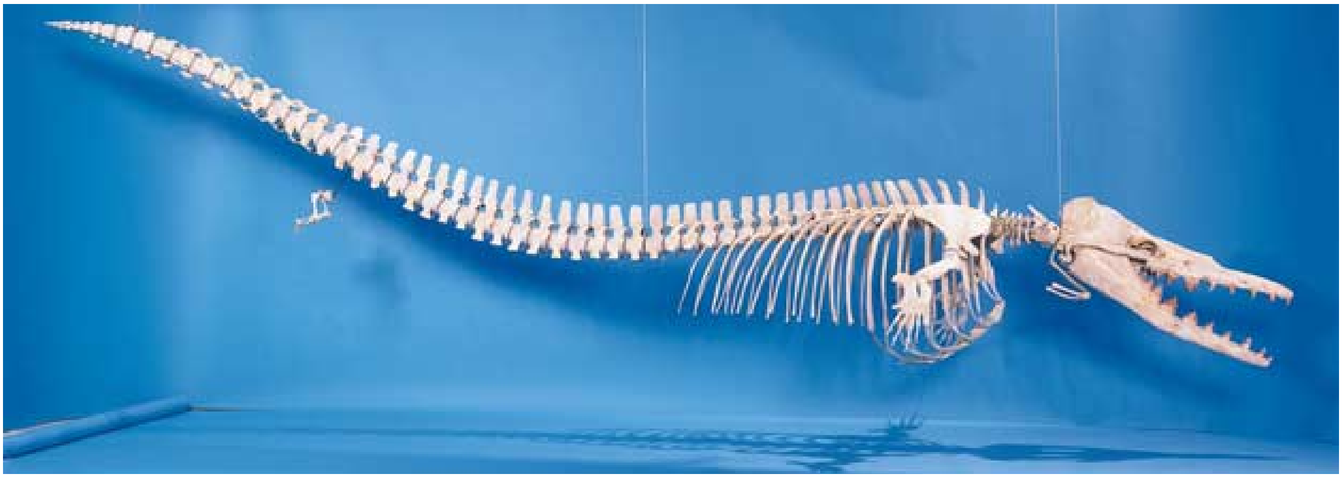
REPRESENTATIVE ARCHAEOCETES in the lineage leading to modern odontocetes and mysticetes trace some of the anatomical changes that enabled these animals to take to the seas (reconstructed bone appears in lavender). In just 15 million years, whales shed their terrestrial trappings and became fully adapted to aquatic life. Notably, the hind limbs diminished, the forelimbs transformed into flippers, and the vertebral column evolved to permit tail-powered swimming. Meanwhile the skull changed to enable underwater hearing, the nasal opening moved backward to the top of the skull, and the teeth simplified into pegs for grasping instead of grinding. Later in whale evolution, the mysticetes' teeth were replaced with baleen.

ocean realm. Some of the earliest of these adaptations to emerge, as *Pakicetus* shows, are those related to hearing. Sound travels differently in water than it does in air. Whereas the ears of humans and other land-dwelling animals have delicate, flat eardrums, or tympanic membranes, for receiving airborne sound, modern whales have thick, elongate tympanic ligaments that cannot receive sound. Instead a bone called the bulla, which in whales has become quite dense and is therefore capable of transmitting sound coming from a denser medium to deeper parts of the ear, takes on that function. The *Pakicetus* bulla shows some modification in that direction, but the animal retained a land mammal–like eardrum that could not work in water.

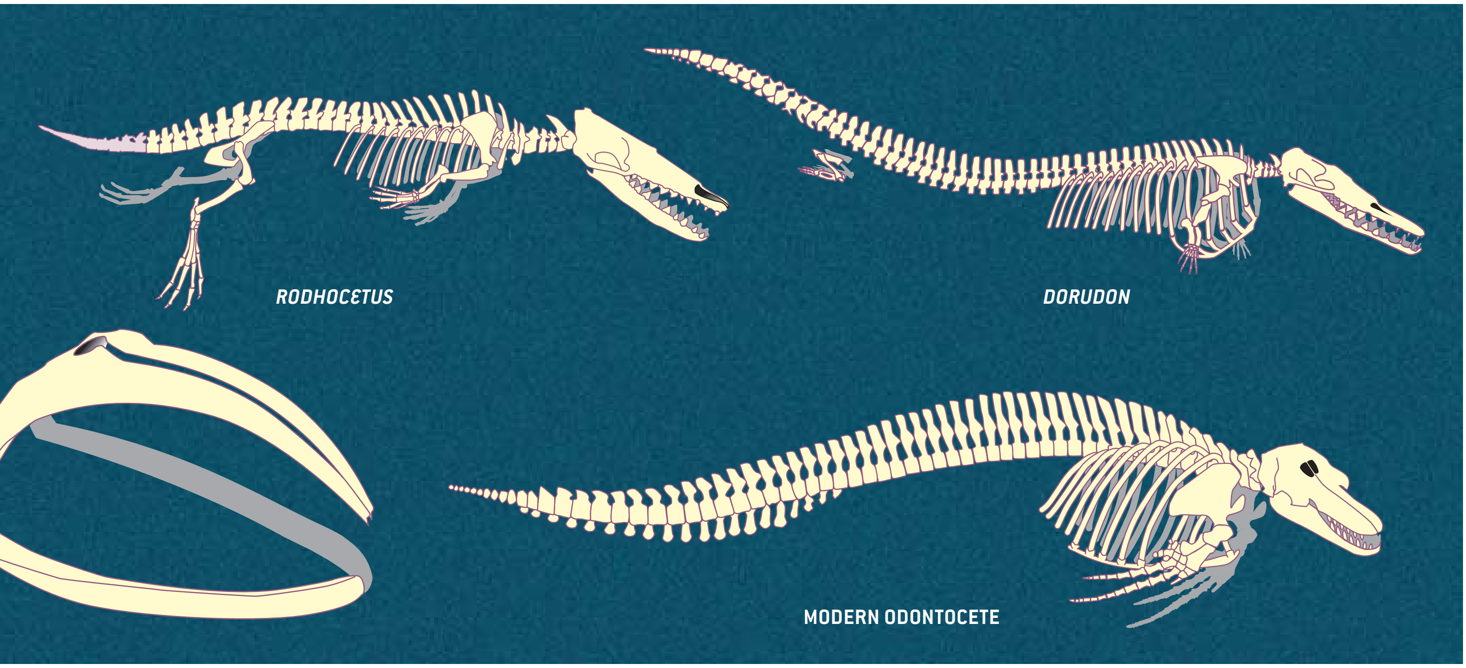
What, then, might *Pakicetus* have used its thickened bullae for? Thewissen suspects that much as turtles hear by picking up vibrations from the ground through their shields, *Pakicetus* may have employed its bullae to pick up ground-borne sounds. Taking new postcranial evidence into consideration along with the ear morphology, he envisions *Pakicetus* as an ambush predator that may have lurked around shallow rivers, head to the ground, preying on animals that came to drink. *Ambulocetus* is even more likely to have used such inertial hearing, Thewissen says, because it had the beginnings of a channel linking jaw and ear. By resting its jaw on the ground—a strategy seen in modern crocodiles—*Ambulocetus* could have listened for approaching prey. The same features that allowed early whales to receive sounds from soil, he surmises, preadapted them to hearing in the water.

Zhe-Xi Luo of the Carnegie Museum of Natural History in Pittsburgh has shown that by the time of the basilosaurines and dorudontines, the first fully aquatic whales, the ropelike tympanic ligament had probably already evolved. Additionally, air

DORUDON, a 4.5-meter-long, dolphinlike archaeocete that patrolled the seas between roughly 40 million and 37 million years ago, may be the ancestor of modern whales.



PORTIA SLOAN (*illustrations*); UNIVERSITY OF MICHIGAN EXHIBIT MUSEUM OF NATURAL HISTORY (*photograph*)



sinuses, presumably filled with spongelike tissues, had formed around the middle ear, offering better sound resolution and directional cues for underwater hearing. Meanwhile, with the external ear canal closed off (a prerequisite for deep-sea diving), he adds, the lower jaw was taking on an increasingly important auditory role, developing a fat-filled canal capable of conducting sound back to the middle ear.

Later in the evolution of whale hearing, the toothed and baleen whales parted ways. Whereas the toothed whales evolved the features necessary to produce and receive high-frequency sounds, enabling echolocation for hunting, the baleen whales developed the ability to produce and receive very low frequency sounds, allowing them to communicate with one another over vast distances. Fossil whale ear bones, Luo says, show that by around 28 million years ago early odontocetes already had some of the bony structures necessary for hearing high-pitched sound and were thus capable of at least modest echolocation. The origin of the mysticete's low-frequency hearing is far murkier, even though the fossil evidence of that group now dates back to as early as 34 million years ago.

Other notable skull changes include movement of the eye sockets from a crocodilelike placement atop the head in *Pakicetus* and *Ambulocetus* to a lateral position in the more aquatic protocetids and later whales. And the nasal opening migrated back from the tip of the snout in *Pakicetus* to the top of the head in modern cetaceans, forming the blowhole. Whale dentition morphed, too, turning the complexly cusped, grinding molars of primitive mammalian ancestors into the simple, pronglike teeth of modern odontocetes, which grasp and swallow their food without chewing. Mysticetes lost their teeth altogether and developed comblike plates of baleen that hang from their upper jaws and strain plankton from the seawater.

The most obvious adaptations making up the whale's protean shift are those that produced its streamlined shape and unmatched swimming abilities. Not surprisingly, some bizarre amphibious forms resulted along the way. *Ambulocetus,* for one, retained the flexible shoulder, elbow, wrist and finger joints of its terrestrial ancestors and had a pelvis capable of supporting its weight on land. Yet the creature's disproportionately large hind limbs and paddlelike feet would have made walking somewhat awkward. These same features were perfect for paddling around in the fish-filled shallows of Tethys, however.

Moving farther out to sea required additional modifications, many of which appear in the protocetid whales. Studies of one member of this group, *Rodhocetus,* indicate that the lower arm bones were compressed and already on their way to becoming hydrodynamically efficient, says University of Michigan paleontologist Bill Sanders. The animal's long, delicate feet were probably webbed, like the fins used by scuba divers. *Rodhocetus* also exhibits aquatic adaptations in its pelvis, where fusion between the vertebrae that form the sacrum is reduced, loosening up the lower spine to power tail movement. These features, says Gingerich, whose team discovered the creature, suggest that *Rodhocetus* performed a leisurely dog paddle at the sea surface and a swift combination of otterlike hind-limb paddling and tail propulsion underwater. When it went ashore to breed or perhaps to bask in the sun, he proposes, *Rodhocetus* probably hitched itself around somewhat like a modern eared seal or sea lion.

By the time of the basilosaurines and dorudontines, whales were fully aquatic. As in modern cetaceans, the shoulder remained mobile while the elbow and wrist stiffened, forming flippers for steering and balance. Farther back on the skeleton, only tiny legs remained, and the pelvis had dwindled accordingly. Analyses of the vertebrae of *Dorudon,* conducted by Mark D. Uhen of the Cranbrook Institute of Science in Bloomfield Hills, Mich., have revealed one tail vertebra with a rounded profile.

Modern whales have a similarly shaped bone, the ball vertebra, at the base of their fluke, the flat, horizontal structure capping the tail. Uhen thus suspects that basilosaurines and dorudontines had tail flukes and swam much as modern whales do, using so-called caudal oscillation. In this energetically efficient mode of locomotion, motion generated at a single point in the vertebral column powers the tail's vertical movement through the water, and the fluke generates lift.

Exactly when whales lost their legs altogether remains unknown. In fact, a recent discovery made by Lawrence G. Barnes of the Natural History Museum of Los Angeles County hints at surprisingly well developed hind limbs in a 27-million-year-old baleen whale from Washington State, suggesting that whale legs persisted far longer than originally thought. Today, however, some 50 million years after their quadrupedal ancestors first waded into the warm waters of Tethys, whales are singularly sleek. Their hind limbs have shrunk to externally invisible vestiges, and the pelvis has diminished to the point of serving merely as an anchor for a few tiny muscles unrelated to locomotion.

WATER, WATER EVERYWHERE

MOST MAMMALS—big ones in particular—cannot live without freshwater. For marine mammals, however, freshwater is difficult to come by. Seals and sea lions obtain most of their water from the fish they eat (some will eat snow to get freshwater), and manatees routinely seek out freshwater from rivers. For their part, cetaceans obtain water both from their food and from sips of the briny deep.

When did whales, which evolved from a fairly large (and therefore freshwater-dependent) terrestrial mammal, develop a system capable of handling the excess salt load associated with ingesting seawater? Evidence from so-called stable oxygen isotopes has provided some clues. In nature, oxygen mainly occurs in two forms, or isotopes: ^{16}O and ^{18}O. The ratios of these isotopes in freshwater and seawater differ, with seawater containing more ^{18}O. Because mammals incorporate oxygen from drinking water into their developing teeth and bones, the remains of those that imbibe seawater can be distinguished from those that take in freshwater.

J.G.M. (Hans) Thewissen of the Northeastern Ohio Universities College of Medicine and his colleagues thus analyzed the oxygen isotope ratios in ancient whale teeth to gain insight into when these animals might have moved from a freshwater-based osmoregulatory system to a seawater-based one. Oxygen isotope values for pakicetids, the most primitive whales, indicate that they drank freshwater, as would be predicted from other indications that these animals spent much of their time on land. Isotope measurements from amphibious *Ambulocetus,* on the other hand, vary widely, and some specimens show no evidence of seawater intake. In explanation, the researchers note that although *Ambulocetus* is known to have spent time in the sea (based on the marine nature of the rocks in which its fossils occur), it may still have had to go ashore to drink. Alternatively, it may have spent the early part of its life (when its teeth mineralized) in freshwater and only later entered the sea.

The protocetids, however, which show more skeletal adaptations to aquatic life, exhibit exclusively marine isotope values, indicating that they drank only seawater. Thus, just a few million years after the first whales evolved, their descendants had adapted to increased salt loads. This physiological innovation no doubt played an important role in facilitating the protocetids' dispersal across the globe. *—K.W.*

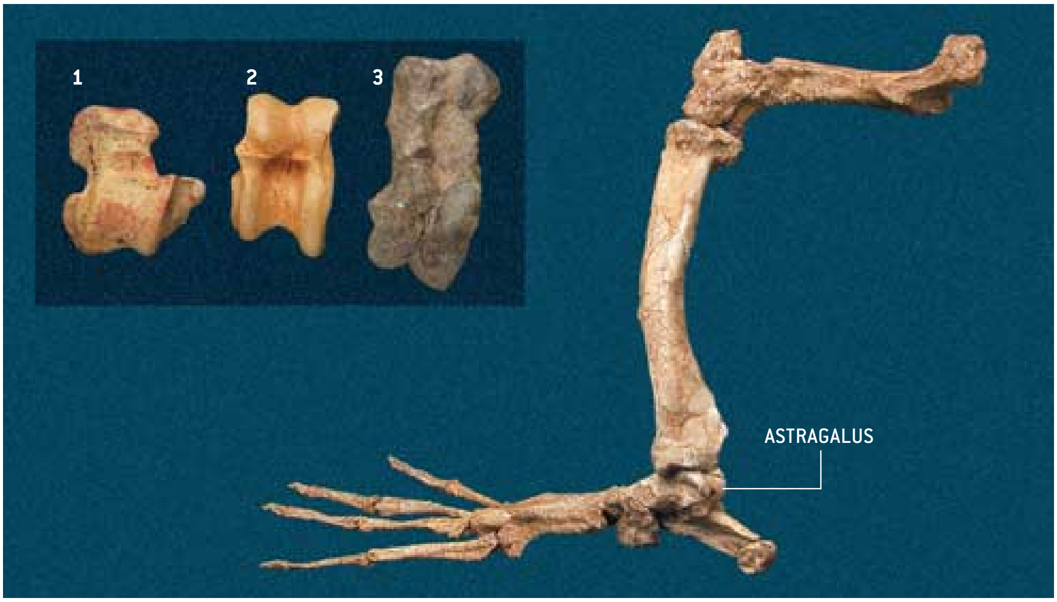
Making Waves

THE FOSSILS UNCOVERED during the 1980s and 1990s advanced researchers' understanding of whale evolution by leaps and bounds, but all morphological signs still pointed to a mesonychid origin. An alternative view of cetacean roots was taking wing in genetics laboratories in the U.S., Belgium and Japan, however. Molecular biologists, having developed sophisticated techniques for analyzing the DNA of living creatures, took Boyden's 1960s immunology-based conclusions a step further. Not only were whales more closely related to artiodactyls than to any other living mammals, they asserted, but in fact whales were themselves artiodactyls, one of many twigs on that branch of the mammalian family tree. Moreover, a number of these studies pointed to an especially close relationship between whales and hippopotamuses. Particularly strong evidence for this idea came in 1999 from analyses of snippets of noncoding DNA called SINES (short interspersed elements), conducted by Norihiro Okada and his colleagues at the Tokyo Institute of Technology.

The whale-hippo connection did not sit well with paleontologists. "I thought they were nuts," Gingerich recollects. "Everything we'd found was consistent with a mesonychid origin. I was happy with that and happy with a connection through mesonychids to artiodactyls." Whereas mesonychids appeared at the right time, in the right place and in the right form to be considered whale progenitors, the fossil record did not seem to contain a temporally, geographically and morphologically plausible artiodactyl ancestor for whales, never mind one linking whales and hippos specifically. Thewissen, too, had largely dismissed the DNA findings. But "I stopped rejecting it when Okada's SINE work came out," he says.

It seemed the only way to resolve the controversy was to find, of all things, an ancient whale anklebone. Morphologists have traditionally defined artiodactyls on the basis of certain features in one of their anklebones, the astragalus, that enhance mobility. Specifically, the unique artiodactyl astragalus has two grooved, pulleylike joint surfaces. One connects to the tibia, or shinbone; the other articulates with more distal anklebones. If whales descended from artiodactyls, researchers reasoned, those that had not yet fully adapted to life in the seas should exhibit this double-pulleyed astragalus.

That piece of the puzzle fell into place last fall, when Gingerich and Thewissen both announced discoveries of new primitive whale fossils. In the eastern part of Baluchistan Province,



HIND LIMB of an ancient whale, *Rodhocetus,* preserves a long-sought anklebone known as the astragalus (*at right*). Shown in the inset beside a mesonychid astragalus (*1*) and one from a modern artiodactyl (*2*), the *Rodhocetus* astragalus (*3*) exhibits the distinctive double-pulley shape that characterizes all artiodactyl astragali, suggesting that whales descended not from mesonychids as previously thought but from an ancient artiodactyl.

DAN ERICKSON

Gingerich's team had found partially articulated skeletons of *Rodhocetus balochistanensis* and a new protocetid genus, *Artiocetus*. Thewissen and his colleagues recovered from a bone bed in the Kala Chitta Hills of Punjab, Pakistan, much of the long-sought postcranial skeleton of *Pakicetus,* as well as that of a smaller member of the pakicetid family, *Ichthyolestes*. Each came with an astragalus bearing the distinctive artiodactyl characteristics.

The anklebones convinced both longtime proponents of the mesonychid hypothesis that whales instead evolved from artiodactyls. Gingerich has even embraced the hippo idea. Although hippos themselves arose long after whales, their purported ancestors—dog- to horse-size, swamp-dwelling beasts called anthracotheres—date back to at least the middle Eocene and may thus have a forebear in common with the cetaceans. In fact, Gingerich notes that *Rodhocetus* and anthracotheres share features in their hands and wrists not seen in any other later artiodactyls. Thewissen agrees that the hippo hypothesis holds much more appeal than it once did. But he cautions that the morphological data do not yet point to a particular artiodactyl, such as the hippo, being the whale's closest relative, or sister group. "We don't have the resolution yet to get them there," he remarks, "but I think that will come."

What of the evidence that seemed to tie early whales to mesonychids? In light of the new ankle data, most workers now suspect that those similarities probably reflect convergent evolution rather than shared ancestry and that mesonychids represent an evolutionary dead end. But not everyone is convinced. Maureen O'Leary of the State University of New York at Stony Brook argues that until all the available evidence—both morphological and molecular—is incorporated into a single phylogenetic analysis, the possibility remains that mesonychids belong at the base of the whale pedigree. It is conceivable, she says, that mesonychids are actually ancient artiodactyls but ones that reversed the ankle trend. If so, mesonychids could still be the whales' closest relative, and hippos could be their closest living relative [*see box on page 74*]. Critics of that idea, however, point out that although folding the mesonychids into the artiodactyl order offers an escape hatch of sorts to supporters of the mesonychid hypothesis, it would upset the long-standing notion that the ankle makes the artiodactyl.

Investigators agree that figuring out the exact relationship between whales and artiodactyls will most likely require finding additional fossils—particularly those that can illuminate the beginnings of artiodactyls in general and hippos in particular. Yet even with those details still unresolved, "we're really getting a handle on whales from their origin to the end of archaeocetes," Uhen reflects. The next step, he says, will be to figure out how the mysticetes and odontocetes arose from the archaeocetes and when their modern features emerged. Researchers may never unravel all the mysteries of whale origins. But if the extraordinary advances made over the past two decades are any indication, with continued probing, answers to many of these lingering questions will surface from the sands of time. SA

Kate Wong is a writer and editor for ScientificAmerican.com

MORE TO EXPLORE

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PROCEEDINGS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Genetic analysis of hair samples attributed to yeti, bigfoot and other anomalous primates

Bryan C. Sykes, Rhettman A. Mullis, Christophe Hagenmuller, Terry W. Melton and Michel Sartori

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Genetic analysis of hair samples attributed to yeti, bigfoot and other anomalous primates

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In the first ever systematic genetic survey, we have used rigorous decontamination followed by mitochondrial 12S RNA sequencing to identify the species origin of 30 hair samples attributed to anomalous primates. Two Himalayan samples, one from Ladakh, India, the other from Bhutan, had their closest genetic affinity with a Palaeolithic polar bear, *Ursus maritimus*. Otherwise the hairs were from a range of known extant mammals.

1. Introduction

Despite several decades of research, mystery still surrounds the species identity of so-called anomalous primates such as the yeti in the Himalaya, almasty in central Asia and sasquatch/bigfoot in North America. On the one hand, numerous reports including eye-witness and footprint evidence, point to the existence of large unidentified primates in many regions of the world. On the other hand, no bodies or recent fossils of such creatures have ever been authenticated. There is no shortage of theories about what these animals may be, ranging from surviving populations of collateral hominids such as *Homo neanderthalensis*, *Homo floresiensis* [1] or Denisovans [2], extinct apes such as *Gigantopithecus* [3] or even unlikely hybrids between *Homo sapiens* and other mammals [4]. Modern science has largely avoided this field and advocates frequently complain that they have been ‘rejected by science’ [5]. This conflicts with the basic tenet that science neither rejects nor accepts anything without examining the evidence. To apply this philosophy to the study of anomalous primates and to introduce some clarity into this often murky field, we have carried out a systematic genetic survey of hair samples attributed to these creatures. Only two ‘tongue-in-cheek’ scientific publications report DNA sequence data from anomalous primates. Milinkovitch *et al*. [6], after analysis of a Nepalese sample, confirmed Captain Haddock’s suspicions that the yeti was an ungulate [7]. The same conclusion was reached by Coltman *et al*. [8] after analysis of sasquatch hair from Alaska.

2. Material and methods

Hair samples submissions were solicited from museum and individual collections in a joint press release issued on 14 May 2012 by the Museum of Zoology, Lausanne and the University of Oxford. A total of 57 samples were received and subjected to macroscopic, microscopic and infrared fluorescence examination to eliminate obvious non-hairs. This excluded one sample of plant material and one of glass fibre. Of the screened samples, 37 were selected for genetic analysis based on their provenance or historic interest. Lengths (2–4 cm) of individual hair shaft

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Table 1. Origin and GenBank sequence matches of hair samples attributed to anomalous primates. (All sequence matches were 100%.)

ref. no.	location	attribution	GenBank sequence match	common name
25025	Ladakh, India	*yeti*	*U. maritimus*	polar bear
25191	Bhutan	*yeti/migyhur*	*U. maritimus*	polar bear
25092	Nepal	*yeti*	*Capricornis sumatraensis*	serow
25027	Russia	*almasty*	*U. arctos*	brown bear
25039	Russia	*almasty*	*Equus caballus*	horse
25040	Russia	*almasty*	*Bos taurus*	cow
25041	Russia	*almasty*	*Equus caballus*	horse
25073	Russia	*almasty*	*Equus caballus*	horse
25074	Russia	*almasty*	*U. americanus*	American black bear
25075	Russia	*almasty*	*P. lotor*	raccoon
25194	Russia	*almasty*	*U. arctos*	brown bear
25044	Sumatra	*orang pendek*	*Tapirus indicus*	Malaysian tapir
25035	AZ, USA	*bigfoot*	*P. lotor*	raccoon
25167	AZ, USA	*bigfoot*	*Ovis aries*	sheep
25104	CA, USA	*bigfoot*	*U. americanus*	American black bear
25106	CA, USA	*bigfoot*	*U. americanus*	American black bear
25081	MN, USA	*bigfoot*	*Erethizon dorsatum*	N. American porcupine
25082	MN, USA	*bigfoot*	*U. americanus*	American black bear
25202	OR, USA	*bigfoot*	*U. americanus*	American black bear
25212	OR, USA	*bigfoot*	*C. lupus/latrans/domesticus*	wolf/coyote/dog
25023	TX, USA	*bigfoot*	*Equus caballus*	horse
25072	TX, USA	*bigfoot*	*Homo sapiens*	human
25028	WA, USA	*bigfoot*	*U. americanus*	American black bear
25029	WA, USA	*bigfoot*	*C. lupus/latrans/domesticus*	wolf/coyote/dog
25030	WA, USA	*bigfoot*	*Bos taurus*	cow
25069	WA, USA	*bigfoot*	*Odocoileus virginianus/hemionus*	white-tailed/mule deer
25086	WA, USA	*bigfoot*	*Bos taurus*	cow
25093	WA, USA	*bigfoot*	*C. lupus/latrans/domesticus*	wolf/coyote/dog
25112	WA, USA	*bigfoot*	*Bos taurus*	cow
25113	WA, USA	*bigfoot*	*C. lupus/latrans/domesticus*	wolf/coyote/dog

were thoroughly cleaned to remove surface contamination, ground into a buffer solution in a glass homogenizer then incubated for 2 h at 56°C in a solution containing proteinase K before extraction with phenol/chloroform/isoamyl alcohol. PCR amplification of the ribosomal mitochondrial DNA 12S fragment corresponding to bps 1093–1196 of the human mitochondrial genome was carried out [9,10]. Recovered sequences were compared to GenBank accessions for species identification.

3. Results and discussion

The table 1 shows the GenBank species identification of sequences matching the 30 samples from which DNA was recovered. Seven samples failed to yield any DNA sequences despite multiple attempts. As the sequence of mitochondrial 12S RNA segment is identical in *H. sapiens* and *H. neanderthalensis*, amplification and sequencing of mitochondrial DNA hypervariable region 1 (bps 16 000–16 400) of no. 25072 was carried out and identified the source as being identical to the revised Cambridge Reference Sequence [11] and thus *H. sapiens* of likely European matrilineal descent. Other submitted samples were of known mammals that in most cases were living within their normal geographical range, the exceptions being sample nos. 25025 and 25191 (*Ursus maritimus*, polar bear) from the Himalayas, no. 25074 (*Ursus americanus*, American black bear) and no. 25075 (*Procyon lotor*, raccoon) that were submitted from Russia even though they are native to North America.

Despite the wide range of age and condition of the submitted hair shafts, which ranged from fresh to museum specimens more than 50 years old, the majority yielded mitochondrial 12S RNA sequences which allowed species identification with 100% sequence identity. Of the recovered sequences, only one (no. 25072) yielded a human sequence, indicating that the rigorous cleaning and extraction protocol had been effective in eliminating extraneous human contamination which often confounds the analysis of old material and may lead to misinterpretation of a sample as human or even as an unlikely and unknown human x mammalian hybrid [4]. The deliberately permissive primer combination used here allowed a wide range of mammalian DNA to be amplified within a single reaction, although this meant that some identification did not go beyond the level of genus. For example, no. 25029 was identified as *Canis* but did not distinguish between *Canis lupus* (wolf), *Canis latrans* (coyote) and *Canis domesticus* (domestic dog).

Sequences derived from hair sample nos. 25025 and 25191 had a 100% match with DNA recovered from a Pleistocene fossil more than 40 000 BP of *U. maritimus* (polar bear) [12] but not to modern examples of the species. Hair sample no. 25025 came from an animal shot by an experienced hunter in Ladakh, India *ca* 40 years ago who reported that its behaviour was very different from a brown bear *Ursus arctos* with which he was very familiar. Hair sample no. 25191 was recovered from a high altitude (*ca* 3500 m) bamboo forest in Bhutan and was identified as a nest of a migyhur, the Bhutanese equivalent of the yeti. The Ladakh hairs (no. 25025) were golden-brown, whereas the hair from Bhutan (no. 25191) was reddish-brown in appearance. As the match is to a segment only 104 bp long, albeit in the very conserved 12S RNA gene, this result should be regarded as preliminary. Other than these data, nothing is currently known about the genetic affinity of Himalayan bears and although there are anecdotal reports of white bears in Central Asia and the Himalayas [13,14], it seems more likely that the two hairs reported here are from either a previously unrecognized bear species, colour variants of *U. maritimus*, or *U. arctos*/*U. maritimus* hybrids. Viable *U. arctos*/*U. maritimus* hybrids are known from the Admiralty, Barayanov and Chicagov (ABC) islands off the coast of Alaska though in the ABC hybrids the mitochondrial sequence homology is with modern rather than ancient polar bears [15]. If they are hybrids, the Ladakh and Bhutan specimens are probably descended from a different hybridization event during the early stages of species divergence between *U. arctos* and *U. maritimus*. Genomic sequence data are needed to decide between these alternatives. If these bears are widely distributed in the Himalayas, they may well contribute to the biological foundation of the yeti legend, especially if, as reported by the hunter who shot the Ladakh specimen, they behave more aggressively towards humans than known indigenous bear species.

With the exception of these two samples, none of the submitted and analysed hairs samples returned a sequence that could not be matched with an extant mammalian species, often a domesticate. While it is important to bear in mind that absence of evidence is not evidence of absence and this survey cannot refute the existence of anomalous primates, neither has it found any evidence in support. Rather than persisting in the view that they have been ‘rejected by science’, advocates in the cryptozoology community have more work to do in order to produce convincing evidence for anomalous primates and now have the means to do so. The techniques described here put an end to decades of ambiguity about species identification of anomalous primate samples and set a rigorous standard against which to judge any future claims.

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Data accessibility. DNA sequences: GenBank accession nos. KJ155696–KJ155724 and KJ607607. Voucher samples of the research materials have been deposited in the Heuvelmans Archive at the Museum of Zoology, Lausanne, Switzerland.

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Molecular analysis of 'anomalous primate' hair samples: a commentary on Sykes *et al.*

Norman MacLeod

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Commentary

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Molecular analysis of 'anomalous primate' hair samples: a commentary on Sykes *et al.*

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While the correct logical formulation of a scientific hypothesis test is taught to virtually every child in their secondary school curriculum, the manner in which scientific researchers approach the resolution of questions concerning the cause(s) of natural phenomena is often misunderstood and/or misconstrued by the general public, usually aided or abetted by media reports that 'scientists have proved' this or that. With the exception of discoveries of species, minerals, compounds, etc., new to science, or known to science but found to occur at a place or time previously unanticipated, very little is 'proved' by science. Indeed, even in these cases all that is proved is that the phenomenon exists or existed at the place and time where it was found. The day-to-day work of most scientists lies not only with the discovery of new phenomena and/or occurrences, but also with the seemingly more mundane, though infinitely more complex, task of interpreting nature: how did the processes and objects we see in nature come to be? How do they function? What influences them and what do they influence? In statistics (which is often used as a tool for testing scientific hypotheses), the hypothetico-deductive formalism scientists most often used to explore these issues is enshrined in the concept of the null hypothesis which states that there is no relationship between two observed or measured phenomena [1]. Thus, in R. A. Fisher's classic 'lady tasting tea' experiment, the ability of the lady in question (Dr Muriel Bristol-Roach) to determine whether the milk was placed in the cup before or after the tea was evaluated by performing a series of randomized blind tests, recording the number of correct identifications and determining whether this number was sufficient to preclude the null hypotheses that they were obtained through random guessing. If the null hypothesis cannot be refuted no alternative hypotheses need be sought.

In the case of cryptozoology, its proponents have, for many years, claimed that the scientific establishment has failed to live up to the tenets of its own philosophy by failing to acknowledge the evidence they have offered for the existence of large species presently unknown to science. In most cases, scientific researchers have regarded this evidence—typically anecdotal observations recounted by individuals backed up on occasion by photographic and/or sound recordings, usually of quite poor quality—as hopelessly ambiguous and so not suitable for rigorous hypothesis testing. In such cases, the 'evidence' that links the observation with an unknown species (the alternative hypothesis) can be attributed reasonably to lack of familiarity of the observer with the regional biota, uncharacteristic behaviour of a known species, unusual lighting or fraudulent staging. In cases of ambiguous evidence such as these the null hypotheses of no link between such evidence and any unknown species is accepted because it cannot be refuted specifically. This stricture also applies to certain types of ephemeral physical evidence (e.g. trackways) that have often been documented photographically.

Of a different character altogether, however, is direct physical evidence in the form of bodies or body parts. These could, in principle, be compared with the body parts of known species and identified unambiguously as either having a combination of characteristics known to occur in a species described previously (the null hypothesis) or a set of characteristics of sufficient novelty to warrant establishment of a new species (the alternative hypothesis). Scientists who have looked into the claims of cryptozoologists have often been struck by the lack of such physical evidence in the form of collected individuals, dead bodies, fossils and/or parts thereof. This lack of direct and unambiguously testable evidence supporting the recognition of animals such as the yeti, Loch Ness Monster, and Morag, not to mention sewer alligators, and the various beasts of Bodmin, Dartmoor, Exmoor and Dean, is the primary reason why many regard cryptozoology as a pseudoscience that accepts the existence of species in the absence of unambiguous physical evidence relying instead on personal observation, anecdote, legend and myth.

Until recently, hair samples reported to have been collected from areas where mammalian cryptozoological species are suspected to have occurred fell into this category of ambiguous evidence owing to the lack of morphological characteristics sufficient to rule out the possibility that they could be derived from extant species. However, owing the recognition that naturally occurring hair samples often include bits of skin and parts of hair follicles, the cells of which contain DNA, along with current DNA sequencing technologies, this physical evidence has moved out of the category of ambiguous, untestable evidence and into the realm of scientifically acceptable physical evidence that can be used, at least in principle, to identify unknown species. The reason for this alteration in the status of hair samples is that DNA sequences recovered therefrom could, in principle, be compared with those of extant species and the null hypothesis that the hair sample was derived from a species already known to science tested empirically.

The results of such tests on a series of 37 hair samples reported anecdotally to come from cryptozoological species is the subject of the Sykes *et al*. [2] article in this issue of the *Proceedings of the Royal Society*. These 37 samples were a subset of 58 samples submitted to the Sykes team for analysis. Of these 58 samples, two were excluded as being non-hair and 37 of the remaining 56 samples were selected for DNA analysis. The 19 samples excluded from DNA analysis were so designated for a variety of reasons including budget constraints, prioritization of samples of particular historical interest and amount of material available. In this reduced sample, seven of the samples selected for sequencing yielded no DNA. However, all of the 30 samples that did yield DNA contained base-pair sequences that were 100% compatible with known mammal species, though in certain instances the hair sample was reported to have been obtained from a region well outside the species' known geographical range. In two instances (samples 25 025 and 25 191), the gene sequence matched not an extant species, but a fossil sequence obtained from a Pleistocene polar bear (*Ursus maritimus*). As polar bears are not known to occur on the Tibetan Plateau, the Sykes team speculate that these samples may have come either from a previously unknown bear species or possibly from a hybrid between *U. maritimus* and the brown bear (*Ursus arctos*). Viable hybrids of these species are known to occur in North America. A hybrid between two known bear species does not conform to the model offered by cryptozoologists to account for these samples, though if a hybrid bear species does occur in this region it may explain some of the anecdotal observations reported by individuals.

Does this evidence disprove the legends of the Yeti, Migyhur, Almasty, Sasquatch/Bigfoot? It does not. Scientific hypothesis testing of this sort is not designed to, and cannot, prove hypotheses alternative to the null hypothesis. All that can be said with confidence is that the results obtained by the Sykes team for the 29 samples that yielded DNA sequences failed to reject the null hypothesis that these samples came from species already known to science. Interestingly, despite the fact that most cryptozoologists have suggested the cryptids in question are unknown primate species, not one of the Sykes team's sequences yielded DNA that could be shown to have come from any non-human primate. Nevertheless, 19 of the original 55 bona fide hair samples submitted originally to the Sykes team did not produce DNA sequences. The taxonomic affinity of these samples remains unknown and science has nothing further to say about them, at least for the moment. From a scientific point of view, these samples return to the category of ambiguous evidence insofar as they cannot offer any unambiguous information that can be used to refute the null hypothesis of no link to any presently unknown (primate) species.

On a more general note, and as the Sykes *et al*. [2] report mentions in its last paragraph, this type of analysis opens the way for cryptozoologists and mainstream biological zoologists to enter into a productive dialogue. Cryptozoologists must now either accept the findings of the Sykes team or show where they are in error. Mainstream zoologists must also now recognize that, in the case of hair samples, the claims of the cryptozoological community are now amenable to scientific testing and potential verification. In this area, these two communities can and should speak the same language, the language of hard scientific data and hypothesis testing. Will this ultimately lead to the recognition of new large mammalian species in out-of-the way corners of the world? No one—certainly no scientist—can say for sure. What we do know is that scientific discoveries just as strange and unexpected as those advocated by cryptozoologists in these cases have happened before (e.g. the coelacanth [3] and the okapi [4]).

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The Impact of Conservation on the Status of the World's Vertebrates

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The Impact of Conservation on the Status of the World's Vertebrates

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Using data for 25,780 species categorized on the International Union for Conservation of Nature Red List, we present an assessment of the status of the world's vertebrates. One-fifth of species are classified as Threatened, and we show that this figure is increasing: On average, 52 species of mammals, birds, and amphibians move one category closer to extinction each year. However, this overall pattern conceals the impact of conservation successes, and we show that the rate of deterioration would have been at least one-fifth again as much in the absence of these. Nonetheless, current conservation efforts remain insufficient to offset the main drivers of biodiversity loss in these groups: agricultural expansion, logging, overexploitation, and invasive alien species.

In the past four decades, individual populations of many species have undergone declines and many habitats have suffered losses of original cover (*1*, *2*) through anthropogenic activity. These losses are manifested in species extinction rates that exceed normal background rates by two to three orders of magnitude (*3*), with substantial detrimental societal and economic consequences (*4*). In response to this crisis, 193 parties to the Convention on Biological Diversity (CBD; adopted 1992) agreed "to achieve by 2010 a significant reduction of the current rate of biodiversity loss at the global, regional, and national level as a contribution to poverty alleviation and to the benefit of all life on Earth" (*5*). That the target has not been met was borne out by empirical testing against 31 cross-disciplinary indicators developed within the CBD framework itself (*1*). However, this does not mean that conservation efforts have been ineffective. Conservation actions have helped to prevent extinctions (*6*, *7*) and improve population trajectories (*8*), but there has been limited assessment of the overall impact of ongoing efforts in reducing losses in biodiversity (*9*, *10*). Here, we assess the overall status of the world's vertebrates, determine temporal trajectories of extinction risk for three vertebrate classes, and estimate the degree to which conservation actions have reduced biodiversity loss.

