Intraspecific morphological and genetic variation of common species predicts ranges of threatened ones

Trevon L. Fuller1, Henri A. Thomassen1,†, Manuel Peralvo2, Wolfgang Buermann1,3, Borja Milá1,4, Charles M. Kieswetter5, Pablo Jarrín-V6, Susan E. Cameron Devitt7, Eliza Mason8,9, Rena M. Schweizer8, Jasmin Schlunegger8, Janice Chan8, Ophelia Wang10,11, Christopher J. Schneider5, John P. Pollinger1,8, Sassan Saatchi1,12, Catherine H. Graham13, Robert K. Wayne8 and Thomas B. Smith1,8

1 Center for Tropical Research, Institute of the Environment and Sustainability, University of California, Los Angeles, La Kretz Hall, Suite 300, 619 Charles E. Young Dr. East, Los Angeles, CA 90095, USA
2 Facultad de Ciencias Ambientales Andinas (FCCA), Consorcio para el Desarrollo Sostenible de la Ecorregión Andina, Diego de Breda E17-169 y Clemente Lévi (Sector Bellavista), PO Box 17-21-1977, Quito, Ecuador
3 School of Earth and Environment, University of Leeds, Leeds LS2 9JT, West Yorkshire, UK
4 Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, José Gutiérrez Abascal 2, Madrid 28006, Spain
5 Department of Biology, Boston University, Boston, MA 02215, USA
6 Yasuní Research Station, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito, Ecuador
7 Department of Wildlife Ecology and Conservation, University of Florida, PO Box 110430, Gainesville, FL 32611-0430, USA
8 Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 621 Charles E. Young Dr. East, Los Angeles, CA 90095, USA
9 Department of Microbiology and Immunology, School of Medicine, University of North Carolina, 804 Mary Ellen Jones Building, Chapel Hill, NC 27599, USA
10 Department of Geography and the Environment, University of Texas at Austin, Mailcode A1100, Austin, TX 78712, USA
11 Center for Sustainable Environments, Northern Arizona University, PO Box 5767, Flagstaff, AZ 86011, USA
12 Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Drive, Pasadena, CA 91109, USA
13 Department of Ecology and Evolution, Stony Brook University, 650 Life Sciences Building, New York, NY 11794, USA

Predicting where threatened species occur is useful for making informed conservation decisions. However, because they are usually rare, surveying threatened species is often expensive and time intensive. Here, we show how regions where common species exhibit high genetic and morphological divergence among populations can be used to predict the occurrence of species of conservation concern. Intraspecific variation of common species of birds, bats and frogs from Ecuador were found to be a significantly better predictor for the occurrence of threatened species than suites of environmental variables or the occurrence of amphibians and birds. Fully 93 per cent of the threatened species analysed had their range adequately represented by the geographical distribution of the morphological and genetic variation found in seven common species. Both higher numbers of threatened species and greater genetic and morphological variation of common species occurred along elevation gradients. Higher levels of intraspecific divergence may be the result of disruptive selection and/or introgression along gradients. We suggest that collecting data on genetic and morphological variation in common species can be a cost effective tool for conservation planning, and that future biodiversity inventories include surveying genetic and morphological data of common species whenever feasible.

1. Introduction

To conserve biodiversity, 168 signatory nations at the 2010 conference of the Convention on Biological Diversity held in Nagoya, Japan agreed to establish...
protected areas comprising 17 per cent of the terrestrial area of the planet by 2020 [1]. Because protecting every species is infeasible, conservation planners typically focus on threatened species. However, identifying the most critical areas for protecting threatened species requires accurate distributional data for these species, most of which are rare and difficult to survey. This challenge has prompted conservation planners to search for surrogates for species of conservation concern [2]. To be effective, surrogate distributions must be easily measured and have a high probability of encompassing areas where threatened species occur [3].

Genetic and morphological variation of common species can be easily measured and are increasingly recognized for their potential utility in designing protected areas [4]. The ability to develop and use molecular markers to assess genetic variation in non-model organisms is increasingly possible owing to advancements in methods and decreases in costs [5]. In addition, measures of intraspecific morphological variation for many species can be collected easily in the field or obtained from museum specimens. If genetic and morphological traits of common species are effective for identifying and protecting areas where species of conservation concern occur, these attributes would make them excellent tools to use as indicators for conservation. For example, in the Atlantic Forest of Brazil, the genetic divergence of common frogs is an effective predictor of endemism in other taxa [6]. Here, we investigateWeb supplement for this paper (13MB).  

