containing 2.5×10^5 PBMCs was added to each well after counting in the presence of trypan blue. The plates were incubated at 37°C for 4 hours before harvesting 20 μl of supernatant. Percentage lysis was estimated as [(experimental counts − media control) × 100]/(detergent counts − media control). Lysis of non-peptide controls was subtracted to give peptide-specific lysis. Media controls were between 10 to 15% of detergent controls.

22. One tetramer-positive $CD8^+$ cell was cloned directly into each well of a 96-well flat-bottomed plate (Nunc) in the presence of 10^6 irradiated PBMCs from at least three donors, together with phytohemagglutinin (5 μg/ml, Wellcome) in RPMI 1640 (Gibco) supplemented with glutamine, penicillin, streptomycin, and 5% human serum. After 4 days, Lymphocult-T (Biotest) was added to a final concentration of 10%. After 3 weeks the cultures were expanded into 24-well plates.
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Biodiversity Assessment and Conservation Strategies

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The efficient representation of all species in conservation planning is problematic. Often, species distribution is assessed by dividing the land into a grid; complementary sets of grids, in which each taxon is represented at least once, are then sought. To determine if this approach provides useful surrogate information, species and higher taxon data for South African plants and animals were analyzed. Complementary species sets did not coincide and overlapped little with higher taxon sets. Survey extent and taxonomic knowledge did not affect this overlap. Thus, the assumptions of surrogacy, on which so much conservation planning is based, are not supported.

Practical conservation uses surrogate information, such as richness of indicator taxa, endemism (taxa restricted to a given area), or higher taxon richness (that is, genus or family richness) to identify possible conservation areas (*1–8*). Although not universally accepted (*9*), there is broad agreement that conservation areas should strive to sample regional features, a goal that is most efficiently accomplished with complementary sets (*10, 11*). These are sets of grids that contain all species in a taxon at least once (*10, 12*); the complementarity principle ensures that conservation areas represent all species efficiently and that rare species are included (*10*). Although the outcome of such a complementarity analysis provides a sound basis for the efficient conservation of the focal taxon, it is commonly assumed that the outcome is more widely applicable to other taxa (*13*).

The value of species richness, species rarity, and higher taxon richness as biodiversity surrogate measures ("traditional" surrogates) has been explored, and the consensus is that richness "hotspots" (highly species-rich areas) and "coldspots" (areas poor in species) rarely coincide; nor do hotspots and rare (restricted range) taxa generally coincide (*6, 14–17*). However, the surrogacy value of complementary sets has not been assessed. Here, the relation between traditional surrogate measures and complementary sets, as well as the degree of overlap among complementary sets across taxa, is investigated.

The study incorporated 9119 species, including well-studied taxa that are frequently used as biodiversity indicators (*4*), such as vascular plants (Plantae), mammals (Mammalia), birds (Aves), and butterflies (Hesperioidea and Papilionoidea), and less well-known taxa, such as termites (Isoptera), antlions (Myrmeleontidae), buprestid beetles (Buprestidae), and scarabaeoid beetles (Scarabaeoidea) (*18*). These taxa vary considerably with regard to survey extent and taxonomic knowledge. For example, birds are surveyed in all grid cells and all species are included, whereas ~20% of antlion species are included and these are surveyed in 8% of the grid cells in the study area. Species that were chosen for inclusion in the poorly surveyed taxa represent either the known fauna for the region (for example, buprestids and scarabs) or, where the majority of the fauna has not been adequately cataloged, a well-known monophyletic unit (antlions). In one instance (termites), only an incomplete set of published data from a systematic survey was available, resulting in poor species coverage (*19*). In none of these cases was there reason to presume that the species chosen are a nonrandom subset of the taxon as a whole with regard to geographic distribution.

Data from the Transvaal region (now including Gauteng, Mpumalanga, Northern, and part of North-West provinces; South Africa) were mapped on a 25 km by 25 km grid ($n = 474$), and complementary sets for each of the taxa were identified by means of a rarity-based algorithm (*12*). The study area is about the size of the United Kingdom and comprises 20% of the surface area of one of the most species-rich countries in the world. Richness hotspots and coldspots reflect the top 5% of species-rich and species-poor 25-km squares, respectively (*14*). Rare species are defined as those occurring in less than 24 squares (5% of 474 squares), and this rarity may be the consequence of a restricted range or inadequate sampling (*20*). The degree of spatial overlap among complementary sets, species richness (hotspots and coldspots), and areas containing rare taxa is expressed by the Jaccard coefficient (Table 1).

As in previous studies (*14*), we found little overlap within taxa using measures of richness (hotspots and coldspots) and rarity (*21*) (Fig. 1 and Table 1). The single exception was richness hotspots and rarity where the mean overlap was 50% (Table 1). This high value was due mostly to high overlap values in plants and in phytophagous insects (plants, buprestids, and butterflies all had overlap values exceeding 75%) (Table 1). Speciose plant regions in southern Africa include large numbers of rare plant species (*22*), and patterns in plant diversity are often a good predictor of patterns in insect diversity (*23*). This may account, at least to some extent, for the high overlap values of richness hotspots and rarity observed within each of these taxa.