Described vertebrates include 5498 mammals, 10,027 birds, 9084 reptiles, 6638 amphibians, and 31,327 fishes (table S1). Vertebrates are found at nearly all elevations and depths, occupy most major habitat types, and display remarkable variation in body size and life history. Although they constitute just 3% of known species, vertebrates play vital roles in ecosystems (*11*) and have great cultural importance (*12*). Under the auspices of the International Union for Conservation of Nature (IUCN) Species Survival Commission, we compiled data on the taxonomy, distribution, population trend, major threats, conservation measures, and threat status for 25,780 vertebrate species, including all mammals, birds, amphibians, cartilaginous fishes, and statistically

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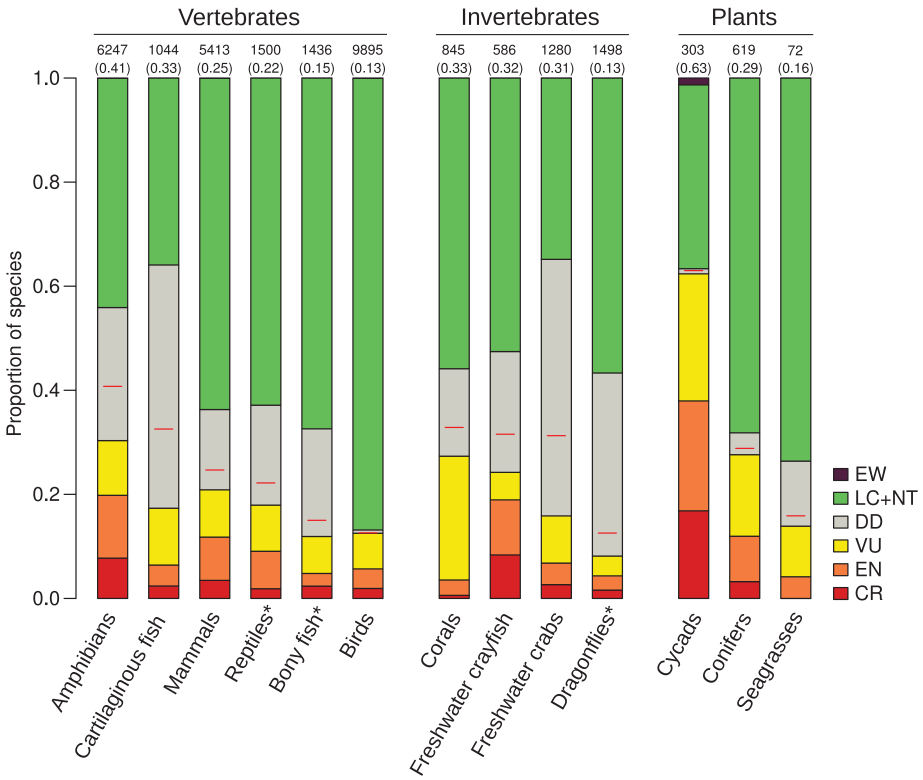

Fig. 1. The proportion of vertebrate species in different Red List categories compared with completely (or representatively) assessed invertebrate and plant taxa on the 2010 IUCN Red List (*15*). EW, Extinct in the Wild; CR, Critically Endangered; EN, Endangered; VU, Vulnerable; NT, Near Threatened; LC, Least Concern; DD, Data Deficient. Extinct species are excluded. Taxa are ordered according to the estimated percentage (shown by horizontal red lines and given in parentheses at tops of bars) of extant species considered Threatened if Data Deficient species are Threatened in the same proportion as data-sufficient species. Numbers above the bars represent numbers of extant species assessed in the group; asterisks indicate those groups in which estimates are derived from a randomized sampling approach.

representative samples of reptiles and bony fishes [~1500 species each (*13*)].

The IUCN Red List is the widely accepted standard for assessing species' global risk of extinction according to established quantitative criteria (*14*). Species are categorized in one of eight categories of extinction risk, with those in the categories Critically Endangered, Endangered, or Vulnerable classified as Threatened. Assessments are designed to be transparent, objective, and consultative, increasingly facilitated through workshops and Web-based open-access systems. All data are made freely available for consultation (*15*) and can therefore be challenged and improved upon as part of an iterative process toward ensuring repeatable assessments over time.

Status, trends, and threats. Almost one-fifth of extant vertebrate species are classified as Threatened, ranging from 13% of birds to 41% of amphibians, which is broadly comparable with the range observed in the few invertebrate and plant taxa completely or representatively assessed to date (Fig. 1 and table S2). When we incorporate the uncertainty that Data Deficient species (those with insufficient information for determining risk of extinction) introduce, the proportion of all vertebrate species classified as Threatened is between 16% and 33% (midpoint = 19%; table S3). [Further details of the data and assumptions behind these values are provided in (*16*) and tables S2 and S3.] Threatened vertebrates occur mainly in tropical regions (Fig. 2), and these concentrations are generally disproportionately high even when accounting for their high overall species

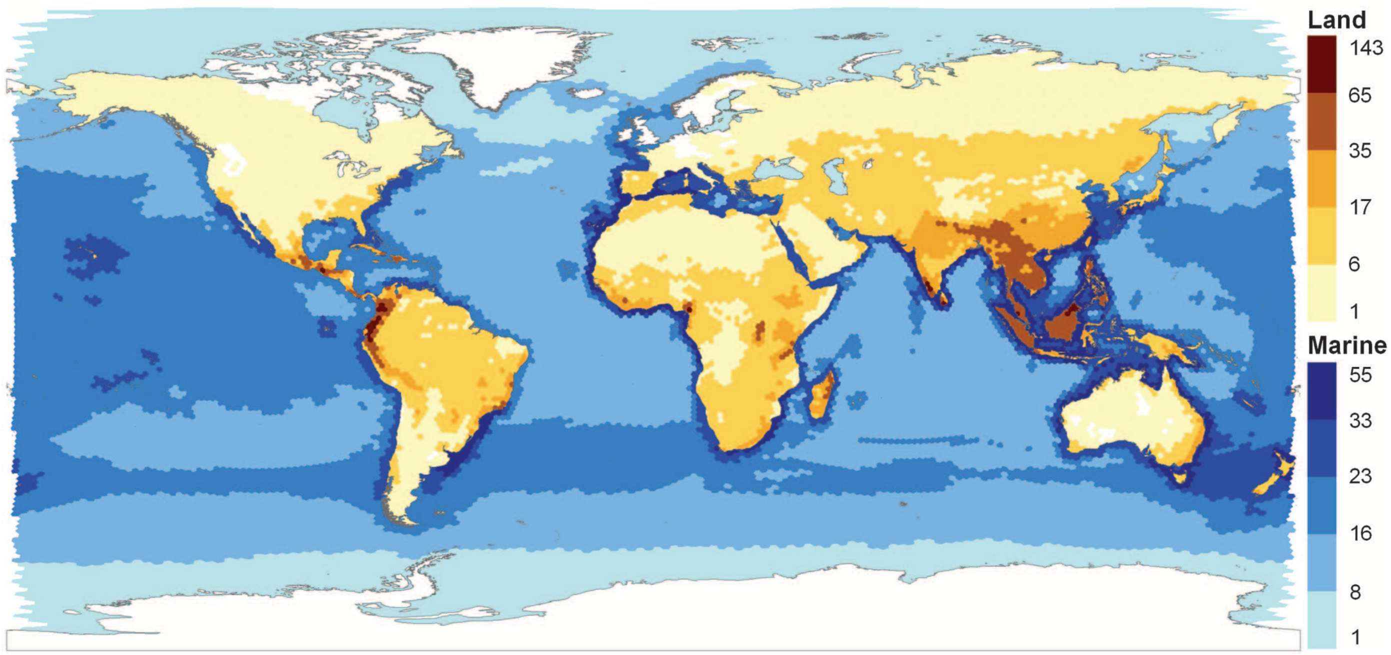

Fig. 2. Global patterns of threat, for land (terrestrial and freshwater, in brown) and marine (in blue) vertebrates, based on the number of globally Threatened species in total.

richness (fig. S4, A and B). These patterns highlight regions where large numbers of species with restricted distributions (*17*) coincide with intensive direct and indirect anthropogenic pressures, such as deforestation (*18*) and fisheries (*19*).

To investigate temporal trends in extinction risk of vertebrates, we used the IUCN Red List Index (RLI) methodology (*20*) that has been

Table 1. Net number of species qualifying for revised IUCN Red List categories between assessments owing to genuine improvement or deterioration in status, for birds (1988 to 2008), mammals (1996 to 2008), and amphibians (1980 to 2004). Category abbreviations are as for Fig. 1; CR(PE/PEW) denotes Critically Endangered (Possibly Extinct or Possibly Extinct in the Wild). CR excludes PE/PEW. Species undergoing an improvement (i.e., moving from a higher to a lower category of threat) are indicated by "+"; species deteriorating in status (i.e., moving from a lower to a higher category of threat) are indicated by "–". Species changing categories for nongenuine reasons, such as improved knowledge or revised taxonomy, are excluded. In the case of birds, for which multiple assessments have been undertaken, values in parentheses correspond to the sum of all changes between consecutive assessments; the same species may therefore contribute to the table more than once [see (*16*)].

			Red List category at end of period							
			EX	**EW**	**CR (PE/PEW)**	**CR**	**EN**	**VU**	**NT**	**LC**
Red List category at start of period	**Birds**	EX		0	0	0	0	0	0	0
		EW	0		0	+1 (+1)	0	0	0	0
		CR (PE/PEW)	0	0		0	0	0	0	0
		CR	–2 (–2)	–2 (–2)	–7 (–7)		+16 (+19)	+1 (+3)	0	0
		EN	0	0	0	–22 (–27)		+4 (+5)	0	0
		VU	0	0	0	–10 (–11)	–34 (–41)		+9 (+10)	0 (+1)
		NT	0	0	0	–4 (–4)	–5 (–2)	–40 (–47)		+1 (+1)
		LC	0	0	0	–1 (0)	–5 (–4)	–5 (–5)	–78 (–81)	
	Mammals	EX		0	0	0	0	0	0	0
		EW	0		0	+1	+1	0	0	0
		CR (PE/PEW)	0	0		0	0	0	0	0
		CR	0	–1	–3		+3	+2	0	0
		EN	0	0	0	–31		+3	+1	0
		VU	0	0	0	–2	–39		+5	+1
		NT	0	0	0	–1	–4	–47		+7
		LC	0	0	0	0	–2	–2	–39	
	Amphibians	EX		0	0	0	0	0	0	0
		EW	0		0	0	0	0	0	0
		CR (PE/PEW)	–2	0		0	0	0	0	0
		CR	–3	–1	–34		0	+2	0	0
		EN	–2	0	–42	–77		0	+2	0
		VU	–2	0	–19	–51	–45		0	0
		NT	0	0	0	–7	–18	–32		0
		LC	0	0	0	–3	–8	–20	–92	

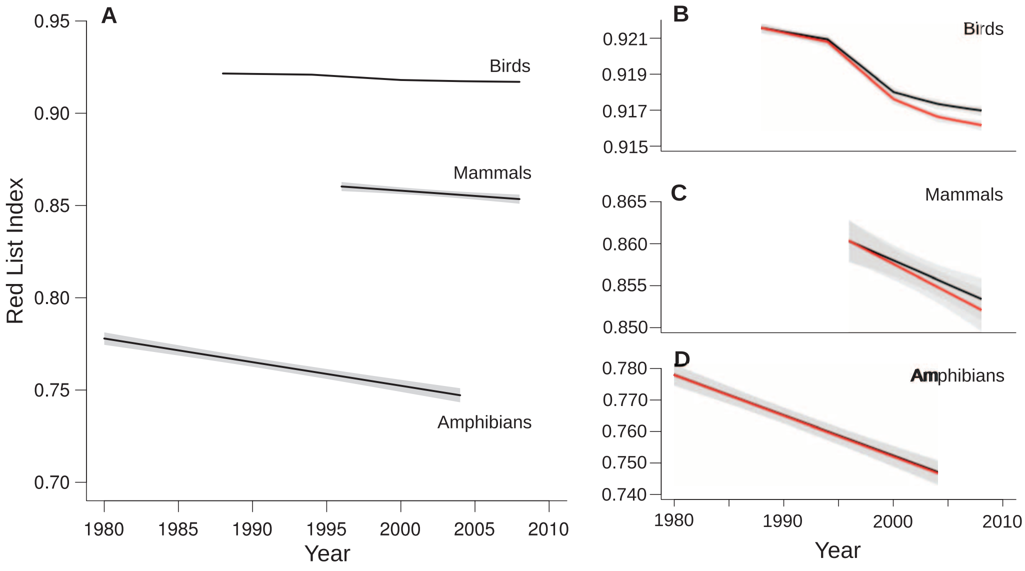

Fig. 3. (**A**) Trends in the Red List Index (RLI) for the world's birds, mammals, and amphibians. (**B** to **D**) Observed change in the RLI for each group (black) compared with RLI trends that would be expected if species that underwent an improvement in status due to conservation action had undergone no change (red). The difference is attributable to conservation. An RLI value of 1 equates to all species being Least Concern; an RLI value of 0 equates to all species being Extinct. Improvements in species conservation status lead to increases in the RLI; deteriorations lead to declines. A downward trend in the RLI value means that the net expected rate of species extinctions is increasing. Shading shows 95% confidence intervals. Note: RLI scales for (B), (C), and (D) vary.

adopted for reporting against global targets (*1*, *2*). We calculated the change in RLI for birds (1988, 1994, 2000, 2004, and 2008), mammals (1996 and 2008), and amphibians (1980 and 2004); global trend data are not yet available for other vertebrate groups, although regional indices have been developed (*21*). The RLI methodology is explained in detail in (*16*), but in summary the index is an aggregated measure of extinction risk calculated from the Red List categories of all assessed species in a taxon, excluding Data Deficient species. Changes in the RLI over time result from species changing categories between assessments (Table 1). Only real improvements or deteriorations in status (termed "genuine" changes) are considered; recategorizations attributable to improved knowledge, taxonomy, or criteria change ("nongenuine" changes) are excluded (*22*). Accordingly, the RLI is calculated only after earlier Red List categorizations are retrospectively corrected using current information and taxonomy, to ensure that the same species are considered throughout and that only genuine changes are included. For example, the greater red musk shrew (*Crocidura flavescens*) was classified as Vulnerable in 1996 and as Least Concern in 2008; however, current evidence indicates that the species was also Least Concern in 1996, and the apparent improvement is therefore a nongenuine change. In contrast, Hose's broadbill (*Calyptomena hosii*) was one of 72 bird species to deteriorate one Red List category between 1994 and 2000, from Least Concern to Near Threatened, mainly because of accelerating habitat loss in the Sundaic lowlands in the 1990s. Such a deterioration in a species' conservation status leads to a decline in the RLI (corresponding to increased aggregated extinction risk); an improvement would lead to an increase in the RLI.

Temporal trajectories reveal declining RLIs for all three taxa. Among birds, the RLI (Fig. 3A) showed that their status deteriorated from 1988 to 2008, with index values declining by 0.49%, an average of 0.02% per year (table S4). For mammals, the RLI declined by 0.8% from 1996 to 2008, a faster rate (0.07% per year) than for birds. Proportionally, amphibians were more threatened than either birds or mammals; RLI values declined 3.4% from 1980 to 2004 (0.14% per year). Although the absolute and proportional declines in RLIs for each taxonomic group were small, these represent considerable biodiversity losses. For example, the deterioration for amphibians was equivalent to 662 amphibian species each moving one Red List category closer to extinction over the assessment period. The deteriorations for birds and mammals equate to 223 and 156 species, respectively, deteriorating at least one category. On average, 52 species per year moved one Red List category closer to extinction from 1980 to 2008. Note that the RLI does not reflect ongoing population changes that are occurring too slowly to trigger change to different categories of threat. Other indicators based on vertebrate population sizes showed declines of 30% between 1970 and 2007 (*1*, *2*, *22*).

Global patterns of increase in overall extinction risk are most marked in Southeast Asia (Fig. 4 and figs. S5A and S6). It is known that the planting of perennial export crops (such as oil palm), commercial hardwood timber operations, agricultural conversion to rice paddies, and unsustainable hunting have been detrimental to species in the region (*23*), but here we show the accelerating rate at which these forces are driving change. In California, Central America, the tropical Andean regions of South America, and Australia, patterns have been driven mainly by the "enigmatic" deteriorations among amphibians (*24*), which have increasingly been linked to the infectious disease chytridiomycosis, caused by the presumed invasive fungal pathogen *Batrachochytrium dendrobatidis* (*25*). Almost 40 amphibians have deteriorated in status by three or more IUCN Red List categories between 1980 and 2004 (Table 1).

Although chytridiomycosis has been perhaps the most virulent threat affecting vertebrates to emerge in recent years, it is not the only novel cause of rapid declines. The toxic effects of the veterinary drug diclofenac on Asian vultures have

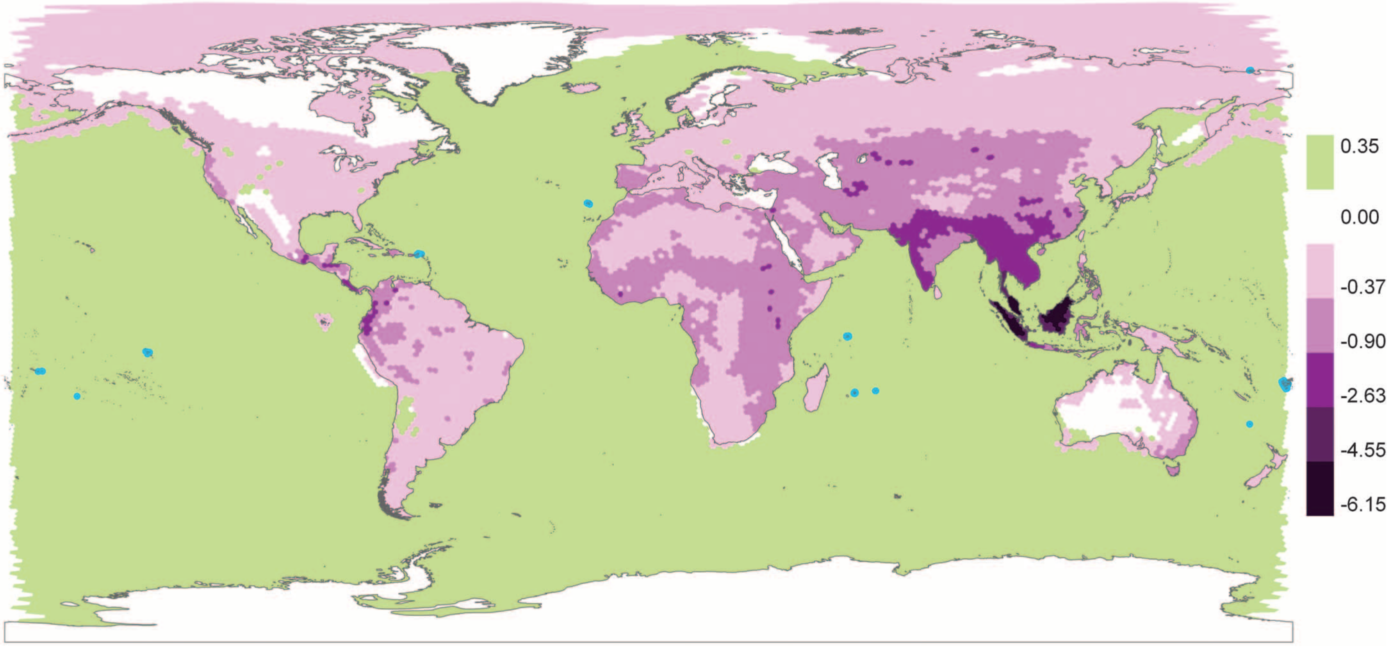

Fig. 4. Global patterns of net change in overall extinction risk across birds, mammals, and amphibians (for the periods plotted in Fig. 3) mapped as average number of genuine Red List category changes per cell per year. Purple corresponds to net deterioration (i.e., net increase in extinction risk) in that cell; green, net improvement (i.e., decrease in extinction risk); white, no change. The uniform pattern of improvement at sea is driven by improvements of migratory marine mammals with cosmopolitan distributions (e.g., the humpback whale). Deteriorations on islands [e.g., the nightingale reed-warbler (*Acrocephalus luscinius*) in the Northern Mariana Islands] and improvements on islands [e.g., the Rarotonga monarch (*Pomarea dimidiata*) in the Cook Islands] are hard to discern; islands showing overall net improvements are shown in blue. Note that the intensity of improvements never matches the intensity of deteriorations.

caused estimated population declines exceeding 99% over the past two decades in certain *Gyps* species, and have resulted in three species moving from Near Threatened to Critically Endangered between 1994 and 2000. Numbers of Tasmanian devils (*Sarcophilus harrisii*) have fallen by more than 60% in the past 10 years because of the emergence of devil facial tumor disease (resulting in three step changes from Least Concern to Endangered). Climate change is not yet adequately captured by the IUCN Red List (*26*, *27*) but has been directly implicated in the deteriorating status of several vertebrates and may interact with other threats to hasten extinction (*28*). However, there is no evidence of a parallel to the systemwide deteriorations documented for reef-building corals affected by bleaching events related to El Niño–Southern Oscillation occurrences (*29*).

Most deteriorations in status are reversible, but in 13% of cases they have resulted in extinction. Two bird species—the kamao (*Myadestes myadestinus*) from Hawaii and the Alaotra grebe (*Tachybaptus rufolavatus*) from Madagascar—became extinct between 1988 and 2008, and a further six Critically Endangered species have been flagged as "possibly extinct" during this period (Table 1 and table S5). At least nine amphibian species vanished during the two decades after 1980, including the golden toad (*Incilius periglenes*) from Costa Rica and both of Australia's unique gastric-brooding frog species (genus *Rheobatrachus*); a further 95 became possibly extinct, 18 of them harlequin toads in the Neotropical genus *Atelopus* (23% of species). No mammals are listed as Extinct for the period 1996 to 2008, although the possible extinction of the Yangtze River dolphin (*Lipotes vexillifer*) would be the first megafauna vertebrate species extinction since the Caribbean monk seal in the 1950s (*30*).

Estimates of conservation success. These results support previous findings that the state of biodiversity continues to decline, despite increasing trends in responses such as protected areas coverage and adoption of national legislation (*1*, *2*). Next, we asked whether conservation efforts have made any measurable contribution to reducing declines or improving the status of biodiversity.

The RLI trends reported here are derived from 928 cases of recategorization on the IUCN Red List (Table 1 and table S6), but not all of these refer to deteriorations. In 7% of cases (68/928), species underwent an improvement in status, all but four due to conservation action. For example, the Asian crested ibis (*Nipponia nippon*) changed from Critically Endangered in 1994 to Endangered in 2000 owing to protection of nesting trees, control of agrochemicals in rice fields, and prohibition of firearms; the four exceptions were improvements resulting from natural processes, such as unassisted habitat regeneration (tables S7 and S8). Nearly all of these improvements involved mammals and birds, where the history of conservation extends farther back and where the bulk of species-focused conservation funding and attention is directed (*31*). Only four amphibian species underwent improvements, because the amphibian extinction crisis is such a new phenomenon and a plan for action has only recently been developed (*32*).

To estimate the impact of conservation successes, we compared the observed changes in the RLI with the RLI trends expected if all 64 species that underwent an improvement in status due to conservation action had not done so (*16*). Our explicit assumption is that in the absence of conservation, these species would have remained unchanged in their original category, although we note that this approach is conservative because it is likely that some would have deteriorated [in the sense of (*6*)]. The resulting difference between the two RLIs can be attributed to conservation. We show that the index would have declined by an additional 18% for both birds and mammals in the absence of conservation (Fig. 3, B and C, and table S4). There was little difference for amphibians (+1.4%; Fig. 3D) given the paucity of species improvements. For birds, conservation action reduced the decline in the RLI from 0.58% to 0.49%, equivalent to preventing 39 species each moving one Red List category closer to extinction between 1988 and 2008. For mammals, conservation action reduced the RLI decline from 0.94% to 0.8%, equivalent to preventing 29 species moving one category closer to extinction between 1996 and 2008.

These results grossly underestimate the impact of conservation, because they do not account for species that either (i) would have deteriorated further in the absence of conservation actions, or (ii) improved numerically, although not enough to change Red List status. As an example among the former, the black stilt (*Himantopus novaezelandiae*) would have gone extinct were it not for reintroduction and predator control efforts, and its Critically Endangered listing has thus remained unchanged (*6*). Among the latter, conservation efforts improved the total population numbers of 33 Critically Endangered birds during the period 1994 to 2004, but not sufficiently for any species to be moved to a lower category of threat (*33*). As many as 9% of mammals, birds, and amphibians classified as Threatened or Near Threatened have stable or increasing populations (*15*) largely due to conservation efforts, but it will take time for these successes to translate into improvements in status. Conservation efforts have also helped to avoid the deterioration in status of Least Concern species. Finally, conservation actions have benefited many other Threatened species besides birds, mammals, and amphibians, but this cannot yet be quantified through the RLI for groups that have been assessed only once [e.g., salmon shark (*Lamna ditropis*) numbers have improved as the result of a 1992 U.N. moratorium on large-scale pelagic driftnet fisheries].

Confronting threats. Species recovery is complex and case-specific, but threat mitigation is always required. We investigated the main drivers of increased extinction risk by identifying, for each species that deteriorated in status, the primary threat responsible for that change. To understand which drivers of increased extinction risk are being mitigated most successfully, we identified, for each species that improved in status, the primary threat offset by successful conservation (table S6).

We found that for any single threat, regardless of the taxa involved, deteriorations outnumber improvements; conservation actions have not yet succeeded in offsetting any major driver of increased extinction risk (fig. S7). On a per-species basis, amphibians are in an especially dire situation, suffering the double jeopardy of exceptionally high levels of threat coupled with low levels of conservation effort. Still, there are conservation successes among birds and mammals, and here we investigate the degree to which particular threats have been addressed.

Conservation actions have been relatively successful at offsetting the threat of invasive alien species for birds and mammals: For every five species that deteriorated in status because of this threat, two improved through its mitigation. These successes have resulted from the implementation of targeted control or eradication programs [e.g., introduced cats have been eradicated from 37 islands since the mid-1980s (*34*)] coupled with reintroduction initiatives [e.g., the Seychelles magpie-robin (*Copsychus sechellarum*) population was 12 to 15 birds in 1965 but had increased to 150 birds by 2005 (fig. S8)]. Many of these improvements have occurred on small islands but also in Australia, owing in part to control of the red fox (*Vulpes vulpes*) (Fig. 4 and fig. S5B). However, among amphibians, only a single species—the Mallorcan midwife toad (*Alytes muletensis*)—improved in status as a result of mitigation of the threat posed by invasive alien species, compared with 208 species that deteriorated. This is because there is still a lack of understanding of the pathways by which chytridiomycosis is spread and may be controlled, and in situ conservation management options are only just beginning to be identified [e.g., (*35*)]. Meanwhile, the establishment of select, targeted captive populations with the goal of reintroducing species in the wild may offer valuable opportunities once impacts in their native habitat are brought under control [e.g., the Kihansi spray toad (*Nectophrynoides asperginis*), categorized as Extinct in the Wild because of drastic alteration of its spray zone habitat].

For mammals and birds, the threats leading to habitat loss have been less effectively addressed relative to that of invasive alien species: For every 10 species deteriorating as a result of agricultural expansion, fewer than 1 improved because of mitigation of this threat. Protected areas are an essential tool to safeguard biodiversity from habitat loss, but the protected areas network remains incomplete and nonstrategic relative to Threatened species (*17*), and reserve management can be ineffective (*36*). Numerous Threatened species are restricted to single sites, many still unprotected (*37*), and these present key opportunities to slow rates of extinction. In the broader matrix of unprotected land, agri-environmental schemes could offer important biodiversity benefits, provided that management policies are sufficient to enhance populations of Threatened species (*38*).

Hunting has been relatively poorly addressed in mammals (62 deteriorations, 6 improvements) when compared with birds (31 deteriorations, 9 improvements). In birds, successes have resulted mainly from targeted protection [e.g., Lear's macaw (*Anodorhynchus leari*) changed from Critically Endangered to Endangered as a result of active protection of the Toca Velha/Serra Branca cliffs in Brazil], but also from enforcement of legislation (e.g., hunting bans) and harvest management measures. Many mammals subject to hunting occur at low densities, have large home ranges, and/or are large-bodied. Although active site-based protection has contributed to an improvement in the status of some of these species, site protection alone is often insufficient if not complemented by appropriate legislation, biological management, and effective enforcement (*39*). For example, a combination of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and enactment of the Vicuña Convention, which prohibited domestic exploitation and mandated the establishment of protected areas, has helped to improve the status of the vicuña (*Vicugna vicugna*) from Near Threatened to Least Concern.

The threat of fisheries has been mitigated relatively more effectively for marine mammals (4 deteriorations, 2 improvements) than for birds (10 deteriorations, 0 improvements), reflecting both the time when drivers first emerged and the past influence of supranational conservation policy. Among historically exploited, long-lived mammals, for example, the humpback whale (*Megaptera novaeangliae*) has benefited from protection from commercial whaling (since 1955) and has improved from Vulnerable to Least Concern. Declines among slow-breeding seabirds (particularly albatrosses and petrels; fig. S9) are mainly a consequence of increasing incidental by-catch resulting from the growth of commercial fisheries, primarily those that use long-line and trawling methods. Legislative tools, such as the recently enacted multilateral Agreement on the Conservation of Albatrosses and Petrels (*40*), may yet deliver dividends by coordinating international action to reduce fisheries mortality of these highly migratory species. Binding legislation and harvest management strategies also are urgently needed to address the disproportionate impact of fisheries on cartilaginous fishes (fig. S10).

We have no data on the relationship between expenditure on biodiversity and conservation success. A disproportionate percentage of annual conservation funding is spent in economically wealthy countries (*41*), where there are generally fewer Threatened species (Fig. 2 and fig. S4B) and the disparity between success and failure appears less evident (Fig. 4). Southeast Asia, by contrast, has the greatest imbalance between improving and deteriorating trends, emphasizing the need there for greater investment of resources and effort.

Conclusions. Our study confirms previous reports of continued biodiversity losses. We also find evidence of notable conservation successes illustrating that targeted, strategic conservation action can reduce the rate of loss relative to that anticipated without such efforts. Nonetheless, the current level of action is outweighed by the magnitude of threat, and conservation responses will need to be substantially scaled up to combat the extinction crisis. Even with recoveries, many species remain conservation-dependent, requiring sustained, long-term investment (*42*); for example, actions have been under way for 30 years for the golden lion tamarin (*Leontopithecus rosalia*), 70 years for the whooping crane (*Grus americana*), and 115 years for the white rhinoceros (*Ceratotherium simum*).

Halting biodiversity loss will require coordinated efforts to safeguard and effectively manage critical sites, complemented by broad-scale action to minimize further destruction, degradation, and fragmentation of habitats (*37*, *39*) and to promote sustainable use of productive lands and waters in a way that is supportive to biodiversity. Effective implementation and enforcement of appropriate legislation could deliver quick successes; for example, by-catch mitigation measures, shark-finning bans, and meaningful catch limits have considerable potential to reduce declines in marine species (*19*). The 2010 biodiversity target may not have been met, but conservation efforts have not been a failure. The challenge is to remedy the current shortfall in conservation action to halt the attrition of global biodiversity.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1194442/DC1
Materials and Methods
Figs. S1 to S10
Tables S1 to S9
References
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Competing interests statement

The authors declare that they have no competing financial interests.

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Global environmental controls of diversity in large herbivores

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Large mammalian herbivores occupy half of the earth's land surface and are important both ecologically and economically[1], but their diversity is threatened by human activities[2]. We investigated how the diversity of large herbivores changes across gradients of global precipitation and soil fertility. Here we show that more plant-available moisture reduces the nutrient content of plants but increases productivity, whereas more plant-available nutrients increase both of these factors. Because larger herbivore species tolerate lower plant nutrient content but require greater plant abundance, the highest potential herbivore diversity should occur in locations with intermediate moisture and high nutrients. These areas are dry enough to yield high quality plants and support smaller herbivores, but productive enough to support larger herbivores. These predictions fit with observed patterns of body size and diversity for large mammalian herbivores in North America, Africa and Australia, and yield a global map of regions with potentially high herbivore diversity. Thus, gradients of precipitation, temperature and soil fertility might explain the global distribution of large herbivore diversity and help to identify crucial areas for conservation and restoration.

Previous studies have linked rainfall, soil fertility and primary productivity to total herbivore community biomass[3–5], plant quality[6–8] and species richness of herbivores[9–12], but have not explained why and how these factors affect herbivore diversity[13]. The ability of large herbivores (mass > 2 kg) to persist probably changes across gradients of plant abundance and quality. Plant productivity and quality are influenced by the availability of two principal plant resources, water and nutrients, and thus change across environmental gradients of these resources[14]. Previous results[15] have shown that plant abundance, as measured by the equilibrium biomass of ungrazed plants, increases linearly with rainfall—a crude measure of plant-available moisture. This increase is stronger at higher nutrient availability (Fig. 1a). However, leaf tissue nitrogen content, an index of plant quality to herbivores, decreases with plant-available moisture even though it also increases with plant-available nutrients (Fig. 1b). Similar patterns occur with plant phosphorus content[15,16].

These combined effects imply that plant abundance and nutrient content show different response surfaces to moisture and nutrients (Fig. 1c, d). Plant abundance is lowest at either low moisture or low nutrient availability, and highest when both are high (Fig. 1c). By contrast, plant nutrient content is lowest at combinations of high plant-available moisture and low nutrients, and highest at combinations of low plant-available moisture and high nutrients. We expect the contours of the response surface for plant nutrient content to be concave at low moisture and relatively horizontal at high moisture (Fig. 1d), because an increase in nutrients will increase plant nutrient content more strongly at low than at high moisture[17] (Fig. 1b).

The two response surfaces for plant abundance and nutrient content can be combined to define potential conditions for the presence of large herbivores. A given herbivore species must encounter plants of both sufficient abundance and quality to persist, and therefore may be constrained to persist only under certain conditions of plant-available moisture and nutrients. These conditions can be defined in a graphical model by two proposed thresholds of combinations of moisture and nutrients that allow plants of sufficient quality and abundance for a herbivore's persistence (Fig. 2a). A specific contour of the plant abundance response surface (Fig. 1c) will correspond to the plant abundance requirements of a herbivore, and represents the 'plant abundance threshold' of the herbivore. Similarly, a specific contour of the plant nutrient content response surface (Fig. 1d) will correspond to the plant quality requirements of a herbivore, and represents the 'plant quality threshold' of the herbivore.

The plant abundance threshold of a herbivore species is the minimum plant-available moisture, for a given nutrient availability, above which plant productivity will be sufficiently high to support a population of that herbivore species. Likewise, the plant quality threshold of a herbivore species is the maximum plant-available moisture, for a given nutrient availability, below which plant tissue is sufficiently nutrient-rich for that herbivore species to persist. Together, the quality and abundance thresholds define a 'wedge' of combinations of moisture and nutrients at which a herbivore species can persist (Fig. 2a).

The predicted potential diversity of different-sized herbivores at a certain combination of moisture and nutrients should reflect how many species can persist at those conditions. Larger herbivores require more abundant plants but can tolerate lower plant quality than smaller herbivores, whereas smaller herbivores can persist on less-abundant plants but only if the plants are of higher quality[3,8,18–20]. Thus, the plant abundance and quality thresholds should differ across orders of magnitude in herbivore body sizes[8]. The plant abundance threshold of larger herbivores will be shifted farther from the origin, but their plant quality threshold will be more horizontal and shifted to wetter conditions (Fig. 2b). Smaller herbivores should have abundance thresholds closer to the origin, plus more sharply concave quality thresholds shifted towards drier, more fertile conditions.

Thus, the occurrence of larger herbivores is expected to increase with greater moisture, but to be relatively independent of plant-available nutrients. In contrast, smaller herbivores should decrease in occurrence with greater moisture and increase with greater nutrient availability. Therefore, the mean body size for all species

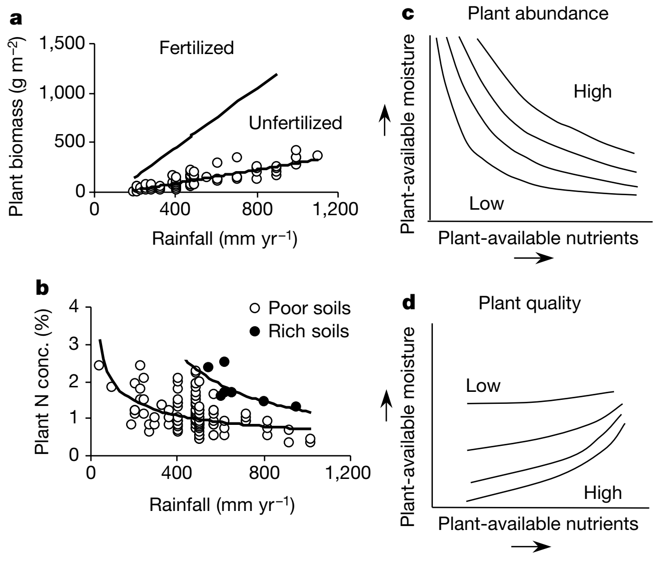

Figure 1 Plant biomass and tissue nitrogen content changes across rainfall gradients in Africa. **a**, Ungrazed plant biomass (*V*, open circles) increases with rainfall (*M*) on poor soils in West Africa ($V = -46.32 + 0.34M$; $n = 77$, $R^2 = 0.70$, $P < 0.001$) and fertilized patches at the same sites (only the regression line is available)[15,16]. **b**, Whole-plant tissue nitrogen content (*N*) at the same sites (open circles) decreases across the same rainfall gradient on poor soils ($N = 15.99M^{-0.45}$; $n = 117$, $R^2 = 0.22$, $P < 0.001$), as it does on rich soils from East Africa ($N = 822.14M^{-0.95}$; $M^2 = 0.57$, $P = 0.02$)[26–30]. Plant tissue phosphorus content on poor West African soils responded similarly to rainfall as tissue nitrogen content[15,16]. **c**, **d**, Hypothetical response surfaces for plant biomass (**c**; abundance) and plant nutrient content (**d**) to plant-available moisture (balance of rainfall and potential evapotranspiration) and plant-available nutrients, inferred from observed data in **a** and **b**. Contour shapes in **c** reflect the joint limitation of plant biomass by water and soil nutrients. Contour shapes in **d** reflect the observed data in **b**, which show that plant nutrient content increases with plant-available nutrients more rapidly at low than at high plant-available moisture.

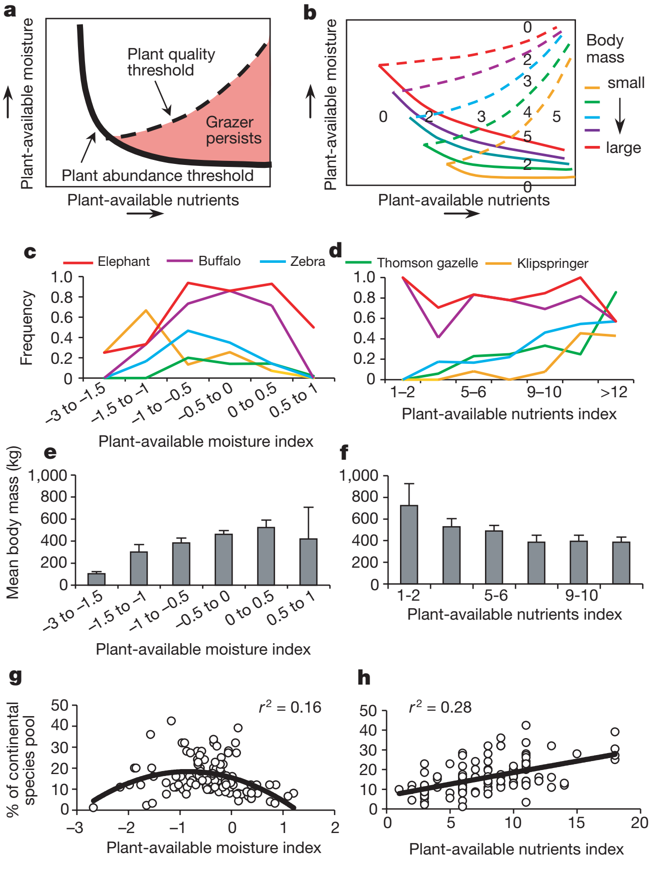

Figure 2 Predicted and observed patterns of herbivore diversity along gradients of plant-available moisture and nutrients. **a**, Threshold combinations of plant-available moisture and nutrients that allow a hypothetical herbivore to persist. Plant abundance and plant quality thresholds reflect shapes of the contours of the response surfaces for plant biomass and plant nutrient content, respectively. **b**, Hypothetical regions of persistence for six different species that differ in body mass, as defined by plant abundance thresholds (solid curves) and plant quality thresholds (dashed curves). Numbers indicate how many herbivore species can persist under different conditions of plant-available moisture and nutrients. Note the greater overlap in regions of persistence at intermediate plant-available moisture and high plant-available nutrients. **c**, **d**, Frequency of occurrence of five different-sized herbivore species (klipspringer, *Oreotragus oreotragus*; Thomson's gazelle, *Gazella thomsoni*; Burchell's zebra, *Hippotigris quagga*; Cape buffalo, *Syncerus caffer*; elephant, *Loxodonta africana*) among 85 African parks in different intervals of indices for plant-available moisture (**c**) and plant-available nutrients (**d**). **e**, **f**, Body mass (mean ± s.e.) of all species present in different intervals of indices for plant-available moisture (**e**) and plant-available nutrients (**f**). **g**, **h**, Observed large herbivore species richness, expressed as a percentage of the continental species pools from 118 sites in North America and Africa versus indices for plant-available moisture ($\log_{10}$[precipitation/potential evapotranspiration]), $y = -3.81x^2 - 6.53x + 14.93$ (**g**), and plant-available nutrients (ref. 25, and Methods), $y = 1.10x + 6.79$ (**h**).

is expected first to increase rapidly with plant-available moisture and then to level off, but to decrease continuously with plant-available nutrients (Fig. 2b).

The trade-off in requirements for plant quantity and quality for different-sized herbivores ultimately predicts general patterns of herbivore diversity across gradients of water availability and soil nutrients. At a given nutrient concentration, herbivore species richness is predicted to peak at intermediate moisture because both small and large species occur together (Fig. 2b). For a given moisture, however, herbivore species richness should increase continuously with greater nutrients because more smaller species are added (Fig. 2b). The highest herbivore diversity is thus expected in locations that are not so wet and/or infertile that average plant quality would be too low to sustain smaller herbivores, and also not so dry and/or infertile that plant productivity would be insufficient to sustain larger herbivores (Fig. 2b). This prediction is insensitive to the shapes of the contours of plant abundance and nutrient content (Fig. 1a–d).