We also compared reserves designed based on genetic and morphological variation in common species to reserves designed based on only environmental variables or occurrences of birds and amphibians (see the electronic supplementary material, figure S1 summarizes the analysis). The analysis was restricted to western Ecuador because this region of dry forest is particularly threatened by anthropogenic impacts (see the electronic supplementary material, figure S2 shows our study region).

### (a) Measurement of genetic and morphological traits in common species and generalized dissimilarity modelling

Three birds, three bats and one frog species were analysed because they were abundant and easily sampled and represent a range of different vagilities and life histories ([4]; table 1). The seven species occupy distinct niches representing a diverse range of altitudes, vegetation communities and feeding ecologies, including insectivory, frugivory and nectarivory. Genetic data were available for the three birds, one bat and the frog (table 1). We developed anonymous loci (amplified fragment length polymorphisms (AFLPs), microsatellites and nuclear sequence) and targeted loci (recombination activating gene 1 (RAG1)) following published methods [4,10] to assess genetic variation among populations of common vertebrates. The number of threatened vertebrates is among the highest in the world [9].

#### Table 1. Measures of intraspecific morphological and genetic variation were developed for seven common species in western Ecuador.

<table>
<thead>
<tr>
<th>species</th>
<th>genetic markers (loci, individuals, sites)</th>
<th>morphological traits (n, sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>birds</td>
<td>amplified fragment length polymorphisms (AFLPs; 136, 178, 15)</td>
<td>wing length, tarsus length, tail length, bill length, bill width and bill depth (195, 15)</td>
</tr>
<tr>
<td>streak-necked flycatcher</td>
<td>microsatellites (10, 106, 9)</td>
<td>wing length, tarsus length, tail length, bill length, bill width and bill depth (122, 8)</td>
</tr>
<tr>
<td>bats</td>
<td>none</td>
<td>centroid size of the skull, angle of curvature of the zygomatic arch and forearm length (167, 43)</td>
</tr>
<tr>
<td>seba’s short-tailed bat</td>
<td>AFLPs (311, 83, 9)</td>
<td>centroid size of the skull, angle of curvature of the zygomatic arch and forearm length (86, 25)</td>
</tr>
<tr>
<td>frog</td>
<td>two anonymous loci and an 840 base-pair region of recombination activation gene 1 (RAG1; 3,76–127 per locus, 9)</td>
<td>snout-vent length, gape width, lengths of the metacarpal phalanges, length of the radio-ulna, lengths of the metatarsal phalanges, length of the tarsus, tibio–fibula length, femur length and lower jaw length (224, 16)</td>
</tr>
</tbody>
</table>

Two anonymous loci (amplified fragment length polymorphisms (AFLPs), microsatellites and nuclear sequence) and targeted loci (recombination activating gene 1 (RAG1)) following published methods [4,10] to assess genetic variation among populations of common
species. We used AFLP loci for the wedge-billed woodcreeper (*Glyphorynchus spirurus*) [10,11] and seba’s short-tailed bat (*Carollia perspicillata*; [4]), whereas for the masked flowerpiercer (*Diglossa cyanon*) and streak-necked flycatcher (*Mionectes stricillolis*), we developed microsatellites [4]. Finally, for the zurucuchu robber frog (*Hyloales buergeri*), we sequenced two anonymous loci and an 840 base-pair region of the RAG1 [4]. We then calculated the genetic distance between sampled populations. We used $D_{0}$ as the distance measure for the wedge-billed woodcreeper, streak-necked flycatcher and the masked flowerpiercer because this measure requires genotypic data, and we had microsatellites and AFLPs for these species. We used $D_{0}$ for the zurucuchu robber frog because this measure uses sequence data, which we had for the frog.

To quantify morphological variation among populations, following published methods [4], we measured morphological traits on each sampled individual (table 1): for birds, wing, tail, tarsus and bill length, bill width and bill depth; for bats, skull size, zygomatic arch and forearm length; and for the frog, snout-vent length, gape width and the length of the metacarpal phalange, radio-ulna, metatarsal phalange, tarsus, tibio-fibula and lower jaw. The frog morphological traits were corrected for size as described elsewhere, but regression analysis indicated that body size corrections were unnecessary for the birds and bats [4]. The morphological distance between pairs of sampled populations was calculated as the Euclidean distance between the mean phenotype in each population. The total number of genetic and morphological traits for the seven common species was 41 (36 morphological and five genetic traits; table 1).