Overlap among taxa for richness hotspots and coldspots is, respectively, highest between butterflies and plants (24%), and scarab and buprestid beetles (13%) (*24*). Overlap among areas containing rare taxa is most

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Table 1. Percentage overlap among types of priority conservation areas, species-based complementary sets for different taxa, and complementary sets representing different taxonomic levels. The overlap was calculated with the Jaccard coefficient [number of grids shared/(number of additional grids selected for taxon A + number of additional grids selected for taxon B)] × 100.

Comparisons/taxa	Mammals	Birds	Plants	Butterflies	Termites	Antlions	Scarab beetles	Buprestid beetles
Priority conservation areas								
Richness hotspots versus rare species	29.2	18.0	82.6	77.8	23.8	60.0	6.7	80.0
Richness coldspots versus rare species	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Complementary sets versus richness hotspots	8.1	20.0	8.6	16.3	11.1	16.7	32.1	19.7
Complementary sets versus richness coldspots	0.0	0.0	1.0	2.9	0.0	9.7	0.0	2.0
Complementary sets versus rare species	21.4	30.0	8.2	16.3	11.1	16.7	10.7	12.7
Complementary species sets								
Mammals		11.9	6.6	8.5	0.0	3.5	9.3	11.8
Birds			7.3	9.8	0.0	6.5	13.6	8.6
Plants				12.7	0.4	2.2	7.3	19.5
Butterflies					0.0	2.0	11.7	20.7
Termites						0.0	0.0	1.5
Antlions							10.0	2.9
Scarab beetles								14.3
Buprestid beetles								
Complementary sets representing different taxonomic levels								
Species versus genus	17.9	34.5	37.6	17.8	20.0	0.0	24.0	34.4
Species versus family	8.0	3.7	7.4	2.3	40.0	12.5	4.0	1.6

pronounced in mammals and birds (37%). Nonetheless, all of these overlap values are low, indicating that different taxa are speciose, species-poor, or have their rare species represented, in different grid cells (*24*).

The mean coincidence between complementary species sets and grids selected on the basis of richness (hotspots and coldspots), and between complementary sets and grids containing rare taxa, is well below 20% (Fig. 1 and Table 1). The highest overlap in complementary sets and richness hotspots is for scarab beetles (32%) and birds (20%); this overlap reached only 8% in mammals. Coincidence between complementary sets and rare taxa was highest in mammals (30%). Thus, grids selected for a single representation of each species tend not to be those with excessively high or unusually low species richness, nor do they include a disproportionate number of rare species (Table 1).

Pairwise comparisons of complementary species sets reveal a mean overlap of less than 10% (Fig. 2 and Table 1); maximum overlap (21%) is between butterflies and buprestid beetles. In multiple comparisons of complementary sets, no grid cell was shared by all taxa, and a maximum of six taxa shared complementary grids (coincidentally, $n = 6$ grids shared). This further emphasizes the lack of overlap of complementary sets across taxa. Thus, different conservation areas are required to conserve different taxa.

Complementary sets that represent genera and families show little overlap with species-based complementary sets across taxa (<30%) (Fig. 2 and Table 1). Maximum overlap between genus- and species-based sets is for plants (38%) and birds (35%), taxa that are well surveyed and systematically well known (*25*), and for buprestid beetles (34%), a group that has not been well surveyed and in which many species remain undescribed (*18, 26*). Similarly, overlap between family- and species-based sets is highest for termites (40%) and antlions (13%), which are either poorly surveyed or represented by few species in this analysis. In contrast, the overlap between well-surveyed and taxonomically well-represented groups, namely plants, birds, and mammals, was minimal, at 7, 4, and 8% respectively (Table 1). Patterns of overlap based on complementary sets were also inconsistent between taxa with changing hierarchical levels (for plants, overlap declines from 38 to 7% from genus to family level, whereas for termites there was an increase from 20 to 40%). Thus, selecting conservation areas by genus- or family-level data cannot result in efficient species-level conservation.

Our results provide little support for the notion that species complementary sets are congruent across taxa or that complementary sets are congruent with richness (hotspots, coldspots, or both) or areas harboring rare taxa, or both. In addition, our results suggest that the use of higher taxa as surrogates (*27*) for species-based complementary set selection holds little promise at a scale relevant to practical conservation planning. This largely undermines hopes for using "indicator taxa" or higher taxon surrogate information as biodiversity planning tools. These data

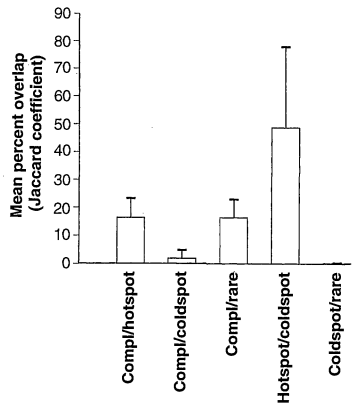

Fig. 1. The degree of spatial overlap (mean ± SD of Jaccard coefficient) between conservation areas generated by means of different prioritization criteria (species-based complementary areas, richness hotspots and coldspots, areas containing rare taxa).