We tested our predictions by compiling a data set of the observed occurrence and species richness of all terrestrial mammalian herbivores with a mass greater than 2 kg (grazers, mixed feeders and browsers) in 33 different protected natural areas in North America and 85 such areas in sub-Saharan Africa (Methods). For every site, we calculated indices for plant-available moisture and nutrients (Methods), and graphed changes in individual species, mean body mass and species richness along these gradients. We expressed species richness as a proportion of the total species richness per continent to standardize for differences between the two continents in size and biogeographical history[21,22].

Observed frequencies of occurrence of five different-sized grazing mammals, chosen as representative examples, in 85 parks in Africa support our predictions for individual species (Fig. 2c, d). Large species (Cape buffalo and elephant) peaked in occurrence at higher plant-available moisture than did intermediate-sized herbivores (zebra, Thomson's gazelle), which in turn peaked in occurrence at higher water availability than did a small species (klipspringer). In addition, logistic regression showed that occurrence of the two largest species was independent of plant-available nutrients ($P > 0.05$), but that occurrence of the smaller three species increased with increasing plant-available nutrients ($P < 0.05$). As we predicted, the mean body mass of all species present at a site increased with increasing plant-available moisture, and decreased with increasing plant-available nutrients (Fig. 2e, f).

Consistent with these results for individual species and mean body mass, and with our predictions of diversity patterns (Fig. 2b), we found that total herbivore species richness (as a percentage of the continental species pool) for Africa and North America together peaked at intermediate plant-available moisture (Fig. 2g) and increased continuously with plant-available nutrients (Fig. 2h). Multiple regression analysis (Table 1) showed that herbivore species richness increased linearly with plant-available nutrients and non-linearly (as a quadratic function) with plant-available moisture, and that each had a significant effect. Separate herbivore diversity patterns for Africa and North America were similar. This pattern is unlikely to be caused by plant diversity (leading to more resource types), because plant diversity is typically highest at low soil fertility[23]. It is also unlikely to be caused by non-food differences between habitats (for example, shelter to predation) as the patterns shown in Fig. 2g and h did not change substantially when the analysis was restricted to include only sites that were primarily grassland.

On a global scale, this empirical regression model (Table 1) predicts that there are regions that can support high herbivore diversity when applied to maps of our indices for plant-available moisture and nutrients (Methods and Fig. 3). To validate our regression model with independent data, we predicted large herbivore species richness (as a percentage of continental pool) for ten preserves and natural areas in Australia on the basis of our global

Table 1 Dependence of species richness on water and soil

Coefficient	Regression coefficient	Standard error	t	P
Constant	8.091	1.483	5.46	< 0.001
Soil fertility index (linear)	1.031	0.181	5.70	< 0.001
Water availability index (linear)†	–3.639	1.489	–2.45	0.016
Water availability index (quadratic)	–2.897	0.877	–3.30	< 0.001

Results of the multiple regression analysis of the dependence of large herbivore species richness (given as a percentage of the continental species pool; see Methods) on indices of water availability and soil fertility are shown.

† This linear coefficient was negative, despite a unimodal relationship (Fig. 2e), because water availability indices were mainly negative (potential evapotranspiration > rainfall).

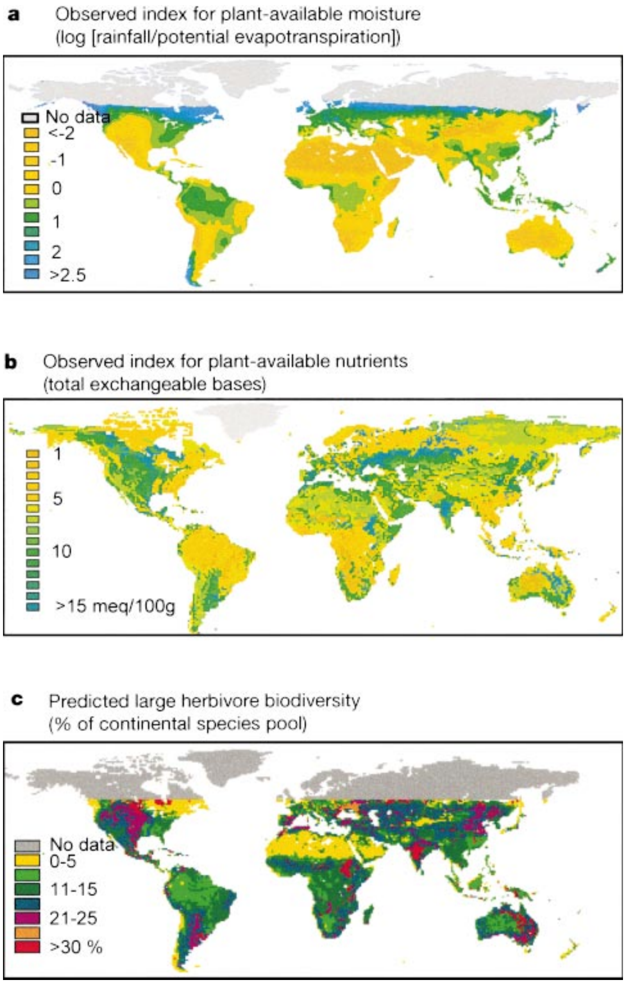

Figure 3 Global distribution of large herbivore diversity, as predicted by indices for plant-available moisture and nutrients using a regression model obtained from data for African and North American parks. **a**, **b**, Maps of observed water supply and soil fertility indices, respectively. **c**, Map of species richness of large herbivores, as a percentage of continental species pool (Methods), predicted from indices for plant-available moisture and nutrients using the multiple regression model (Table 1, Fig. 2d). Continental species pools are North America, 25; Africa, 99; Central and South America, 18; Europe, 5; Middle East, 11; North Africa, 8; India, 10; Northern Asia and Far East, 31; southeast Asia and Indonesian archipelago, 10; Australia, 59. All maps represent a planar projection, at a resolution of 0.5° longitude/latitude (**a**) or 1° longitude/latitude (**b**, **c**). No data for potential evapotranspiration are available for the boreal zones in **a**, hence no diversity predictions could be made for this region (**c**).

map of plant-available moisture and nutrient indices. We found a strong correspondence between predicted and observed diversity ($R^2 = 0.69$, $P = 0.003$, $n = 10$). Regions of known high herbivore diversity in other regions and continents[1,10,22] also seem to correspond to areas that are classified as having high potential diversity by our global map. These include the Argentinian pampa, Gir Forest of India, steppes of Khazakstan and Mongolia, Cordillera of Spain, and the coastal region of Morocco and Algeria (Fig. 3c).

Extrapolating the predictions of our model to the global map yields potentially important insights about the global status of large herbivore conservation. For example, the prime regions for large herbivore diversity can host potentially more than 25% of the species in a continental species pool, but comprise only about 5% of the investigated land of the world (see Fig. 3c). Fewer than 2% of the prime regions for large herbivore diversity overlap with regions designated as 'general purpose' biodiversity hotspots[24]. Current land-use practices[25] suggest that more than half of the area of these prime regions has been already converted to agriculture and lost its herbivore diversity. Another 25% of these prime regions may be converted to agriculture in the next 25 yr. Thus, less than 1.2% of the earth's surface might remain to support uniquely diverse, grazing ecosystems by 2025. Some regions, such as the northern Great Plains in North America, might be highly suitable for restoring large herbivore diversity if agriculture were to be abandoned.

Our approach is powerful because it identifies how plant resources constrain the distribution of herbivores of different sizes. We can use this functional relationship to predict patterns in large herbivore diversity on a global scale. Similar approaches might be applied to other groups of organisms to help to identify crucial areas for current conservation and future restoration of biodiversity. □

Methods

Data sources

Main data sources for species occurrences in protected areas in North America (34 sites) and Africa (85 sites) were the Man and Biosphere Species Database (http://ice.ucdavis.edu/mab) and the UNEP-WCMC Protected Areas Database (http://www.unep-wcmc.org). Only mammalian herbivores > 2 kg that eat graminoids, forbs and/or woody plants were recorded. We restricted the analysis to this size class because the records of smaller herbivores (small mammals, insects) in these areas are incomplete. Species that eat mostly seeds and fruits were not included as it is unclear whether the food abundance and quality patterns shown in Fig. 1a and b also hold for these food types. We included only wilderness areas, national parks and national monuments and wildlife management areas (International Union for the Conservation of Nature (IUCN)) categories I, II or III or IV).

Plant-available moisture index

The plant-available moisture index for each protected area was calculated as the monthly average of the $\log_{10}$ of the ratio of actual rainfall over potential evapotranspiration using published maps[26]. Data of potential evapotranspiration and therefore our moisture index and diversity prediction were not available for the polar region, as the calculation method is inappropriate for areas with long-term snow cover.

Plant-available nutrients index

Data on plant-available nutrients are based on the FAO-UNESCO Soil Map of the World, assigned[25] to 1° by 1° cells. Plant-available nutrients were assumed to be proportional to the sum of soil cations Ca^{2+}, Mg^{2+}, Na^+ and K^+ or total exchangeable bases (TEB), which is calculated from base saturation, $BS\% = [(TEB/CECsoil) \times 100]$, and soil exchange capacity (soil CEC) according to $TEB = (BS\%/100) \times 3.5OC\% + [(Clay\% \text{ x } CECclay)/100)]$, where OC% is the percentage of organic carbon in the soil, Clay% is the percentage of clay content and CECclay is the approximate cation exchange capacity for the dominant clay mineral.

Species frequency of occurrence

The frequency of occurrence of individual herbivore species is the proportion of parks that contain a particular species in each of six intervals of plant-available moisture index, and seven intervals of plant-available nutrients index. Patterns were robust to our choice of interval sizes. For each interval, we also calculated the mean body mass of all species present. Because Africa (99 large herbivore species) and North America (25 large herbivore species) differ in their continental species pools and local species richness, owing in part to extinction of 50% of the species in North America since the last glaciation, the species richness at each park was expressed as a percentage of the continental species pool. This crudely standardizes diversity relative to the potential number of species that could be present theoretically at a site.

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Ecology, Sexual Selection, and the Evolution of Mating Systems

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Ecology, Sexual Selection, and the Evolution of Mating Systems

Stephen T. Emlen and Lewis W. Oring

Mating systems (*1*) were first discussed in evolutionary terms by Darwin (*2*). Since then, major developments in genetic theory have allowed a better understanding of sex ratios, sexual dimorphism, and differential patterns of parental care (*3–7*). Important milestones toward an ecological understanding of mating systems have also been reached (*8–16*). Nevertheless, attempts at synthesizing natural history data into a unified theory of mating system evolution have lagged behind the development of population genetics theory.

One factor hindering development of a sociobiological framework of mating system theory has been a recurring tendency for field workers to search for and to discuss "adaptiveness" in the context of the survival or well-being of the population or species. To understand mating systems, we must abandon species- or group-selection viewpoints and return to the evolutionary tenet of natural selection operating at the level of the individual genome (*17*).

Fitness, in genetic theory, measures the reproductive success of an individual (or a genotype) measured relative to the reproductive success of other individuals (or genotypes) in the same or in other populations. Thus, we should expect a strong competitive element in many aspects of reproductive behavior. Darwin was fully aware of this intraspecific competition when he introduced the theory of sexual selection (*2*). Stated simply, when one sex becomes a limiting factor for the other, the result is an increase in intrasexual competition among members of the available sex for access to mates of the limiting sex. The greater the "shortage" of one sex, the more intense the sexual selection. Sexual selection is relatively slight in monogamous groups while it is intense in highly polygamous societies. A better understanding of the causes of sexual selection is thus pivotal to the development of any mating system theory.

What accounts for the differing intensities of sexual selection found in different species and, frequently, between populations of the same species? We hypothesize that one important cause is the *ability of a portion of the population to control the access of others to potential mates*. This control can be direct, as in the physical herding of potential mates, and the physical exclusion of other members of the same sex from these mates, or indirect, by controlling resources that are critical either for mate attraction or for successful reproduction. The greater the degree of control or monopolization, the greater the resulting variance in mating success. As the difference in reproduction success of "haves" and "have nots" increases, so too does intrasexual competition to be in the former category. It becomes crucial to understand the manner in which access to mates can be controlled.

One of the emerging findings of sociobiology is that many aspects of an animal's social organization can be predicted on the basis of an understanding of a limited set of environmental variables. We propose that certain environmental factors determine the degree to which mates can be defended or monopolized. In effect, *ecological constraints impose limits on the degree to which sexual selection can operate*. The greater the potential for multiple mate monopolization, the greater should be the potential intensity of sexual selection and the tendency for polygamy.

In this article, we discuss various ecological factors that influence the degree and form of polygamous mating systems. Our intention is to develop a theory adequate for predicting environmental influences on the evolution and expression of avian mating systems. We believe these predictions are also broadly applicable to many mammalian groups, as well as to certain insects and lower vertebrates. They are less applicable to strongly "r"-selected species (*18*), or for species that normally live in permanent groups with restricted membership where the potential for reciprocal interactions is high (*19*).

Economic Monopolizability and the Environmental Potential for Polygamy

There are two preconditions for the evolution of polygamy (see Fig. 1). First, multiple mates, or resources sufficient to attract multiple mates, must be energetically defendable by individuals. Many environmental factors affect the potential for such control. But primary among these are the spatial and temporal patterns of resource dispersion. The potential for controlling access to multiple mates should be viewed in a cost-benefit context. Stated simply, the prerequisite for a monogamous mating system is the economic defendability of a mate. The prerequisite for a polygamous mating system is the economic monopolizability of several mates. The *environmental potential for polygamy* depends on the degree to which multiple mates, or resources critical to gaining multiple mates, are economically defendable.

A second prerequisite is the ability of the animals to utilize this potential. Critical resources might be distributed such

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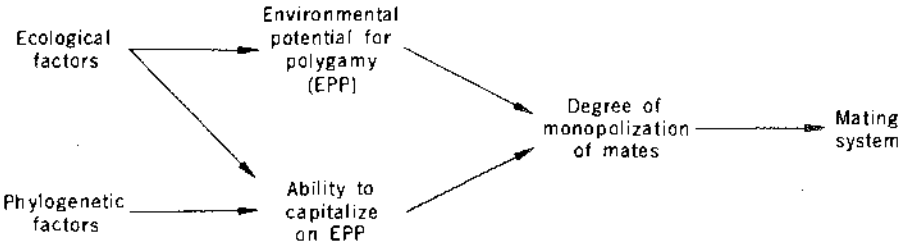

Fig. 1. A general schema of the determinants of a mating system.

that they are economically defendable for an individual that expends most of its time budget on such defense, but not for one that devotes considerable time to alternative activities. The degree to which an animal can take advantage of the "polygamy potential" of the environment depends in large part on the degree of parental care required for successful rearing of young.

Recent attempts at synthesizing the literature on mating systems have focused on this latter point. Polygamy is more prevalent in species where one sex is freed from parental care duties. Members of this sex can expend increased time and energy on intrasexual competition for resources and mates. Several predictions logically follow and have been stressed by others: polygamy should be more common among animals where (i) one sex is predisposed to assume most of the parental care (for example, mammals); (ii) parental care requirements are minimal (for example, birds with precocial as compared with altricial young); and (iii) a superabundant food resource enables a single parent to provide full parental care (*7, 11–13*). Emancipation by itself, however, need not lead to the evolution of polygamy. It merely assures that one sex can fully exploit the potential for mate monopolization inherent in the environment (see discussion on resource defense polygyny, below).

The spatial distribution of resources. Brown introduced the concept of economic defendability to the study of animal spacing patterns (*20*). We extend his approach to encompass mating systems.

When important resources are distributed uniformly in space, there is little opportunity for resource monopolization. If the resources are sufficiently abundant and stable through time, territoriality typically occurs. Members of the breeding population would tend toward even dispersion and the potential for multiple matings would be low. Sexual selection would be minimal, and the fitness of individuals might be maximized by sharing equally in parental care duties (see monogamy).

As critical resources become unevenly distributed in nature, the potential for obtaining additional mates increases. This is because some individuals may be able to control a larger quantity or better quality of resource than other individuals. To the degree that this influences mate choice, polygamous matings may be expected (Fig. 2, horizontal axis).

When important resources are highly clumped, the possibility arises for a small percentage of the population to monopolize a large proportion of the available resources. Sexual selection and variance in reproductive success should be high, and we speak of the environment as having a high polygamy potential (EPP).

The temporal distribution of mates. In our model, the benefits derived from resource defense lie in an increased probability of mate attraction and acquisition. The magnitude of this benefit is strongly influenced by the temporal pattern of availability of sexually receptive partners.

If females in a population become sexually receptive in unison, there is little potential for individual males to monopolize multiple females. This will be especially true if each female is sexually active for only a brief period. By the time the sexes have located one another, and normal courtship and mating have taken place, most of the remaining available females have already been inseminated and few new partners are available. As long as the time involved in servicing a single sexual partner constitutes a significant portion of the total time that potential mates are available, trends toward polygamy will be minimal.

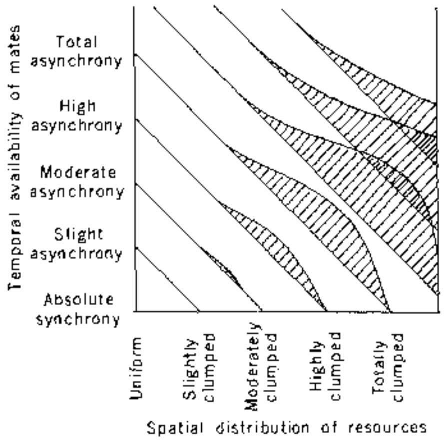

Fig. 2. Graphic representation of the environmental potential for polygamy (indicated by the perpendicular height of the shaded area) and its relation to the spatial distribution of resources and temporal availability of receptive mates.

With increasing degrees of asynchrony among members of one sex, the potential for individuals of the other sex to accumulate multiple mates increases. Among species in which both sexes contribute to parental care, a moderate degree of asynchrony is essential for the expression of polygamy. In species where one sex is largely freed from parental duties, individuals of this sex should remain sexually active for the duration of the period during which members of the other sex become sexually receptive. The intensity of sexual selection then will be determined by the degree to which critical resources are differentially controlled by members of the limited sex, by the availability of members of the limiting sex (*21*), and by the process of mate selection.

As the degree of asynchrony becomes extreme, the rate of appearance of new potential mates reaches a point at which the cost of continued resource defense necessary for attracting an additional mate exceeds the additional benefits gained. Continued mate accumulation would no longer be energetically practical and tendencies toward polygamy will decrease (see Fig. 2, vertical axis).

The concept of operational sex ratios. To understand the intensity of sexual selection it is not the overall population ratio of males to females that is of importance but rather what we term the operational sex ratio (OSR)—defined as the average ratio of fertilizable females to sexually active males at any given time (*22*). This ratio is strongly affected by the degree of spatial and temporal clumping of the limiting sex. For example, continuous long periods of sexual activity by males, coupled with brief and asynchronous periods of receptivity by females, will produce a strong skew in the OSR.

The OSR provides an empirical measure of the degree of monopolizability of mates. The greater the degree of imbalance in the OSR, the greater the expected variance in reproductive success among members of the limited sex and the greater the degree of polygamy. Where the OSR is skewed toward males, polygyny is expected; when the skew is toward females, polyandry should occur (*23*).

Types of Mating Systems

Attempts to categorize mating systems have been hampered by a lack of generally accepted terminology. Classically, mating systems have been defined according to the number of mates that one sex can accumulate. Sometimes this is modified to separate simultaneous from sequential multiple mate acquisition. Selander (14) has proposed a new classification based, in part, on the duration of the pair bond or mate association. The difficulty with these classification schemes is that they leave functionally or causatively unrelated situations (or both) in the same category. They ignore the importance of environmental pressures on parental care and sexual selection, and the influence of these factors on mating system evolution. In this article we have characterized mating systems on the basis of the ecological and behavioral potential to monopolize mates, and by the means through which such monopolization takes place. Where male and female strategies conflict, we use terms appropriate for the sex that controls the resource base or monopolizes multiple mates (or both) (see Table 1).

Table 1. An ecological classification of mating systems.

Monogamy: Neither sex has the opportunity of monopolizing additional members of the opposite sex. Fitness often maximized through shared parental care.
Polygyny: Individual males frequently control or gain access to multiple females.
  Resource defense polygyny: Males control access to females *indirectly*, by monopolizing critical resources.
  Female (or harem) defense polygyny: Males control access to females *directly*, usually by virtue of female gregariousness.
  Male dominance polygyny: Mates or critical resources are *not economically monopolizable*. Males aggregate during the breeding season and *females select mates* from these aggregations.
    Explosive breeding assemblages: Both sexes converge for a short-lived, highly synchronized mating period. The operational sex ratio is close to unity and sexual selection is minimal.
    Leks: Females are less synchronized and males remain sexually active for the duration of the females' breeding period. Males compete directly for dominant status or position within stable assemblages. Variance in reproductive success and skew in operational sex ratio reach extremes.
Rapid multiple clutch polygamy: Both sexes have substantial but relatively *equal* opportunity for increasing fitness through multiple breedings in rapid succession. Males and females each incubate separate clutches of eggs.
Polyandry: Individual females frequently control or gain access to multiple males.
  Resource defense polyandry: Females control access to males *indirectly*, by monopolizing critical resources.
  Female access polyandry: Females do not defend resources essential to males but, through interactions among themselves, may limit access to males. Among phalaropes, both sexes converge repeatedly at ephemeral feeding areas where courtship and mating occur. The mating system most closely resembles an explosive breeding assemblage in which the OSR may become skewed with an excess of females.

Monogamy

Neither sex has the opportunity to monopolize additional members of the opposite sex, directly or through resource control. Multiple breedings per season may occur in sequence.

Monogamy is expected to occur when (i) there is no environmental "polygamy potential," or (ii) there is no opportunity to take advantage of what "polygamy potential" the environment affords. Monogamy is the dominant avian mating system, occurring in more than 90 percent of the species studied (24), but it is believed rare among mammals (25).

The prevalence of monogamy among birds is due primarily to the inability of most species to take advantage of any environmental "polygamy potential." Considerable parental care by both parents often is required for successful rearing of young. Thus, losses to an individual parent accrued by withholding care from one set of offspring while courting and mating with additional mates may be greater than the gains resulting from such behavior.

In mammals, the preponderance of female parental care allows males of most species to exploit whatever "polygamy potential" exists. Nevertheless, monogamy may be more common than is usually supposed, especially in populations where individuals are widely dispersed over relatively uniform environments (26). As was mentioned previously, male emancipation by itself should only lead to polygyny under permissive environmental conditions.

If the potential of, or the gain from, mate monopolization is nonexistent, an individual should benefit by remaining with its initial mate and acting in such a manner as to maximize the survival chances of its offspring. Recent studies of long-lived birds show a clear advantage to long-term mate fidelity (27). Birds breeding with former mates show low levels of aggression and a high degree of within-pair synchronization, allowing them to breed more rapidly and efficiently and leading to a demonstrable increase in reproductive success. The longer the period of mate fidelity, the more the future physical condition of a mate becomes of importance to its partner. It then becomes adaptive to equalize the energetic burden of reproduction and to share in parental care.

Forms of Polygyny

Polygyny occurs if environmental or behavioral conditions bring about the clumping of females, and males have the capacity to monopolize them. Types of polygyny are classified according to the means that males use to control females.

1) *Resource defense polygyny.* Males defend resources essential to females. To the degree that males can monopolize these resources, they can monopolize females.

When important resources are unevenly distributed or spatially clumped, certain males can defend areas containing a larger quantity or better quality of resource than others. If these resources are critical for female reproduction, then competition among males should revolve around subdividing and defending these resources. Female choice of mate should be influenced both by the quality of the defending male and the resources under his control (territory quality). The extent of polygyny will tend to increase with increasing variance in territory quality among the males of the population.

Among bird species where both sexes provide at least some parental care for offspring, females stand to lose if their mates take on additional females. Polygynous matings will be advantageous to the female only if the benefits gained by genetic access to a high-quality male or to the resources controlled by him more than offset her compensatory costs for the decreased contribution of the male in parental care. As stressed by Verner, Willson, and Orians, polygyny is expected when the distribution of resources is sufficiently irregular that a female mating with an already paired male on a superior-quality territory will have equal or better reproductive success than if she mated with an unpaired male occupying a poorer quality territory (8, 12, 13).

Studies on mating systems of North American passerines support these views. Resource defense polygyny is most prevalent in habitats with uneven resource distribution that results in a mosaic of male territories of different qualities (*12, 28*). Demographic studies further show that females forming polygynous pair bonds realize a reproductive success as great or greater than monogamous females (*29–31*). The specific resource attributes that comprise "territory quality" are expected to vary between species. Similarly, the extent of difference between territories that is sufficient to favor polygyny [the "polygyny threshold" of Orians (*13*)] will vary with such factors as the distribution of male parental care among his various mates, the total amount of parental care required by the young (for example, precocial versus altricial young), and the degree of dependency on the territory itself (for example, whether just for nesting or also for feeding).

Under certain environmental situations, the cost to the male of resource control or mate accumulation might be relatively low, while the potential benefit is extremely high. The cumulative advantage of multiple matings to the male could far outweigh the decreased reproductive success of individual females. In such conflict situations, females could be forced into assuming a larger fraction of the total parental care, even if it necessitated such long-term adaptations as reduced brood size or decreased growth rates of young. We suggest that some cases of male emancipation might best be viewed as an evolutionary result of a high environmental potential for polygamy rather than as an independently derived precursor to the evolution of polygamy.

Many passerine species believed to be monogamous show disparate parental investment by the male and female. (The female alone incubates the eggs while both sexes bring food for the nestlings.) Many of these species are sexually dimorphic, the male assuming a conspicuous breeding plumage while the female remains cryptically colored. The decreased male involvement in parental care preadapts such species to respond to slight changes in the environmental potential for polygamy by enabling them to become opportunistically or facultatively polygynous (*31*). We expect that many additional cases of such facultative polygyny will be discovered when individually marked populations are studied in areas where the feeding or nesting resources are limited or highly localized in space.

When male parental investment is minimal or nonexistent, a limited and clumped resource distribution can lead to extreme development of polygyny. In the orange-rumped honeyguide (*Indicator xanthonotus*), beeswax forms an essential part of the diet. Males do not provide defense of the nest site or food for the young, but they do maintain year-around territories at the locations of bee nests. These bee nests are found only on exposed cliffs; they are in short supply and a small proportion of the male population is able to control access to this resource. When females become sexually active, they enter the male territories and feed on the wax of the bee comb. Courtship is centered at the locations of bees' nests and copulation success is high for territory owners. One male was observed to copulate 46 times with at least 18 different females while nonterritorial males had minimal, if any, copulatory success. This species exemplifies an extreme degree of polygyny, based primarily on the ability of a small number of males to monopolize access to a critical resource (*32*).

Analogous, but less extreme, examples of resource defense polygyny have been reported for fiery-throated (*Panterpe insignis*) and Anna hummingbirds (*Calypte anna*) (*33, 34*). Many aspects of hummingbird biology are closely linked to their habit of nectar feeding. When suitable flowers are sufficiently clumped and nectar production is high, territorial defense becomes economically feasible (*34–36*). Females incubate and rear the young alone, but they require a reliable nectar source to do so. In several species males allow females nesting within their territory to feed therein, but aggressively exclude all other hummingbirds (*34, 35*). The extent of polygyny presumably is determined by the distribution pattern of nectar-producing flowers which leads to differences in territory quality among males.

The yellow-bellied marmot (*Marmota flaviventris*) provides a mammalian example of resource defense polygyny. Overwintering sites constitute a monopolizable resource. Studies by Armitage and Downhower (*37*) suggest that areas of rock outcroppings provide the most suitable underground retreats for predator escape and for hibernation. Such sites are limited in number and are vigorously defended by males. High-quality locations are occupied by a colony including one or (rarely) a few dominant males, a variable number of mature females, plus young and juveniles. These units have been called "harems," but we feel they are better understood in the context of resource defense polygyny. Male marmots achieve high reproductive success not by forcibly accumulating a harem of females but rather by economically monopolizing a resource that leads to female clumping.

2) *Female (or harem) defense polygyny.* Females are gregarious for reasons unrelated to reproduction. Their self-clumping tendencies facilitate direct monopolization by males.

If females themselves are defendable, we might expect males to forcibly accumulate females and to herd or maintain them under their jurisdiction by aggressively excluding all other males from the area. Such harem formation or "female defense polygyny" does occur, but usually in conjunction with male resource defense.

In many ungulates, females and young aggregate into small herds for part or all of the year. During times of parturition and sexual receptivity, these groups move into areas of preferred habitat (generally affording increased cover or abundant food, or both). Among the African impala (*Aepyceros melampus*) and waterbuck (*Kobus defassa*), for example, males at this time of year divide the habitat into defended territories. "The frequency with which a male has females in his territory is related to the amount of preferred habitat for that season that his territory contains. This may mean that a restricted number of territories may attract all the females for the duration of a limited mating season, and holders of those territories alone will contribute to breeding. In such circumstances it may be found that the marginal territories are held by young or old, rather than prime males" (*38*).

The *reasons* for female clumping may be totally unrelated to reproduction. Ungulates gain through increased predator detection and avoidance; other groups might gain through information exchange about locations of unpredictable food resources, increased foraging efficiency by observational learning, or cultural transmission of learned habits. But one *result* of the clumping is to increase the male potential for differential access and control of multiple mates.

Among many pinnipeds, females are sexually active shortly after giving birth (*39*). Females "haul out" onto land or ice to give birth, and the combination of female gregariousness, a shortage of suitable parturition locations, and a tendency to return annually to traditional areas gives rise to dense aggregations of sexually active females. By monopolizing access to these sites, dominant males are able to realize phenomenal numbers of copulations. Competition among males is extremely intense and the resulting sexual selection presumably has led to the marked sexual dimorphism and intensity of aggressive behavior found in these species (*10, 40*).

The result of female clumping in pinnipeds may result in mates being directly defended as a resource (for example, in elephant seals) or may greatly increase the benefits derived from resource (site) defense (in many otariids such as the fur seals). In either case it leads to intensified sexual selection and increased imbalances in the reproductive success of different males in the population.

Female defense polygyny is rare among birds, being known for certain only in the greater rhea, a few tinamous, and several pheasants. The rhea provides one of the best known examples, but since males incubate and females sometimes mate with several males in succession, this system is described in the section on female selection and the evolution of polyandry.

In a number of pheasants and possibly in peafowl and chachalacas, female self-clumping occurs in combination with male resource defense. Female ring-necked pheasants (*Phasianus colchicus*) form groups that are attracted to certain male territories during the breeding season. Males directly defend female groups, and limited evidence suggests that larger harems and harems comprised of experienced females associate preferentially with males on prime territories (*41*). Membership in female groups is variable, and new individuals join throughout the summer. The result is a staggering of female breeding readiness (that is, asynchrony) enabling a single male to inseminate all sexually receptive members of the female group.

3) *Male dominance polygyny.* Males do not directly defend females or resources essential to females, but rather sort out among themselves their relative positions of dominance. Females choose males primarily on the basis of male status.

Communal displaying is frequent among species in which the male is totally emancipated from parental care and the *environment provides little potential for resource or mate control*. This is expected (i) when critical resources are superabundant but widely dispersed, or (ii) when they are sufficiently unpredictable in space and time as to be economically undefendable. It is also expected (iii) when resources or mates are clumped in a defensible pattern but the cost of successful defense is too high. High population density (increasing the number of competitors) and increased intensity of competition (resulting from extreme limitation of resources or from strong skew in the OSR) can lead to situations where successful defense is energetically unfeasible. Cases where territorial defense is abandoned as the cost of defense increases have been documented in a variety of taxa (*34, 36, 42*). When access to females cannot be controlled through resource defense, male competition may take the form of direct male-male encounters, resulting in differential dominance relationships between the male members of the population.

Under what conditions should individual males aggregate and display communally? Such aggregations provide a forum for male-male competition and should increase the variance in reproductive performance among males. The copulation success of a high-ranking male would be increased by joining such a group; that of a low-ranking individual might decrease. Why then should subordinate males enter communally displaying groups?

If female movements or concentration areas are predictable, encounter rates would be high for males that position themselves in these areas, leading to localized concentrations of males. As males start to aggregate, their advertising and courtship signals become pooled, creating an enhanced stimulus situation that attracts females preferentially to larger aggregations (*22, 43, 44*). If isolated males have minimal chances of successfully attracting males, low-ranking males will be expected to adopt alternative, cryptic, or satellite strategies for obtaining females within the communal display areas rather than avoiding male aggregations altogether (*45*).

Females also benefit by male clumping since they can better compare a large number of potential mates in a minimum period of time. By relying on the male-male interactions to have done a preliminary sorting for them, females can preferentially select from a predetermined group of "tested" and "proven" males.

Other advantages have been suggested for male display aggregations. These include increased alertness and defense against potential predators as well as pooling of information about locations of patchy or ephemeral food resources (*24, 46, 47*). We believe that these are secondary advantages, accruing after the development of group displaying and being of greatest importance in species that remain sexually active and aggregated for long periods of time.

The form of male dominance polygyny will depend on the degree of synchronization of sexual activity among females of the population. Where females are highly synchronized and converge at the male aggregation within a short period of time, a highly promiscuous, "explosive" breeding situation will occur. This is typical of many singing insects and chorusing amphibians (*16, 48*). As was discussed previously, the synchrony of the females restricts the potential for individual males to monopolize matings. Breeding activity is frenzied, but the operational sex ratio should not be highly skewed and sexual selection should not be intense (*49*).

If the females of a population are relatively asynchronous in their periods of sexual receptivity (*50*), the operational sex ratio becomes increasingly skewed and sexual selection intensifies. Males generally remain active for the duration of the population's breeding season. The intensity of male-male competition, together with the longer duration of the mating period, result in the establishment of stable dominance or position effects among the advertising males. The result is an organized aggregation usually referred to as a *lek*.

A lek is defined as a communal display area where males congregate for the sole purpose of attracting and courting females and to which females come for mating (*51*). Males jockey for a status or position that conveys maximal attractiveness to females. Frequently, central positions are occupied by older, more dominant, males that achieve a disproportionate share of female copulations (*22, 44, 46, 47, 52, 53*). Females visit the lek when sexually receptive and "sample" numerous males before selecting a mate. After copulation, the female leaves the display area and proceeds, on her own, to rear the young.

Lek mating systems have been described for several species of insects, mouth breeding fish, bullfrogs, a scattering of mammals and approximately 30 species of birds (*54–56*). We hypothesize that all cases will be typified by a fairly long breeding season, a heavily skewed operational sex ratio, and by *the inability of individual males to economically control or monopolize the resources essential for female acquisition.*

Male Incubation, Female Emancipation

Among most animals, female parental investment greatly exceeds that of males (*3, 5–7*). Consequently, most cases of polygamy involve male emancipation and the development of polygyny. However, a significant portion of avian species share rather equally in parental care duties, including incubation of the eggs. And, in a very small percentage of species, males have assumed the full burden of incubation and brood rearing, emancipating the female and increasing the possibility of her mating repeatedly.

Males should assume the bulk of parental care only when their individual fitness is increased through such an action. This might occur in two general situations: (i) when the future physical condition of the female is of direct importance to the male (discussed under Monogamy), and (ii) when the lack of dependability of breeding conditions places a premium on female ability to produce additional or replacement clutches for the male. Lack of dependability can result either (i) from great fluctuations in environmental suitability for breeding or (ii) from very low success rates of reproductive attempts (caused, for example, by high predation rates).

From a female's viewpoint, the principal advantage of male incubation is a lessening of her metabolic burden, freeing her to devote increased time and energy to replenish nutrient and mineral reserves drained through egg production *(57, 58)*.

From the male's point of view, female emancipation can be viewed as an insurance strategy—when the failure rate is sufficiently high, the cost involved in incubation may be less, on average, than the gain accrued by having a mate physiologically able to rapidly produce new clutches of eggs.

Complete male parental care is most likely to develop in groups with slight to moderate parental care needs (precocial young) and a phylogenetic history of shared incubation. It should also be more prevalent among determinate than indeterminate egg layers *(59)*.

Rapid multiple clutch polygamy. Both sexes have substantial but relatively equal opportunity for increasing fitness through multiple breedings in rapid succession. Simultaneous brooding of two clutches usually occurs.

In some shorebirds and galliforms, the female may lay a first clutch that is incubated solely by the male, and a second clutch that she herself incubates. When conditions are good, the result is a doubling of reproductive potential with only a minimal increase in breeding time; when conditions are poor, the result is an increased ability to produce replacement clutches.

Species exemplifying such rapid multiple clutch polygamy include red-legged partridge (*Alectoris rufa*), sanderling (*Calidris alba*), mountain plover (*Charadrius montanus*), and Temminck's stint (*Calidris temminckii*) *(58, 60, 61)*. All are ground-nesting birds whose precocial young suffer moderate to extremely high predation losses. The mountain plover inhabits marginal short-grass prairies and is further subjected to severe fluctuations in environmental suitability due to great variations in rainfall. The short arctic breeding season of the sanderling is characterized by great fluctuations in suitability for breeding. The California quail (*Lophortyx californicum*), which, at least occasionally, practices this mating system *(62)* also is subject both to severe fluctuations in environmental suitability and to extreme predation *(63)*.

Rapid multiple clutch polygamy is probably more common than is currently known, especially among shorebirds. It may also prove to be common among small phasianids, where males of a number of species are known to sometimes incubate or brood young (or both) without female help *(64)*.

Advantages of male incubation to males are maximized (i) when females preferentially return to their original mates to lay a subsequent clutch of eggs, and (ii) when males remain sexually active for as long as possible during or before the initiation of incubation.

Preferential treatment by females for their original mates has been investigated in red-legged partridge, mountain plover, and Temminck's stint [see also *(77)*]. In the former two species, females lay second clutches in the territories of their original mate and copulate with him providing he is sexually active. In Temminck's stint, on the other hand, mate fidelity between first and second clutch is reported from one locality (U.S.S.R.) but not from another (Finland) *(58, 65)*.

Males exhibiting rapid multiple clutch polygamy continue to actively court additional females for a period following the completion of the first clutch. Such behavior is rarely reported among species that share incubation. In the mountain plover, this is accomplished by maintaining sexual activity during part of incubation, while in the Temminck's stint, sanderling, and red-legged partridge, males frequently delay incubation, leaving the first clutch untended for as long as 6 to 12 days while continuing to court additional females.

As a result of this behavior, rapid multiple clutch polygamy can lead to a doubling of reproductive potential *without producing a strong skew in the operational sex ratio*.

Female Sexual Selection and the Evolution of Polyandry

Male incubation preadapts a species for possible evolution toward polyandry. Emancipation allows the female opportunity to increase her fitness through continued production of multiple clutches. Female fitness can be increased only to the degree that males are sexually receptive and available to assume incubation of these additional clutches.

Since an individual male is not able to maintain sexual activity indefinitely during incubation, males sitting on eggs normally must be considered sexually "unavailable." To the degree that females can produce more clutches than can be serviced by males (caused either by high production rates of females or low failure rates of existent clutches), the operational sex ratio will become skewed with a shortage of males. These conditions lead to increased intrasexual competition among females for access to available males. The degree to which polyandry will develop then depends on the intensity of female sexual selection and the environmental potential for monopolization of mates (in this case female monopolization of males).

True polyandry is extremely rare among birds, being found in less than 1 percent of the species studied to date *(24, 66)*. Most documented cases are restricted to the avian orders Gruiformes and Charadriiformes. Comprehensive studies involving individually marked, wild birds have been conducted on only three species, the spotted sandpiper (*Actitis macularia*), the American jacana (*Jacana spinosa*), and the northern phalarope (*Phalaropus lobatus*). The mating systems of the first two are considered resource defense polyandry, while the latter represents an explosive type of female access polyandry. There are no documented examples of female dominance polyandry with stable organization analogous to that typical of leks.

1) *Resource defense polyandry*. Females compete for and defend resources essential to males. To the degree that these resources are clumped and monopolizable, females can monopolize multiple males.

In the spotted sandpiper, males often perform all incubation and brood care. Incubation begins during egg laying, and by the time of clutch completion males are sexually disinterested. Predation losses are extremely high *(67, 68)*, and the ability of females to rapidly lay replacement clutches is impressive *(69)*.

Spotted sandpipers breed throughout

the mid- and lower latitudes of North America. Under favorable conditions, the long breeding season, coupled with a high egg-producing ability, results in the female reproductive output exceeding the availability of males to assume incubation responsibilities. Because of male incubation, the operational sex ratio is skewed in favor of females, competition exists for available mates, and female sexual selection is moderately strong.

The extent of polyandry seems to be determined, in part, by the "availability" of males and by the quality and the spatial dispersion pattern of essential resources. In one population in Minnesota, male density was low and breeding females on average mated with 1.17 males. In contrast, in dense populations in Minnesota and New York, where birds were concentrated on patches of high-quality habitat, successful females averaged 2.4 mates (*67, 70*). High nest predation and asynchronous patterns of arrival of males both influenced the incidence of polyandry through their effect on increasing the "availability" of males to accept additional clutches of eggs (*71*).