We used generalized dissimilarity modelling (GDM), a matrix regression technique [12], to identify environmental variables that were significantly correlated with genetic and morphological distance. We used the implementation of GDM for the ArcView, and SPLS software packages to predict value of each of the 36 genetic and morphological traits across western Ecuador. For each trait, we assessed the importance of 14 independent variables that represent climate, vegetation phenology derived from satellite data and elevation [4,10]. The contributions of independent variables to explaining each trait were tested by permutation (models for 36 traits performed better than random [4,10]).

(b) Species of conservation concern

Our dataset on species of conservation concern comprised a total of 29 plants and vertebrates. Peralvo et al. [13] detail the species selection criteria, which we will summarize here. For vertebrates, we started with 315 species classified as conservation targets by TNC-Ecuador’s technical staff because the species are under threat and restricted to the Equatorial Pacific ecoregion of western Ecuador and northwestern Peru. The list was filtered to retain species that were in the 2012 IUCN Red List and had at least 10 records in our vertebrate occurrence database [13]. The refined list comprised five birds and six mammals (table 2). We required 10 or more occurrences because we constructed geographical distribution models for each species (see below), and the modelling technique that we used needed 10 observations to make accurate predictions [14]. For plants, we began with TNC-Ecuador’s list of vascular plants of conservation concern, which comprises 1050 species. Botanists specializing in Ecuador (B. León and D. Padilla) identified one diagnostic species that was representative of each vegetation community in the Equatorial Pacific (communities were defined as per [15]). To keep the analysis tractable, we filtered the list to retain just these diagnostic species. Last, we excluded diagnostic species with less than 10 records in the Missouri Botanical Garden’s VAST database [13]. This resulted in a list of 18 plants (table 2), all of which are restricted to the Equatorial Pacific and five of which are in Ecuador’s national plant Red List [16]. We did not limit our list of plants to Red-listed species because the exclusive use of the Red List may not adequately represent plants [17], especially in a hotspot of floral endemism such as Ecuador, and may not capture the threat of land clearing in dry forest vegetation communities west of the Andes. Instead, we selected plants that were deemed to be of conservation concern based on expert knowledge and were also representative of vegetation communities that have a high risk of being deforested. Hereafter, the term ‘threatened species’ refers to the 18 plants, five birds and six mammals selected using the aforementioned criteria.

Next, we calculated the number of threatened species occurring in reserves designed based on genetic and morphological variation of common species. We aggregated the occurrence data to the 100 km² resolution (see the electronic supplementary material, table S1) because the correlation between surrogates and threatened species is typically highest at this resolution [18]. The resulting dataset consisted of 639 sites that abut each other and occupy a continuous swath of land in western Ecuador (see the electronic supplementary material, figure S2). The same spatial scale was used for the analyses based on environmental variables, threatened species and genetic and morphological traits. In addition to analysing raw data on the known occurrences of threatened species, we also examined models of the geographical distribution of threatened species, which predict species’ niches using occurrences and environmental variables. The models used here were constructed by Peralvo et al. [13] with the GARP software package using environmental data comprising temperature and precipitation variables, elevation and vegetation type. We calculated the number of threatened species that had 10 per cent of their predicted distributions included in reserves designed based on genetic and morphological variation of common species.

(c) Modelling the geographical range of common birds and amphibians

Since the occurrence of common species are effective surrogates in some geographical regions [19], we also calculated the number of threatened species in reserves designed based on occurrences of common birds and amphibians. This aspect of the analysis did not incorporate genetic and morphological variation within common species. We computed occurrences of common birds and amphibians in western Ecuador from maps available from the NatureServe INFONATURA database (www.natureserve.org/infonatura; [20–22]). The dataset comprised 1397 amphibian and bird species (Columbiformes and suboscine passerines) mapped at a 1 km resolution, which we aggregated to 100 km². For each 100 km² site, the dataset indicated whether each of the 1397 species was present or absent in the site.

(d) Modelling environmental variables

Certain climatic, edaphic and topographic features can be effective surrogates for predicting the occurrence of some threatened species [23]. We therefore compared the predictive performance of genetic and morphological divergence with that of environmental variables. Environmental variables (aspect, climate, elevation and slope) available at the global scale were obtained from online global databases and clipped to our study region (see the electronic supplementary material, table S3). With the exception of soil type, the environmental variables were continuous. We discretized each continuous variable into several categorical variables. In general, the effectiveness of an environmental variable as a surrogate for threatened species is expected to increase with the number of categories. This is because a large number of categories tend to capture fine-scale environmental variation in the sites occupied by the species. However, the computational difficulty of the area prioritization problem also increases with the
Table 2. Ecuadorian species of conservation concern included in the analysis (D, decreasing; S, stable; U, unknown; MOBOT, Missouri Botanical Garden).