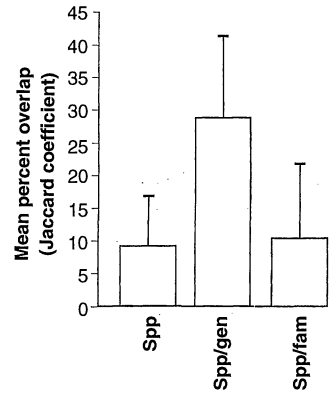

Fig. 2. The degree of spatial overlap (mean ± SD of Jaccard coefficient) among species-based complementary sets across higher taxonomic groupings (that is, species-based surrogacy) and overlap between the species-based priority conservation sets and sets generated by means of genus and family level data (that is, higher taxon surrogacy).

also support findings from a recent study that adopted a different approach and was conducted at a very different scale, yet also concluded that the prospects for indicator taxa are poor (*28*). Furthermore, conservation areas identified by means of traditional prioritization criteria [richness hotspots and coldspots and areas containing rare taxa (*21*)] are unlikely to be useful surrogates for representative complementary conservation networks. This lack of coincidence between taxa, hierarchical levels, and traditional criteria for priority conservation areas implies that all available species-based information should be incorporated into regional conservation assessments (*6*). Moreover, these results underscore the value of sound species-related distribution data for conservation planning and emphasize the necessity for survey research in conservation biology (*29*).

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Competition in Retinogeniculate Patterning Driven by Spontaneous Activity

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When contacts are first forming in the developing nervous system, many neurons generate spontaneous activity that has been hypothesized to shape appropriately patterned connections. In *Mustela putorius furo*, monocular intraocular blockade of spontaneous retinal waves of action potentials by cholinergic agents altered the subsequent eye-specific lamination pattern of the lateral geniculate nucleus (LGN). The projection from the active retina was greatly expanded into territory normally belonging to the other eye, and the projection from the inactive retina was substantially reduced. Thus, interocular competition driven by endogenous retinal activity determines the pattern of eye-specific connections from retina to LGN, demonstrating that spontaneous activity can produce highly stereotyped patterns of connections before the onset of visual experience.

The circuitry of the adult nervous system emerges from diffuse sets of early connections (*1*). Although molecular cues guide initial axonal projections, activity-dependent competition is thought to sculpt precise connections (*1–3*). Activity-dependent competition driven by visual input from each eye shapes the ocular dominance columns of the visual cortex (*3*). Likewise, activity-dependent competition between motor neurons shapes connections at the neuromuscular junction (*4*). Yet precise connections can form in the central nervous system before there is any external sensory input (*1*, *5*). Because many of these connections are highly stereotyped, it is generally thought that they are established in response to molecular cues, rather than by activity-dependent mechanisms (*1*). However, endogenous neural activity could pattern these connections through a competitive mechanism.

In the mammalian visual system, retinal ganglion cell inputs from each eye, initially intermixed within the lateral geniculate nucleus (LGN), become segregated during development before vision (*6–9*). Correlated bursts of spontaneous action potentials sweep in "waves" across the retina and are transmitted to the postsynaptic neurons in the LGN during this segregation (*10–12*). Segregation of eye-specific layers is dependent on neural activity. When all action potentials in the LGN are blocked by tetrodotoxin (TTX), layers fail to form (*13*). To directly investigate the role of activity-dependent competition between the inputs from the two eyes in the formation of eye-specific LGN layers, we analyzed the effects of a prolonged and selective blockade of the retinal waves in ferret kits (*Mustela putorius furo*).

Synaptic activation of neuronal nicotinic receptors (nAChR) on ganglion cells is necessary for the generation and propagation of retinal waves (*10*). Fluorescence imaging experiments (*14*) were used to monitor large numbers of retinal ganglion cells simultaneously during bath application of cholinergic agents to determine which compounds would block the retinal waves and therefore be potentially useful for in vivo intraocular application. Periodic increases in intracellular calcium concentration $\{[Ca^{2+}]_i\}$, which are known to be associated with cholinergic synaptic currents measured in ganglion cells (*10*), are also correlated with bursts of action potentials recorded from ganglion cells (Fig. 1A). Most action potential bursts occurred simultaneously with changes in fluorescence associated with waves propagating through the surrounding tissue [96%; n = 44 of 46 bursts recorded in nine cells from five retinas; animals' ages ranged from birth (P0) to postnatal day 9 (P9)].

Both 10 μM nicotine and 100 μM curare can block the periodic increases in $[Ca^{2+}]_i$ (*10*), but these agents are short-acting (*10*, *15*) and thus are poor candidates for intraoc-

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