The American jacana exhibits the most extreme polyandry known. In parts of Costa Rica their breeding habitat is severely limited, and only a small fraction of either the male or female population is believed to breed in any one year. The few suitable ponds or lagoons are subdivided into small territories by males. Females control "super-territories," often encompassing the nesting areas of several males. Females frequently have multiple mates incubating clutches simultaneously. Predation of nests is high, and females readily provide replacement clutches for their males, who perform most parental care duties (*66, 72*).

As the degree of female sexual selection increases, so too does behavioral and size dimorphism. In spotted sandpipers the female is 25 percent larger than the male, both sexes defend territories although the female is dominant, and the female frequently shares incubation with her last mate of the year. In the jacana, breeding females weigh 50 to 75 percent more than males, are totally dominant over males in aggressive interactions, and provide minimal parental care for eggs or young. In essence, female jacanas are specialists in egg production.

2) *Male defense or "harem" polyandry?* Several species of tinamous as well as the greater rhea exhibit unique mating systems that have been termed harem polyandry. On the basis of underlying resource control, we interpret these cases not as polyandry but rather as specialized cases of polygyny coupled with male incubation.

Tinamous are primitive, ground-nesting birds that inhabit the neotropics. Most defend all-purpose territories from which they advertise vocally. Females of some species are loosely gregarious and travel between male territories. Males may have pair bonds simultaneously with several females who lay a communal clutch of eggs. Males then perform all incubation and care for the precocial young without female help. Competition for females appears to exist and indirect evidence suggests a considerable differential in reproductive success of neighboring males (*73–76*).

Male tinamous recoup the cost of incubation in three ways. (i) Females freed from parental duties are better able to provide replacement clutches when predation does occur (*73, 77*). (ii) If several females lay communally, the male obtains a compound clutch rapidly, thereby minimizing the times during which eggs are unattended and, hence, exposed to extreme predation pressure. (iii) A male can inseminate many females and increase his reproductive success if he is able to attract groups of synchronized females. Variance in clutch size is large among tinamous, and individual male *Tinamus* and *Rhynchotus* have been seen incubating as many as 12 eggs (*76, 78*).

Once incubation has begun, a male ceases calling and becomes sexually unavailable. Females maximize their fitness by continuing to produce additional eggs, but they now must seek out additional males that are sexually active and able to accept a new clutch. Because of the long, tropical breeding season, females are frequently able to breed with a number of males in sequence. The wandering of females between neighboring males can be viewed as a strategy that is maximally advantageous to both sexes.

Female gregariousness is highly pronounced in the greater rhea (*79*). At the onset of the breeding season, older males compete for possession of a self-clumping group of females. Males defend no resources other than females and nest sites. Subordinate males are physically driven from the vicinity of females. In this way, a dominant male gains access to or monopolizes a group of females that then communally lay a clutch of eggs. The male assumes full incubation. The emancipated females continue to produce eggs and move on, laying a communal clutch for a second, third, or even fourth male. Many aspects of this breeding situation, including the reasons underlying male incubation, are presumed to be basically similar to those in the tinamous.

There is little evidence for sexual selection among females in the tinamous and rhea. Females are subordinate to males and sexual dimorphism is slight. All of this suggests a specialized form of resource defense polygyny (tinamous) or mate-defense polygyny (rheas) rather than an ecologically based polyandry.

3) *Female access polyandry*. Females do not directly defend resources essential to males but, through interactions among themselves, may limit access to males. Females sometimes defend males. This defense is longer than the reciprocal defense noted in male dominance polygyny since a female must remain with a male until a clutch is completed and incubation begins.

Phalaropes are highly specialized shorebirds that breed at mid- and high latitudes. They feed on a relatively small variety of insects and aquatic invertebrates in or adjacent to wetlands. They utilize a narrow range of nest sites located near these feeding areas. Because of the extremely unpredictable and ephemeral nature of their food resource, courting areas shift from year to year and even from week to week. Males perform all parental care, but there is no opportunity for a stable resource defense.

In all three phalarope species, males and females congregate at bodies of water where they feed, display, and copulate. In our opinion, the mating system is most analogous to an explosive breeding assemblage with the difference that the females can be the limited, and males the limiting, sex. We predict that the operational sex ratio will become increasingly skewed and polyandry will occur when (i) males arrive asynchronously on the breeding grounds, (ii) the breeding season is sufficiently long to allow renesting attempts, and (iii) nesting failure is frequent, increasing the importance of such replacement nestings. Female competition then occurs, and polyandry has been reported (*80*). Pair bonds are brief, and females attempt to maximize fitness through repeated matings (*81*). At high population levels, female-female interactions become severe and some members of these ephemeral aggregations are prevented from breeding (*82*). To the degree that certain females can influence the access of others to males during the period of copulation and nest initiation, the mating system can be called female access polyandry (*83*).

Plasticity of Mating Systems

Throughout this article, we have stressed that ecological variables influence or constrain the intensity of sexual selection. This, in turn, profoundly influences the form of the resulting mating system. Certain individual strategies for resource or mate control are adaptive to one set of ecological conditions, but not necessarily to another. As stated by Emlen ". . . ecological parameters impose limits on the range of types of social organization that will be adaptive. With differences in the dispersion of a critical resource, the availability of mates, or other factors, optimal social strategies shift, resulting in a fine tuning of social organization to ecological constraints" (*84*).

If the productivity or the spatial or temporal distribution of a critical resource changes from year to year or from area to area, we should expect corresponding changes in the environmental potential for polygamy. Similarly, if the energetic cost of resource or mate monopolization changes as a result of changes in population density, length of breeding season, and the like, we should expect corresponding changes in the ability to take advantage of the environmental potential for polygamy.

Considerable lability in mating systems is thus expected between different populations of a given species in different environmental or density situations. The form of the plasticity, as well as the conditions under which it should occur, should in themselves be predictable on the basis of the ecological framework presented here.

Shifts from monogamy to facultative polygyny (resource defense polygyny) and shifts in degree of polygyny have been documented in a wide variety of avian species—invariably in accordance with ecological predictions (*8, 30, 31, 85*). Similar variability in the amount of polyandry and its dependence on male availability and degree of resource monopolization has already been mentioned among spotted sandpipers. Predictable shifts along the continuum of monogamy (with shared incubation)—rapid multiple clutch polygamy—resource defense polyandry have also been suggested in both sanderlings and spotted sandpipers (*61, 67*).

Several organisms, including dragonflies, bullfrogs, turkeys, puku, topi, and Uganda kob, are known to exhibit lekking behavior at high population densities, but shift to a resource defense polygyny or mate defense polygyny at low densities (*53, 54, 86, 87*). We hypothesize that these shifts are due to the changing energetic costs of mate or resource defense associated with density-related changes in the intensity of intramale competition (*87*).

Until recently, many field biologists have worked under a preconception that species specificity was a characteristic not only of courtship behavior but of mating systems as well. We are now coming to realize that variability in social organization, including mating systems, is widespread. The ecological model presented in this article should provide a basis for generating testable predictions concerning the expected form of such mating system variability.

Summary

We have attempted to provide an ecological framework for understanding and predicting the forms of animal mating systems. The underlying assumption is that intrasexual interactions associated with mating are basically competitive. An individual member of the limited sex is expected to maximize its inclusive fitness by attempting to control access to mates of the limiting sex. The degree to which this is possible depends on the costs and benefits associated with such control. Certain environmental factors, particularly the spatial dispersion pattern of key resources and the temporal availability of receptive mates, are important determinants of these costs and benefits. The greater the potential for individuals to monopolize resources or mates, the greater the intensity of sexual selection and the greater the environmental potential for polygamy.

The precise form of the mating system will depend on which sex is limiting and on the manner and the degree to which the limited sex controls the resource base or monopolizes mates (or both).

An ecological categorization of mating systems is presented (Table 1) that allows a better understanding of the selective forces shaping one mating system over another. Within this ecological framework, specific examples are discussed ranging from the occurrence of leks to the evolution of polyandry.

References and Notes

1. The term "mating system" of a population refers to the general behavioral strategy employed in obtaining mates. It encompasses such features as: (i) the number of mates acquired, (ii) the manner of mate acquisition, (iii) the presence and characteristics of any pair bonds, and (iv) the patterns of parental care provided by each sex.
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4. J. S. Huxley, in *Evolution: Essays on Aspects of Evolutionary Biology Presented to Professor E. S. Goodrich on His 70th Birthday*, G. R. DeBeer, Ed. (Clarendon, Oxford, 1938), pp. 11–42; *Am. Nat.* **72**, 416 (1938); J. M. Smith, in *A Century of Darwin*, S. A. Barnett, Ed. (Heinemann, London, 1958), pp. 231–244; W. D. Hamilton, *Science* **156**, 477 (1967); R. L. Trivers, *Am. Zool.* **14**, 249 (1974).
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14. R. K. Selander, in *Sexual Selection and the Descent of Man, 1871–1971*, B. Campbell, Ed. (Aldine, Chicago, 1972), p. 180.
15. F. A. Pitelka, R. T. Holmes, S. F. MacLean, Jr., *Am. Zool.* **14**, 185 (1974).
16. R. D. Alexander, in *Insects, Science, and Society*, D. Pimental, Ed. (Academic Press, New York, 1975), p. 35.
17. We intend this to encompass the concept of inclusive fitness, as developed by W. D. Hamilton [*J. Theor. Biol.* **7**, 1 (1964); *ibid.*, p. 17] and M. J. West-Eberhard [*Q. Rev. Biol.* **50**, 1 (1975)], among others.
18. "r"-selected species generally tend to maximize quantity rather than quality of propagules. Parental care is relatively unimportant, if present at all. Hence the form of the mating system is less influenced by minor differences in resource control or mate assistance in the rearing of young.
19. The effects of competition or selfish behavior may not be fully expressed among long-lived animals that spend much of their lives in small, closed groups. This may be due to (i) the effects of kin selection, whereby selfish behavior might reduce the actor's inclusive fitness as a result of its negative effects on close relatives within the group, (ii) reciprocal interactions including reciprocal altruism, sensu R. L. Trivers [*Q. Rev. Biol.* **46**, 35 (1971)], or (iii) the general advantages of group living being sufficiently great that selfish behavior is not carried to the point where it results in subordinate individuals leaving the group, sensu R. D. Alexander [*Ann. Rev. Ecol. Syst.* **4**, 325 (1974)].
20. J. L. Brown, *Wilson Bull.* **76**, 160 (1964).
21. In species where one sex provides the bulk of parental investment, this sex becomes temporarily "unavailable" for further reproductive attempts (for example, during incubation of eggs or lactation for young). This can cause a skew in the operational sex ratio that will lead to intensified competition for access to the few remaining partners of the limiting sex.
22. S. T. Emlen, *Behav. Ecol. Sociobiol.* **1**, 283 (1976).
23. The operational sex ratio is measured by censusing the number of sexually active males and fertilizable females on different dates throughout the breeding season and combining these values to arrive at an average ratio. Direct measurement of OSR in the field can be difficult, since intrasexual competition often takes the form of exclusion of a portion of the population from the breeding area. Censuses of the breeding animals then would underrepresent the number of the limited sex. The problems of empirically measuring the OSR, however, are separate from, and should not detract from, its heuristic value in understanding and predicting the degree of polygamy to be expected under different environmental situations.
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26. Certain mammalian carnivores that hunt dispersed prey provide examples of species with low EPP. Such carnivores are rarely polygamous. Where males play a role in parental care, monogamy frequently results (among many canids and several felids and mustelids).
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36. See also F. B. Gill and L. L. Wolf, *Ecology* **56**, 333 (1975); F. L. Carpenter and R. E. MacMillen, *Science* **194**, 639 (1976).
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38. The quotation is from P. J. Jarman [*Behaviour* **48**, 215 (1974)], p. 248; the same point is made also by C. A. Spinage [*J. Zool.* **159**, 329 (1969)].
39. In phocid seals, estrus occurs near the end of the lactation period and lasts for several days or longer. Most otariid seals exhibit a very brief estrus, early in the lactation period.
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49. To the extent that certain males limit the access of other males to females, some polygynous matings would occur. This situation could be called "male access polygyny" and would be analogous to the mating situation described for phalaropes under the section on female access polyandry.
50. Degree of asynchrony in a female population should be characterized relative to the time necessary for a male to attract, court, and service an individual female of the species in question—not in absolute units such as hours, days, or weeks. Asynchrony generally results from differences in the times at which individual females first become sexually active; but it may be augmented by high failure rates of early nests, causing females to return to the "sexually active" pool at later dates.
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57. A clutch of eggs in many shorebirds may weigh 80 to 90 percent of the female's body weight and contain up to 200 percent of the female's total calcium [S. F. MacLean, Jr., *Ibis* **116**, 552 (1974); L. W. Oring, personal communication].
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69. Interclutch intervals can be as short as 3 days. As many as 20 eggs (totaling more than 400 percent of the female's body weight) have been laid by one female in a period of 42 days (*68*).
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71. On an island in Minnesota, all nests were lost to predators in 1975. Males kept recycling into sexual activity and successful females laid for an average of 2.0 males, while males bred with an average of 1.5 females. In 1976, predators were artificially removed (making established males less available), but a number of late-arriving new males entered the breeding population asynchronously (increasing availability of new males). As a result, all males bred with only one female, but each female laid for an average of 2.0 males and copulated with an average of 3.7 males. In each year, the high incidence of polyandry resulted from the high and prolonged availability of males to accept new clutches—but the reasons underlying this availability differed in the two seasons (*68*).
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83. The nesting habitat of phalaropes comprises prairie wetlands and arctic tundra. Environmental and ecological conditions vary greatly from year to year in these habitats, and we should expect a corresponding amount of variability in the degree of polyandry shown by the phalaropes. (See section on plasticity of mating systems.)
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87. S. T. Emlen, in preparation.
88. We thank members of Cornell's Graduate Seminar in Social Behavior who offered both insights and criticisms, and D. A. Jenni who provided intellectual stimulation throughout his semester as a visiting scientist in Ithaca. D. Lancaster and the Cornell Laboratory of Ornithology aided this work through discussions and provision of space to L.W.O. H. F. Mayfield, E. H. Miller, G. H. Orians, and F. A. Pitelka kindly offered helpful comments and criticisms on the manuscript. Supported, in part, by NSF grants BMS-75-18905 and BMS-76-81921 to S.T.E. and GB-42255 to L.W.O.

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Risk-disturbance overrides density dependence in a hunted colonial rodent, the black-tailed prairie dog *Cynomys ludovicianus*

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Summary

1. Traditional understanding of how hunting affects vertebrate populations emphasizes competitive release and density dependence of vital rates, but more recent thinking has proposed complex non-lethal responses to hunting disturbance and predation risk. Colonial species have been proposed to be more vulnerable than dispersed, solitary species to disturbance and perceived risk from hunting. However, empirical comparisons of density dependence vs. risk disturbance in hunted species are few.
2. To compare density dependence with risk-disturbance effects of hunting on individuals and populations of a colonial species, we tested the response of black-tailed prairie dogs *Cynomys ludovicianus* to shooting in a before–after, treatment–control experiment. We subjected five colonies to a pulse of shooting, and compared individual and colony attributes to those of five control colonies, protected from shooting.
3. Surviving prairie dogs increased alert behaviours eightfold and reduced both aboveground activity and time spent foraging by 66%. Changes in behaviour lowered the body condition of surviving adults by 35%. Survivors of shooting, especially juveniles, exhibited elevated stress levels; faecal corticosterone concentrations increased by 80% among juveniles. Unexpectedly, overwinter survival rates did not increase in response to reduced prairie dog density. Colonies subjected to shooting experienced reproductive near-collapse the summer after shooting; pregnancy rates declined by 50% and reproductive output fell by 82%.
4. Risk-disturbance overwhelmed any possible density-dependent effects of shooting in prairie dogs, which exhibited additive mortality in response to hunting, and reproductive failure 1 year after shooting. Risk-disturbance was the predominant mechanism whereby individuals and colonies were affected by hunting.
5. *Synthesis and applications.* Because of their coloniality, prairie dogs possess certain life-history traits that predisposed them to be particularly susceptible to hunting-associated disturbances, which had cascading effects on population-level processes. Our findings contradict the general belief that small-bodied mammals quickly rebound from hunting exploitation via compensatory mortality and reproduction. Managers should consider measures to reduce recreational shooting intensity and duration in regions where black-tailed prairie dog colony growth and persistence is desired, yet allow shooting in areas where colonies conflict with landowner interests.

Key-words: Allee effect, coloniality, density dependence, hunting, prairie dog, risk–disturbance, Wyoming.

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Introduction

Ecologists increasingly realize that predators, including human hunters, can affect populations of prey in indirect but important ways. Under the risk of predation, animals may alter activity regimes, habitat-use patterns or other behaviours (Lima 1998). Such decision-making occurs when perceived risk of predation constrains other fitness-enhancing behaviours: animals trade-off various kinds of risk – such as starvation, being killed by a predator and not finding a mate – in order to maximize fitness (Lima 1998). These trade-offs have been postulated to result in cascades of effects that can extend to population size, demography and even the structure of ecosystems (Lima 1998; Ripple & Beschta 2004).

The predation–risk paradigm has been applied to animals responding to humans as the risk-disturbance hypothesis (Frid & Dill 2002). In response to hunting or other human disturbance, vertebrates may increase vigilance (Kilgo, Labisky & Fritzen 1998), alter foraging regimes (Roy & Woolf 2001), shift migration routes (Béchet *et al.* 2003) or use resource-poor habitats (Madsen 1998). Such behaviours have been shown to be capable of lowering body condition (Féret *et al.* 2003) or reducing reproductive output (Mainguy *et al.* 2002). Animals unable to avoid risk or disturbance may exhibit other symptoms such as physiological stress (Bateson & Bradshaw 1997). Clearly, these hypotheses and mechanisms have given us a fuller understanding of how animals with adaptive behaviours maximize fitness in the face of human disturbance, or suffer fitness losses in response to unavoidable perceived risk.

At the population level, positive growth ($\lambda > 1$) might counteract the effects of perceived risk, and has been attributed commonly to density dependence. Following hunting, populations limited by resources should enjoy higher survival and reproduction, and such density-dependent responses have been shown for a wide range of hunted taxa (Fowler 1987). Generally, reducing populations below carrying capacity should increase rates of somatic growth, survival and reproduction (Kokko 2001). Physiological stress may decline in response to reduced social interactions, and levels of intraspecies conflict are also expected to decrease. Therefore, human hunting and associated disturbance can be postulated to have contradictory effects on populations, primarily via the two mechanisms of risk-disturbance and density dependence.

Which of these mechanisms is more important might depend on degree of sociality and site fidelity. Gill, Norris & Sutherland (2001) hypothesized that vulnerability to disturbance should be related inversely to capacity to relocate to alternative habitats, and FitzGibbon (1998) reviewed the mechanisms whereby some colonial species are more vulnerable to hunting disturbance than solitary ones. Colonial species cannot relocate to areas away from disturbance, tend to communicate threats to each other and are prone to social disruption (Stephens *et al.* 2002). Such hypothesized vulnerabilities are additive to the fundamental one: that colonies represent high and predictable local concentrations of the species sought. Therefore, the risk-disturbance costs of hunting to surviving animals – perhaps extending to population processes – should be higher among colonial species than solitary, dispersed ones.

In the absence of human hunting, the ecological costs and benefits of colonial living have been well described (Hoogland & Sherman 1976; Hoogland 1979). Colonial animals benefit from cooperative breeding, shared vigilance, decreased predation, increased foraging efficiency and shared habitat enhancement and maintenance. Colony members also pay certain costs: increased disease transmission, intensified intraspecific competition for resources, easier detection by predators and heightened sensitivity to behavioural disturbances. It is unknown, however, how and to what degree an evolutionarily novel perturbation, such as modern human hunting, alters these costs and benefits of coloniality.

The black-tailed prairie dog *Cynomys ludovicianus*, a colonial sciurid rodent of the plains of North America, is an ideal model for examining responses to hunting in a colonial species. Studied intensively throughout their range, in part because of the ecologically pivotal role they play in grassland systems (Miller, Ceballos & Reading 1994), the natural history and coloniality of this species have been well documented (e.g. Hoogland 1995). Prairie dogs have only recently been subject to hunting and, in contrast to most other hunted taxa, are rarely killed for meat or fur but are used as targets by recreational shooters, who typically use high-velocity rifles effective at ≤ 500 m (Reeve & Vosburgh 2005). Most states impose no seasonal restrictions, harvest limits or licensing requirements on prairie dog shooting (Reeve & Vosburgh 2005), and a single shooter can shoot scores of prairie dogs in a single session (Vosburgh & Irby 1998). In recent years, recreational shooters have reported killing > 2 000 000 black-tailed prairie dogs year^{-1} from three states combined (Reeve & Vosburgh 2005). Therefore, shooting is a widespread population influence across the range of *Cynomys*.

Although previous studies (Knowles 1982; Vosburgh & Irby 1998) have described some population and behavioural effects of shooting on prairie dogs, none has evaluated the relative importance of risk-disturbance vs. density-dependent effects. We experimentally tested the response of black-tailed prairie dogs to recreational shooting, and examined some mechanisms that might contribute to the effects hypothesized. We measured population and environmental attributes of 10 colonies, subjected one-half of the colonies to a pulse of shooting, and compared prairie dog attributes between shot and protected colonies over two consecutive summers. Specifically, we quantified shooting-related changes in abundance, survival, reproduction and demography of colonies subjected to vs. protected from shooting. We also compared the behaviours, growth rates and stress levels of the survivors on hunted colonies with those on protected colonies. We predicted effects of both risk-disturbance and density dependence, but favoured the former because of the hypothesized vulnerability of colonial species. Specifically, we predicted that surviving prairie dogs would spend less time foraging and allocate more time to vigilance or hiding underground in burrows (Blumstein & Pelletier 2005), and recognized that such effects might cascade to body condition and population-level attributes. At the same time, we expected to observe increased overwinter survival on colonies subjected to shooting, and a pulsed increase in reproduction the following summer as a result of competitive release.

Materials and methods

STUDY SITE AND SELECTION OF COLONIES

We conducted fieldwork in summers 2003–04 on private lands around Thunder Basin National Grassland (TBNG), north-eastern Wyoming (43°45′ N, 105°00′ W). TBNG encompasses > 230 000 ha of federal land in a mosaic of public and private lands. The region is characterized by rolling hills of mixed-grass prairies and sagebrush steppe habitats, dissected locally into small areas of badlands. Dominant plant species included blue grama (*Bouteloua gracilis*), western wheatgrass (*Agropyron cristatum*), needle-and-thread grass (*Stipa comata*), big sagebrush (*Artemisia tridentata*) and pricklypear cactus (*Opuntia polyacantha*). Deciduous trees, primarily plains cottonwood (*Populus deltoides*) and willows (*Salix* spp.), occur along drainages and at some springs. Ponderosa pine (*Pinus ponderosa*) is common at higher elevations. TBNG has hot, dry summers and cold, dry winters. Mean monthly temperatures range from –7 °C in January to 22·8 °C in July Average annual precipitation is about 33 cm, 70% falling during April–August (National Weather Service, Weather Station no. 487810).

We selected 10 black-tailed prairie dog colonies on private land near TBNG for study, excluding colonies that had experienced poisoning, recreational shooting or plague in the previous 10 years. Landowners agreed not to poison or allow shooting on study colonies during our study; we placed signs at each colony prohibiting shooting and monitored for compliance. All colonies were sufficiently isolated that they functioned independently; mean distance to another study colony was 6·5 km (minimum = 2·3 km; see Fig. S1 in Supplementary material) and no dispersals between colonies were detected during fieldwork.

EXPERIMENTAL DESIGN

To control for potentially confounding variation, colonies were paired based on colony area, vegetation attributes, density of prairie dogs and grazing regime in spring 2003 (Appendix S1, see Supplementary material). One colony from each pair was randomly assigned as the treatment and subjected to recreational shooting during midsummer 2003, while the other served as the control, with no shooting.

We entered standardized values (Krebs 1999) of colony area, vegetation, prairie dog density and grazing regime into Horn's (1966) equation to estimate pairwise similarity indices for the 10 colonies. Pairwise distances (PWD) were estimated as $\mathrm{PWD}_{i,j} = 1 - \mathrm{PWS}_{i,j}$, where $\mathrm{PWS}_{i,j}$ is Horn's similarity index for colonies *i* and *j*. We generated a cluster tree using the unweighted pair group method with arithmetic averages (UPGMA). Colonies clustered into distinct clades when analysed with pairwise distances (Fig. S1). We paired colonies sharing the smallest pairwise distance and assigned randomly one colony from each pair as the treatment (Fig. S1). In spring 2004, a plague epizootic reduced prairie dog abundance on one control colony by 95% (Pauli *et al.* 2006). Therefore, data from this colony (5C) and its paired treatment colony (5T) were excluded from analyses requiring data from 2004.

Five volunteers participated in shooting prairie dogs on treatment colonies during 15 June–21 July 2003. Shooters used high-velocity rifles of various models and calibres, with variable-power telescopic sights and various ammunition types, most frequently the 0·223 (5·56 × 45 mm). Shooters (1–2) fired shots opportunistically from positions 15–150 m away from a colony perimeter. An observer recorded the number of prairie dogs killed, the number of rounds fired and the length of each session (Table 1). At the end of our treatment in July 2003, shooters had reduced prairie dog abundance on each treatment colony by our objective of 25–30%. Although invasive, such experimentation was necessary to rigorously quantify prairie dog responses to recreational shooting. Further, mortality rates incurred by our treatment were nominal compared to those on colonies subjected to unregulated shooting and poisoning, which is common on private lands in this region.

We recovered, mapped and determined the sex and age (using the premolar gap method of Cox & Franklin 1990) of 68% ($n = 324$) of shot prairie dogs. Following collection of data from carcasses, we returned them to the colony to mimic typical shooting events. We evaluated whether our treatment was dispersed evenly across each colony by transferring mapped locations of shot prairie dogs to ArcGIS (ESRI, Redlands, CA, USA) where we calculated dispersion using a nearest neighbour algorithm. Nearest-neighbour values of carcasses (1T = 2·33, 2T = 2·70, 3T = 1·15, 4T = 2·21, 5T = 1·79) were > 1·00 (all $Z > 4{\cdot}93$ and $P < 0{\cdot}001$) for all colonies, showing that animals were shot in a spatially uniform pattern (Krebs 1999).

BEHAVIOUR, BODY CONDITION AND STRESS

We randomly established 100 × 100 m marked grids on each colony and recorded prairie dog activity levels and behaviour within each of them twice daily [1·5 h after sunrise and 2 h before sunset, coinciding with peak above-ground activity (Powell *et al.* 1994)] for five consecutive days. Observations were conducted concurrently with trapping, and paired colonies were observed

Table 1. Number of rounds fired, time spent shooting and number of black-tailed prairie dogs killed by recreational shooters on treatment colonies, Thunder Basin National Grassland, Wyoming, June–July 2003. The percentage of the population killed was estimated by dividing the number killed by the estimated population size

Colony	No. rounds fired	Time spent shooting (person h^{-1})	No. killed	% of population killed
1T	323	8·0	67	31
2T	146	14·3	27	32
3T	697	27·6	209	30
4T	174	9·4	37	30
5T	502	21·2	138	25

simultaneously to control for temporal variation. Observations were postponed during periods of rain or wind speed > 40 km h^{-1}. Observations followed the protocol of scan sampling (Martin & Bateson 1993) and were taken from an elevated position with a 20 × telescope. Following a 20-min delay after assuming our position to allow behaviour to stabilize (Powell *et al*. 1994), we observed and recorded the number and behaviour of prairie dogs on the grid at 10-min intervals for 70 min. We categorized behaviour as: (1) foraging, (2) alert (vigilant or calling), (3) moving, (4) resting, (5) socializing or (6) other. Because few prairie dogs were observed socializing or moving, these two behaviours were pooled with the behavioural category 'other' for subsequent statistical analyses.

Above-ground counts and behaviour were each highly autocorrelated within observation periods (all $r^2 > 0{\cdot}50$). Therefore, we averaged these two variables for each observation period. We expressed behavioural data as the percentage of total time spent in a particular behaviour type. We also expressed above-ground activity as the percentage of animals estimated to occur in a colony (prairie dogs ha^{-1}) above ground at a given time. We used a repeated-measures analysis of variance (RM-ANOVA) to test for the effect of recreational shooting on behaviours over the three trapping sessions and analysed within- and between-subject effects with *post-hoc* comparisons. Prior to each RM-ANOVA, we tested data for normality and homoscedasticity (Zar 1999), transforming non-conforming data using arcsine square-root transformations. In addition, we used Mauchly's *W*-test statistic to test for assumed sphericity (von Ende 2001).

In May–June 2003 (predisturbance), we placed trapping grids randomly on each colony; these same grids were re-established in July–August 2003 and May–June 2004 (post-disturbance). Live traps (Model 203, Tomahawk Live Traps, Tomahawk, WI, USA) were arranged in a 9 × 9 grid, 15 m apart. Paired colonies were trapped simultaneously for 6 consecutive days. During each session, traps were set, wired open and prebaited for 24 h, then baited and set at sunrise and checked and closed at sunset of each day's trapping. We marked captured prairie dogs with fingerling ear tags (National Band and Tag, Newport, KY, USA) and, because we observed during preliminary studies that males tended to lose ear tags, also injected males with subcutaneous passive integrated transponders (Biomark, Boise, ID, USA). We measured weight and hind foot length of each captured animal, using a ratio of the two measurements (g cm^{-1}) as an index of body condition (Krebs & Singleton 1993). Values for an individual were averaged within a trapping session. We tested for changes in the body conditions of juveniles and adults over the study period and between control and treatment colonies with an RM-ANOVA.

We collected scat samples from trapped prairie dogs in 2003 for analysis of faecal corticosterone concentration, a metric of physiological stress (Harper & Austad 2000). Although trapping and handling elevates corticosterone levels, faecal corticosterone levels reflect stress experienced about 6–12 h before defecation (Harper & Austad 2000). Therefore, we collected scat samples only from animals that had been held in traps < 5 h, and that had not been captured the previous day. Scat samples were air-dried in the field and stored at –20 °C. Corticosterone was extracted from faeces using methods described by Monfort *et al*. (1998) and assayed using a radioimmunoassay kit (ICN Biomedical Inc., Costa Mesa, CA, USA) and scintillation counter. The inter- and intra-assay coefficients of variation were 10% and 8%, respectively, and the detection threshold of the assay was 2·6 ng g^{-1}. We analysed variation in corticosterone levels for two age groups (adults and juveniles), before and after shooting, and on control and treatment colonies with two-way ANOVAS.

DENSITY, DEMOGRAPHY AND VITAL RATES

We determined the reproductive status of adult females in May and June at first capture. Females were considered to have been reproductively active earlier in the year if their nipples were enlarged and turgid. We entered capture histories into program CAPTURE to estimate juvenile, adult female and adult male abundances for each colony and trapping session. We estimated densities by dividing abundance estimates by the effective area trapped (Wilson & Anderson 1985), using half the mean maximum distance moved (averaging the maximum distance between recaptures for captured animals) and adding it to the perimeter of the trapping grid. Separate values were calculated for each age–sex group (juvenile, adult female, adult male), colony and trapping session, and variances for densities were calculated using the approach of Otis *et al*. (1978). We analysed changes in the densities of each prairie dog age–sex group over the study period and between control and treatment colonies with RM-ANOVA.

We evaluated changes in the proportions of juveniles, adult males and adult females in each colony with log-likelihood ratios. We compared the proportion of males and females that were yearlings and adults, and the proportional number of reproductively active females

Table 2. Mean percentage and standard error of prairie dogs foraging, alert and resting on control ($\bar{x}_C$) and treatment ($\bar{x}_T$) colonies from three sampling periods, Thunder Basin National Grassland, Wyoming, 2003–04. Prior to testing, percentages were converted using the arcsine transformation (Zar 1999) and compared between control and treatment colonies for each sampling period with paired *t*-tests (d.f. for all tests = 39). Treatment colonies were subjected to intensive shooting in June–July 2003

	Foraging						Alert						Resting					
Date	$\bar{x}_C$	SE	$\bar{x}_T$	SE	*t*	*P*	$\bar{x}_C$	SE	$\bar{x}_T$	SE	*t*	*P*	$\bar{x}_C$	SE	$\bar{x}_T$	SE	*t*	*P*
May–June 2003 (pre-disturbance)	76·2	1·3	78·7	1·3	–1·23	0·23	7·4	1·2	3·7	1·0	1·65	0·11	6·0	1·1	6·8	1·5	0·60	0·55
July–August 2003 (post-disturbance)	91·1	1·0	66·1	1·2	14·5	<0·001	3·2	0·7	29·2	1·1	–22·1	<0·001	2·7	0·9	0·7	1·1	3·55	0·01
May–June 2004 (post-disturbance)	74·7	1·4	71·0	1·4	1·20	0·24	10·3	1·1	15·6	1·2	–3·45	0·001	1·1	1·1	0·7	1·1	0·85	0·40

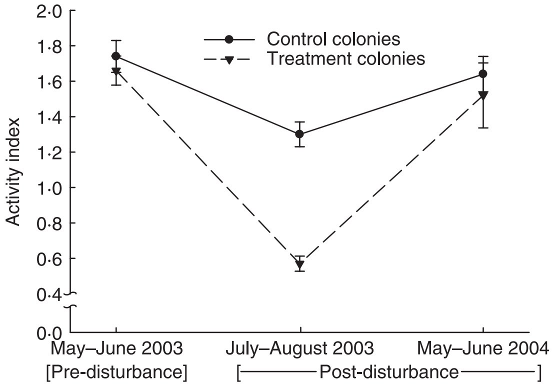

Fig. 1. Mean above-ground activity indices (± 1 SE) for black-tailed prairie dogs on control and treatment colonies, Thunder Basin National Grassland, Wyoming, 2003–04. Activity indices were calculated by dividing the number of prairie dogs above ground (prairie dogs ha^{-1}) by density estimates (prairie dogs ha^{-1}) for that colony. Prior to shooting, activity indices did not differ between control and treatment colonies. Following shooting in June–July 2003, activity on treatment colonies was lower than on control colonies. Above-ground activity on treatment colonies rebounded to control levels in 2004.

between control and treatment colonies in 2003 and 2004 with log-likelihood ratios, corrected for continuity. Indices of reproductive rate (juveniles × adult $female^{-1}$) were compared between control and treatment colonies in 2003 and 2004 with a paired *t*-test.

Using the robust design model in program MARK (White & Burnham 1999), we modelled apparent survival ($\hat{S}_i$), temporary immigration (γ_i') and emigration (γ_i''), conditional capture ($\hat{p}_{ij}$) and recapture ($\hat{c}_{ij}$) probabilities. Parameter estimates in MARK are maximum likelihood estimates with 95% confidence intervals. To remove the estimates of population size from the likelihood, we used Huggins' estimator (Huggins 1991). We developed a series of 12 *a priori* models based on a number of factors that we believed would influence one or more of the parameter estimates. Factors that we hypothesized would affect parameter estimation included the intervals between trapping occasions, the dates of capture, age and sex of animals, colony, colony pair and experimental status (control, treatment). Because we assumed that temporary immigration and emigration were not occurring on any of the colonies, the parameters γ_i' and γ_i'' were set at 0.

We ranked models of survival rate using Akaike's information criterion corrected for small samples (AIC_c; Burnham & Anderson 2002). We ranked AIC_c values relative to the model with the lowest AIC_c value. Comparisons among models were made using ΔAIC_c (Burnham & Anderson 2002). Normalized Akaike weights (w_i) were also computed for each model as an additional assessment of the strength of evidence for each model.

Results

BEHAVIOUR, BODY CONDITION AND STRESS

Behaviour and time spent above ground were not influenced by the time (morning or evening) of observations (all $F_{2,39} < 0·70$, $P > 0·65$); therefore, morning and evening observations were pooled. Before shooting, prairie dog behaviour did not differ between control and treatment colonies (Table 2); most behaviour was foraging (76%), followed by resting (7%) and alertness (5%). Shooting caused several behaviour types to differ (foraging: $F_{2,39} = 39·5$, $P < 0·001$; resting: $F_{2,39} = 2·90$, $P = 0·033$; alertness: $F_{2,39} = 89·7$, $P < 0·001$) between control and treatment colonies. Shortly after shooting, alertness on treatment colonies increased to 29%, while on control colonies it decreased to 3% over the same period (Table 2). This coincided with reduced foraging (to 66%) and resting (to < 1%). By contrast, on control colonies foraging increased to 91%, while resting decreased slightly to 3% (Table 2). In 2004, the year after shooting, behaviours on treatment colonies returned to control levels for foraging (73%) and resting (1%), but alertness remained slightly elevated (Table 2). Time spent above ground was also affected by recreational shooting. Activity indices did not differ between control and treatment colonies prior to shooting ($t_{39} = 0·69$, $P = 0·50$), but diverged after shooting ($F_{2,39} = 5·95$, $P = 0·003$; Fig. 1). Above-ground activity on treatment colonies declined later the same summer by 66%, while corresponding values on control colonies declined by only 22% ($t_{39} = 8·72$, $P < 0·001$). The summer after shooting, above-ground activity recovered on treatment colonies, so that it did not differ ($t_{39} = 0·65$, $P = 0·52$; Fig. 1) from control-colony values.

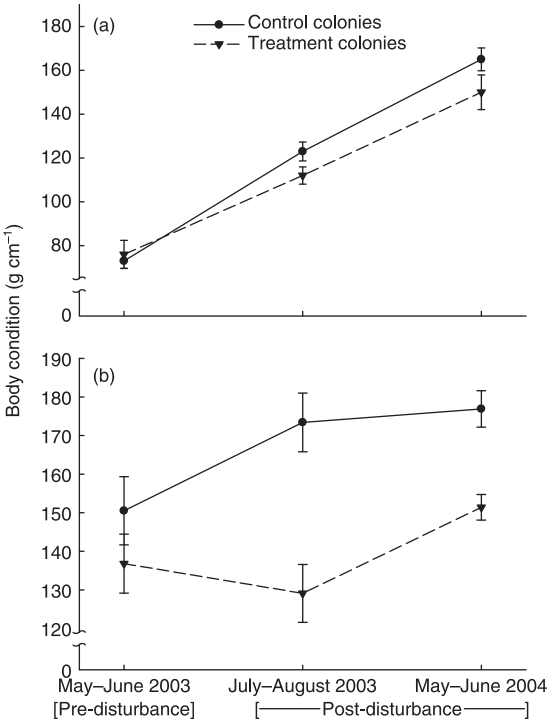

Fig. 2. Mean body condition (± 1 SE) for (a) juvenile and (b) adult black-tailed prairie dogs on control and treatment colonies over three sampling periods, Thunder Basin National Grassland, Wyoming, 2003–04. Treatment colonies were subjected to intensive recreational shooting in June–July 2003. On both control and treatment colonies, juveniles exhibited improved body condition over the course of the study. After shooting, adult body condition on treatment colonies declined, while adults on control colonies exhibited improved body condition. In 2004, adult body condition on treatment colonies partially recovered, but remained lower than on control colonies.

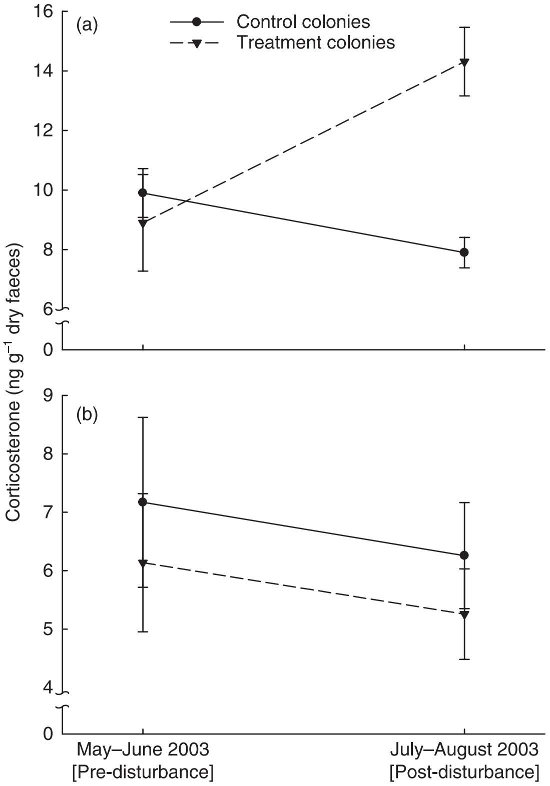

Fig. 3. Mean faecal corticosterone concentrations (± 1 SE) from (a) juvenile and (b) adult black-tailed prairie dogs on control and treatment colonies before and after treatment colonies were subjected to recreational shooting, Thunder Basin National Grassland, Wyoming.

Recreational shooting did not affect body condition of surviving juveniles ($F_{2,56} = 2\cdot37$, $P = 0\cdot11$). On both control and treatment colonies, body condition of animals that were juveniles in 2003 increased approximately linearly from May–June 2003–04 ($F_{2,56} = 176\cdot1$, $P < 0\cdot001$; Fig. 2a). In contrast, recreational shooting altered the body condition of surviving adults ($F_{2,38} = 3\cdot23$, $P = 0\cdot050$; Fig. 2b). Before shooting, adult body condition did not differ between control and treatment colonies ($t_{18} = 0\cdot46$, $P = 0\cdot65$). After shooting, adult body condition decreased on treatment colonies and increased on control colonies, resulting in a 35% difference in body condition between control and treatment colonies later in the summer of shooting ($t_{18} = 2\cdot78$, $P = 0\cdot012$). By the following summer, body condition of adults on treatment colonies had recovered somewhat, to 17% below control values ($t_{18} = 3\cdot00$, $P = 0\cdot008$; Fig. 2b).