<table>
<thead>
<tr>
<th>family</th>
<th>scientific name</th>
<th>common name</th>
<th>occurrences</th>
<th>IUCN Red List</th>
<th>IUCN population trend</th>
<th>Ecuador's plant Red List</th>
<th>MOBOT plants of Ecuador database</th>
</tr>
</thead>
<tbody>
<tr>
<td>birds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cracidae</td>
<td>Ortalis erythroptera</td>
<td>rufous-headed</td>
<td>16</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fringillidae</td>
<td>Carduelis siemiradzki</td>
<td>saffron siskin</td>
<td>15</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picidae</td>
<td>Campephilus</td>
<td>guayaquil woodpecker</td>
<td>24</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psittacidae</td>
<td>Aratinga erythroguara</td>
<td>red-masked parakeet</td>
<td>27</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fringillidae</td>
<td>Carduelis siemiradzki</td>
<td>saffron siskin</td>
<td>15</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picidae</td>
<td>Campephilus</td>
<td>guayaquil woodpecker</td>
<td>24</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psittacidae</td>
<td>Aratinga erythroguara</td>
<td>red-masked parakeet</td>
<td>27</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mammals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canidae</td>
<td>Pseudalopex sechuran</td>
<td>sechuran zorro</td>
<td>21</td>
<td>X</td>
<td>U</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Felidae</td>
<td>Leopardus pardalis</td>
<td>ocelot</td>
<td>45</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puma concolor</td>
<td>Puma concolor</td>
<td>puma</td>
<td>28</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muridae</td>
<td>Oryzomys xantholus</td>
<td>yellowish rice rat</td>
<td>35</td>
<td>X</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muridae</td>
<td>Oryzomys xantholus</td>
<td>yellowish rice rat</td>
<td>35</td>
<td>X</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phyllotomidae</td>
<td>Antibus alpinus</td>
<td>fraternal fruit-eating bat</td>
<td>40</td>
<td>X</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sciurus xantheolus</td>
<td>guayaquil squirrel</td>
<td>51</td>
<td>X</td>
<td>U</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vascular plants</td>
<td>Fabaceae</td>
<td>Albizia multiflora</td>
<td>none</td>
<td>28</td>
<td>U</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caesalpinia julibrissina</td>
<td>none</td>
<td>none</td>
<td>25</td>
<td>U</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centrolobium ochysmium</td>
<td>amarillo</td>
<td>16</td>
<td>U</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Geoffroea spinosa</td>
<td>almandon</td>
<td>24</td>
<td>U</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leucena triodes</td>
<td>capsia</td>
<td>45</td>
<td>U</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Machaerium millei</td>
<td>chiche</td>
<td>27</td>
<td>U</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pithecellobium excelsum</td>
<td>chiquin</td>
<td>28</td>
<td>U</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swartsia haughtii</td>
<td>none</td>
<td>38</td>
<td>X</td>
<td>D</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anacardiaceae</td>
<td>Mauria heterophylla</td>
<td>fenogreco</td>
<td>23</td>
<td>U</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anacardiaceae</td>
<td>Anthurium</td>
<td>none</td>
<td>42</td>
<td>U</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Anacardiaceae</td>
<td>dolichostachyum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bombacaceae</td>
<td>Eriotheca ruzii</td>
<td>pasallo</td>
<td>19</td>
<td>U</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boraginaceae</td>
<td>Corda lutea</td>
<td>yellow geiger</td>
<td>26</td>
<td>U</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capparaceae</td>
<td>Capparis heterophylla</td>
<td>caper shrub</td>
<td>17</td>
<td>X</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capparaceae</td>
<td>Capparis heterophylla</td>
<td>caper shrub</td>
<td>17</td>
<td>X</td>
<td>D</td>
<td></td>
</tr>
</tbody>
</table>

(Continued.)
number of categories. Sarkar et al. [18] analysed the effect of the number of categories on the effectiveness of environmental surrogates for threatened species on three continents, and identified the optimal number of classes into which climatic and topographic variables should be partitioned to represent threatened species effectively while remaining computationally tractable [18,24]. Our analysis uses their partitioning scheme, which divides each climatic variable into 4–10 equal interval classes (see the electronic supplementary material, table S3). Next, we designed reserves to include at least 10 per cent of the sites in each class of each environmental variable. We then compared the models of the predicted distributions of the threatened species with these reserves. We determined the percentage of threatened species that had 10 per cent of their predicted distributions included in reserves designed based on the environmental variables.

(e) Assessing the effectiveness of surrogates for threatened species when reserves are prioritized without areal constraints

We used a complementarity-based algorithm in the ResNet v. 1.2 software package [25] to select sites containing each surrogate (genetic and morphological traits of common species, occurrences of common amphibians and birds and environmental variables). Complementarity chooses sites iteratively such that the site selected in each iteration contains the largest number of surrogates not included at the targeted levels in sites chosen in previous iterations ([3]; The targeted level is the percentage of the surrogates’ occurrences that we want to include in reserves.) Since reserve selection algorithms require discrete data [26], we divided the GDM predictions about genetic and morphological divergence into 50 distinct categories per trait (the use of a slightly different number of classes yields similar results). Fifty were selected so that the reserve selection algorithm would remain computationally tractable. We required the selected sites to satisfy a conservation target of 10 per cent for each surrogate [26], meaning that the reserves included at least 10 per cent of the occurrences of each surrogate. We selected 10 per cent because this target has seen widespread use in conservation planning exercises [18,24], but the use of higher targets gave similar results (see the electronic supplementary material, figure S4). However, in this stage of the analysis, there was no constraint on the amount of land in western Ecuador that could be included in the selected sites.

After designing reserves, we compared the number of threatened species included in sites selected based on genetic and morphological variation of common species, environmental variables and occurrences of amphibian and bird species using a one-tailed, unequal variance t-test. If we had used a two-tailed test, the rejection of the null hypothesis would have meant that the number of threatened species is different in areas selected based on genetic and morphological traits, and areas selected based on environmental variables or occurrences of common species without indicating the direction of the difference. Since we hypothesized that there would be more threatened species at sites selected based on genetic and morphological variation of common species, we used a one-tailed test. The rejection of the null hypothesis in a one-tailed test implies that the number of threatened species in areas prioritized based on genetic and morphological variation is greater than the number of threatened species in sites selected based on environmental variables or occurrences of common birds and amphibians. The test assumed unequal variance because the number of sites selected based on environmental variables, species occurrences and genetic and morphological variation of common species were different. Owing to the unequal sample sizes, the unequal variance test was the most appropriate because it adjusts the test statistic and degrees of freedom based on the number of samples in the two groups [16].
(f) Assessing the effectiveness of surrogates for threatened species when reserves are prioritized subject to areal constraints

Since land cost is an important determinant of which sites may be appropriate for inclusion in a conservation plan [27, 28], and analysing cost makes our planning exercise more realistic, we incorporated a budgetary ceiling on the total area of reserves selected based on genetic and morphological traits of common species, occurrences of common birds and amphibians and environmental variables (see the electronic supplementary material analyses other measures of land cost, including proximity to infrastructure). For each surrogate, we constructed a curve called a surrogacy curve using the SURROGACY software package [29, 30]. A surrogacy curve is a generalization of a species accumulation curve that lists how the number of threatened species increases as more land is selected based on a surrogate [23]. Each curve describes the properties of 17 different reserve networks selected based on the surrogate. We first selected reserves based on a surrogate subject to the constraint the reserves could only occupy 1 per cent of the land in western Ecuador and calculated the percentage of threatened and endangered species in the selected sites. Next, we selected sites based on a surrogate subject to the constraint that the sites could only occupy 2 per cent of western Ecuador and calculated the percentage of threatened and endangered species that were included in the selected sites. The procedure was repeated at 1 per cent increments up to 17 per cent of the land in western Ecuador. The upper target of 17 per cent was used because the parties to the Convention on Biological Diversity agreed to establish protected areas comprising 17 per cent of the terrestrial area of the planet [1]. This target is arbitrary to the extent that one should protect whatever per cent of a species’ range is sufficient to ensure its survival. However, owing to the difficulty of estimating this percentage, the 17 per cent target is used by many countries [31].