Stress responses to shooting depended on age (Fig. 3). For adults, recreational shooting did not affect stress; corticosterone levels were 10·8 ± 0·7 (± 1 SE) ng g^{-1} dry faeces for both control and treatment colonies, before and after shooting ($F_{1,27} < 0\cdot001$, $P = 0\cdot99$). Also for adults, corticosterone levels did not change from May–June 2003 to July–August 2003 ($F_{1,27} = 0\cdot60$, $P = 0\cdot45$). In contrast, juveniles exhibited elevated stress in response to shooting ($F_{1,41} = 7\cdot42$, $P = 0\cdot009$); before shooting, corticosterone levels did not differ between control and treatment colonies ($t_8 = 0\cdot47$, $P = 0\cdot65$). After shooting, however, values increased on treatment colonies to levels 80% higher than those on control colonies ($t_{33} = 4\cdot95$, $P < 0\cdot001$; Fig. 3).

DENSITY, DEMOGRAPHY AND VITAL RATES

Prior to shooting in 2003, prairie dog densities were similar between control and treatment colonies for juveniles ($t_3 = -0\cdot33$, $P = 0\cdot76$), adult females ($t_3 = 0\cdot26$, $P = 0\cdot81$) and adult males ($t_3 = 1\cdot17$, $P = 0\cdot33$; Fig. 4). Densities of all three age–sex groups on treatment and control colonies diverged following our shooting treatment (juveniles: $F_{2,7} = 11\cdot83$, $P = 0\cdot001$; adult females: $F_{2,7} = 4\cdot54$, $P = 0\cdot034$; adult males $F_{2,7} = 6\cdot43$, $P = 0\cdot013$; Fig. 4). After shooting in 2003, densities of juveniles born in 2003 did not differ between treatment and control colonies ($t_3 = -2\cdot27$, $P = 0\cdot11$), but 10 months later the 2004 cohort on treatment colonies was 85% smaller than that of the previous year, and 89% smaller than the 2004 cohort on control colonies ($t_3 = -4\cdot96$, $P = 0\cdot016$; Fig. 4a). Shooting reduced adult female densities by 40% in the short term, to values lower than those on control colonies at the same time ($t_3 = -3\cdot40$, $P = 0\cdot042$). Ten months later, densities of adult females on treatment

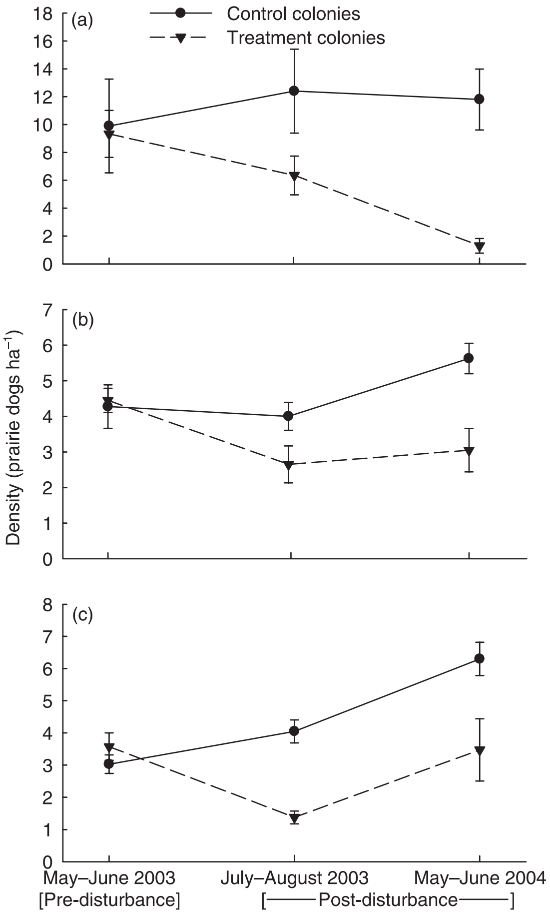

Fig. 4. Density estimates (± 1 SE) for (a) juvenile, (b) adult female and (c) adult male black-tailed prairie dogs on control and treatment colonies during three trapping occasions, Thunder Basin National Grassland, Wyoming, 2003–04. Treatment colonies were subjected to intensive recreational shooting in June–July 2003. For juveniles, shooting did not immediately reduce their densities, but 1 year later juvenile densities fell by 85%. Shooting reduced female densities by 40% in the short term and these densities remained lower 1 year later, in 2004. Adult males were most susceptible to shooting, exhibiting a 62% decline in densities, but were capable of rebounding to predisturbance and control-level densities in 2004.

colonies remained marginally lower than on control colonies ($t_3 = -2{\cdot}99$, $P = 0{\cdot}058$; Fig. 4b). For adult males the pattern differed; densities declined by 62% in short-term response to shooting ($t_3 = -9{\cdot}05$, $P = 0{\cdot}003$), but recovered by 10 months later, when densities did not differ from those on control colonies ($t_3 = -2{\cdot}23$, $P = 0{\cdot}11$; Fig. 4c). Changes in the density of each age–sex group is explained partially by the selectivity of shooting: juveniles represented 59% of animals shot, a value proportional to their abundance (Appendix S2), while adult females were the least vulnerable, composing only 15% of animals shot, but 22% of the colony members. Adult males were the most susceptible age–sex group, constituting 26% of animals shot, but only 19% of the animals on the colony (Appendix S2).

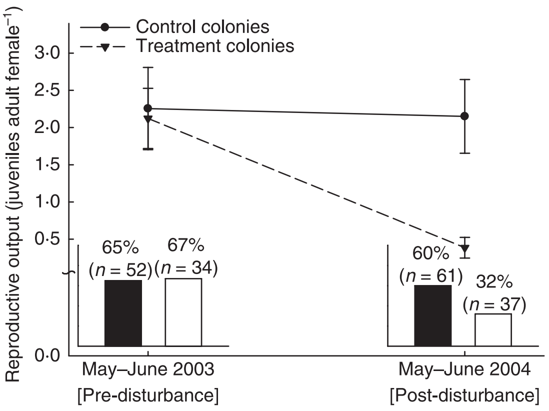

Fig. 5. Mean reproductive output (± 1 SE; main graph) and pregnancy rates (inset bar graphs) for adult female black-tailed prairie dogs on control (solid shading, inset bar graphs) and treatment colonies (open shading, inset bar graphs), Thunder Basin National Grassland, Wyoming, 2003–04. After shooting treatment colonies in June–July 2003, pregnancy rates fell by 50% and reproductive output fell by 82%.

Age structure did not differ between control and treatment colonies before shooting ($G2 = 2{\cdot}46$, $P = 0{\cdot}88$); juveniles dominated age–sex groups (53% of animals), followed by adult females (27%) and adult males (20%). Immediately after shooting, age structure did not differ between control and treatment colonies ($G_2 = 3{\cdot}10$, $P = 0{\cdot}21$). However, 10 months after shooting, age structure had changed ($G_2 = 11{\cdot}7$, $P = 0{\cdot}003$), with juveniles (born in 2004) less prevalent on treatment colonies (16% vs. 49% on control colonies). The proportion of the population that was adult female (39% treatment, 24% control) and adult male (45% treatment, 27% control) increased, reflecting the major decline in proportional abundance of juveniles.

Before shooting, age structure for prairie dogs > 1 year of age did not differ between control and treatment colonies for females ($G_1 = 2{\cdot}47$, $P = 0{\cdot}12$) or males ($G_1 = 0{\cdot}14$, $P = 0{\cdot}71$); 31% of adult females and 30% of adult males were yearlings. One year after shooting, the percentage of yearling females increased (53%), but did not differ between control and treatment colonies ($G_1 = 0{\cdot}01$, $P = 0{\cdot}94$). For males in 2004, however, age structure diverged between control and treatment colonies ($G_1 = 4{\cdot}89$, $P = 0{\cdot}027$); on control colonies, the percentage of yearlings increased to 54%, while on treatment colonies the percentage of yearlings more than doubled, to 76%.

In 2003, reproductive output was 2·2 juveniles × adult female^{-1}, similar between control and treatment colonies ($t_3 = -0{\cdot}41$, $P = 0{\cdot}71$; Fig. 5). The summer following shooting reproductive output remained similar on control colonies, but fell by 82%, to 0·4 juveniles × adult female^{-1}, on treatment colonies ($t_3 = 3{\cdot}33$, $P = 0{\cdot}044$; Fig. 5). This reduction reflected a concomitant decline in pregnancy rates, which did not differ between control and treatment colonies in 2003 ($G_1 < 0{\cdot}01$, $P = 0{\cdot}96$); 66% of females showed evidence of recent reproduction (Fig. 5). In 2004, however, 60% of females on control colonies gave birth, in comparison with just 32% of females on treatment colonies ($G_1 = 6{\cdot}62$, $P = 0{\cdot}010$; Fig. 5).

Table 3. Twelve a priori models from survival analyses using program MARK (White & Burnham 1999) for black-tailed prairie dogs from eight paired study colonies, Thunder Basin National Grasslands, captured in Wyoming, 2003 and 2004. We modelled capture ($\hat{p}$) and recapture probabilities ($\hat{c}$) from five variables: age (juvenile, adult), date (date of capture or recapture), session (primary trapping session), colony (the eight colonies) and treatment (control vs. treatment colonies). We modelled prairie dog survival ($\hat{S}$) from six variables: interval (period between trapping sessions), age (juvenile, adult), age/sex (juvenile, adult male, adult female), block (colony pairs), shot-acute (shooting effects on treatment colonies from May–August 2003) and shot-chronic (shooting effects on treatment colonies from August 2003 to May 2004). For all models temporary emigration (γ'') and immigration (γ') were set at 0 and therefore not depicted below. Models were ranked using Akaike's information criterion (AIC$_c$). Also provided with each model are ΔAIC$_c$ (the difference between the best model's AIC$_c$ and subsequent models), w_i (weight of evidence in favour of each model), K (number of parameters) and log (L) (maximum log-likelihood)

Model	AICc	ΔAICc	w_i	K	log(L)
$\hat{S}$ (interval, age, shot-acute) $\hat{p}$ (age,date*session, colony,treatment) $\hat{c}$ (age,date*session,colony)	7952·6	0	0·410	29	–3945·9
$\hat{S}$ (interval,age/sex,shot-acute) $\hat{p}$ (age,date*session, colony,treatment) $\hat{c}$ (age,date*session,colony)	7954·2	1·6	0·184	30	–3945·6
$\hat{S}$ (interval,age,shot-acute,shot-chronic) $\hat{p}$ (age,date* session,colony,treatment) $\hat{c}$ (age,date*session,colony)	7954·6	2·0	0·151	30	–3945·8
$\hat{S}$ (interval,age,block,shot-acute) $\hat{p}$ (age,date* session,colony,treatment) $\hat{c}$ (age,date*session,colony)	7955·5	2·9	0·095	32	–3944·0
$\hat{S}$ (interval,age/sex,shot-acute,shot-chronic) $\hat{p}$ (age,date *session,colony,treatment) $\hat{c}$ (age,date*session,colony)	7956·3	3·7	0·064	31	–3945·5
$\hat{S}$ (interval,age/sex,block,shot-acute) $\hat{p}$ (age,date* session,colony,treatment) $\hat{c}$ (age,date*session,colony)	7957·2	4·7	0·040	33	–3943·8
$\hat{S}$ (interval,age,block,shot-acute,shot-chronic) $\hat{p}$ (age,date* session,colony,treatment) $\hat{c}$ (age,date*session,colony)	7957·2	4·7	0·040	33	–3943·8
$\hat{S}$ (interval,age/sex,block,shot-acute,shot-chronic) $\hat{p}$ (age,date* session,colony,treatment) $\hat{c}$ (age,date*session,colony)	7959·1	6·5	0·016	34	–3943·6
$\hat{S}$ (interval,age,block) $\hat{p}$ (age,date*session,colony, treatment) $\hat{c}$ (age,date*session,colony)	7968·4	15·8	0·000	31	–3951·6
$\hat{S}$ (interval,age) $\hat{p}$ (age,date*session,colony,treatment) $\hat{c}$ (age,date*session,mix*adult,colony)	7968·0	15·4	0·000	28	–3954·7
$\hat{S}$ (interval,age/sex,block) $\hat{p}$ (age,date*session,colony,treatment) $\hat{c}$ (age,date*session,colony)	7970·3	17·7	0·000	32	–3951·4
$\hat{S}$ (interval,age/sex) $\hat{p}$ (age,date*session,colony,treatment) $\hat{c}$ (age,date*session,colony)	7969·8	17·2	0·000	29	–3954·5

Table 4. Survival estimates and associated standard errors for black-tailed prairie dog age–sex groups in Thunder Basin National Grassland, Wyoming, 2003–04. Modelling was performed in program MARK. Treatment colonies were subjected to a pulse of recreational shooting in June–July 2003; control colonies were protected from shooting. Shown, in order, are estimates from the three most competitive models ranked by AIC$_c$. For details on models and model ranking see Table 3

	June–August 2003				August 2003–June 2004			
	Control		Treatment		Control		Treatment	
Age–sex group	$\hat{S}$	SE	$\hat{S}$	SE	$\hat{S}$	SE	$\hat{S}$	SE
Model 1								
Juvenile	0·725	0·047	0·438	0·06	0·423	0·042	–*	–*
Adult	0·677	0·055	0·383	0·06	0·368	0·044	–*	–*
Model 2								
Juvenile	0·726	0·047	0·438	0·06	0·423	0·042	–*	–*
Adult male	0·647	0·069	0·351	0·07	0·337	0·057	–*	–*
Adult female	0·697	0·06	0·404	0·07	0·390	0·054	–*	–*
Model 3								
Juvenile	0·727	0·048	0·438	0·06	0·450	0·071	0·414	0·045
Adult	0·678	0·055	0·381	0·06	0·392	0·069	0·358	0·048

*Survival estimates and standard error did not differ from those computed for control colonies.

Variation in survival was best explained by a model (Table 3) that included parameters for trapping sessions, age–sex group and shooting (ΔAIC$_c$ = 0·0, w_i = 0·410; Table 3). Estimates of survival from June to August 2003 on control colonies were 0·73 for juveniles and 0·68 for adults. Corresponding estimates for treatment colonies (juvenile: 0·44; adult: 0·38) were about 30% lower (Table 4). Overwinter survival (August 2003–

June 2004), however, was not affected by shooting; our best model estimated juvenile survival as 0·42 and adult survival as 0·37 for both control and treatment colonies (Table 4).

We found less, and approximately equal, support for two other models (Table 3) that, in addition to accounting for short-term effects of shooting, also (1) differentiated survival rates among sexes ($\Delta AIC_c = 1{\cdot}6$, $w_i = 0{\cdot}184$); and (2) accounted for shooting reducing overwinter survival on treatment colonies ($\Delta AIC_c = 2{\cdot}0$, $w_i = 0{\cdot}151$). However, both models appeared suboptimal because log-likelihood estimates remained similar even though the number of estimable parameter increased (Table 3). Further, uncertainty associated with the survival estimates from these suboptimal models resulted in considerable overlap between survival estimates, making their utility ambiguous (Table 4). As predicted, models that did not account for changes in survival from shooting fitted our data poorly (ΔAIC_c range = 15·8–17·2, all $w_i < 0{\cdot}0001$; Table 3).

Discussion

Prairie dogs subjected to hunting exhibited risk-disturbance effects that overwhelmed and obscured any possible density-dependent ones. As observed in some other hunted vertebrates, surviving prairie dogs altered behaviour to reduce their vulnerability to shooting at the expense of other fitness-enhancing activities. They increased their alertness and decreased above-ground activity, time spent foraging and time spent resting above ground. In general, these behavioural responses were transient, returning to preshooting and control-colony levels the summer after shooting. However, alert behaviours remained slightly elevated on treatment colonies 10 months later. This persistently elevated alertness may reflect a confounding influence, however. Loughry (1992) showed that adult prairie dogs exhibited higher alertness than juveniles, so the increase in alertness we observed could be attributable to changes in age structure. Although qualitatively similar behavioural responses have been reported for hunted waterfowl (Madsen & Fox 1995; Féret *et al.* 2003) and ungulates (Kilgo *et al.* 1998), prairie dog responses to shooting were particularly dramatic.

Behavioural responses of prairie dogs to shooting may have been influenced by factors in addition to risk-avoidance. First, prairie dogs exhibit complex social networks and deaths due to shooting could have disrupted important social interactions and individual behaviour. Indeed, Shier (2006) showed that among translocated prairie dogs, group cohesion strongly affected foraging efficiency: intact family groups foraged more and were vigilant less than those composed of mostly unrelated individuals. Shier (2006) found that these changes extended to survival and reproductive output, as did we. Secondly, in contrast to more traditional forms of hunting, recreational shooting involves many rounds fired over hours (Table 1; Vosburgh & Irby 1998). Such a disturbance contributed presumably to the dramatic behavioural responses we observed in prairie dogs. Thus, the behavioural sensitivity of prairie dogs to shooting seems a combination of their coloniality, low mobility and sensitivity to social disruption as well as the duration and intensity of the disturbance.

Reduced foraging and above-ground activity resulted in lowered body condition of surviving adult prairie dogs, which had body conditions 35% poorer than those of control animals. Although adult body condition on treatment colonies improved the following year, it remained 17% lower than on control colonies. So, effects of shooting on body condition via foraging persisted into the next growing season for adults. In contrast, shooting did not affect the body condition of surviving juveniles, for reasons that are suggested by our stress-response data. Unlike adults, juveniles exhibited a distinct stress-response to shooting, but no response of body condition. We hypothesize that juveniles tended to remain above ground and forage during and after shooting, unlike adults. Prairie dogs survive winter on somatic stores (Lehmer & Van Horne 2001), and with especially limited stores, juveniles must increase body mass rapidly in the first summer of life to survive their first winter (Rayor 1985). Therefore, juveniles may need to forage during times of increased risk, including during shooting, more than do adults, thereby exposing them to disturbance and resulting physiological stress (Lima 1998). Despite the adaptiveness of corticosterone in managing short-term stressors, chronically high levels can lead to reproductive failure, immune suppression and poor body condition (Sapolsky 1992). Therefore, chronically elevated corticosterone levels could cascade to reduced survival and recruitment.

Such cascades have been shown for other hunted species. Féret *et al.* (2003) and Roy & Woolf (2001) found that game birds subjected to intensive hunting increased the time spent flying and decreased the time spent foraging, with negative consequences for body condition. Hjeljord & Histøl (1999) found that the body mass of moose (*Alces alces*) in Norway was correlated negatively with hunting intensity. The changes reported by these studies were less dramatic than those we describe here for prairie dogs. Again, the coloniality and low mobility of prairie dogs and the intense and disruptive nature of recreational shooting seem to account for this difference.

Age–sex groups differed in their vulnerabilities and responses to shooting. Adult females were not particularly susceptible to shooting, but failed to recover to preshooting densities the summer after the treatment. Conversely, adult males were susceptible to shooting, but recovered to preshooting and control-colony densities the following summer. Juvenile proportional abundances were not affected in the short term, but were reduced by 85% the following summer. Juveniles, the dominant age group (54%) on colonies before shooting, were the smallest age group (< 20%) the following year. This delayed effect, of shooting on juvenile abundance the following summer, was mediated largely by reproduction. On colonies subjected to shooting, reproductive output decreased by 82% from 2003 to 2004, whereas control colonies showed little change over the same period. The proportion of females producing young on treatment colonies declined by ~0·5. Knowles (1987) found a fairly constant 0·66 of females giving birth, similar to values from our control colonies.

Two mechanisms could explain reduced pregnancy and reproductive rates on treatment colonies. First, reduced body condition of surviving adult females could have reduced litter production. We found that the body condition of prairie dogs > 1 year of age decreased by 35% in response to shooting. Somatic stores are important for both survival and reproduction among ground squirrels (Woods & Armitage 2003), and female prairie dogs must accumulate sufficient reserves to reproduce successfully (Seabloom & Theisen 1990). At northern latitudes, black-tailed prairie dogs copulate in February–March (Hoogland 1995), when prairie dogs have the lowest body masses and are most stressed nutritionally (Lehmer & Van Horne 2001). Therefore, adult females on colonies subjected to shooting could have been physiologically unable to carry a litter to parturition, as a result of foraging opportunities lost (Knowles 1987). Secondly, changes in age–sex structure could have reduced the availability of reproductively competent males. The summer after shooting abundance of males rebounded, but ages were lower than before shooting; most mature males had been replaced by presumably immigrant, yearling males. As in larger mammals (Wielgus & Bunnell 1994), yearling male prairie dogs are less competent breeders than older males (Seabloom & Theisen 1990). This form of Allee effect, resulting from reduced availability to breeding-age females of competent adult males (Stephens & Sutherland 2000), could be an important consequence of male-biased hunting, particularly among polygynous, colonial animals for which females are mate-selective (Halliday 1983). Thus, nutritional deficiencies in adult females or the influx of yearling males after shooting – or both – might have contributed to the reproductive near-collapse that we observed the summer following shooting.

Surprisingly, shooting did not improve overwinter survival. If anything, we saw evidence for decreased survival among treatment animals. One top-ranked model accounted for reduced overwinter survival on treatment colonies, but those survival estimates overlapped considerably with values for control colonies. None the less, the importance of incorporating a parameter for shooting was apparent; models that accounted for an effect of shooting in reducing survival were competitive, while models that did not do so performed poorly.

COMPARATIVE ASPECTS

Generally, populations of small-bodied mammalian herbivores, such as lagomorphs (Rose 1977) and squirrels (Mosby 1969), recover quickly from hunting via density-dependent vital rates. In contrast, prairie dogs in our study showed no evidence of density dependence in overwinter survival or next-year natality. Rather, we found that for prairie dogs, hunting induced not only additive effects on survival, but also led to reproductive near-collapse the summer following the shooting. We attribute these remarkable effects to costs represented by shooting to a highly colonial species: shooting reduced the usual benefits and increased some of the common costs of coloniality. In unperturbed colonies, animals benefit from cooperative vigilance, allowing a relaxation of individual vigilance, which increases foraging efficiency and reduces susceptibility to predation (Hoogland 1981). Coloniality also facilitates adult reproduction and juvenile survival through enhanced mate-finding and cooperative breeding (Jennions & MacDonald 1994). These common benefits of coloniality, however, appeared to be reduced by recreational shooting. Intense, prolonged shooting increased alertness at the expense of foraging, so that a primary benefit of coloniality was reduced. Coloniality presumably facilitates reproduction by providing easy access to mates, and some communal care of neonates. However, the effects that we observed suggest a shift in the net fitness costs and benefits of coloniality.

Among costs, coloniality causes all above-ground animals to be disturbed by a single shot that is fired; animals that escape to below ground forego foraging opportunities. Under most circumstances, access to mature male mates is not limiting to female prairie dogs (Hoogland & Foltz 1982), but shooting selectively removed adult males, so that female access to competent mates may have been reduced. This may have contributed to the reproductive near-collapse the summer after shooting. Thus, coloniality appears to make prairie dogs more sensitive to hunting than other small mammalian herbivores.

CONSERVATION IMPLICATIONS

Prairie dogs are ecologically pivotal members of North American grassland systems (Miller *et al.* 1994), supporting predators, including the obligate prairie dog predator, the endangered black-footed ferret (*Mustela nigripes*). Prairie dog burrows also provide habitat structure for burrowing owls (*Athene cunicularia*), prairie rattlesnakes (*Crotalus viridis*) and various small mammals. Through herbivory, prairie dogs alter vegetation and cycle nutrients (Holland & Detling 1990). Therefore, the population biology of prairie dogs and the viability of their colonies have broad implications for North American grassland communities. Future research needs to elucidate the large-scale effects of shooting on prairie dog populations and colony viability as well as its effects on other species that depend on prairie dog colonies. Wildlife managers should consider measures to reduce recreational shooting intensity and duration in regions where black-tailed prairie dog colony growth and persistence is desired, such as recovery sites for the black-footed ferret, yet allow shooting to continue in areas where colonies conflict with private-landowner interests.

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Supplementary material

The following supplementary material is available for this article.

Appendix S1. Attributes of 10 black-tailed prairie dog colonies, Thunder Basin National Grassland, Wyoming, summer 2003.

Appendix S2. Mean standardized selection indices and associated standard errors for prairie dog cohorts shot on treatment colonies, Thunder Basin National Grassland, Wyoming, 2003.

Fig. S1. (a) Locations of 10 black-tailed prairie dog colonies used in an experimental study of recreational shooting around Thunder Basin National Grassland (TBNG), Wyoming, 2003-2004. (b) Cluster tree depicting the relative similarity of colonies using the unweighted pair group method with arithmetic averages.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2664.2007.01337.x
(This link will take you to the article abstract.)

xylem sap were high under N-starved conditions but lower under N-rich conditions.

Altogether, the available evidence from molecular and physiological analyses of CEP–CEPR ligand receptor pairs suggests that CEP acts as a root-derived ascending N-demand signal to the shoot, where its perception by CEPR leads to the production of a putative shoot-derived descending signal that up-regulates nitrate transporter genes in the roots. This mechanism supports N acquisition, especially when NO_3^- is unevenly distributed within the soil. CEP family peptides induced on one side of the roots by local N starvation mediate up-regulation of nitrate transporter genes in the distant part of the roots exposed to N-rich conditions to compensate for N deficiency.

The systemic mode of action of CEP family peptides in N-demand signaling is reminiscent of that of *Rhizobium*-induced, xylem-mobile CLE peptides that suppress excess nodulation in legume plants, although CEP plays a role opposite to that of CLE in terms of lateral organ formation (*5*, *12*, *13*). Plants, as sessile organisms, continuously face a complex array of environmental fluctuations and have evolved sophisticated responses to cope with them. Given that CEP family peptides are conserved throughout vascular plants except for ferns (*8*, *9*), peptide-mediated root-to-shoot-to-root long-distance signaling is likely to be a general strategy employed by all higher plants for environmental adaptation.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/346/6207/343/suppl/DC1
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References (*14*–*18*)

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TROPHIC CASCADES

Large carnivores make savanna tree communities less thorny

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Understanding how predation risk and plant defenses interactively shape plant distributions is a core challenge in ecology. By combining global positioning system telemetry of an abundant antelope (impala) and its main predators (leopards and wild dogs) with a series of manipulative field experiments, we showed that herbivores' risk-avoidance behavior and plants' antiherbivore defenses interact to determine tree distributions in an African savanna. Well-defended thorny *Acacia* trees (*A. etbaica*) were abundant in low-risk areas where impala aggregated but rare in high-risk areas that impala avoided. In contrast, poorly defended trees (*A. brevispica*) were more abundant in high- than in low-risk areas. Our results suggest that plants can persist in landscapes characterized by intense herbivory, either by defending themselves or by thriving in risky areas where carnivores hunt.

The observation that most ecosystems support abundant plant life, despite the existence of herbivores that eat plants, has motivated a great deal of research and debate in ecology. Two broad hypotheses have been advanced to explain this phenomenon. The green world hypothesis (*1*) posits that predators indirectly benefit plants by suppressing herbivory; such trophic cascades occur when carnivores consumptively reduce herbivore densities or trigger risk-avoidance behaviors (such as increased vigilance or refuge-seeking) that reduce plant consumption (*2*, *3*). In contrast, the plant defense hypothesis contends that the world is green because plants have evolved structural and chemical defenses that inhibit consumption (*4*, *5*), often at a cost to their growth and competitive ability (*6*, *7*). Although traditionally viewed as alternatives, these hypotheses are no longer thought to be mutually exclusive (*7*, *8*). A key challenge for contemporary ecology is to understand how plant defense and predation interact across landscapes to shape a green world (*8*).

We evaluated how the combination of plant defense and risk avoidance by a common African ungulate (impala, *Aepyceros melampus*) determined the outcome of a trophic cascade in an East African savanna. Impala consume a mixture of grasses and trees ("browse") (*9*) and are preyed upon by several carnivores, especially leopards (*Panthera pardus*) and African wild dogs (*Lycaon pictus*) (fig. S1). We tested three hypotheses (Fig. 1) to explain the structure of this food web: (i) Predation risk drives habitat selection by impala; (ii) impala prefer to eat less-thorny tree species, thereby suppressing their abundance; and (iii) predation risk thus differentially influences the distribution of thorny versus less-thorny *Acacia* trees (table S1).

To test our first hypothesis, we quantified habitat selection by impala, using resource selection functions, global positioning system (GPS) telemetry, and high-resolution (0.36-m^2) satellite imagery (*10*) (fig. S2). Specifically, we quantified the selection of woody cover, which represents forage for impala (*9*) but could also increase risk by concealing predators (*11*, *12*). We also tracked how impala used two discrete habitat features typified by low versus high woody cover (fig. S3): (i) "glades," which are ~0.5-ha clearings (with 8% mean tree cover) derived from abandoned cattle corrals, covered with nutrient-rich grasses, and maintained through grazing by wildlife (*13*, *14*); and (ii) "thickets," which are <100-m-wide strips of woody vegetation (with 25% cover) along the edges of dry channels. We then quantified the relationship between woody cover and two components of risk: (i) relative probability of encountering predators, assessed using resource-selection functions of leopards and wild dogs for woody cover; and (ii) per-capita risk of mortality from predation, measured as the spatial distribution of kill sites relative to the spatial distribution of impala (*10*).

Impala avoided woody cover (Fig. 2A) and aggregated in glades and other open habitats, especially during times of the day when their predators are most active (tables S2 and S3). Both the relative probability of encountering predators (Fig. 2A) and the per-capita risk of mortality from predation (Fig. 2B) increased with increasing woody cover. Leopards and wild dogs accounted for 83% of impala kills (52 and 31% respectively; fig. S1), and kill sites from all carnivore species occurred in areas with similar amounts of woody cover ($F_{2,51} = 0.765$, $P = 0.47$).

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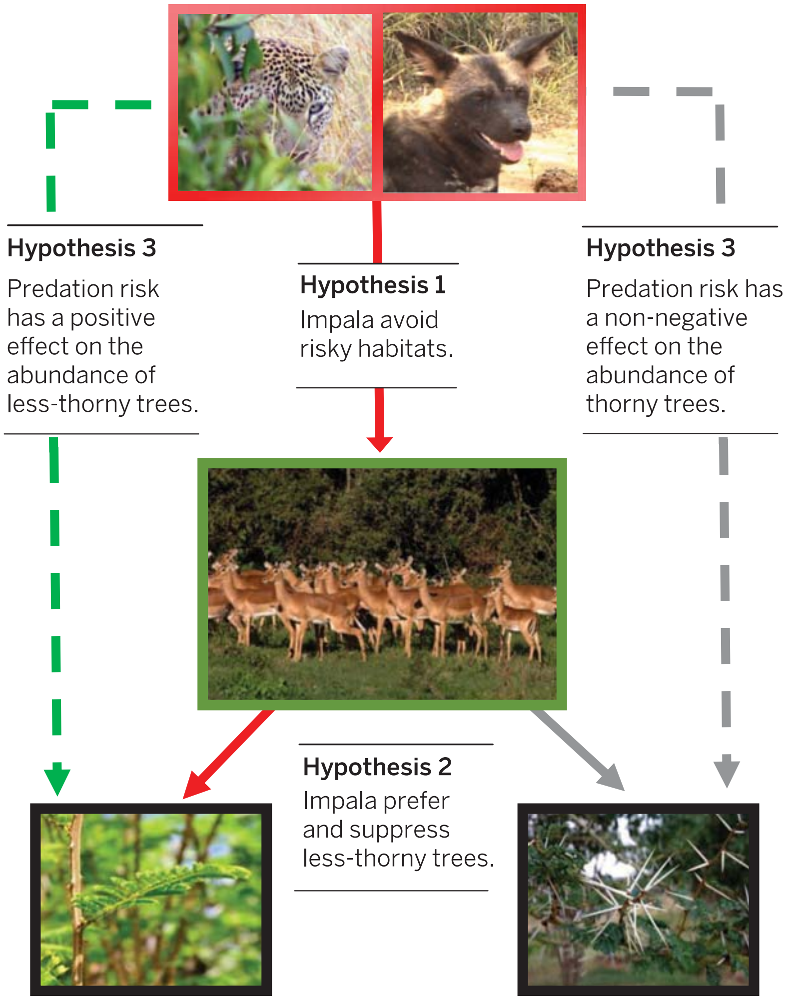
Thus, a single cue—woody cover—integrated two components of risk (encounters and mortalities) arising from the two major predators of impala.

Although impala avoided risky areas, this behavior might be explained by selection for the nutrient-rich grasses that characterize glades and open habitats (*14*). We tested this alternative hypothesis by experimentally removing all woody cover from five 0.5-ha plots, thereby

Fig. 1. Food web hypotheses tested in our study. Solid and dashed arrows represent direct and indirect effects, respectively. Red arrows represent negative effects, green arrows represent positive effects, and gray arrows represent either neutral or positive effects. Hypothesis 1: The risk of predation from large carnivores drives habitat selection of impala. Hypothesis 2: Impala both prefer and suppress the densities of poorly defended plants. Hypothesis 3: Predation risk increases the abundance of poorly defended trees in high-risk areas.



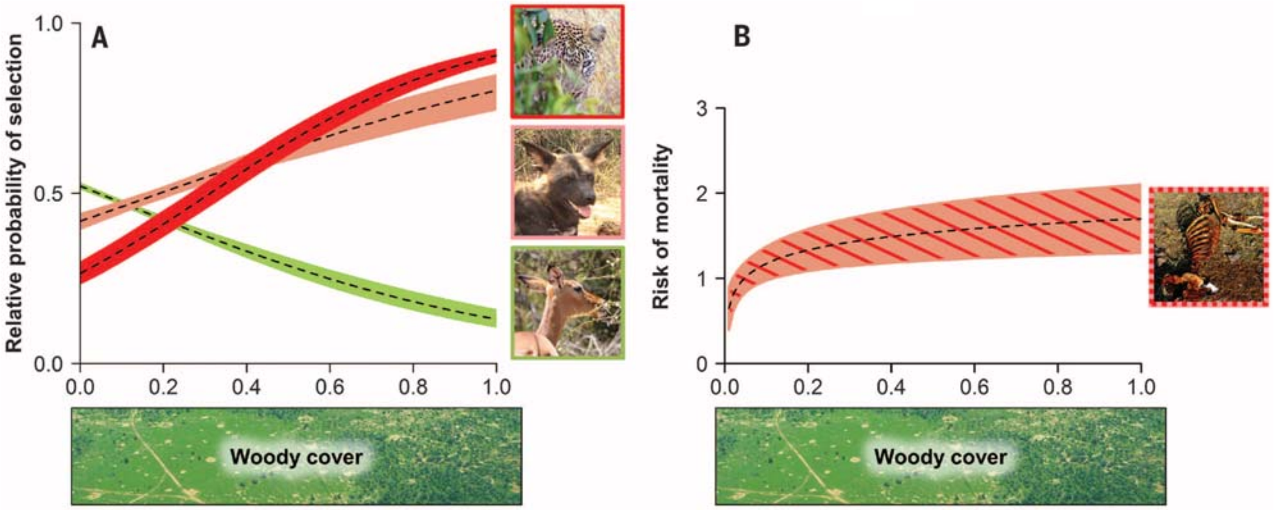
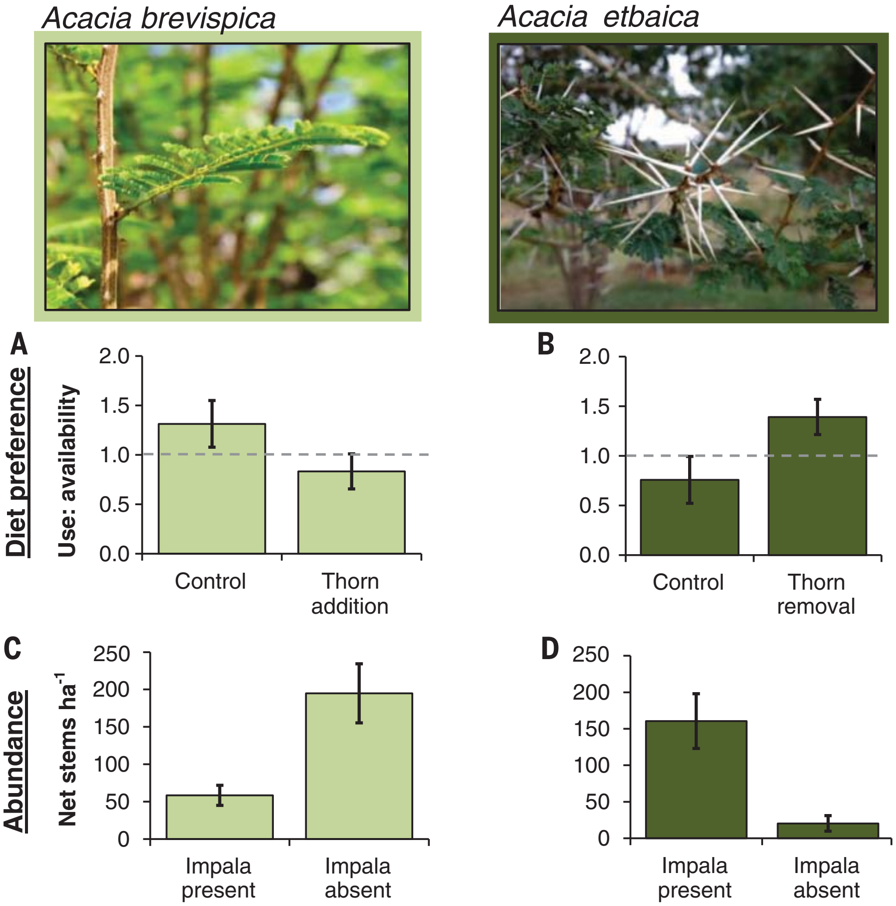


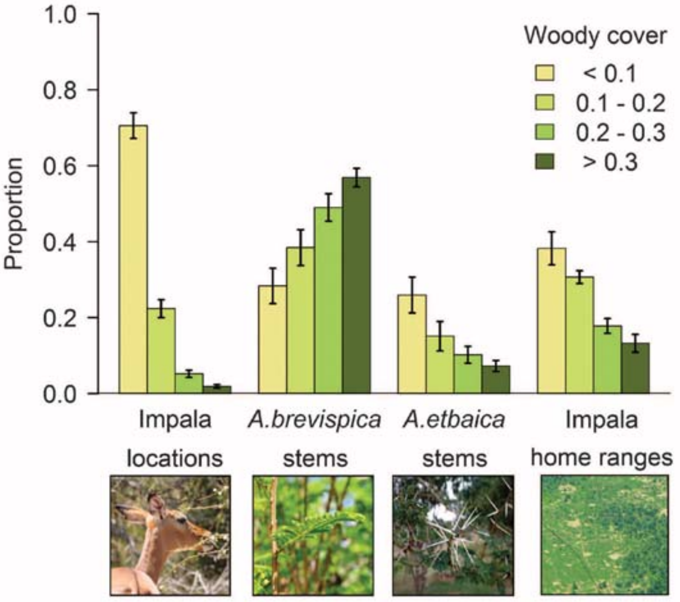
Fig. 2. Impala avoid risky areas, characterized by increasing woody cover. (A) Habitat selection by impala (green, $\beta = -1.99 \pm 0.14$, $n = 20$ impala, $P < 0.001$), leopards (red, $\beta = 3.42 \pm 0.14$, $n = 4$ leopards, $P < 0.001$), and wild dogs (pink, $\beta = 1.64 \pm 0.19$, $n = 5$ wild dogs, $P < 0.001$), where the βs represent population-level coefficients from resource selection functions for woody cover. Positive and negative coefficients indicate selection and avoidance of woody cover, respectively. (**B**) The predicted per-capita risk of mortality from predation [$1.70 + 0.228 \times \ln(\text{woody cover})$], coefficient of determination based on pooled kill sites from all large carnivores (fig. S2). Values <1 and >1 indicate that kill sites occur less or more than expected, respectively, relative to the spatial distribution of impala. Shading indicates 95% prediction intervals.

Fig. 3. Impala both preferentially consume and suppress *Acacia* spp. lacking large thorns. The presence of long thorns significantly reduced impala's preference for (**A**) *A. brevispica* and (**B**) *A. etbaica* in feeding experiments [likelihood ratio (*LR*) = 4.76, $P = 0.029$)]. The effects of species and species × thorns on preference were nonsignificant (*10*). A value of 1 (dashed line) indicates that diet preference (leaf consumption) occurred randomly among the four treatments, whereas values >1 indicate selection and values <1 indicate avoidance. Over a 5-year impala exclusion experiment, the net density (stems/ha) of (**C**) *A. brevispica*, which lacks long thorns, increased in plots where impala were absent (*LR*: $\chi^2_1 = 127.13$, $P < 0.001$); in contrast, (**D**) *A. etbaica* decreased in plots where impala were absent (*LR*: $\chi^2{}_1 = 158.88$, $P < 0.001$). Error bars indicate ±1 SEM.



mimicking the lowered risk of glades, but without potential confounds associated with forage quality. We monitored the movements of five GPS-collared impala herds for 60 days before and after creating these clearings. Impala's use of these areas increased by 160 to 576% after the removal of woody cover (table S4), indicating that forage quantity and quality cannot fully explain impala's selection of open areas. Additionally, impala typically increase their consumption of woody plants during the dry season when grass quality is poor (*9*), yet we detected no significant influence of season on their use of open habitat (tables S2 and S3). Hence, risk avoidance appears to drive habitat selection by impala.