To provide a comparison with the three surrogate sets (genetic and morphological traits, occurrences of common species or environmental variables), we also randomly selected sites 10 000 times. The ‘random’ curve in the surrogacy plot shows the mean number of threatened and endangered species included in sites chosen randomly at targets ranging from 1 to 17 per cent. In the curves representing the other surrogates in the surrogacy plot, the y-coordinate of each point is the areal constraint (1–17%) on the sites selected based on the surrogate. The y-coordinate of each point is the percentage of the 29 threatened species that are included in sites selected subject to the areal constraint. When a curve representing a surrogate is above the curve for random sites, the surrogate does better than random. When a curve for one surrogate is above the curve for another surrogate, the first surrogate is more effective than the second surrogate.

3. Results

(a) The effectiveness of genetic and morphological traits as surrogates for threatened species when reserves are prioritized without areal constraints

When we designed reserves without restricting the amount of land that could be put under a conservation plan, sites selected based on genetic and morphological divergence of common species contained a higher proportion of threatened species than sites selected at random or regions selected using environmental variables or bird and amphibian occurrences as a proxy (figure 1). In particular, sites selected based on genetic and morphological traits contain 2.1 times as many threatened species as sites selected based on environmental variables, and 1.7 times as many as sites based on bird and amphibian occurrences (see the electronic supplementary material, figure S2). The number of threatened and endangered species in sites selected at random or regions selected using environmental variables or bird and amphibian occurrences is significantly greater than in sites selected based on bird and amphibian occurrences (one-tailed, unequal variance t-test, $t = 6.495$, d.f. = 116.043, $p = 1.076 \times 10^{-7}$) or environmental variables (one-tailed, unequal variance t-test, $t = 2.102$, d.f. = 171.355, $p = 0.0185$). Sites chosen based on genetic and morphological variation of common species contained steeper elevational gradients than unsampled sites in the study region (one-tailed, unequal variance t-test, $t = 3.582$, d.f. = 14.409, $p = 0.00144$). These steep altitudinal gradients also contained a high number of threatened species. Finally, we found significant spatial overlap between reserves selected based on genetic and morphological traits of common species and models of the geographical distributions of threatened species.
(Cramer–Von Mises test: null hypothesis of significant overlap, $p > 0.05$; electronic supplementary material, table S2).

(b) The effectiveness of genetic and morphological traits as surrogates for threatened species when reserves are prioritized subject to areal constraints

When the maximum amount of land that could be put under a conservation plan ranged from 1 to 17 per cent of western Ecuador, intraspecific variation of the seven common species remained the most effective surrogate for species of conservation concern. First, we compared the reserves selected based on genetic and morphological data of common species with models of the geographical distributions of threatened species. We found that genetic and morphological traits of the seven common species were the most effective surrogate for threatened species because the curve that represents genetic and morphological traits is consistently higher than the curves for random sites, environmental variables or bird and amphibian occurrences (figure 2a). Of the 29 threatened species analysed, 27 have at least 17 per cent of their ranges represented in regions identified based on intraspecific morphological, and genetic variation exhibited by the seven common species. In every case, regions selected to represent genetic and morphological divergence in the common species we surveyed performed better than random, though the benefit of the method is limited as one approaches the 17 per cent target (figure 2a). Second, we compared reserves selected based on genetic and morphological traits of common species with known occurrences of threatened species. We found that reserves selected based on genetic and morphological traits include a significantly higher percentage of threatened species than sites selected at random. In the corresponding surrogacy plot, the curve that represents genetic and morphological traits is consistently higher than the curve for random sites (figure 2b). In addition, when we incorporated land cost into the prioritizations, sites selected based on intraspecific genetic and morphological divergence included significantly more threatened species than random sites or sites prioritized based on environmental parameters or bird and amphibian occurrences (see the electronic supplementary material, figure S3).

4. Discussion

(a) Processes that generate divergence and threatened species along gradients

Our results show that the genetic and morphological variation exhibited by common species effectively predicts the
occurrence of threatened species in western Ecuador. In particular, when we prioritized reserves without areal constraints, we found that elevational gradients supported higher intraspecific divergence of genetic and morphological traits in common species and higher numbers of threatened species than other sites, such as lowlands. Genetic and morphological traits of common species may predict the ranges threatened species well simply because the two overlap spatially along gradients of elevation. Why should this be the case? Below we examine the processes acting along gradients that may help explain patterns.