Fig. 4. Tree-community composition as a function of predation risk. Impala avoid woody cover because it increases the risk of predation (Fig. 1), thereby shifting tree communities toward dominance by the less thorny species (*A. brevispica*) as woody cover increases. Shown are (left) the mean proportions of GPS relocations per individual ($n = 20$ adult female impala located at 20-min intervals in 2011–2012) within each of four classes of woody cover; the proportions of poorly defended *A. brevispica* (middle left) and well-defended *A. etbaica* (middle right) among the total number of trees within 108 randomly located 200 m^2 transects; and (right) the availability of woody cover within impala home ranges. Additionally, in Poisson regressions, woody cover had a positive effect on the number of *A. brevispica* stems [1.96 + exp(3.74 × woody cover); $P < 0.001$] and a negative effect on the number *A. etbaica* stems [1.52 + exp(−1.03 × woody cover); $P = 0.011$]. Error bars indicate ±1 SEM.



We next tested our second hypothesis: that impala prefer and consequently reduce the abundance of poorly defended plants. We started by quantifying the effect of plant defenses on diet preference, focusing on two common *Acacia* species (*A. brevispica* and *A. etbaica*) that together constitute ~80% of trees in the study area (*13*) and differ in traits that may affect the diet preference of herbivores (*4*–*8*): *A. brevispica* has shorter thorns (≤0.6 cm versus ≤6.0 cm) but higher condensed-tannin concentrations than *A. etbaica* (table S5). To measure the impact of these traits on diet preference, we removed thorns from *A. etbaica* branches and attached them to *A. brevispica* branches; we then presented both types of manipulated branches alongside unmanipulated controls of each species to free-ranging impala in a cafeteria-style feeding experiment. Mean leaf selection by impala was 1.4 times greater for unmanipulated *A. brevispica* branches than for unmanipulated *A. etbaica* (Fig. 3, A and B). This preference for *A. brevispica* was due to differential thorniness: The removal of

A. etbaica's long thorns increased leaf selection to levels commensurate with that of unmanipulated *A. brevispica*, whereas selection for thorn-addition *A. brevispica* was roughly equal to that of unmanipulated *A. etbaica* (Fig. 3, A and B). Thus, we conclude that *A. brevispica* is preferred relative to *A. etbaica* and that this preference is determined by thorns rather than tannins or other species-specific attributes.

Next, we considered whether the diet preference of impala could alter the abundance of *Acacia* species. We therefore measured the net change in the density of tree stems from 2009–2014 within nine replicate sets of 1-ha herbivore exclosures that independently manipulated megaherbivores [elephants (*Loxodonta africana*) and giraffes (*Giraffa camelopardalis*)], mesoherbivores [impala and eland (*Taurotragus oryx*)], and small browsers [dik-dik (*Madoqua guentheri*)], using electrified wires at different heights (*15*). We isolated the effects of impala on *Acacia* species by comparing the megaherbivore and mesoherbivore-exclusion treatments; we attributed mesoherbivore-driven effects on tree density to impala because they account for ~87% of browser biomass in this size class (*9*). The exclusion of impala increased the net stem density of the preferred and poorly defended *A. brevispica* by 233% (Fig. 3C). Conversely, net stem density of well-defended *A. etbaica* increased by 692% in plots accessible to impala as compared to impala-exclusion plots (Fig. 3D). This increase in *A. etbaica* in plots where impala were present is perhaps due to reduced competition with *A. brevispica* (*15*, *16*). Thus, although impala consumed leaves from both *Acacia* species (Fig. 3, A and B), the long thorns of *A. etbaica* enabled them to avoid suppression by impala.

To evaluate our third and final hypothesis, we related spatial patterns in the abundance of these two *Acacia* species to satellite-derived estimates of woody cover. We counted all trees in 108 transects (200 m^2) located near randomly selected glades and thickets throughout our 140-km^2 study area. The abundance of *A. brevispica* increased monotonically with satellite-derived estimates of woody cover (i.e., risk) across these transects, whereas *A. etbaica* became scarcer as woody cover increased (Fig. 4 and fig. S4). Risk avoidance by impala (Fig. 2) was functionally analogous to impala exclusion by electrified fences (Fig. 3, C and D): Our results consistently showed that the absence of impala herbivory increased the prevalence of poorly defended trees but not the prevalence of well-defended trees. Thus, tree communities became less thorny as predation risk arising from large carnivores increased (Fig. 4).

Collectively, our results show that the nature of trophic control is contingent on biotic context: namely predation risk and plant defenses (Fig. 1). Both mechanisms enable plants to thrive in different parts of the landscape: Where risk is high, poorly defended trees are released from browsing, resulting in a trophic cascade; where risk is low, intense herbivory increases the benefit of defenses, creating communities dominated by thorny trees. Consequently, the thorniness of tree communities decreased across the landscape because of the way in which impala responded to spatial variation in predation risk, and also because of the way plant defenses affected impala's diet preference.

Human activities—both past and present—exert a major influence on the interactions between carnivores, impala, and the tree community. Glades represent the legacy of traditional livestock production (*17*), generating a constellation of refugia that has shaped the spatial distribution of impala herbivory. However, the loss of large carnivores will make landscapes less risky (*18*), decoupling the spatial interplay of risk avoidance and herbivory. The loss of carnivores will also render obsolete the need for pastoralists to corral their cattle nightly, eliminating the formation of glades. Consequently, human-driven extirpation of large carnivores from African savannas (*2*) will reduce spatial variation in plant communities, leading to a world that is thornier, but still green. As large-carnivore populations continue to decline globally, understanding the context in which predators shape key ecosystem processes is an urgent priority (*19*). Studies integrating risk of predation and plant defenses will constitute a major step toward this goal.

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SUPPLEMENTARY MATERIALS

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CLIMATE CHANGE

Increased variability of tornado occurrence in the United States

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Whether or not climate change has had an impact on the occurrence of tornadoes in the United States has become a question of high public and scientific interest, but changes in how tornadoes are reported have made it difficult to answer it convincingly. We show that, excluding the weakest tornadoes, the mean annual number of tornadoes has remained relatively constant, but their variability of occurrence has increased since the 1970s. This is due to a decrease in the number of days per year with tornadoes combined with an increase in days with many tornadoes, leading to greater variability on annual and monthly time scales and changes in the timing of the start of the tornado season.

Separating nonmeteorological effects in the official database of tornadoes in the United States from actual meteorological ones has made interpreting the existence and causes of long-term physical changes in tornado occurrence extremely difficult (*1*). Nonmeteorological effects in the database result from changes in the emphasis on, and methodology of, collecting reports, and from how tornadoes are observed. The mean occurrence of well-reported aspects of the database, such as the mean annual

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Report

Defensive Plant-Ants Stabilize Megaherbivore-Driven Landscape Change in an African Savanna

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Summary

Tree cover in savanna ecosystems is usually regarded as unstable, varying with rainfall, fire, and herbivory [1–4]. In sub-Saharan Africa, elephants (*Loxodonta africana*) suppress tree cover, thereby maintaining landscape heterogeneity by promoting tree-grass coexistence. In the absence of elephants, tree encroachment may convert savannas into closed-canopy woodlands [5, 6]; when elephants increase in abundance, intensified browsing pressure can transform savannas into open grasslands [5–8]. We show that symbiotic ants stabilize tree cover across landscapes in Kenya by protecting a dominant tree from elephants. In feeding trials, elephants avoided plants with ants and did not distinguish between a myrmecophyte (the whistling-thorn tree [*Acacia drepanolobium*]) from which ants had been removed and a highly palatable, nonmyrmecophytic congener. In field experiments, elephants inflicted severe damage on whistling-thorn trees from which ants had been removed. Across two properties on which elephants increased between 2003 and 2008, cover of whistling-thorn did not change significantly inside versus outside large-scale elephant exclusion fences; over the same period of time, cover of nonmyrmecophytes differed profoundly inside versus outside exclusion fences. These results highlight the powerful role that symbioses and plant defense play in driving tree growth and survival in savannas, ecosystems of global economic and ecological importance.

Results and Discussion

Within African savannas, elephants are powerful drivers of landscape-level habitat heterogeneity, capable of inflicting intensive and extensive damage to woody plants [4–8]. Trees and shrubs employ various mechanisms to reduce such catastrophic herbivory, including vigorous resprouting of broken stems, the development of heavy buttresses, growth to large sizes, and the production of a variety of chemical and spinescent defenses that may reduce palatability [9]. In African savanna ecosystems, including the Laikipia plateau in central Kenya, many trees in the widespread genus *Acacia* are defended with spines and digestibility-reducing secondary compounds (tannins). Despite such defenses, these plants often suffer intense bouts of elephant herbivory that may severely damage or kill mature trees (Figure 1). In striking contrast, a co-occurring congener defended by ant bodyguards (*Acacia drepanolobium*) is seldom browsed by elephants and occurs in dense near-monocultures (800–1100 individuals/hectare) throughout many portions of its range [10–13]. We hypothesized that the protective ant symbionts of *A. drepanolobium* serve as an effective defense against elephant herbivory and thus play a strong role in buffering this species from fluctuations in abundance in the face of variation in elephant numbers.

We investigated the role of symbiotic ants in determining levels of tree cover following manipulated and natural changes in numbers of large mammalian herbivores in central Kenya. Our study sites in Laikipia, Kenya (see Figure S1 available online) are underlain by one of two well-defined soil types: black clayey vertisols of volcanic origin (hereafter "clayey soils"), comprising ~35% of Laikipia, and red sandy aridosols derived from quartzite (hereafter "sandy soils"), comprising the remaining 65% of Laikipia [14]. Although elephant abundances are similar between clayey and sandy soils [15], each soil type harbors a distinctive community of woody plants. On clayey soils, *A. drepanolobium* occurs in virtual monoculture, typically accounting for ≥95% of the overstory vegetation [16]. *Acacia drepanolobium* is a myrmecophyte (ant-plant), providing both housing (swollen thorn domatia) and food (extrafloral nectar) for symbiotic ants. Four species of ants (*Crematogaster mimosae*, *C. nigriceps*, *C. sjostedti*, and *Tetraponera penzigi*) compete for exclusive access to host plants and protect host trees (to varying degrees) by swarming, biting, and stinging intruders [17]. Tree communities occurring on sandy soils are more diverse, with the 3–5 most common woody plant species typically accounting for no more than 80% of the canopy in a given locale. *Acacia drepanolobium* is virtually absent from sandy soils, constituting <0.1% of the overstory.

Between 1992 and 2002, elephant abundances throughout the Laikipia ecosystem increased approximately 5-fold [18] (P. Omondi, personal communication) and continued to increase over the course of our study (unpublished data; W. Giesen, personal communication; Figure S2). To assess the impact of increased elephant densities on tree assemblages, we quantified changes in tree cover both inside and outside of plots excluding megaherbivores (elephants and less-common giraffe [*Giraffa camelopardalis*]) on sandy and clayey soils at the Lewa Wildlife Conservancy in central Kenya (37°41′E, 0°2′N, Figure S1). Changes in tree cover were determined by comparing high-resolution (60 cm) Quickbird satellite images (Digital Globe) acquired in 2003 and 2008. Between 1992 and 2002, six double-strand, electrified fences were erected on Lewa to exclude megaherbivores from parcels of land while allowing other wildlife species to freely pass beneath the 2 m high fence strands. Four fences were established in sandy soil, and two fences were established in clayey soil (Table S1). Hereafter, we refer to megaherbivore exclosures as elephant exclosures, because elephants (and not giraffes) were responsible for the vast majority of differences arising from megaherbivore browsing on both clayey and sandy soils (Tables S2 and S3). Control plots paired with

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Figure 1. Elephant Herbivory on *Acacia* spp.

Recent catastrophic herbivory by elephants on the nonmyrmecophyte *Acacia mellifera* (foreground) surrounded by unbrowsed individuals of the myrmecophyte *A. drepanolobium*.

elephant exclosures were sampled from 200 m wide buffer strips around each exclosure (see "Satellite Imagery and Aerial Photos" in Experimental Procedures).

To increase our sample sizes within clayey soil habitats, we further quantified changes in tree cover at the Kenya Long-term Exclusion Experiment (KLEE) at the Mpala Research Centre in central Kenya (36°52′E, 0°17′N, Figure S1) using a single Quickbird satellite image from June 2003 (Digital Globe) and a high-resolution (30 cm) aerial photograph (Ramani Communications) from December 2007. Established in 1995, KLEE consists of three replicate blocks, each of which contains two plots accessible to all wildlife, two plots accessible to all wildlife except megaherbivores, and two plots from which all wildlife are excluded. KLEE occurs entirely on clayey soil.

Between 2003 and 2008, tree cover diverged significantly between elephant exclosures and control plots on sandy soil at Lewa ($F_{1,6} = 9.27$, $p = 0.02$). Absolute tree cover increased by 6.0% in response to elephant exclusion (from 25.3% in 2003 to 31.3% in 2008; Figure 2) while simultaneously decreasing by 8.3% within control plots (from 24.7% in 2003 to 16.6% in 2008; Figure 2). The decline in tree cover in control plots coincided with an ∼2.5-fold increase in elephant densities at Lewa (Figure S2). In 2003, tree cover within elephant exclosures and control plots on clayey soils did not differ between Lewa and KLEE, nor did change in tree cover between 2003 and 2008 differ significantly between Lewa and KLEE (see "Analysis of Remotely-Sensed Imagery" in Supplemental Experimental Procedures). Thus, we pooled sites in our analysis for tree cover within elephant exclosures on clayey soils. Between 2003 and 2008 (2007 at KLEE), tree cover did not change significantly between elephant exclosures and control plots on *A. drepanolobium*-dominated clayey soil at Lewa and KLEE (exclosures: 26.5%–23.9%; controls: 22.5%–22.8%; $F_{1,8} = 0.90$, $p = 0.37$; Figure 2), nor did the change in tree cover inside versus outside elephant exclosures differ significantly from zero, despite increasing elephant numbers on both Lewa and Mpala (Figure S2).

To explore whether differential change in tree cover was due to ants or other factors associated with sandy versus clayey soils, we conducted ground surveys for the incidence of browse on clayey soils. Ground surveys revealed that elephants preferred to browse on nonmyrmecophytes (Tables S2 and S3), thereby reducing tree cover of subordinate (i.e., nonmyrmecophytic) woody plant species (multivariate analysis of variance [MANOVA] for megaherbivore effect on subordinate species: Wilks' $\lambda_{9,4} = 0.003$, $p < 0.0001$; $p < 0.01$ for univariate F tests on five most abundant nonmyrmecophytes; Figure 3). Further, and consistent with analysis of remotely sensed data, ground surveys confirmed relatively low levels of browsing on *A. drepanolobium* by elephants (Tables S2 and S3) and nonsignificant impacts of elephants on tree cover of *A. drepanolobium* ($p = 0.27$; Figure 3). Other (nonelephant) browsers reduced cover of a single subordinate species (*Rhus natalensis*; Wilks' $\lambda_{9,4} = 0.05$, $p = 0.03$; univariate F test for *R. natalensis*: $p = 0.01$).

To directly establish whether plant defense by *Acacia* ants influenced elephant browsing of host *A. drepanolobium* trees, we conducted a 12 month in situ ant removal experiment 2.5 km east of KLEE. We reduced ant abundances on host plants by removing approximately 100%, 60%, or 30% of existing colony members on individual trees, and then we assessed levels of elephant damage on these trees relative to unmanipulated plants after a 1 year period. The level of

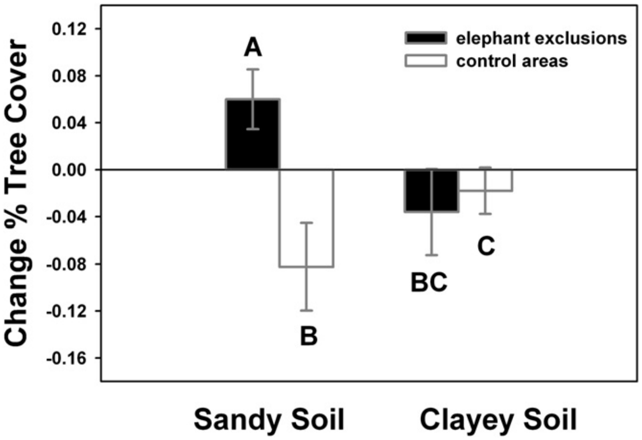

Figure 2. Landscape Change on Lewa Downs Conservancy and Mpala

Change in tree cover in elephant exclusions (black bars) and paired controls (white bars), 2003–2008. Virtually all trees on sandy soil are nonmyrmecophytes; ∼95% of trees on clayey soil are the myrmecophyte *A. drepanolobium*. Means (±95% confidence intervals) from sandy soil represent averages across four exclusion plots and their paired controls from Lewa. Means from clayey soil represent averages across six exclusion plots and their paired controls at KLEE and two exclusion plots and their paired controls at Lewa. Letters associated with bars represent statistically significant differences between groups ($p < 0.05$).

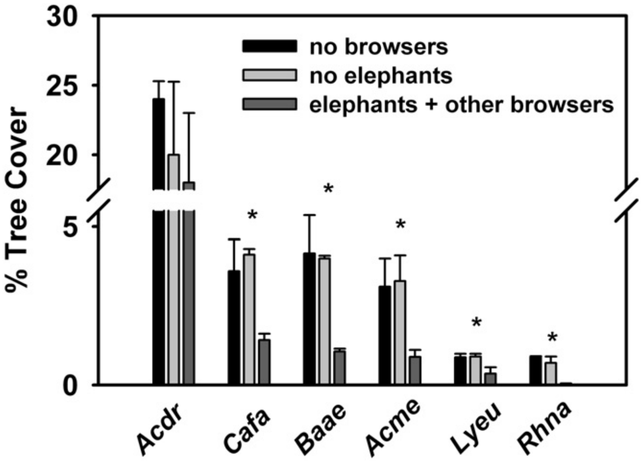

Figure 3. Differences in Tree Cover as a Function of Herbivore Treatment

Percent tree cover of the myrmecophyte *A. drepanolobium* (Acdr) and nonmyrmecophytes *Cadaba farinosa* (Cafa), *Balanites aegyptiaca* (Baae), *A. mellifera* (Acme), *Lycium europaeum* (Lyeu), and *Rhus natalensis* (Rhna) by herbivore treatment at KLEE in 2008. Black bars (±standard error of the mean) represent plots from which all browsers have been excluded, light gray bars represent plots from which only elephants have been excluded, and dark gray bars represent plots accessible to all browsers. *p < 0.01 is statistically significant between plots.

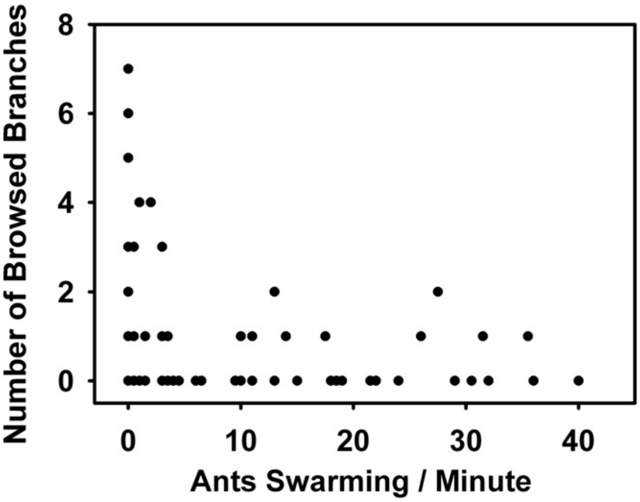

Figure 4. Responses of Elephant Browsing to In Situ Ant Removal from Trees

The number of *A. drepanolobium* branches browsed by elephants as a function of ant activity levels on trees from the ant-removal experiment ($\chi^2_1 = 28.41$, $p < 0.01$).

elephant browsing on host plants was significantly and negatively related to ant abundances on host plants (negative binomial regression: $\chi^2_1 = 28.41$, $p < 0.01$; Figure 4).

To further investigate whether protection by ant symbionts was the causal mechanism underlying observed patterns of landscape change, we conducted free-choice feeding trials on six 8-year-old elephants at the Sheldrick Wildlife Trust Reintegration Centre in Tsavo National Park, Kenya. We presented elephants with four groups comprised of ∼20 1.5–2 m branches: (1) *Acacia drepanolobium* control, (2) *A. drepanolobium* ant removal, (3) *A. mellifera* control, and (4) *A. mellifera* ant addition. Browse surveys on sandy soils from Lewa demonstrated that elephants neither prefer nor avoid *A. mellifera* relative to other tree species on sandy soil (Table S4); thus, *A. mellifera* represents a typical nonmyrmecophyte from the perspective of an elephant. Groups of branches were spaced 10 m apart from each other, and their position in the elephant corral was determined randomly. Elephants were equally likely to feed on *A. drepanolobium* and *A. mellifera* in the absence of ants (Cox regression: $\beta = 0.33$, $p = 0.57$), indicating that, without its ants, *A. drepanolobium* is fundamentally palatable to elephants. Similarly, elephants avoided branches of both tree species if ants were present (Cox regression: $\beta = 3.61$, $p < 0.01$), demonstrating that symbiotic ants can deter elephant herbivory when alternative food plants are available (Figure 5).

Elephants are known to avoid swarming attack by other hymenopterans (bees [19]). The efficacy of ant defense may result from a combination of high densities of ants on host plants (up to 90,000 workers on some trees [20]), the species of ant occupant (*C. mimosae* and *C. nigriceps* swarm equally aggressively in response to disturbance [21] and occupy ∼70% and 80% of trees at KLEE [22] and Lewa ["Lewa Browse Surveys" in Supplemental Experimental Procedures]), and the tendency of ants to attack areas of thin skin and mucous membranes by biting down and holding fast with their mandibles. Further, elephants are unique in that their nostrils are located away from their mouths at the apex of their feeding apparatus (trunk), rendering them vulnerable to swarming insects. In contrast, giraffes use their long, prehensile tongues to swipe away ants from their muzzles (unpublished data). Thus, despite their thick dermis, elephants are highly sensitive

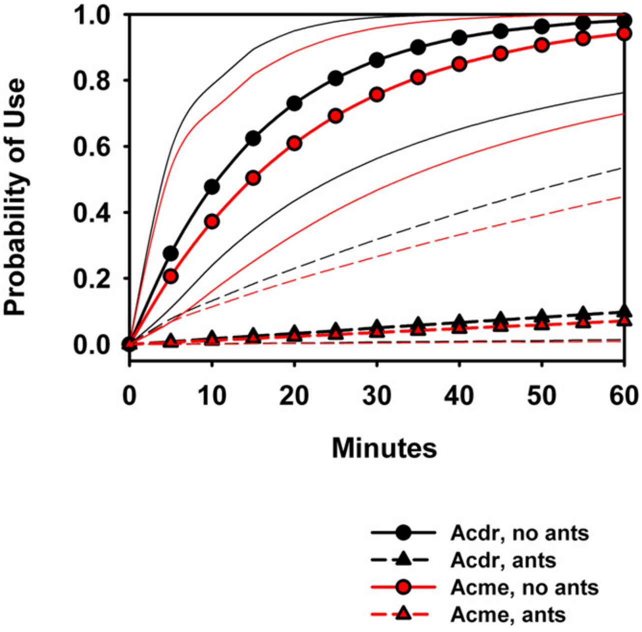

Figure 5. Free-Choice Feeding Trials with Elephants and *Acacia* spp.

Best-fitting Cox regression models as a function of food type: control *A. drepanolobium* (black triangles), ant-removal *A. drepanolobium* (black circles), control *A. mellifera* (red circles), ant-addition *A. mellifera* (red triangles). Solid and dashed thin lines represent 95% confidence intervals for probability of use of branches with and without ants, respectively. Elephants preferred branches without ants ($\beta = 3.61$, $p < 0.01$) but did not distinguish between tree species ($\beta = 0.33$, $p = 0.57$). The points in the graph are fitted from the model.

around their eyes and on the inner membranes of their trunks [23]; attack by scores of biting ants probably serves as a strong deterrent.

Classic experiments by Janzen [24] and subsequent studies by others [25–28] have elegantly demonstrated the capacity of symbiotic ants to prevent or greatly reduce herbivory and competition for light, thereby promoting the growth and survival of individual host plants. Ours is the first study to demonstrate that ant-plant symbioses can stabilize landscape structure at larger spatial scales by protecting adult trees from catastrophic herbivory. Because ants reduce palatability of their host trees, selective browsing on nonmyrmecophytes is at least partly responsible for creating the virtual monocultures of *A. drepanolobium* that typify black clayey soils in Laikipia [27, 29] and other regions of East Africa [10–13, 28]. A major challenge for the future is elucidating why *A. drepanolobium* is restricted to clayey vertisols, such that a diversity of nonmyrmecophytes thrives on other soil types. We hypothesize that characteristics of sandy soils (e.g., particle size, nutrient content, infiltration, etc.) favor nonmyrmecophytes and interact strongly with browsing to promote segregation of trees (*A. drepanolobium* and other myrmecophytes versus nonmyrmecophytes) across soil types (e.g., see [30]). In light of this, we expect that, on clayey soils, other determinants of savanna structure (i.e., rainfall and fire) or mortality factors affecting early life stages of trees (e.g., seeds, seedlings) will override browsing as drivers of tree populations, where plant defenses nullify elephants as important agents of mortality on adult trees [31].

Savannas typically are envisaged as unstable or disequilibrial systems in which climatic variability or disturbances generate the tree-grass mixtures that typify these ecosystems [1–3]. In Africa, browsing and killing of trees by elephants is often a critical force underlying the coexistence of trees and grasses [4–8]. Throughout much of their historic range, however, declines in elephant populations have triggered extensive increases in tree numbers, shifting open savannas to closed-canopy woodlands [8]. Elsewhere, and typically in response to confinement within protected areas, elephants have become "compressed," have overexploited trees, and have shifted savannas toward structurally simplified grasslands [8, 32]. Our study highlights the stabilizing effect that ant symbionts can confer on tree cover over expansive spatial scales. In sum, our experiments show that ant symbionts protect against catastrophic herbivory, effectively buffering a dominant tree against top-down control by megaherbivores. Because tree cover strongly regulates a host of ecosystem processes, including carbon storage, fire-return intervals, food web dynamics, nutrient cycling, and soil-water relations in our system [33, 34] and others [35, 36], these tiny bodyguards likely exert powerful indirect effects at very large spatial and temporal scales. As elephants and other large mammals in Africa exhibit chronic declines in some habitats and overabundance in others, identifying the ecological consequences of such landscape change remains an important challenge for wildlife managers in the future.

Experimental Procedures

Satellite Imagery and Aerial Photos

At Lewa, control plots paired with elephant exclosures were sampled from 200 m wide buffer strips around each exclosure, subject to the constraint that the buffer strip occurred entirely within Lewa boundaries. When an elephant exclosure abutted a neighboring property, we expanded the width of buffer strips to compensate for the area not sampled in that property. At KLEE, elephant exclosures consisted of the central hectare (ha) within each 4 ha fence.

KLEE Browse Surveys

From July 2007 to September 2007 at KLEE, we recorded canopy breadth, height, and diameter at breast height (DBH) on all individuals of the subordinate woody species (i.e., nonmyrmecophytes; n = 721). We paired each of the 721 individual trees with the nearest neighboring *A. drepanolobium*, subject to the constraint that the diameter of the *A. drepanolobium* was within 5 cm of the subordinate individual with which it was paired, and we recorded canopy breadth, height, DBH, and incidence of browsing. We used MANOVA to test for the effects of megaherbivores, wildlife, and cattle on percent tree cover of *A. drepanolobium* and the five most common, subordinate woody species: *A. mellifera*, *Balanites aegyptiaca*, *Cadaba farinosa*, *Lycium europaeum*, and *Rhus natalensis*. In addition, we included replicate as a fixed effect (random effects are extremely difficult to implement and interpret in MANOVA) in our analysis, because tree cover at KLEE increases from north to south. For each individual in the six plots accessible to megaherbivores (n = 332), we recorded the incidence of browsing by megaherbivores (elephant and giraffe). We ignored elephant browsing >1 year old, as evidenced by chalky, dull-colored wood. We used log-linear models to calculate odds ratios of browse by elephant and giraffe on each of the five most common nonmyrmecophytes (Table S3).

Ant-Removal Experiment

We removed ant colonies from host plants by inundating the host plant with smoke generated by burning dry grass in a bucket underneath the tree. *Crematogaster mimosae* displays an evacuation behavior when inundated by the smoke from burning grass, in which workers carry the majority of brood, eggs, pupae, winged reproductives, and queens from swollen thorn domatia into cracks in the soil at the base of the host plant over the course of 45–60 min. During smoke inundation, we made a rough approximation of the total number of workers on each tree. We then imposed treatments immediately following smoke inundation, either completely barring ants from recolonizing trees by applying a Tanglefoot sticky barrier at the base of the host plant (full ant removal) or allowing approximately 1/3, 2/3, or the entire colony to recolonize the host plant prior to applying a Tanglefoot barrier (for the 2/3 removal, 1/3 removal, and control treatments, respectively). On control trees, two small (6 cm) dead branches were wired to trees across the sticky barrier to allow ants to move freely across the sticky barrier.

Following the imposition of treatments, we then assayed trees for relative levels of ant defense at 6 and 12 months by disturbing a randomly chosen swollen thorn on each of two branches per tree (one in each of the north and south cardinal directions) and counting the number of workers swarming onto the tip of the uppermost spine of the disturbed swollen thorn over a 30 s period. Two researchers conducted these assays so that swollen thorns on two separate branches could be disturbed simultaneously. Overall levels of ant activity on trees were calculated as the average of these activity assays. Ant activity at 12 months was significantly correlated with ant activity at 6 months ($r = 0.64$, $p < 0.0001$), and our treatments were effective in generating a range of ant activities (Table S5). We then resurveyed each tree at the end of 12 months for browse damage inflicted by both elephants and nonelephant browsers.

Free-Choice Feeding Trials

We cut 1.5–2.0 m branches of *A. drepanolobium* and *A. mellifera* from trees at the periphery of Tsavo National Park near the town of Voi. At the time of collection, all *A. drepanolobium* in our feeding trials were inhabited by the ant *C. nigriceps*. We removed ants and swollen thorns from *A. drepanolobium* with wire cutters. Branches were transported to the holding corral and were presented to elephants within 2 hr of collection. Ants were added to *A. mellifera* by immersing branches for 5 min in a metal drum into which the ants and swollen thorns from the ant removal branches had been collected. A single observer, positioned ~20 m from groups of branches, recorded data on the number, type, and order of foods taken over a 1 hr period. Groups of branches were randomly placed 10 m apart within 10 m of the entrance of the holding corral.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, two figures, and five tables and can be found with this article online at doi:10.1016/j.cub.2010.08.015.

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The dilution hypothesis provides an alternative framework with which to explain observations of the apparent recalcitrance of DOC and lends a physiological meaning to the operationally defined "semi-labile" and "semi-refractory" fractions (*16*, *17*). We hypothesize that under the dilution hypothesis, very heterogeneous mixtures of labile compounds appear semirefractory, whereas increasingly less diverse DOM assemblages containing larger concentrations of some substrates will present higher microbial growth and DOC turnover rates, resulting in increasing degrees of apparent lability. The microbial generation of apparently recalcitrant material (*18*) from labile substrates in a process recently dubbed the "microbial carbon pump" (*19*) can also be explained with the dilution hypothesis. Microbial utilization of abundant, labile compounds results in hundreds of different metabolites (*20*), which are subsequently consumed down to the lowest utilizable concentration. This mechanism explains observations of relatively concentrated, labile materials being transformed into apparently recalcitrant matter through microbial consumption (*18*) but does not necessarily imply the formation of structurally recalcitrant molecules. Indeed, "recalcitrant" DOC is not defined structurally, but operationally, as the DOC pool remaining after long experimental incubations or as the fraction transported in an apparently conservative manner with the ocean circulation (*1*). Thus, the dilution hypothesis severely limits the feasibility of geoengineering efforts to enhance carbon storage in the deep ocean (*21*) by using the microbial carbon pump.

FT-ICR-MS characterization of DOC from different oceans (*13*, *14*, *22*, *23*) and also from this study (fig. S5) shows no indication of prevalent, intrinsically recalcitrant compounds accumulating in substantial amounts. Conversely, FT-ICR-MS data show that oceanic DOC is a complex mixture of minute quantities of thousands of organic molecules, which is in good agreement with the dilution hypothesis. Mean radiocarbon ages of deep oceanic DOC in the range of 4000 to 6000 years have been considered as evidence for its recalcitrant nature (*24*, *25*). However, these are average ages of a pool containing a mixture of very old molecules >12,000 years old but also featuring a large proportion of contemporary materials (*26*). Moreover, elevated radiocarbon ages only demonstrate that these old molecules are not being newly produced at any appreciable rate—because that would lower their isotopic age—but does not necessarily imply that they are structurally recalcitrant. Furthermore, it is unlikely that natural organic molecules can accumulate in the ocean in substantial concentrations and remain recalcitrant or be preserved for millennia when degradation pathways for novel synthetic pollutants evolve soon after these compounds are released in nature (*27*).

Although there might be a truly recalcitrant component in deep oceanic DOC, our results clearly show that the concentration of individual labile molecules is a major factor limiting the utilization of a substantial fraction of deep oceanic DOC. These results provide, therefore, a robust and parsimonious explanation for the long-term preservation of labile DOC into one of the largest reservoirs of organic carbon on Earth, opening a new avenue in our understanding of the global carbon cycle.

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SUPPLEMENTARY MATERIALS

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SOCIAL EVOLUTION

Oxytocin-gaze positive loop and the coevolution of human-dog bonds

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Human-like modes of communication, including mutual gaze, in dogs may have been acquired during domestication with humans. We show that gazing behavior from dogs, but not wolves, increased urinary oxytocin concentrations in owners, which consequently facilitated owners' affiliation and increased oxytocin concentration in dogs. Further, nasally administered oxytocin increased gazing behavior in dogs, which in turn increased urinary oxytocin concentrations in owners. These findings support the existence of an interspecies oxytocin-mediated positive loop facilitated and modulated by gazing, which may have supported the coevolution of human-dog bonding by engaging common modes of communicating social attachment.

Dogs are more skillful than wolves and chimpanzees, the closest respective relatives of dogs and humans, at using human social communicative behaviors (*1*). More specifically, dogs are able to use mutual gaze as a communication tool in the context of needs of affiliative help from others (*2*). Convergent evolution between humans and dogs may have led to the acquisition of human-like communication modes in dogs, possibly as a by-product of temperament changes, such as reduced fear and aggression (*1*). This idea yields interesting implications that dogs were domesticated by coopting social cognitive systems in humans that

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are involved in social attachment. The development of human-unique social cognitive modes may depend on specific temperament and social affiliation changes and may have consequently evolved differently from those of chimpanzees and bonobos (*3*). Thus, although humans and dogs exist on different branches of the evolutionary tree, both may have independently acquired tolerance of one another because of alterations in neural systems that mediate affiliation (*1*). These alterations may be related to paedomorphic characteristics in dogs, which enabled them to retain a degree of social flexibility and tolerance similar to that of humans (*4*, *5*); therefore, it is plausible that a specific affiliative relationship developed between humans and dogs despite interspecies differences. This common social relationship change may have enabled cohabitation between humans and dogs and the eventual development of human-like modes of social communication in dogs.

Gaze plays an important role in human communication. Gaze not only facilitates the understanding of another's intention but also the establishment of affiliative relationships with others. In humans, "mutual gaze" is the most fundamental manifestation of social attachment between a mother and infant (*6*), and maternal oxytocin is positively associated with the duration of mother-to-infant gaze (*7*). Oxytocin plays a primary role in regulating social bonding between mother and infants and between sexual partners in monogamous species (*8*, *9*). Moreover, activation of the oxytocin system enhances social reward (*10*) and inhibits stress-induced activity of the hypothalamic-pituitary-adrenal axis (*11*). It has therefore been suggested that these functions may facilitate dyadic interaction, such as an oxytocin-mediated positive loop of attachment and maternal behaviors between mother and infant (*12*, *13*): Maternal nurturing activates the oxytocinergic system in the infant, thus enhancing attachment; this attachment then stimulates oxytocinergic activity in the mother, which facilitates further maternal behavior (*9*). Because the establishment of such an oxytocin-mediated positive loop requires the sharing of social cues and recognition of a particular partner, the study of oxytocin-mediated bonding has been restricted to intraspecies relationships.

The human-dog relationship is exceptional because it is an interspecies form of attachment. Dogs can discriminate individual humans (*14*, *15*). Furthermore, dogs show distinctly different behavior toward caregivers as compared with hand-raised wolves (*14*), and interaction with dogs confers a social buffering effect to humans. Likewise, dogs also receive more social buffering effects from interacting with humans than from conspecifics (*16*). Tactile interaction between humans and dogs increases peripheral oxytocin concentrations in both humans and dogs (*17*, *18*). Further, social interaction initiated by a dog's gaze increases urinary oxytocin in the owner, whereas obstruction of the dog's gaze inhibits this increase (*19*). These results demonstrate that the acquisition of human-like social communication improves the quality of human-dog affiliative interactions, leading to the establishment of a human-dog bond that is similar to a mother-infant relationship. We hypothesized that an oxytocin-mediated positive loop, which originated in the intraspecies exchange of social affiliation cues, acts on both humans and dogs, is coevolved in humans and dogs, and facilitates human-dog bonding. However, it is not known whether an oxytocin-mediated positive loop exists between humans and dogs as has been postulated between mother and infants, and whether this positive loop emerged during domestication.

We tested the hypothesis that an oxytocin-mediated positive loop exists between humans and dogs that is mediated by gaze. First, we examined whether a dog's gazing behavior affected urinary oxytocin concentrations in dogs and owners during a 30-min interaction. We also conducted the same experiment using hand-raised wolves, in order to determine whether this positive loop has been acquired by coevolution with humans. Second, we determined whether manipulating oxytocin in dogs through intranasal administration would enhance their gazing behavior toward their owners and whether this gazing behavior affected oxytocin concentrations in owners.

In experiment 1, urine was collected from the dogs and owners right before and 30 min after the interaction, and the duration of the following behaviors was measured during the interaction: "dog's gaze at owner (dog-to-owner gaze)," "owner's talking to dog (dog-talking)," and "owner's touching of dog (dog-touching)." Dog owners were assigned to one of two groups: long gaze or short gaze (fig. S1). Wolves were tested with the same procedure and were compared with the two dog groups. Dogs in the long-gaze group gazed most at their owners among the three groups. In contrast, wolves rarely showed mutual gazing to their owners (Fig. 1A and fig. S2). After a 30-min interaction, only owners in the long-gaze group showed a significant increase in urinary oxytocin concentrations and the highest change ratio of oxytocin (Fig. 1, B and C). The oxytocin change ratio in owners correlated significantly with that of dogs, the duration of dog-to-owner gaze, and dog-touching. Moreover, the duration of the dog-to-owner gaze correlated with dog-talking and dog-touching (table S2A); however, through multiple linear regression analysis, we found that only the duration of dog-to-owner gaze significantly explained the oxytocin change ratio in owners. The duration of dog-touching showed a trend toward explaining oxytocin concentrations in owners (Table 1A). Similarly, a significantly higher oxytocin change ratio was observed in the dogs of the long-gaze group than in those of the short-gaze group (Fig. 1, D and E). The duration of dog-to-owner gaze also significantly explained the oxytocin change ratio in dogs, and the duration of dog-touching showed a trend toward explaining oxytocin concentrations in dogs by multiple linear regression analysis (Table 1A). In wolves, in contrast, the duration of wolf-to-owner gaze did

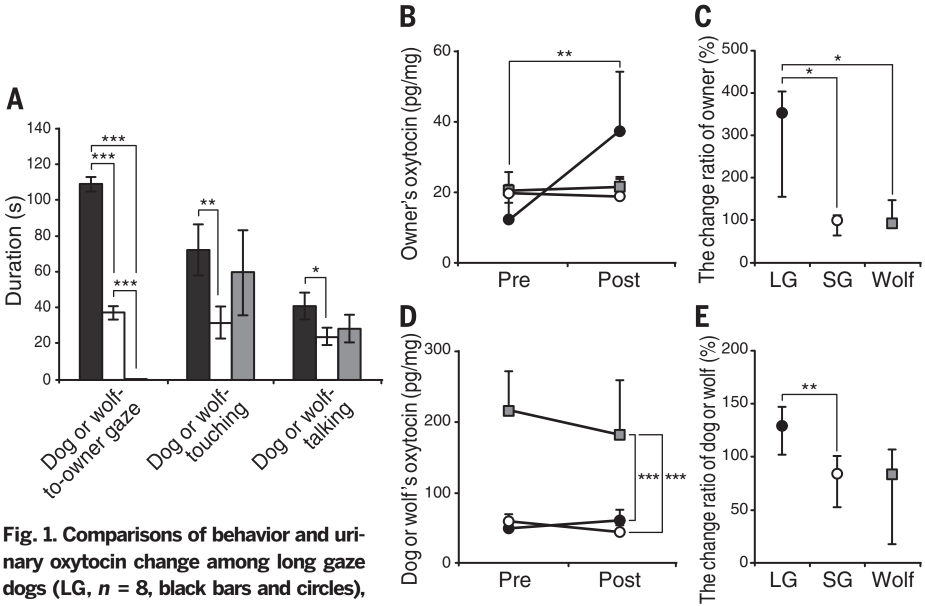

Fig. 1. Comparisons of behavior and urinary oxytocin change among long gaze dogs (LG, n = 8, black bars and circles), short gaze dogs (SG, n = 22, white bars and circles), and wolves (wolf, n = 11, gray bars and square). (**A**) Behavior during the first 5-min interaction. (**B**) and (**D**) Changes of urinary oxytocin concentrations after a 30-min interaction. Urinary oxytocin concentrations in owners (B) and dogs or wolves (D) collected before and after a 30-min interaction are shown. (**C**) and (**E**) Comparisons of the change ratio of urinary oxytocin among LG, SG, and wolf for owners (C) and dogs or wolves (E). The results of (A), (B), and (D) are expressed as mean ± SE. (C) and (E) reflect median ± quartile. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

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not correlate with the oxytocin change ratio in either owners or wolves, and wolf-to-owner gaze did not explain the oxytocin change ratio in owners and wolves (tables S2B and S3). These results suggest that wolves do not use mutual gaze as a form of social communication with humans, which might be expected because wolves tend to use eye contact as a threat among conspecifics (*20*) and avoid human eye contact (*21*). Thus, dog-to-owner gaze as a form of social communications probably evolved during domestication and triggers oxytocin release in the owner, facilitating mutual interaction and affiliative communication and consequently activation of oxytocin systems in both humans and dogs in a positive loop.

In experiment 2, we evaluated the direct evidence of whether oxytocin administration enhanced dog gazing behavior and the subsequent increase in urinary oxytocin concentration in owners. This experiment involved 27 volunteers and their dogs, and participants unfamiliar to the dogs. A solution containing oxytocin or saline was administered to the dog and the dog then entered the experimental room, where the owner and two unfamiliar people were seated (fig. S4). Human behavior toward dogs was restricted to prevent the influence of extraneous stimuli on dog behavior and/or urinary oxytocin concentration. They were forbidden to talk to each other or to touch the dog voluntarily. Urine samples from the owner and the dog were collected before and after the interaction and were later compared. The total amount of time that the dog gazed at, touched, and was close to the owner and the unfamiliar participants was also measured.

Oxytocin administration to dogs significantly increased the duration that the dog gazed at the owner in female dogs but not male dogs (Fig. 2A). Further, urinary oxytocin concentration significantly increased in the owners of female dogs that received oxytocin versus saline, even though oxytocin was not administered to the owners (Fig. 2D). No significant effect of oxytocin administration was observed in the other measured dog behaviors (Fig. 2, B and C). Furthermore, multiple linear regression analysis revealed that the duration of gazing behavior significantly explained the oxytocin change ratio in owners (Table 1B). Thus, oxytocin administration enhances the gazing behavior of female dogs, which stimulates oxytocin secretion in their owners. Conversely, when interaction from humans was limited, no significant difference in urinary oxytocin concentrations in dogs was observed after the interaction in either the oxytocin or the saline conditions, and no significant oxytocin change ratio was found in dogs (Fig. 2, F and G). These results thus suggest that, although oxytocin administration may enhance dog gazing behavior and lead to an oxytocin increase in owners, limited owner-to-dog interaction may prevent the increased oxytocin secretion in dogs by breaking the oxytocin-mediated positive loop.

Table 1. Results of multiple linear regression analysis of oxytocin change ratio and behavioral variables in owners and dogs. $^{*}P < 0.05$, $^{\dagger}P < 0.1$; R, multiple correlation coefficient; **, $P < 0.01$.

(A) Experiment 1

	Oxytocin change ratio	
	Owners	**Dogs**
Owner talking to dog	−0.107	−0.264
Owner touching dog	$0.321^{\dagger}$	$0.335^{\dagger}$
Dog-to-owner gaze	0.458*	0.388*
R	0.619	0.575
Adjusted R^2	0.306	0.247
P	0.008	0.020

(B) Experiment 2

	Oxytocin change ratio	
	Owners	**Dogs**
Dog's sex	0.090	0.138
Oxytocin administration	0.202	0.234
Dog-to-owner gaze	0.458**	0.030
Dog touching owner	−0.040	−0.054
Proximity to owner	0.048	−0.023
R	0.574	0.275
Adjusted R^2	0.248	−0.046
P	0.005	0.686

Sex: Female = 1, male = 0; oxytocin administration: oxytocin = 1, saline = 0.

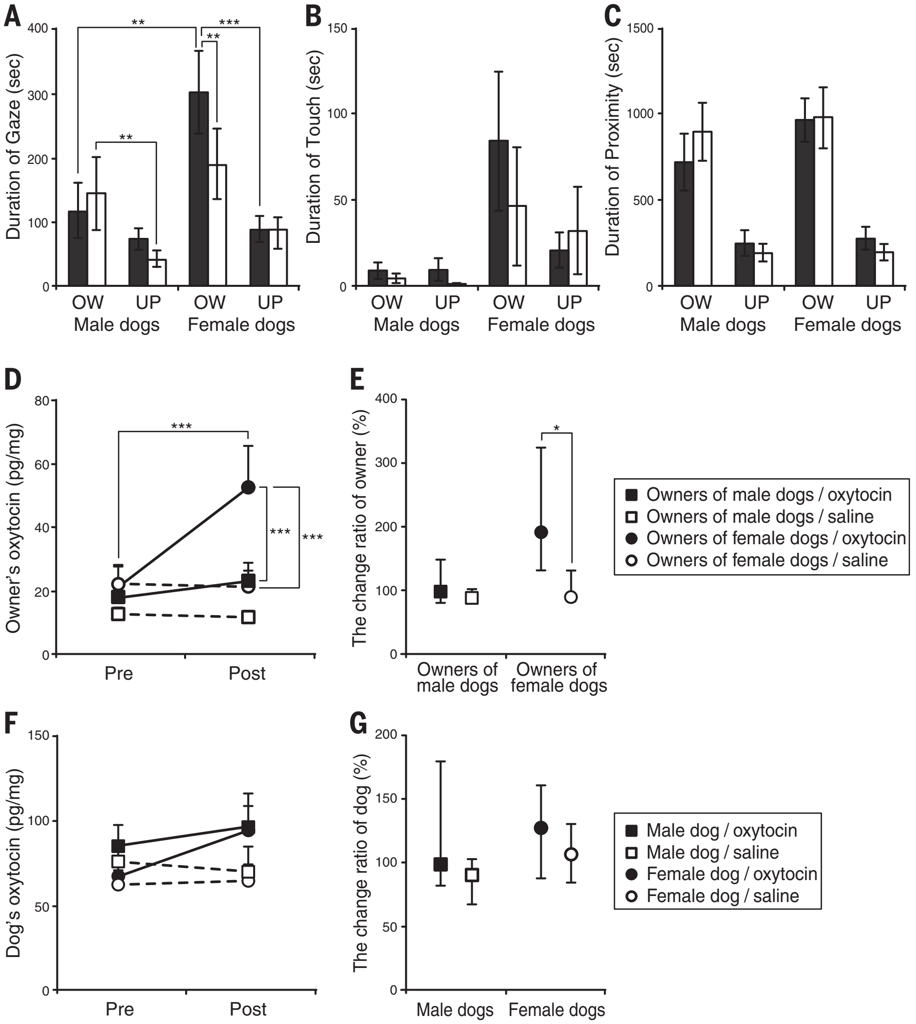

Fig. 2. Comparisons of behavior and urinary oxytocin between oxytocin and saline treatment conditions. (A) to (C) The effects of oxytocin administration on dog behaviors. Panels show the mean duration of dogs' gaze at participants (**A**), touching participants (**B**), and time spent in the proximity of less than 1 m from each participant (**C**). Black and white bars indicate, respectively, oxytocin- and saline treatment conditions. OW, owner; UP, unfamiliar person. (D) to (G) Change in urinary oxytocin concentrations after a 30-min interaction after oxytocin or saline administration. Urinary oxytocin concentrations of owners (**D**) and dogs (**F**) before and after a 30-min interaction are shown for oxytocin and saline groups. The change ratio of urinary oxytocin in owners (**E**) and dogs (**G**) is compared between male and female dogs. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. The results of (A) to (D) and (F) are expressed as mean ± SE. (E) and (G) reflect median ± quartile.

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Interestingly, oxytocin administration only increased mutual gaze duration in female dogs, whereas sex differences were not observed in experiment 1, which did not include unfamiliar individuals. Sex differences in the effects of intranasal oxytocin have been reported in humans as well (*22*), and it is possible that females are more sensitive to the affiliative effects of oxytocin or that exogenous oxytocin may also be activating the vasopressin receptor system preferentially in males. Oxytocin and the structurally related vasopressin affect social bonding and aggression in sexually dimorphic manners in monogamous voles (*8*, *9*), and oxytocin possibly increases aggression (*23*, *24*). Therefore, the results of experiment 2 may indicate that male dogs were attending to both their owners and to unfamiliar people as a form of vigilance. The current study, despite its small sample size, implies a complicated role for oxytocin in social roles and contexts in dogs.

In human infants, mutual gaze represents healthy attachment behavior (*25*). Human functional magnetic resonance imaging studies show that the presentation of human and canine family members' faces activated the anterior cingulate cortex, a region strongly acted upon by oxytocin systems (*26*). Urinary oxytocin variation in dog owners is highly correlated with the frequency of behavioral exchanges initiated by the dogs' gaze (*19*). These results suggest that humans may feel affection for their companion dogs similar to that felt toward human family members and that dog-associated visual stimuli, such as eye-gaze contact, from their dogs activate oxytocin systems. Thus, during dog domestication, neural systems implementing gaze communications evolved that activate the humans' oxytocin attachment system, as did gaze-mediated oxytocin release, resulting in an interspecies oxytocin-mediated positive loop to facilitate human-dog bonding. This system is not present in the closest living relative of the domesticated dog.

In the present study, urinary oxytocin concentrations in owners and dogs were affected by the dog's gaze and the duration of dog-touching. In contrast, mutual gaze between hand-raised wolves and their owners was not detected, nor was there an increase of urinary oxytocin in either wolves or their owners after a 30-min experimental interaction (experiment 1). Moreover, the nasal administration of oxytocin increased the total amount of time that female dogs gazed at their owners and, in turn, urinary oxytocin concentrations in owners (experiment 2). We examined the association between our results and early-life experience with humans in dogs and wolves in order to test the possibility that our results were due to differences in early-life experience with humans. The results did not indicate a significant association between the animals' early-life experiences with humans and the findings of the current study (see the supplementary methods). Moreover, there were no significant differences between dogs in the long-gaze group and wolves in either the duration of dog/wolf-touching and dog/wolf-talking, suggesting that the shorter gaze of the wolves was not due to an unstable relationship. These results support the existence of a self-perpetuating oxytocin-mediated positive loop in human-dog relationships that is similar to that of human mother-infant relations. Human-dog interaction by dogs' human-like gazing behavior brought on social rewarding effects due to oxytocin release in both humans and dogs and followed the deepening of mutual relationships, which led to interspecies bonding.

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SUPPLEMENTARY MATERIALS

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PLANT ECOLOGY

Anthropogenic environmental changes affect ecosystem stability via biodiversity

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Human-driven environmental changes may simultaneously affect the biodiversity, productivity, and stability of Earth's ecosystems, but there is no consensus on the causal relationships linking these variables. Data from 12 multiyear experiments that manipulate important anthropogenic drivers, including plant diversity, nitrogen, carbon dioxide, fire, herbivory, and water, show that each driver influences ecosystem productivity. However, the stability of ecosystem productivity is only changed by those drivers that alter biodiversity, with a given decrease in plant species numbers leading to a quantitatively similar decrease in ecosystem stability regardless of which driver caused the biodiversity loss. These results suggest that changes in biodiversity caused by drivers of environmental change may be a major factor determining how global environmental changes affect ecosystem stability.

Human domination of Earth's ecosystems, especially conversion of about half of the Earth's ice-free terrestrial ecosystems into cropland and pasture, is simplifying ecosystems via the local loss of biodiversity (*1*, *2*). Other major global anthropogenic changes include nutrient eutrophication, fire suppression and elevated fire frequencies, predator decimation, climate warming, and drought, which likely affect many aspects of ecosystem functioning, especially ecosystem productivity, stability, and biodiversity (*1*, *3*–*7*). However, to date there has been little evidence showing whether or how these three ecosystem responses may be mechanistically

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Dynamic biogeography and conservation of endangered species

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As one moves from the core to the periphery of a species' geographical range, populations occupy less favourable habitats and exhibit lower and more variable densities[1–4]. Populations along the periphery of the range tend to be more fragmented and, as a result, are less likely to receive immigrants from other populations. A population's probability of extinction is directly correlated with its variability and inversely correlated with density and immigration rate[5–9]. This has led to the prediction that, when a species becomes endangered, its geographical range should contract inwards, with the core populations persisting until the final stages of decline[2,10]. Convinced by these logical but untested deductions, conservation biologists and wildlife managers have been instructed to avoid the range periphery when planning conservation strategies or allocating resources for endangered species[11–13]. We have analysed range contraction in 245 species from a broad range of taxonomic groups and geographical regions. Here we report that observed patterns of

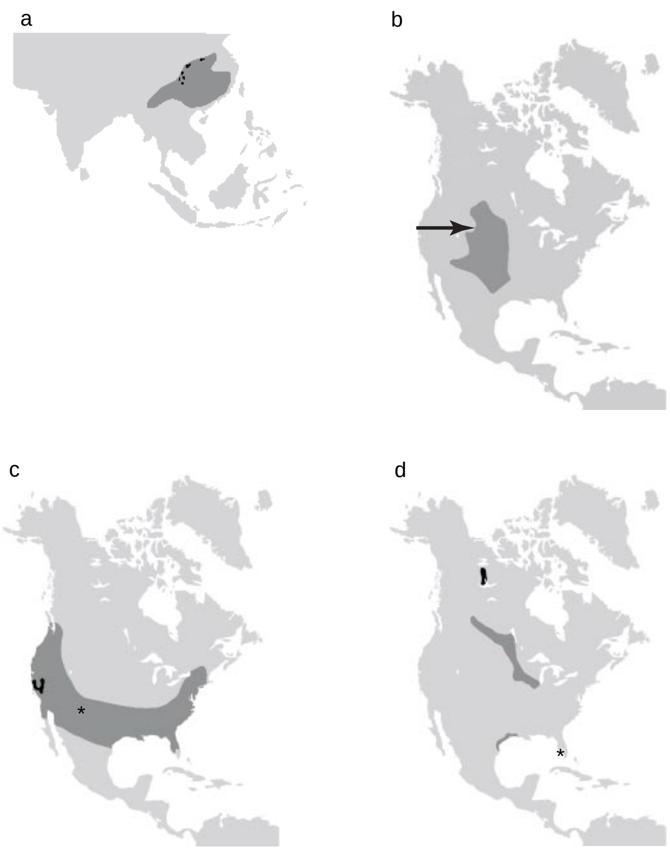

Figure 1 Patterns of range contraction in four endangered species. **a**, Giant panda, *Ailuropoda melanoleuca*; **b**, black-footed ferret, *Mustela nigripes*; **c**, California condor, *Gymnogyps californianus*; **d**, whooping crane, *Grus americana*. Historical range is in grey, extant range is in black or indicated by an arrow, and asterisks mark the locations of recent re-introduction sites for the California condor and the whooping crane.

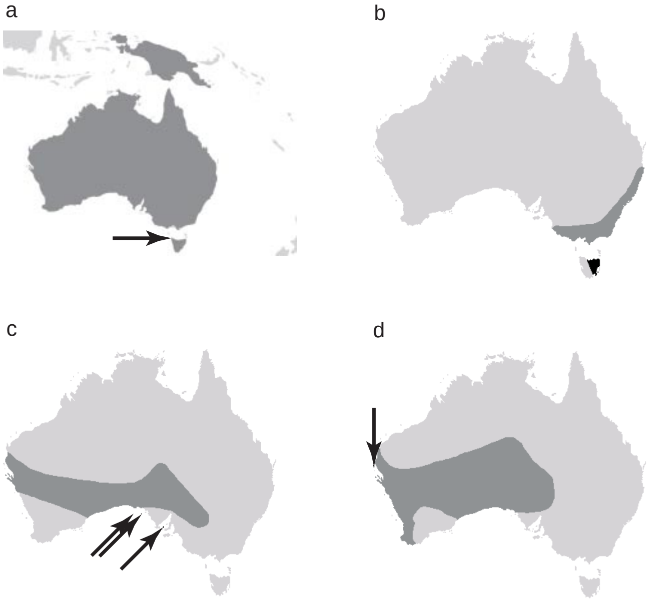

Figure 2 Patterns of range contraction in four species whose historical range included islands as well as much larger areas on the Australian mainland. **a**, Tasmanian tiger, *Thylacinus cynocephalus*; **b**, Tasmanian bettong, *Bettongia gaimardi*; **c**, greater stick-nest rat, *Leporillus conditor*, **d**, Shark Bay mouse, *Pseudomys fieldi*. Historical range in grey, and extant or final range is in black or indicated by an arrow.

Table 1 Number of species studied from different taxonomic groups and geographical regions

	North America	Australia	Eurasia	South America	Africa	Islands	Subtotal
Birds	12	6	19	2	3	45	87
Mammals	8	36	30	5	20	1	100
Reptiles	1		2	1		1	5
Amphibians	3		1				4
Fishes	1		1				2
Mollusks	1		1			20	22
Arthropods	2		1		1		4
Plants	4					17	21
Subtotal	32	42	55	8	24	84	245

See ref. 24.

range contraction do not support the above predictions and that most species examined persist in the periphery of their historical geographical ranges.

Table 1 shows the number of species studied and their geographical distribution. We found that 240 (98%) of the 245 species maintained populations in at least a portion of their peripheral range. Furthermore, 167 (68%) maintained a greater than expected portion of their range in the periphery, not the core ($P < 0.001$, binomial test). In fact, remnant populations of 91 species occurred exclusively in the periphery of their historical range, whereas populations of only five species persisted solely in the core of their historical range ($P < 0.001$, binomial test). We detected no significant difference in the patterns of range contraction between birds and mammals (63 (72%) of 87 birds and 70 (70%) of 100 mammals exhibiting greater persistence along the periphery). Most species, including some of the flagship species of conservation biology (Fig. 1), persist along the edge of their range.

Consistent with contemporary theory in ecology[6,7,9], persistence was greater for populations occupying larger patches of their historical range. On the mainland, 12 (75%) of 16 species persisted in larger patches of their historical range, whereas 15 (83%) of 18 insular species persisted in larger patches. However, if a species' historical range included both mainland and insular sites, population persistence was highest on the islands, despite their smaller size (23 [68%] of 34 species exhibited greater than expected persistence on islands; $P = 0.029$, binomial test; Fig. 2).

We found two additional patterns that seem contrary to the general tendency for greater persistence along the range periphery—Africa and the Hawaiian Islands. Africa was the only continent with an adequate sample size whose species failed to exhibit a significant peripheral bias in persistence (14 (58%) of 24 species persisted in the periphery; $P = 0.271$, binomial test). In contrast, 42 (78%) of 54 Eurasian species, 34 (81%) of 42 Australian species and 26 (81%) of 32 North American species persisted in their range peripheries ($P < 0.001, 0.001, 0.001$, respectively, binomial tests). In a similar fashion, whereas 11 (92%) of the 12 species we studied from New Zealand, and all of the 6 species from the Mariana Islands (including Guam) persisted more in the periphery than expected by chance, only 43% of the 54 Hawaiian species exhibited a peripheral bias.

These apparently exceptional results and the more general tendency for persistence along the periphery indicate that range contraction is strongly influenced by anthropogenic extinction forces (for example, habitat degradation, biocides and introduced species) which render historical density patterns irrelevant. Populations that persist the longest are those last affected by the contagion-like spread of extinction forces; that is, those along the edge of the range, on an isolated and undisturbed island, or at high elevations. African species failed to show any peripheral bias in range decline because, instead of moving across species' geographical ranges like a contagion, humans having a significant ecological effect became established in many places across the continent before the earliest record of historical extinctions. We actually predicted this result for Africa, based largely on Martin's[14,15] explanation for the absence of a post-Pleistocene collapse of the African megafauna: large mammals and birds shared a long evolutionary and ecological history with prehistoric humans. The 'exceptional' pattern for Hawaiian species is also entirely consistent with the above hypothesis concerning the contagion-like spread of extinction forces. Polynesians and, later, Europeans colonized most of the beach front and lowlands of these islands, and then spread, along with their commensals, upward. Persistent populations of Hawaiian species are either those that can cope with these anthropogenic disturbances, or those whose final populations remain in the least disturbed and most isolated sites; that is, in the montane areas. In short, the geography of recent extinctions is largely the geography of humanity. Thus, our ability to understand patterns in recent extinctions and to predict those of future ones depends to a very large degree on our ability to reconstruct and predict the spatial dynamics of humans and associated extinction forces.

These results have strong implications for conservation biology. Although they may have represented suboptimal habitats in historical times, areas along the range periphery and on remote islands and mountain ranges often provide valuable opportunities for conserving endangered species[16,20]. We find it very encouraging, therefore, that a number of recent conservation programmes have broadened their options by including peripheral sites for re-introductions and areas to search for undiscovered populations of endangered species (asterisks in Fig. 1c, d). Although once viewed as the land of the living dead[21,22], sites along the range periphery may now hold great promise for conserving endangered species and biological diversity in general. ☐

Methods

We obtained range maps for 245 species from the literature or through personal correspondence with authorities (see Supplementary Information). We include only those species with maps available for both historical and extant ranges (or final site in the case of extinct species), and with extant ranges that were less than 25% of the species' historical distribution. We digitized the range maps into Idrisi, a geographical information system[23]. For each species, we first located the centre, which was the point within the species' historical range that was most distant from all edges of the range. The distance from this point to the nearest edge was then calculated. We defined the region that was within half of this distance to an edge as periphery and the remaining portion of the range as central. We then calculated an index of centrality (C), which is a measure of the proportion of the extant or final range that fell within the central region of the historical range.

First, we calculated the area of the extant range expected to occur within the central region (C_{EE}) as follows:

$$C_{\mathrm{EE}} = \left(\frac{C_{\mathrm{H}}}{T_{\mathrm{H}}}\right) T_{\mathrm{E}},$$

where T_{E} is the total area of the extant (or final) range; T_{H} is the total area of the historical range; and C_{H} is the area of the central region of the historical range. We then calculated C as follows. If $C_{\mathrm{EO}} \leq C_{\mathrm{EE}}$, where C_{EO} is the area of the extant range obsrved within the historical central region, then

$$C = \left(\frac{C_{\mathrm{EO}}}{C_{\mathrm{EE}}}\right) 0.5$$

If $C_{\mathrm{EO}} > C_{\mathrm{EE}}$, then

$$C = 0.5 + \left[0.5\left(\frac{C_{\mathrm{EO}} - C_{\mathrm{EE}}}{T_{\mathrm{E}}}\right)\right].$$

The index of centrality (C) ranged from 0, where the extant range fell completely outside the central portion of the historical range, to 1, where the extant range fell completely within the central portion of the historical range. We designated species with C values greater than 0.5 as 'central species', and those species with C values less than 0.5 as 'peripheral species'. We then used a binomial test to determine whether the ratio of central to peripheral species differed significantly from 1 : 1.

We used maps for species with multiple patches in their historical range to test whether persistence was higher for populations inhabiting larger patches. We first assigned patches to one of two size categories ('large' or 'small'), based on their area relative to the median patch size. If a species had an odd number of patches in its historical range, the median-sized patch was excluded from the analysis. For each species, we counted the number of large and small patches maintaining persistent populations (P_l and P_s, respectively). We counted the number of species (S_l) for which P_l was greater than P_s and the number of species (S_s) where P_s was greater than P_l. Species with ties ($P_l = P_s$) were excluded from analysis. We used a binomial test to determine whether the ratio of S_l to S_s differed significantly from 1 : 1. This analysis was done for 124 continental and 44 insular species[24].

To compare the relative persistence of mainland and island patches, we first calculated the total area of all of the historical patches (A_{TH}) and the area of the historical mainland patches (A_{MH}) for 44 species. We multiplied $A_{\mathrm{MH}}/A_{\mathrm{TH}}$ by the total number of persisting patches (P_{TP}) to generate the expected number of patches persisting on the mainland. If the number of patches persisting on the mainland (P_{MP}) was greater than expected, we classified the species as a mainland species, otherwise it was classified as an island species. There were no ties (P_{MP} = expected number of patches). We tested whether the ratio of mainland species and island species differed significantly from 1 : 1 using a binomial test.

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Reduced vas deferens contraction and male infertility in mice lacking $P2X_1$ receptors

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$P2X_1$ receptors for ATP are ligand-gated cation channels, present on many excitable cells including vas deferens smooth muscle cells[1–5]. A substantial component of the contractile response of the vas deferens to sympathetic nerve stimulation, which propels sperm into the ejaculate, is mediated through P2X receptors[1]. Here we show that male fertility is reduced by ~90% in mice with a targeted deletion of the $P2X_1$ receptor gene. Male mice copulate normally—reduced fertility results from a reduction of sperm in the ejaculate and not from sperm dysfunction. Female mice and heterozygote mice are unaffected. In $P2X_1$-receptor-deficient mice, contraction of the vas deferens to sympathetic nerve stimulation is reduced by up to 60% and responses to P2X

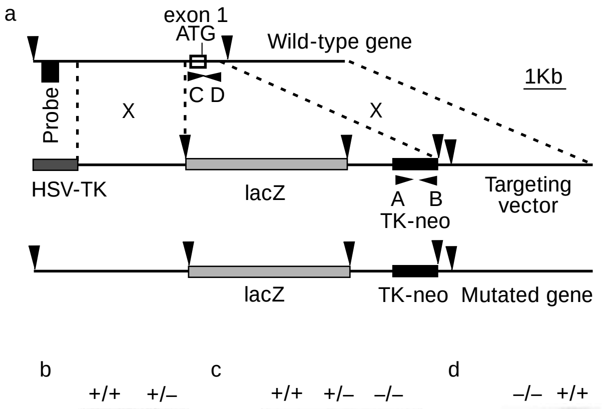

Figure 1 Generation of $P2X_1$-receptor-deficient mice. **a**, Genomic maps of the wild-type gene, targeting vector and mutated gene. *Bam*HI sites (indicated by arrows) and the probe used for detection of the homologous recombination events by Southern analysis are shown. Polymerase chain reaction (PCR) primers used for genotyping of mouse-tail DNA are indicated (A–D). **b**, Southern blot analysis of tail genomic DNA from +/+ and –/– animals. Genomic DNA was digested with *Bam*HI and hybridized with the probe indicated in **a** which detects a 4.8-kb band in +/+ DNA and a 3.7-kb band in –/– DNA. WT, wild-type; KO, knock-out. **c**, PCR genotyping of mouse-tail DNA. Primers A, B, C and D were used in one PCR reaction to genotype mouse-tail genomic DNA. Primers A and B amplify a 519-bp product from the *neo*$^{\mathrm{R}}$ gene, whereas primers C and D amplify a 317-bp product from the deleted region of the $P2X_1$ receptor gene. **c**, RT-PCR analysis. A PCR product of 442 bp from the $P2X_1$-receptor gene was amplified from bladder complementary DNA from a +/+ animal but not from bladder cDNA of a –/– animal. As a control, amplification of 199-bp product from the actin gene was detected in both samples.

The Evolution of Maximum Body Size of Terrestrial Mammals

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The Evolution of Maximum Body Size of Terrestrial Mammals

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The extinction of dinosaurs at the Cretaceous/Paleogene (K/Pg) boundary was the seminal event that opened the door for the subsequent diversification of terrestrial mammals. Our compilation of maximum body size at the ordinal level by sub-epoch shows a near-exponential increase after the K/Pg. On each continent, the maximum size of mammals leveled off after 40 million years ago and thereafter remained approximately constant. There was remarkable congruence in the rate, trajectory, and upper limit across continents, orders, and trophic guilds, despite differences in geological and climatic history, turnover of lineages, and ecological variation. Our analysis suggests that although the primary driver for the evolution of giant mammals was diversification to fill ecological niches, environmental temperature and land area may have ultimately constrained the maximum size achieved.

For the first 140 million years of their evolutionary history, mammals were small and occupied a fairly narrow range of body sizes and niches (*1*, *2*). Although diverse feeding adaptations evolved by the middle Mesozoic, and larger mammals may have preyed on small dinosaurs (*3*, *4*), their body size range extended only from ~3 to 5 g to ~10 to 15 kg (*4*, *5*). This restricted range almost certainly constrained the ecological roles of early mammals in paleocommunities. For example, herbivory was probably limited; allometric, anatomical, and physiological constraints set a lower threshold of ~5 kg for ruminant herbivores (*6*). The Cretaceous/Paleogene (K/Pg) mass extinction, which eliminated nonavian dinosaurs as well as many vertebrate, plant, and invertebrate taxa, was followed by a wholesale reorganization of ecological communities (*7*). It marked the onset of rapid morphological, ecological, and phylogenetic diversification in terrestrial mammals that led to an expansion in mass by four orders of magnitude and the occupation of a full range of ecological roles (*8*).

Here we analyze maximum size of terrestrial mammals across different continents, taxonomic groups, phylogenetic lineages, and feeding guilds. We compiled and analyzed data on the maximum body size of each taxonomic order in each sub-epoch on each continent over their entire evolutionary history (*9*). Information about body mass was obtained for fossil taxa from primary sources or estimated directly from taxon-specific allometric regressions based on measurements of teeth or limbs (table S1). Because of taphonomic considerations, we focused on the maximum size achieved by each order; it tends to be reported in the literature and is robustly related to the overall body size distribution and hence to the mean and median body size (*10*). Fossil ages were standardized using the midpoint for each Cenozoic sub-epoch on the Gradstein geological time scale (*11*). Diversity estimates were extracted from the Paleobiology Database (*12*), using the range-through option for each interval of time. We conducted simulations to assess the potential effect of sampling on the probability of detecting the largest mammal; including as few as 10% of fossil sites yielded nearly 100% probability of recovering the largest mammal on a continent (fig. S1).

The data show that the pattern of body size evolution was similar across continents, lineages, and trophic groups. Globally, and on each continent, maximum body mass increased rapidly during the early Cenozoic (Fig. 1). By the late Eocene [42.9 million years ago (Ma)], maximum body mass was three orders of magnitude larger than at the beginning of the Cenozoic. Our results are consistent with a previous analysis of North American mammals (*5*, *8*). The upper limit of ~17 tons was reached in the early Oligocene of Eurasia, with the evolution of *Indricotherium transouralicum* (Perissodactyla) and again in the Miocene by several *Deinotherium* species (Proboscidea) in Eurasia and Africa (Fig. 1B; fig. S2); North America never supported a mammal of this size. Strikingly, the overall pattern was not driven by a single taxon or an individual continent. At one time or another, six different orders and three of the four continents contained the largest mammal. Because of the current paucity of data for South America, body mass values for this continent should be considered an underestimate; nonetheless, results illustrate the same general trends. Contrary to earlier suggestions (*13*–*15*), increases in body mass were not driven by increasing generic or ordinal diversity: Mammals were not consistently larger when they were more diverse (*9*) (fig. S3).

We tested two hypotheses for the evolution of maximum body size. The first is a simple growth model, in which maximum body size (M) evolves following a geometric Brownian motion, that is, an unconstrained random walk on the logarithmic scale. This model implicitly assumes that niche space is uniformly distributed. Under a random walk, M is predicted to increase as a power law of the form $\log M = M_0 t^{\gamma}$, where M_0 is initial maximum body size, t is time, and $\gamma = 1/2$, so that maximum body size increases as the square root of time (*15*).

The second model has growth saturating over time, reflecting limits of resources or physiological, allometric, biomechanical, or ecological constraints, such as the slower life histories of larger mammals. Thus, the initial change in body mass M with time is proportional to body mass (that is, $\frac{dM}{dt} \propto M$) and increases at some intrinsic rate α. However, as maximum body size evolves, the evolutionary possibilities for increasing size are progressively exhausted. Consequently, the rate of change is also proportional to the availability of open niche space, which is captured by the difference between asymptotic (K) and current log body mass [that is, $\log(K) - \log(M)$], or $\log\left(\frac{K}{M}\right)$. Combining these ecological and evolutionary growth dynamics yields the Gompertz equation $\frac{dM}{dt} = \alpha M \log\left(\frac{K}{M}\right)$, a sigmoidal growth model often used in time series analyses. The integrated form is $\log M = \log K - \log\left(\frac{K}{M_0}\right)e^{-\alpha t}$, where M_0 is initial maximum body size. The Gompertz model is more biologically plausible than the random walk model, because it captures both the multiplicative nature of body size evolution and the saturating effects of exponentially decreasing niche space availability at larger body sizes.

We compared model fits using corrected Akaike information criteria (AICc). The results suggested that the random walk was not an appropriate model (Table 1). Although a power function provided a reasonable fit to the data, the fitted exponent γ was 0.25, significantly less than the predicted value of 0.50. Moreover, after the initial growth phase, the residuals were not normally distributed. This was probably because maximum

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body size approaches a plateau as opposed to increasing monotonically. The Gompertz model provided a much better fit to the data throughout the time series and yielded the lowest AICc (Table 1 and fig. S2). The inflection point between the growth phase and the saturating phase occurred during the late Eocene at 42.9 Ma, at a body mass of 4850 kg.

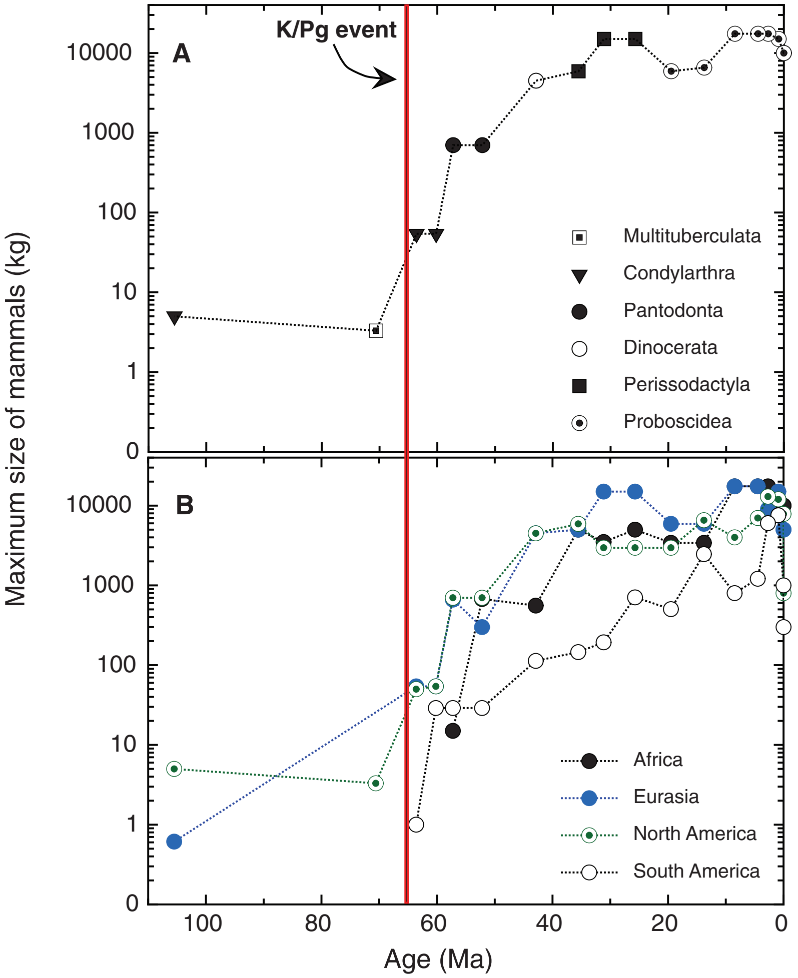

Fig. 1. Maximum body mass of terrestrial mammals over time and space. (**A**) Maximum body mass over time examined globally at the sub-epoch level over the past 110 million years. (**B**) Maximum body mass for the largest continents (South America, North America, Africa, and Eurasia) over the same time interval. The overall trend is not driven by a single taxonomic order or an individual continent; six different orders and three of the four continents depicted have at one time or another housed the largest mammal. Data for Australia (not shown) and South America were particularly difficult to obtain because of limited material and/or collecting; thus, estimates for these continents should be considered underestimates. Data are binned at the resolution of sub-epochs using the Gradstein time scale (*12*).

Table 1. Model fits for global, continental and trophic level body size trajectories. The power law is of the form $\log M = c_0 t^{\gamma}$ and the Gompertz equation $\log M = \log K - \log(\frac{K}{M_0})e^{-\alpha t}$.

Model	Parameters	AICc	R^2 value	*P* value
All data				
Power law	$c_0 = 1.504$, $\gamma = 0.25$	9.3	0.92	<0.001
Gompertz	$K = 13182.57$, $M_0 = 6.92$, $\alpha = 0.08$	8.2	0.94	<0.001
Eurasia				
Gompertz	$K = 15977.18$, $M_0 = 25.14$, $\alpha = 0.05$	—	0.83	<0.001
Africa				
Gompertz	$K = 12900.31$, $M_0 = 0.44$, $\alpha = 0.06$	—	0.86	<0.001
North America				
Gompertz	$K = 6675.75$, $M_0 = 8.78$, $\alpha = 0.07$	—	0.85	<0.001
Carnivores				
Gompertz	$K = 710.56$, $M_0 = 14.62$, $\alpha = 0.10$	—	0.76	<0.001

The Gompertz model also provided good fits for the trajectories of maximum body size on each continent (Table 1 and fig. S2). Fifteen different lineages, representative of different archaic and modern orders (such as Proboscidea, Perissodactyla, Artiodactyla, Dinocerata, Pantodonta, Condylarthra, Xenarthra, etc.) evolved similar maximum size at different times and on different continents. These results show that the sigmoidal or saturating trajectory of maximal size evolution for Cenozoic mammals in North America (*5*, *8*) occurred independently in multiple lineages on all the large continents. These results support the interpretation that similar niches were available to and filled by comparably sized giant mammals on each continent after 35 to 40 Ma. Because these niches were occupied by multiple different lineages at different times and on different continents, the patterns suggest that large mammals convergently evolved to fill similar ecological roles. Consistent with this idea, the largest mammals after the beginning of the Cenozoic were always herbivores. These patterns are also congruent with arguments relating the maximum body size of contemporary herbivorous mammals to constraints of diet and digestive physiology (*16*).

Carnivorous mammals showed similar saturating trajectories but attained smaller maximum sizes than coexisting megaherbivores (Fig. 2). Large mammal-eating mammals were effectively absent in the early Paleocene; instead, birds, terrestrial crocodiles, snakes, and large lizards were the dominant carnivores (*17*). Once carnivorous mammal guilds began to diversify, however, they showed a similar trajectory to that of the herbivores—also well fit by a Gompertz function (Table 1). Although carnivores and herbivores started from a similar size immediately after the K/Pg, after ~30 million years the largest carnivores approached an asymptotic maximum about one order of magnitude smaller than that of the largest herbivores (Fig. 2). As with herbivores (Fig. 1A), the carnivores convergently evolved similar maximum sizes in different lineages: the archaic orders Creodonta and Mesonychia, and the modern order Carnivora. Although the duration of these clades overlapped, there was turnover in the ordinal affiliation of the largest carnivore, with each sequentially evolving to a maximum body mass of ~1000 kg (Fig. 2). After the initial size increase, the ratio of body masses of coexisting carnivorous and herbivorous mammals remained similar across the entire Cenozoic (Pearson correlation = 0.819, $P < 0.000$; fig. S4). This suggests at least an indirect relation in which the maximal sizes of carnivores followed the overall size distribution of mammals, but not necessarily a direct causal relation between the largest carnivores and herbivores. Indeed, the largest carnivores probably did not prey on the largest herbivores. The disparity in maximum size between carnivores and herbivores persists in contemporary mammals: Lions, tigers, and bears are about an order of magnitude smaller than elephants and rhinos. The asymptotic maximum size of carnivores of

~1000 kg is consistent with the recent prediction that this represents an upper limit for flesh-eating terrestrial mammals because of physiological and ecological constraints (*18*).