Elevational gradients typically have substantial ecological turnover because some ecological communities are restricted to a small altitudinal range [4,10,32]. The high number of communities along an elevational gradient could promote diversifying selection as populations adapt to differing abiotic and biotic conditions. This could generate high levels of intraspecific genetic and morphological variation within common species. For example, since temperature and precipitation generally decrease with elevation, selection could lead to both phenotypes adapted to dry, cold environments at the higher ends of gradients and hot, wet environments towards the lowlands [33,34]. The outcome would be high levels of variation in morphological traits related to thermal and water tolerance across the gradient. Such patterns of selection across ecological gradients may ultimately lead to reproductive isolation and speciation [35–38].

Elevational gradients may also be hotbeds of genetic divergence within common species because they undergo strong cyclical fluctuations in the environment. Quantitative genetic models predict that when the environment varies in a cyclical manner, having high additive genetic variance will significantly increase a population's average fitness [39]. Over decadal time scales, there is significant variation in temperature along elevational gradients in Ecuador that is triggered by the El Niño–Southern Oscillation, and this variation is more pronounced along gradients than at lower altitudes [40]. In the light of this, populations that diverge genetically as a means of adapting to cyclically varying climate along elevational gradients may have higher fitness, which could result in high intraspecific genetic variation along gradients.

Moreover, introgression, hybridization between species followed by backcrossing with parents, might also result in genetic divergence along elevational gradients because hybrids carry alleles from both parental lineages [41]. Since introgression has been shown to occur along elevational gradients when high- and low-altitude species hybridize at intermediate altitudes [42–44], elevational gradients in western Ecuador may have high genetic divergence among populations of common species because these gradients are hybrid zones.

Finally, because elevational gradients have high ecological turnover, species along the gradient may exhibit high levels of specialization [45]. These specialist species would be confined to relatively small ranges, because only a narrow elevational band is ecologically suitable for them, and consequently they may be more easily threatened since extinction risk decreases with range size [46]. Anthropogenic disturbance would further increase the risk of extinction of rare, specialized species along gradients. An example of such disturbance is deforestation. Significant clearing of forests in western Ecuador began in the 1950s [8]. The main drivers of deforestation in this region have been the expansion of cattle pasture, cropland and timber extraction [47].

We hypothesize that elevational gradients, because they are harder to reach than lowlands, contain some of the last forest remnants west of the Andes but that deforestation is expanding to steeper areas. When we analysed a satellite-derived map of western Ecuador's land cover [48], we found that the proportion of land recently converted to cropland and artificial areas is greater along steep elevational gradients than in flat areas (one-tailed \( t = 5.6109, \ d.f. = 19.953, \ p = 1.044 \times 10^{-5} \)). This could be due to the fact that flat areas were largely cleared decades ago, or owing to the expansion of infrastructure in steep areas such road development that results in deforestation of previously inaccessible sites. For example, in the Atlantic Forest of Brazil, a disproportional amount of road construction in steep areas from the 1960s to the 1980s contributed significantly to forest fragmentation [49]. If roads have expanded in steep areas in Ecuador in a similar manner, threatened species may concentrate along gradients, because gradients are undergoing high rates of road-associated land clearing.

Although testing this hypothesis remains an important area for future research, findings from elevational gradients in other regions may provide insight into whether deforestation could have led to a high number of threatened species along elevational gradients in western Ecuador. For example, deforestation along elevational gradients in the Columbian Andes during the first half of the twentieth century resulted in a significant decrease in insect diversity [50]. Furthermore, in the Sierra Nevada mountain range of California during the past century, animal populations occurring along altitudinal gradients have experienced range contraction owing to anthropogenic effects such as land clearing and climate change [51,52]. A similar process may have occurred in steep areas in western Ecuador, leading to a high number of threatened species along altitudinal gradients.

(b) Implications for biodiversity monitoring

Surveys of biodiversity in remote areas often have the goal of inventorying all species in a region. However, such approaches are costly because they require that experts be dispatched to survey multiple taxonomical groups in the field and funding for subsequent analyses. Our analyses show that genetic and morphological traits of common species may be used as effective surrogates for the occurrence of species of conservation concern. Given that they are effective surrogates, future rapid assessments of biodiversity might be improved or supplemented by also sampling amphibian, avian and chiropteran genetic and morphological divergence of selected common species. This has the potential to significantly decrease the time and cost of biodiversity assessments, making it possible to carry out a greater number of assessments each year. Lastly, surveying genetic and morphological variation could lead to direct tests about the processes resulting in biodiversity and how it may respond to climate change [53,54].

Since our conclusion that intraspecific variation of birds, bats and a frog is an effective surrogate for threatened species is based on analysis of only seven species, this raises the question of how generalizable our conclusions are. A search of the literature reveals that intraspecific diversity has also been shown to be correlated with diversity and endemism at the species level in a number of other taxa and geographical regions, including birds in the West Indies [55], butterflies in Indonesia [56], stream fishes in the midwestern US [57] and
beetles in the Aegean archipelago [58]. The association between species diversity and intraspecific variation in diverse taxa suggests that our results may be useful for other taxa. However, future work should test whether the association between genetic and morphological traits of common species and threatened species is limited to areas where there are steep altitudinal gradients. Given the relative ease associated with assaying genetic variation in non-model organisms, we believe the approach presented here represents a potentially important new tool for the conservation of biodiversity. Whether this tool can be implemented in real-world conservation planning will depend largely on economic cost: if collecting genetic samples from common species and analysing genetic and morphological data is less expensive than surveys to detect threatened species, then our method will have practical utility for governmental agencies and conservation NGOs. Comparing the cost-efficiency of these two methods remains an important area for future research.

We thank R. Calsbeek, R. Harrigan, B. Larison, R. E. Ricklefs, three reviewers and Associate Editor Daniel Rabosky for comments that improved the manuscript. This work was supported by NSF grant no. IRECBE9979702 to T.B.S., R.K.W. and C.J.S.; and NASA grant nos ID/03–0169–0347 to T.B.S., R.K.W., and C.J.S.; and NCC05GB37G to C.H.G.

References


21. Garson J, Sarkar S. 2002 Surrogacy manual ver 1.1. Austin, TX: Biodiversity and Biocultural Conservation Laboratory, Section of Integrative Biology, University of Texas at Austin.


Correction

http://dx.doi.org/10.1098/rspb.2013.1237

The scientific name of the zurucuchu robber frog should be changed from ‘Hylodes buergeri’ to ‘Pristimantis w-nigrum’ in the following places:

(1) Table 1, column 2, row 7. Corrected table:
(2) Page 3, column 1, paragraph one. ‘Finally, for the zurucuchu robber frog (Hylodes buergeri), we sequenced two anonymous loci and an 840 base-pair region of the RAG1’ should be ‘Finally, for the zurucuchu robber frog (Pristimantis w-nigrum), we sequenced two anonymous loci and an 840 base-pair region of the RAG1’.

<table>
<thead>
<tr>
<th>species</th>
<th>genetic markers (loci, individuals, sites)</th>
<th>morphological traits (n, sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>birds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wedge-billed woodcreeper (Glyphorynchus spirurus)</td>
<td>amplified fragment length polymorphisms (AFLPs; 136, 178, 15)</td>
<td>wing length, tarsus length, tail length, bill length, bill width and bill depth (195, 15)</td>
</tr>
<tr>
<td>masked flowerpiercer (Diglossa cyanea)</td>
<td>microsatellites (10, 102, 12)</td>
<td>wing length, tarsus length, tail length, bill length, bill width, and bill depth (90, 10)</td>
</tr>
<tr>
<td>streak-necked flycatcher (Mionectes striaticollis)</td>
<td>microsatellites (10, 106, 9)</td>
<td>wing length, tarsus length, tail length, bill length, bill width, and bill depth (122, 8)</td>
</tr>
<tr>
<td>bats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>silky short-tailed bat (Carollia brevicauda)</td>
<td>none</td>
<td>centroid size of the skull, angle of curvature of the zygomatic arch and forearm length (167, 43)</td>
</tr>
<tr>
<td>chestnut short-tailed bat (Carollia castanea)</td>
<td>none</td>
<td>centroid size of the skull, angle of curvature of the zygomatic arch and forearm length (86, 25)</td>
</tr>
<tr>
<td>seba’s short-tailed bat (Carollia perspicillata)</td>
<td>AFLPs (311, 83, 9)</td>
<td>centroid size of the skull, angle of curvature of the zygomatic arch and forearm length (160, 44)</td>
</tr>
<tr>
<td>frog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>zurucuchu robber frog (Pristimantis w-nigrum)</td>
<td>two anonymous loci and an 840 base-pair region of recombination activation gene 1 (RAG1; 3,76–127 per locus, 9)</td>
<td>snout-vent length, gape width, lengths of the metacarpal phalanges, lengths of the radio-ulna, lengths of the metatarsal phalanges, length of the tarsus, tibia-fibula length, femur length and lower jaw length (224, 16)</td>
</tr>
</tbody>
</table>