We compared the overall global trajectory of maximum body mass with time series of three major abiotic factors: global temperature (*19*), atmospheric oxygen levels (*20*), and terrestrial land area (*21*) (Fig. 3 and table S1). Each of these variables has been hypothesized theoretically and sometimes shown empirically to affect body size evolution in mammals: temperature by affecting how mammals dissipate heat through Bergmann's rule (*22*–*24*); greater land area by allowing larger populations and reducing extinction probabilities for the largest mammals (*25*, *26*); and higher atmospheric oxygen concentrations by allowing higher rates of metabolism and biomass production (*27*–*29*). We averaged the abiotic values, which were generally reported at a finer scale, using the durations for each geological sub-epoch so we could compare against the trajectory of global body mass over the Cenozoic (table S1). Binned values are superimposed over the finer-scale data shown in Fig. 3. Our analyses were not based on specific values and slopes of these curves at specific times. We varied bin widths and averaging techniques; results were robust with regard to the binning technique employed (*9*). These abiotic records are based on proxies (*19*, *21*) or on modeling of carbon isotopic records (*20*); hence, they contain significant unresolved uncertainties, which complicate interpretations of the patterns.

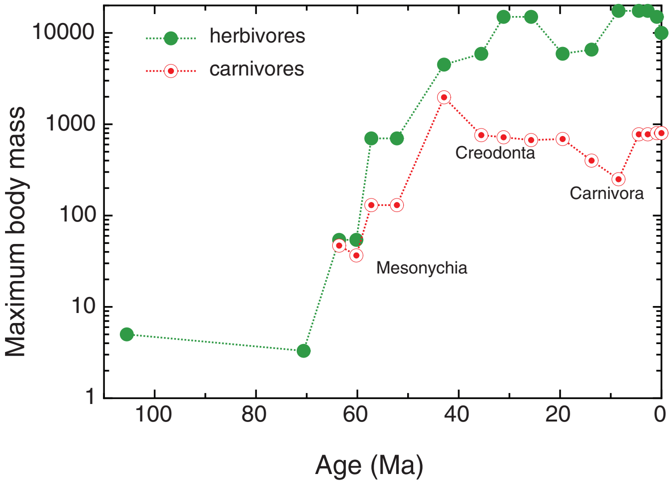

Fig. 2. The trajectory of body mass evolution of selected trophic guilds over the Cenozoic. Green solid circles, herbivores; red open circles with dots, carnivores. Carnivore maximum body mass closely tracks that of herbivores (fig. S4). The ceiling for maximum size is different for herbivores and carnivores (~10 to 15 tons versus ~1 ton) but consistent over time within a trophic group, irrespective of taxonomic affiliation. The largest mammals before the K/Pg may have been omnivorous rather than strict herbivores; our interpretations are based solely on patterns for the Cenozoic.

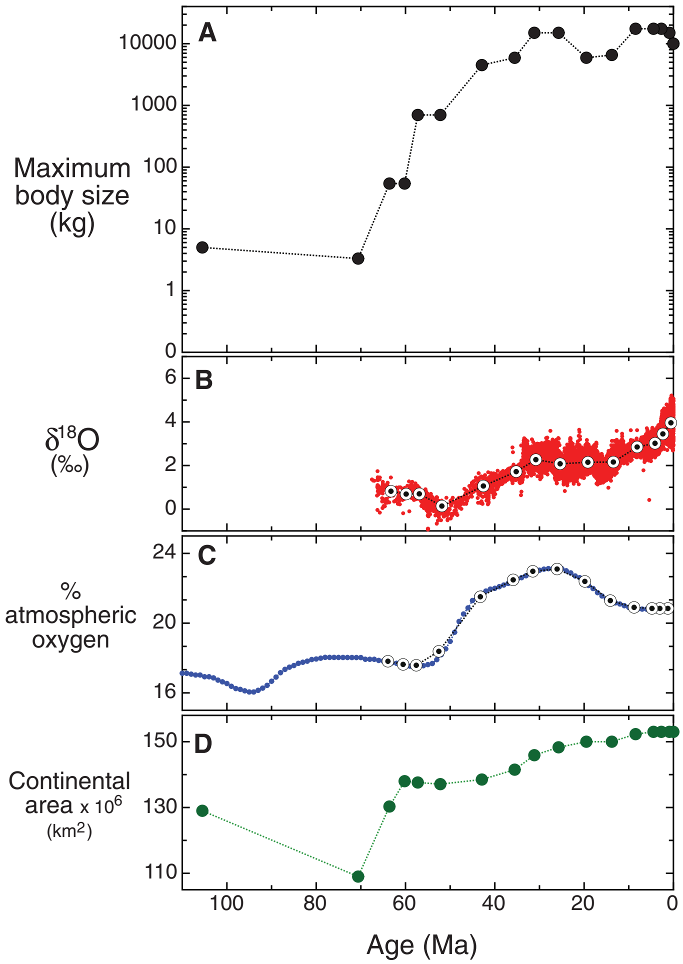

Fig. 3. Global fluctuations in maximum body size and various abiotic factors over the Cenozoic. (**A**) Maximum body mass (this study). (**B**) δ^{18}O adjusted in parts per thousand (*19*). (**C**) Percent atmospheric oxygen concentration (*20*). (**D**) Variations in terrestrial land area in square kilometers (*21*). Abiotic factors chosen were those that have been demonstrated or postulated to influence the body mass of mammals. Linear regression yields highly significant fits between all factors and global mammalian body mass over the Cenozoic; all but atmospheric oxygen concentration remain significant when data are restricted to the past 42.9 million years (table S3). Values for the Mesozoic were excluded because data are fairly scarce; data were truncated at the terminal Pleistocene to yield roughly comparable sub-epoch durations. Data vary in the fineness of their resolution; abiotic variables were binned to obtain values representing the averages of the temporal span represented by each body mass estimate. These binned values are superimposed on the finer-scale data.

All abiotic factors were significantly related to mammalian body mass over the Cenozoic (Fig. 3 and table S3). To determine whether significance was driven by the initial exponential phase, we also ran analyses using the temporal interval from the late Eocene through the Pleistocene (42.9 to 0.9 Ma; results were similar when early or middle Eocene values were chosen). Both global temperature and terrestrial land area remained highly significant: The largest mammals evolved when Earth was cooler and terrestrial land area was greater (table S3), but atmospheric oxygen level dropped out (table S3). However, as might be expected, temperature and land area were significantly related (Pearson correlation = 0.904, $P < 0.001$, df = 13): Lower global environmental temperatures (indexed by ^{18}O) corresponded to more water stored in ice caps, lower sea levels, and increased land areas, and probably to changes in vegetation cover and primary productivity.

That temperature and/or land area may have influenced the evolution of body mass in mammals is consistent with several well-established biogeographic principles. The influence of temperature is consistent with Bergmann's rule, a well-known ecogeographic trend of larger body mass in cooler habitats across space (*24*), and in a few instances, across time (*30*). Bergmann's rule probably reflects physiological adaptations to prevent heat loss, because larger animals have a reduced surface-to-volume ratio; or alternatively, to promote heat dissipation at smaller body masses (*24*). Our results are also consistent with the hypothesis that available land area constrains the upper body mass limit of mammals by limiting population through the size or number of home ranges that can be "packed in" or by reducing energy acquisition (*25*, *26*). Among contemporary mammals, maximum body mass is strongly influenced by terrestrial land area, with larger-bodied mammals being found in larger insular or continental "islands" (fig. S5). Thus, constraints on maximum body size potentially imposed by both abiotic factors ultimately may be traced to physiological processes related to endothermy.

However, some caution should be used in the interpretation of our results. Quantitative analyses of these abiotic variables were complicated by a lack of resolution, potential collinearities, and a lack of statistical power that precluded the use of more-rigorous tests to fully explore the relationships between the predictor variables. Moreover, for some of these abiotic factors the uncertainties are not well characterized, and we currently have no way of knowing how these may interact to influence our results. For example, the oxygen isotope curve is confounded by changes in the terrestrial ice volume, atmospheric oxygen concentration is related to temperature through fluctuations in carbon dioxide and carbon sequestration (*19*) and potentially to global land area through changes in primary productivity, and global land area is clearly related to temperature and sea level. Moreover, other factors such as changes in seasonality and precipitation were not explicitly incorporated; the late Cenozoic saw a global trend toward cooler, drier, and more seasonal climates (*19*, *31*). Nevertheless, the potential role of abiotic factors in the overall trajectory of mammalian evolution cannot be ignored, and the available data suggest interesting and important trends, which should be explored further.

Our analysis implies that the increase in the maximum mass of mammals over the Cenozoic was neither a statistical inevitability driven by increasing species richness nor a random evolutionary walk from a small initial size, but rather reflected processes operating consistently across trophic and taxonomic groups, and independent of the physiographic history of each continent. We find no support for other hypotheses for the evolution of maximum body mass (*9*), including the expected increase in variance due to random divergence from a common ancestor or to increasing species richness (*13*–*15*); nor do terrestrial mammals ever approach sizes that might invoke biomechanical constraints (*32*). The K/Pg extinction provided the ecological opportunity for mammals to become larger. Terrestrial mammals did so in an exponentially decreasing fashion, reaching a more or less maximal size by 40 Ma as evolutionary possibilities for increasing body size were progressively exhausted and abiotic factors began constraining the upper limit.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6008/1216/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S3
References

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Modular Organic Structure-Directing Agents for the Synthesis of Zeolites

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Organic structure-directing agents (OSDAs) are used to guide the formation of particular types of pores and channels during the synthesis of zeolites. We report that the use of highly versatile OSDAs based on phosphazenes has been successfully introduced for the synthesis of zeolites. This approach has made possible the synthesis of the elusive boggsite zeolite, which is formed by 10- and 12-ring intersecting channels. This topology and these pore dimensions present interesting opportunities for catalysis in reactions of industrial relevance.

Zeolites are crystalline microporous and mesoporous materials (*1*–*4*) that offer a wide range of applications because of their well-defined structures, which are formed by channels with pore apertures of molecular dimensions. An important objective during the synthesis of zeolites is to achieve control of the pore dimensions and their connectivity through the use of organic structure-directing agents (OSDAs) that, at the limit, could act as template molecules. A large variety of quaternary organic ammonium salts have been successfully used as OSDAs (*2*, *4*–*6*) as well as analogous molecules, such as phosphonium-derived organic cations (*7*–*10*). However, rather than design new molecules for each zeolite target it could be more efficient to have a type of OSDA that could be easily built by blocks similar to Legos, with a large variety of substituents. Potential new structures could be simulated with molecular modeling techniques, and an OSDA that directs its synthesis by minimizing the energy of the zeolite-OSDA system could be predicted or at least can be selected from a limited number of candidates.

The described procedure requires having a tool box of OSDA molecules that are easy to prepare and adapt while having the adequate polarity and basicity. We present a type of OSDA molecule with a nearly unlimited synthesis flexibility that is based on building-block units. These molecules are based on phosphazenes that can mobilize silica, have the adequate polarity and stability, and offer more structural possibilities than quaternary ammonium or phosphonium cations. We used these OSDAs for the synthesis of new zeolite structures,

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Brain size predicts problem-solving ability in mammalian carnivores

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Despite considerable interest in the forces shaping the relationship between brain size and cognitive abilities, it remains controversial whether larger-brained animals are, indeed, better problem-solvers. Recently, several comparative studies have revealed correlations between brain size and traits thought to require advanced cognitive abilities, such as innovation, behavioral flexibility, invasion success, and self-control. However, the general assumption that animals with larger brains have superior cognitive abilities has been heavily criticized, primarily because of the lack of experimental support for it. Here, we designed an experiment to inquire whether specific neuroanatomical or socioecological measures predict success at solving a novel technical problem among species in the mammalian order Carnivora. We presented puzzle boxes, baited with food and scaled to accommodate body size, to members of 39 carnivore species from nine families housed in multiple North American zoos. We found that species with larger brains relative to their body mass were more successful at opening the boxes. In a subset of species, we also used virtual brain endocasts to measure volumes of four gross brain regions and show that some of these regions improve model prediction of success at opening the boxes when included with total brain size and body mass. Socioecological variables, including measures of social complexity and manual dexterity, failed to predict success at opening the boxes. Our results, thus, fail to support the social brain hypothesis but provide important empirical support for the relationship between relative brain size and the ability to solve this novel technical problem.

brain size | problem-solving | carnivore | social complexity | intelligence

Animals exhibit extreme variation in brain size, with the sperm whale's brain weighing up to 9 kg (1), whereas the brain of the desert ant weighs only 0.00028 g (2). Although body mass is the single best predictor of brain size (1, 3), some species have much larger brains than expected given their body size (e.g., humans and dusky dolphins), whereas other species have much smaller brains than expected (e.g., hippopotamus and blue whale) (1). Brain tissue is energetically costly (4–6), and therefore, large brains are presumed to have been favored by natural selection, because they confer advantages associated with enhanced cognition (3). However, despite great interest in the determinants of brain size, it remains controversial whether brain size truly reflects an animal's cognitive abilities (7–9).

Several studies have found an association between absolute or relative brain size and behaviors thought to be indicative of complex cognitive abilities. For example, brain size has been found to correlate with bower complexity in bower birds (10), success at building food caches among birds (11), numerical abilities in guppies (5), and two measures of self-control in a comparative study of 36 species of mammals and birds (12). Additionally, larger-brained bird species have been found to be more innovative, more successful when invading novel environments, and more flexible in their behavior (13–16). Although there is circumstantial evidence suggesting an association between problem-solving ability and brain size, experimental evidence is extremely rare. To experimentally assess the relationship between brain size and any cognitive ability across a number of species in a standardized way is challenging because of the unique adaptations each species has evolved for life in its particular environment (17). In this study, we investigate whether larger-brained animals do, indeed, exhibit enhanced problem-solving abilities by conducting a standardized experiment in which we present a novel problem-solving task to individuals from a large array of species within the mammalian order Carnivora.

Carnivores often engage in seemingly intelligent behaviors, such as the cooperative hunting of prey (18, 19). Nevertheless, with the exception of domestic dogs, carnivores have largely been ignored in the animal cognition literature (20). Mammalian carnivores comprise an excellent taxon in which to assess the relationship between brain size and problem-solving ability and test predictions of hypotheses forwarded to explain the evolution of large brains and superior cognitive abilities, because they exhibit great variation in their body size, their brain size relative to body size, their social structure, and their apparent need to use diverse behaviors to solve ecological problems. Although most carnivores are solitary, many species live in cohesive or fission–fusion social groups that closely resemble primate societies (21–23). Furthermore, experiments with both wild spotted hyenas (24) and wild meerkats (25) show that members of these species are able to solve novel problems, and in spotted hyenas, those individuals that exhibit the greatest behavioral diversity are the most successful problem-solvers (24).

Here, we presented steel mesh puzzle boxes, scaled according to subject body size, to 140 individuals from 39 species in nine families of zoo-housed carnivores and evaluated whether individuals in each species successfully opened the boxes to obtain a food reward inside (Fig. 1*A* and Dataset S1). In addition to testing whether larger-brained carnivores are better at solving a novel technical problem, we inquired whether species that live in larger social groups exhibit enhanced problem-solving abilities compared with species that are solitary or live in smaller social groups. We also asked whether species exhibiting greater behavioral diversity are better at solving

Significance

Intelligence presents evolutionary biology with one of its greatest challenges. It has long been thought that species with relatively large brains for their body size are more intelligent. However, despite decades of research, the idea that brain size predicts cognitive abilities remains highly controversial; little experimental support exists for a relationship between brain size and the ability to solve novel problems. We presented 140 zoo-housed members of 39 mammalian carnivore species with a novel problem-solving task and found that the species' relative brain sizes predicted problem-solving success. Our results provide important support for the claim that brain size reflects an animal's problem-solving abilities and enhance our understanding of why larger brains evolved in some species.

Author contributions: K.E.H. designed research; G.S. performed research; B.D., E.M.S., and S.B.-A analyzed data; S.B.-A., B.D., E.M.S., and K.E.H. wrote the paper; and S.B.-A. extracted video data.

The authors declare no conflict of interest.

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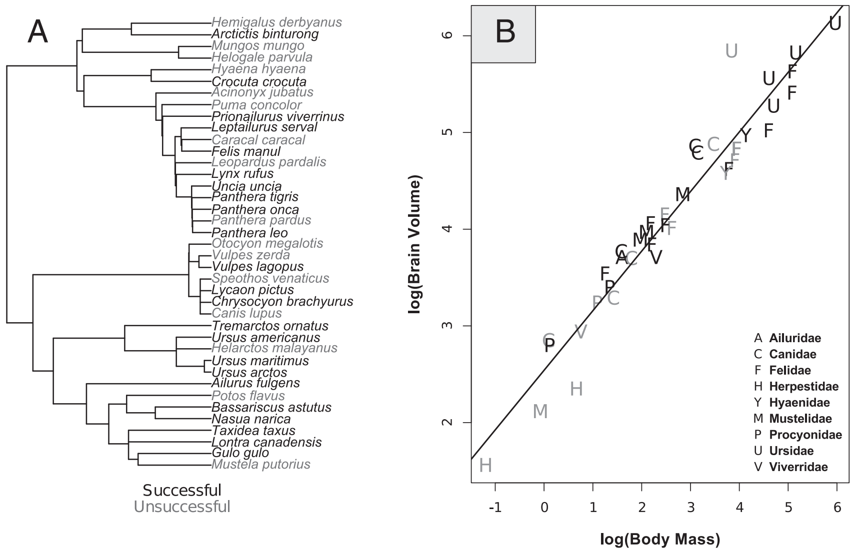

Fig. 1. (*A*) We tested the performance of zoo-housed individuals in 39 species from nine carnivore families by exposing them to our puzzle box problem, with the box scaled to accommodate body size. (*B*) The relationship between body mass (kilograms) and brain volume (milliliters) in 39 mammalian carnivore species. (*A*) Species in gray and (*B*) family names in gray represent species in which no tested subjects opened the box. Note that, in *B*, two species in the family Felidae (*Panthera pardus* and *Puma concolor*) have overlapping points.

problems than species exhibiting less behavioral diversity. Additionally, carnivores exhibit an impressive range of manual dexterity from the famously dexterous raccoons and coatis to the much less dexterous hyenas and cheetahs (26). Therefore, to ensure that our measure of problem-solving ability was not solely determined by manual dexterity and ensure that our problem-solving test was equivalently difficult across a range of species, we also examined the impact of manual dexterity on problem-solving success in this study.

Finally, the relative sizes of specific brain regions might be more strongly predictive of problem-solving ability than overall brain size relative to body size. Recently, Swanson et al. (27) used virtual brain endocasts to show that, although mammalian carnivore species with a higher degree of social complexity did not have larger total brain volumes relative to either body mass or skull size, they did have significantly larger cerebrum volumes relative to total brain volume. Therefore, we used deviance information criterion (DIC) model selection analysis to inquire whether any of four gross regional brain volumes (total cerebrum, posterior cerebrum, anterior cerebrum, and hindbrain) better predicted performance in our puzzle box trials than total brain size in a subset of 17 carnivore species for which these data were available from virtual brain endocasts (Dataset S1).

We retrieved data on brain size and the sizes of gross brain regions from published literature and used phylogenetic comparative statistics to assess relationships among these measures, social complexity, behavioral diversity, manual dexterity, and performance measures obtained during box trials. We used social group size as our proxy for social complexity, because in an earlier comparative study of mammalian carnivores, Swanson et al. (27) found that group size was just as effective of a proxy as the first axis from a principal component analysis of several different measures of social complexity in carnivores. We used an established measure of behavioral diversity, which we obtained by calculating the number of different behaviors exhibited by individuals from each species while interacting with the puzzle box (24, 28–30). To assess manual dexterity, we recorded occurrences of 20 types of forelimb movements following the work by Iwaniuk et al. (26). Finally, we used measures taken from virtual brains to analyze the effects of the size of specific gross brain regions on performance in puzzle box trials. These measures allowed us to inquire whether specific neuroanatomical or socioecological measures can help explain variation in problem-solving ability across species.

Results

We tested one to nine individuals in each of 39 species (mean = 4.9 individuals; median = 5) (Table S1). Of 140 individuals tested, 49 individuals (35%) from 23 species succeeded at opening the puzzle box (Fig. 1*A*, Table S1, and Movie S1). The proportion of individuals within each species that succeeded at opening the box varied considerably among families, with species in the families Ursidae (69.2% of trials), Procyonidae (53.8% of trials), and Mustelidae (47.1% of trials) being most successful at opening the puzzle box and those within the family Herpestidae (0%) being the least successful (Table S1). Total brain volume corrected for body mass varied among the species that we tested, with Canid and Ursid species having the largest brains for their body mass and Viverrid, Hyaenid, and Herpestid species having the smallest brains for their body mass (Fig. 1*B* and Table S1).

It appeared that the majority of subjects in our study actually gained an understanding of the puzzle and how to open it. If individuals were only using brute force to open the box or emitting exploratory behaviors without any understanding of how the puzzle works, then we should not have seen any evidence of learning the solution over time. To investigate whether the test subjects were actually learning the solution to the problem, we ran a non-phylogenetically corrected generalized linear mixed-effects model to examine how work time changed over successive trials for successful individuals. Work time significantly decreased as trial number increased ($F_{9,97} = 2.57$; $P = 0.01$), indicating that successful individuals improved their performance with experience.

The top model based on DIC model selection was one that contained brain volume, body mass, latency to approach the puzzle box, time spent trying to open the box, manual dexterity, behavioral diversity, and group size (Table 1). The only statistically indistinguishable model (i.e., $\Delta\mathrm{DIC} < 2$) did not include group size but was otherwise the same (Table 1). Individuals from carnivore species with both larger absolute brain volumes and larger brain volumes relative to their overall body mass were better than others at opening the puzzle box, but only relative brain volume was a statistically significant predictor [*P* value from Markov Chain Monte Carlo (pMCMC) = 0.013] (Figs. 2 and 3, Table 2, and Table S2). Our results were insensitive to variation in both the total number of individuals tested per species and the minimum number of trials conducted per individual. Specifically, we obtained the same qualitative results if we limited our analyses to only species in which at least three (398 trials on 112 individuals from 23 species) (Table S3) or four individuals (348 trials on 97 individuals from 18 species) (Table S4) were tested per species, and if we restricted our analyses only to individuals to which we administered at least three separate trials (total number of trials per individual was 3–10) (Table S5). Additionally, if we restricted our analyses only to trials 1–3 for individuals that were tested at least three times (388 trials with 39 species), we found that individuals from species with a larger brain volume for their body mass tended to be more likely to open the puzzle box (pMCMC = 0.052) (Table S6).

Individuals from species with large average group sizes, such as banded mongoose (average group size = 23.7 individuals), were

Table 1. Model comparisons using DIC model selection analysis to investigate the predictors of success in opening the puzzle box in 39 carnivore species

Fixed effects	λ-Posterior mode	λ-Mean (95% credible interval)	DIC	ΔDIC
BV + BM + L + WT + D + BD + GS	0.94	0.85 (0.49–0.99)	283.2	0
BV + BM + L + WT + D + BD	0.93	0.82 (0.33–0.99)	284.9	1.7
L + WT + D + BD + GS	0.95	0.87 (0.62–0.99)	286.4	3.2
L + WT + D + BD	0.96	0.85 (0.56–0.99)	288.5	5.3
WT + D + BD	0.93	0.84 (0.54–0.99)	288.5	5.3
BV + BM + L + GS	0.97	0.91 (0.76–0.99)	293.3	10.1
BV + BM + L	0.95	0.88 (0.65–0.99)	294.3	11.1
BV + BM + GS	0.98	0.91 (0.73–0.99)	294.5	11.3
L + GS	0.97	0.92 (0.78–0.99)	296.4	13.2
BV + BM	0.96	0.88 (0.65–0.99)	296.6	13.4
GS	0.97	0.91 (0.73–0.99)	298.1	14.9
Intercept	0.96	0.90 (0.71–0.99)	299.9	16.7

Model terms are behavioral diversity (BD), body mass (BM), brain volume (BV), dexterity (D), group size (GS), latency to approach puzzle box (L), and time spent working trying to open the puzzle box (WT).

no more successful at opening the puzzle box (pMCMC = 0.79) (Table 2) than individuals from solitary species, such as black bears (group size = 1) or wolverines (group size = 1). To further test whether social complexity affected carnivores' ability to open the puzzle box, we also compared success at opening the puzzle box between solitary species (group size = 1) and social species (group size > 1) where group size was a binary predictor. This comparison indicated that social species were no better at opening the puzzle box than solitary species (pMCMC = 0.99) (Table S7).

Surprisingly, individuals from species with larger body sizes were less successful than smaller-bodied species at opening the puzzle box (pMCMC = 0.036) (Table 2). Individuals that were more dexterous (pMCMC = 0.08) (Table 2) and those that spent more time attempting to open the puzzle box (pMCMC = 0.08) (Table 2) tended to be more successful, although neither of these were statistically significant. Individuals that more quickly approached the puzzle box (pMCMC = 0.57) (Table 2) or those that used a greater diversity of behaviors when interacting with the puzzle box (pMCMC = 0.39) (Table 2) were no more successful than others at opening the box. In nine of the puzzle box trials, individuals opened the box door but did not retrieve the food reward, which might reflect underlying differences in motivation. We included these trials in our main analyses (Table 2), but also, we ran our analyses without these nine trials and obtained the same qualitative results (Table S8).

In our brain region analyses, there was no obvious top model that best explained success at opening the puzzle box (Table 3). Models containing relative anterior cerebrum volume (anterior to the cruciate sulcus; **Δ**DIC = 0) and posterior cerebrum volume (posterior to the cruciate sulcus; **Δ**DIC = 0) were the two models with the lowest DIC values (Table 3). However, models containing hindbrain volume (which includes both cerebellum and brainstem volumes; **Δ**DIC = 0.2) or total cerebrum volume (**Δ**DIC = 0.3) were not considerably worse. Notably, models containing body mass and total brain volume in addition to the volume of one of four specific brain regions all had lower DIC values than a model containing only body mass and total brain volume (**Δ**DIC ranged from 1.9 to 2.2) (Table 3). This result suggests that the addition of the volume of a brain region to the model improved its ability to predict performance in the puzzle box trials over a model containing only total brain volume (Table 3). In none of the models using the reduced dataset were the relative sizes of any specific brain region associated with success in opening the puzzle box (Table S9).

Discussion

The connection between brain size and cognitive abilities has been called into question by both a study pointing out the impressive cognitive abilities of small-brained species, such as bees and ants (7), and another study doubting that overall brain size is a valid proxy for cognitive ability (9). In the former case, Chittka and Niven (7) argue that larger brains are partially a consequence of the physical need for larger neurons in larger animals and partially caused by increased replication of neuronal circuits, which confers many advantages for larger-brained species, such as enhanced perceptual abilities and increased memory storage. Chittka and Niven (7) conclude that neither of these properties of larger brains necessarily enhance cognitive abilities. Interestingly, our results actually show that carnivore species with a larger average body mass performed worse than smaller-bodied species on the task that we presented to

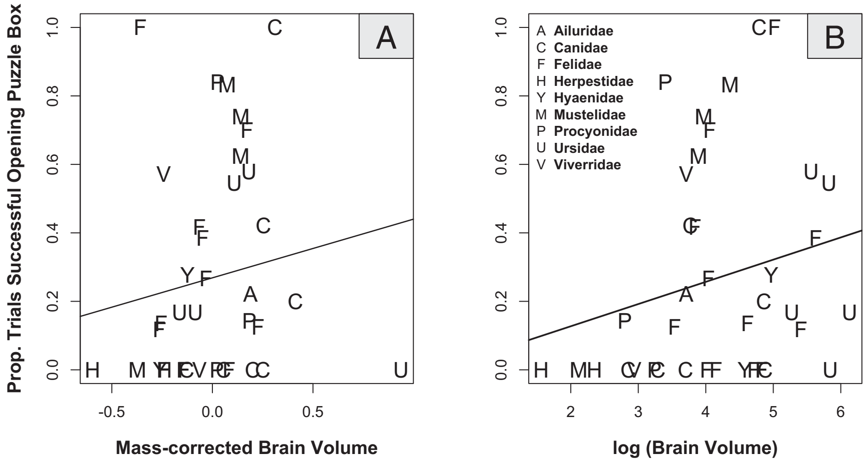

Fig. 2. (*A*) Carnivore species with larger brain volumes for their body mass were better than others at opening the puzzle box. (*B*) There was no significant relationship between absolute brain volume and success at opening the puzzle box in carnivore species when body mass was excluded from the statistical model. Data presented represent the average proportion of puzzle box trials in which species were successful and are for presentation purposes only, whereas statistical results from our full model used for our inferences are shown in Table 2. Mass-corrected brain volume in *A* is from a general linear model and for presentation purposes only; statistical results from the full model are shown in Table 2.

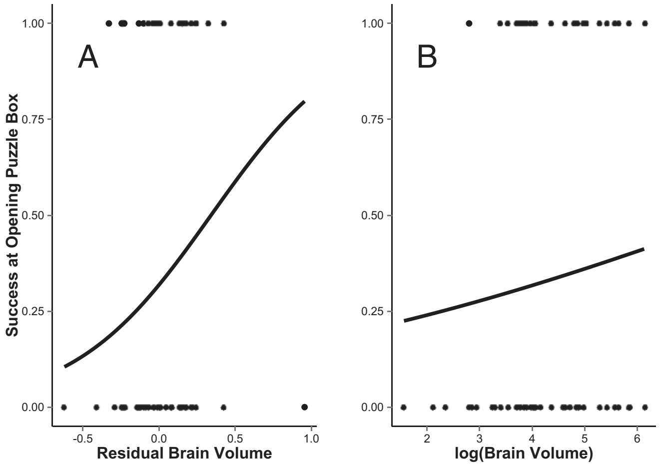

Fig. 3. (*A*) Individuals from carnivore species with larger brain volumes relative to their body mass were significantly better than others at opening the puzzle box (Table 2). (*B*) There was no significant relationship between absolute brain volume and success at opening the puzzle box in our individual-level analyses in which body mass was excluded (Table S2). Individuals with success equal to one opened the box, whereas those with success equal to zero did not. Mass-corrected brain volume in *A* is from a general linear model and for presentation purposes only; full statistical results are shown in Table 2 and Table S2. Regression lines represent predicted relationships from statistical models investigating the association between (*A*) brain volume relative to body mass or (*B*) log (brain volume) and success at opening the puzzle box.

them. Thus, it truly does seem that a larger brain size relative to body size is an important determinant of performance on this task, and it is not the case that larger animals are more successful simply because their brains are larger than those of smaller species.

Regarding whether overall brain size is a valid proxy for cognitive abilities, the use of whole-brain size as a predictor of cognitive complexity in comparative studies is questioned, because the brain has different functional areas, some of which are devoted to particular activities, such as motor control or sensory processing. Given this high degree of modularity in the brain, Healy and Rowe (8, 9) argue that overall brain size is unlikely to be a useful measure when examining how evolution has shaped the brains of different species to perform complex behaviors. Although the brain has functional modules, such as the hippocampus or the olfactory bulbs, which may be under specific selection pressures (31), these modules may also exhibit coordinated changes in size because of constraints on ways in which the brain can develop (32). In addition to functionally specialized modules, the brain also contains broad areas, such as the mammalian neocortex, that control multiple processes. Thus, there are reasons to believe that overall brain size may be an informative proxy for cognitive abilities, despite the modular nature of the brain.

Here we examined relationships between relative brain size, size of specific brain regions, and problem-solving success. Although none of the regional brain volumes that we examined significantly predicted success on this task (Table S9), the addition of the volume of these brain regions improved the ability of our models to explain performance in the puzzle box task over a model containing only total brain volume (Table 3). We emphasize, however, that only 17 species were included in that analysis. Nevertheless, relative brain size was a significant predictor of problem-solving success across species, and this result was robust in all of our analyses. Thus, our data provide important support for the idea that relative brain size can be useful in examining evolutionary relationships between neuroanatomical and cognitive traits and corroborate results from artificial selection experiments showing that larger brain size is associated with enhanced problem solving (5). It will be important in future work to use more detailed noninvasive brain imaging methods rather than endocasts to evaluate whether hypothetically important brain areas, such as prefrontal and cingulate cortexes, contribute to the relationship between brain size and performance during problem solving.

Assessment of the ecological and neuroanatomical predictors of problem-solving ability has some important implications for hypotheses proposed to explain the adaptive value of large brains and sophisticated cognition. One such hypothesis that has garnered much support in primate studies is "the social brain hypothesis" (33, 34), which proposes that larger brains evolved to deal with challenges in the social domain. This hypothesis posits that selection favored those individuals best able to anticipate, respond to, and perhaps even manipulate the actions of conspecific group members. However, a major shortcoming of the social brain hypothesis (35, 36) is its apparent inability to explain the common observation that species with high sociocognitive abilities also excel in general intelligence (37, 38). There is, in fact, a long-standing debate as to whether animal behavior is mediated by cognitive specializations that have evolved to fulfill specific ecological functions or instead, domain-general mechanisms (38, 39). If selection for social agility has led to the evolution of domain-general cognitive abilities, then species living in social groups should solve technical problems better than solitary species. However, we found that carnivore species living in social groups performed no better on our novel technical problem than solitary species. Thus, whereas social complexity may select for enhanced ability to solve problems in the social domain (40), at least in carnivores, greater social complexity is not associated with enhanced ability to solve a novel technical problem.

Table 2. Results from Bayesian phylogenetic generalized linear mixed-effects models to investigate the predictors of success in opening the puzzle box in 39 mammalian carnivore species

	Effective sample size	Posterior mean (95% CI)	Posterior mode	pMCMC
Random effect				
Species	3,094	13.8 (0.0007–40.4)	4.3	—
Individual identification	2,791	21 (7.6–38.2)	16.1	—
Fixed effect				
Intercept*	3,284*	−36.5 (−60.7 to −16.1)*	−30.6*	0.0003*
Brain volume*	3,284*	8.5 (1.3–16.3)*	7.8*	0.013*
Body mass*	3,720*	−4.6 (−9.2 to −0.2)*	−4.9*	0.036*
Latency to approach	3,284	−0.12 (−0.5–0.3)	−0.1	0.57
Work time	2,493	0.34 (−0.04–0.7)	0.4	0.08
Behavioral diversity	3,018	1.7 (−1.9–6)	1.2	0.39
Dexterity	3,284	2.7 (−0.3–5.8)	2.2	0.08
Group size	3,284	−0.04 (−0.3–0.2)	−0.02	0.79

pMCMC is the Bayesian *P* value. Sample sizes are 495 trials on 140 individuals from 39 different species. 95% CI, 95% credible interval.
*Statistically significant.

Table 3. Model comparisons using DIC model selection to investigate whether the volumes of specific brain regions better predicted success in opening the puzzle box than total brain volume in 17 mammalian carnivore species

Model name	Fixed effects	λ-Posterior mode	λ-Mean (95% CI)	DIC	ΔDIC
Anterior cerebrum	AC + BM + BV	0.006	0.42 (0.0003–0.99)	88.4	0
Posterior cerebrum	PC + BM + BV	0.004	0.37 (0.0002–0.98)	88.4	0
Brainstem/cerebellum	BS/CL + BM + BV	0.006	0.42 (0.004–0.99)	88.6	0.2
Cerebrum	C + BM + BV	0.006	0.41 (0.0003–0.99)	88.7	0.3
Brain	BV + BM	0.005	0.36 (0.0002–0.98)	90.6	2.2

Model terms are volume of anterior cerebrum (AC), body mass (BM), volume of brainstem and cerebellum (BS/CL), volume of total brain (BV), volume of total cerebrum (C), and volume of posterior cerebrum (PC). 95% CI, 95% credible interval.

Our results are similar to those obtained in the work by MacLean et al. (12), which examined relationships among brain size, social complexity, and self-control in 23 species of primates. In both that study and our own study, species with the largest brains showed the best performance in problem-solving tasks. However, in neither primates nor carnivores did social complexity predict problem-solving success. This finding is also consistent with results obtained in the work by Gittleman (41), with analysis of 153 carnivore species that revealed no difference in brain size relative to body size between social and solitary species. Nevertheless, in this study, we were only able to present carnivores with a single problem-solving task, and we were only able to test one to nine individuals per species. Ideally, future studies will present a large array of carnivores with additional cognitive challenges and will test more individuals per species.

A second hypothesis forwarded to explain the evolution of larger and more complex brains, the cognitive buffer hypothesis (42, 43), posits that large brains evolved to allow animals to cope with socioecological challenges and thus, reduce mortality in changing environments. Previous work has shown convincingly that diet is a significant predictor of brain size in carnivores (27), as it is in primates (12), and this study shows that carnivore species with larger brains are more likely to solve a novel technical problem. However, an explicit test of the cognitive buffer hypothesis has not yet been attempted with mammalian carnivores.

Overall, our finding that enhanced problem solving is related to disproportionally large brain size for a given body mass is important for several reasons. First, although there is correlational evidence for an association between absolute or relative brain size and problem-solving abilities, experimental evidence is extremely rare. The lack of experimental evidence has led to criticisms of the use of brain size as a proxy for problem-solving abilities (8, 9, 44). We offer experimental evidence that brain size is, indeed, a useful predictor of performance, at least in the single problem-solving task that we posed to our carnivore subjects. Although only brain size relative to body mass was a significant predictor of success with our puzzle box, species with larger absolute brain volumes also tended to be better than others at opening the puzzle box (Figs. 2 and 3 and Table S2). Second, the vast majority of work on this topic has focused on primates, fish, and birds (5, 10, 11, 13–16). Our results offer new evidence for the relationship between brain size and problem-solving abilities in mammalian carnivores. The previous lack of support for this relationship across a diverse set of taxa has limited both its validity and its generality. Thus, the findings presented here represent an important step forward in our understanding of why some animals have evolved large brains for their body size.

Materials and Methods

From 2007 to 2009, we presented puzzle boxes to myriad carnivores housed in nine North American zoos (Fig. 1*A* and Dataset S1). Because we were testing animals that ranged in size from roughly 2 to 300 kg, we used two steel mesh puzzle boxes; the larger box was 63.5 × 33 × 33 cm, and the smaller box was one-half that size. The smaller box was presented to species with an average body mass of <22 kg, such as river otters, kinkajous, sand cats, and other small-bodied carnivores (Dataset S1). The larger box was presented to species with an average body mass >22 kg, including snow leopards, wolves, bears, and other large-bodied species (Dataset S1). For cheetahs (species average body mass = 50 kg) and wild dogs (species average body mass = 22.05 kg), both large and small boxes were used with some subjects, but their performance did not vary with box size (additional details are given in *SI Text*).

We videotaped all trials and extracted performance measures from videotapes using methods described elsewhere (24, 28, 45) (Movie S1). Extracted behaviors included the latency to approach the puzzle box, the total time spent trying to open the box, the number of different behaviors used in attempting to open the box, and a measure of manual dexterity (all described in *SI Text*). We then brought together data on success and performance measures during zoo trials with previously published data on total brain size and body mass (46).

We used Bayesian phylogenetic generalized linear mixed-effects models based on a Markov Chain Monte Carlo algorithm implemented in the R package MCMCglmm (47–49) to identify the variables predicting success or failure in solving this puzzle. These models allowed us to assess the effects of predictor variables on carnivores' success at opening the puzzle box after controlling for shared phylogenetic history.

For our analyses of how brain volume affected the ability of carnivores to open the puzzle box, we constructed 12 different models containing different combinations of the morphological, behavioral, and social characteristics of tested species or individuals (Table 1). In all models except that shown in Table S2, we included species' average body mass as a covariate so that we could assess the effects of brain volume on puzzle box performance relative to body mass (50, 51). We used DIC (51) to examine the relative degree of fit of the different models. DIC is analogous to Akaike's information criterion (52), and lower values for DIC suggest a better fit. We present DIC values for all models (Table 1) but only present results from the model with the lowest DIC (Table 2) (53).

In separate analyses, we performed five different Bayesian phylogenetic generalized linear mixed-effects models to determine whether the volume of any specific brain region better predicted success in opening the puzzle box than overall endocranial volume (Table 3). These models also included species' average body mass and total brain volume as covariates (27). Computed tomography data were available documenting both total endocranial volume and the volumes of specific brain regions from 17 different carnivore species in six families (Dataset S1). Overall endocranial volume was subdivided into (*i*) cerebrum anterior to the cruciate sulcus, (*ii*) cerebrum posterior to the cruciate sulcus, (*iii*) total cerebrum, and (*iv*) hindbrain, which includes both cerebellum and brainstem. The cerebrum anterior to the cruciate sulcus is comprised mainly of frontal cortex. Additional methodological details on the estimation of these brain region volumes can be found elsewhere (54–56) (*SI Text*).

Our response variable was binary (did or did not open puzzle box); therefore, we used a categorical error structure in MCMCglmm, and we fixed the prior for the residual variance to one ($V = 1$; fix = 1). We included random effects for species and individual identity in these models. We used weakly informative inverse γ-priors with a low degree of belief ($V = 1$; $\mu = 0.002$) for the random effect variance. All models were run for appropriate numbers of iterations, burn-ins, and thinning intervals to generate a minimum effective sample size of >2,000 for all parameters in all of the different models. We provide the mean, mode, and 95% credible interval from the posterior distribution of each parameter. We considered parameters to be statistically significant when the 95% credible intervals did not overlap zero and pMCMC was <0.05 (47). Detailed statistical methods are in *SI Text*.

Appropriate ethical approval was obtained for this work. This work was approved by Michigan State University Institutional Animal Care and Use Committee (IACUC) Approval 03/08-037-00 and also, the IACUCs at all nine zoos (St. Louis Zoo, Bergen County Zoo, Binder Park Zoo, Potter Park Zoo, Columbus Zoo, The Living Desert, Wild Canid Survival and Research Center, Turtle Back Zoo, and Denver Zoo) where testing was done.

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