

Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data

Malin C. Rivers · Steven P. Bachman · Thomas R. Meagher ·
Eimear Nic Lughadha · Neil A. Brummitt

Received: 9 May 2009 / Accepted: 25 February 2010 / Published online: 18 March 2010
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Abstract Despite the ecological and economic importance of plants, the majority of plant species and their conservation status are still poorly known. Based on the limited knowledge we have of many plant species, especially those in the tropics, the use of GIS techniques can give us estimates of the degree of population subdivision to be used in conservation assessments of extinction risk. This paper evaluates how best to use the IUCN Red List Categories and Criteria to produce effective and consistent estimates of sub-population structure based on specimen data available in the herbaria around the world. We assessed population structure through GIS-based analysis of the geographic distribution of collections, using herbarium specimen data for 11 species of *Delonix sensu lato*. We used four methods: grid adjacency, circular buffer, Rapoport's mean propinquity and alpha hull, to quantify population structure according to the terms used in the IUCN Red List: numbers of subpopulations and locations, and degree of fragmentation. Based on our findings, we recommend using the circular buffer method, as it is not dependent on collection density and allows points to be added, subtracted and/or moved without altering the buffer placement. The ideal radius of the buffer is debatable; however when dispersal characteristics of the species are unknown then a sliding scale, such as the 1/10th maximum inter-point distance, is the preferred choice, as it is species-specific and not sensitive to collection density. Such quantitative measures of population structure provide a rigorous means of applying IUCN criteria to a wide range of plant species that hitherto were inaccessible to IUCN classification.

M. C. Rivers (✉) · S. P. Bachman · E. Nic Lughadha · N. A. Brummitt
Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK
e-mail: m.rivers@kew.org

M. C. Rivers · T. R. Meagher
Centre for Evolution, Genes & Genomics, School of Biology, University of St Andrews,
Harold Mitchell Building, St Andrews, Fife KY16 9TH, UK

Present Address:

N. A. Brummitt
Department of Botany, Natural History Museum, Cromwell Road, London SW7 5BD, UK

Keywords Extinction risk · Fragmentation · GIS · Herbarium specimens · IUCN red list · Leguminosae · Location · Madagascar · Subpopulation

Introduction

Plant diversity is essential for our food security, medicines and ecosystem services, as well as having cultural and aesthetic value. Despite the importance of plants, our current knowledge of their diversity and conservation status is limited and patchy. To date only less than 4% (12,151) of plant species have so far had their conservation status assessed by current international criteria and appear on the IUCN Red List (IUCN 2009; www.iucnredlist.org). To be able to make well informed conservation decisions, there is an urgent need to increase the knowledge of plant diversity and to assess the conservation status of many more plant species worldwide. In 1992, the need for conservation of natural resources was brought to the world's attention at the Earth Summit in Rio de Janeiro, through the establishment of the UN Convention on Biological Diversity. It called for the conservation of biological resources, their sustainable use and the fair and equitable sharing of benefits. The importance of assessing plant diversity specifically was highlighted 10 years later by the Global Strategy for Plant Conservation (GSPC). The GSPC recognises the need for plant conservation assessments and calls for the “preliminary assessment of the conservation status of all known plant species, at national, regional and international levels” (UNEP 2002).

There is a general, although not comprehensive, understanding of why species go extinct (e.g. range collapse, reduction in number of individuals, severe fluctuation in numbers and/or range), even though factors causing threats to those species will clearly differ. These known characteristics also form the basis for measuring extinction risk according to the IUCN Red List Categories and Criteria version 3.1 (IUCN 2001), hereafter referred to as the IUCN Red List. The IUCN Red List aims to give a warning sign that a species is at risk of going extinct and to give a chance to implement appropriate conservation actions. However, the best methods to measure these early warning signs, especially in poorly known groups like plants, are not fully developed. Some attention has been given to comparing different range measures (Burgman and Fox 2003; Willis et al. 2003; Hernandez et al. 2006; Callmender et al. 2007; Gaston and Fuller 2009), however, there is no consensus on how best to measure population structure. This paper addresses the immediate need to develop a consensus on the techniques used to measure subpopulation structure against the criteria of the IUCN Red List as well as contributing to broader fields; describing and measuring population structure is highly relevant not only to conservation assessments and conservation biology, but also to population biology more generally, and central to ecology and evolutionary biology (e.g. Waples and Gaggiotti 2006).

The IUCN Red List (IUCN 2001) recognises the role population structure plays in extinction risk; and three terms of population subdivision—subpopulations, locations and fragmentation—are included in its categories and criteria (Box 1). Ideally, assigning divisions of population structure requires good knowledge of a species' biology, including information on distribution, ecology, reproductive isolation, the degree of genetic exchange and dispersal ability. For most plant species, there is little or no information available from field studies that can be used to determine population structure in such detail. The paucity of such population level data represents a severe constraint to the production of conservation assessments for plants. Where field observations to underpin

Box 1 Use of terms relating to population structure used by the IUCN Categories and Criteria v 3.1 (IUCN 2001)

Subpopulation—Subpopulations are defined as groups geographically or otherwise distinct in the population between which there is little demographic or genetic exchange (typically one successful migrant individual or gamete per year or less).

Location—The term ‘location’ defines a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon present. The size of the location depends on the area covered by the threatening event and may include part of one or many subpopulations. Where a taxon is affected by more than one threatening event, location should be defined by considering the most serious plausible threat.

Severely fragmented—The term ‘severely fragmented’ refers to the situation in which increased extinction risk to the taxon results from the fact that most of its individuals are found in small and relatively isolated subpopulations (in certain circumstances this may be inferred from habitat information). These small subpopulations may go extinct, with a reduced probability of recolonization.

conservation assessments are lacking, georeferenced herbarium specimens can play an important role (Willis et al. 2003). GIS techniques are already being used to assess geographical range using such information; however, they can also be employed for analysing population structure, on the underlying assumption that increased geographical distance between collections means increased genetic separation of subpopulations. GIS analyses are objective and repeatable; data from new collections can be added or old records from now-extinct subpopulations can be omitted, and the data reanalysed.

In order for a species to be listed in one of the Threatened categories of the IUCN Red List (Vulnerable, Endangered and Critically Endangered), it needs to fulfil at least one of five criteria (A–E; IUCN 2001). References to the terms of population subdivision (subpopulation, location and severe fragmentation) are found in three criteria, namely Criterion B (geographic range), Criterion C (small and declining population size) and Criterion D

Box 2 Detailed description of the incorporation of population subdivision in the IUCN Categories and Criteria v 3.1

Measures of subpopulations are used in Criterion B and Criterion C. In Criterion B, the term subpopulation is included in two subcriteria: continuing decline in the number of subpopulations (subcriterion b) and extreme fluctuation in the number of subpopulations (subcriterion c). Species that fulfil Criterion B and show a decline or extreme fluctuation in subpopulation numbers are listed as B1/B2b(iv) or as B1/B2c(iii), respectively. In Criterion C, species that have 90–100% of individuals in a single subpopulation can be listed as threatened under subcriterion C2a(ii). Criterion C requires detailed information on the number of mature individuals which is often not available for plant species. However, species with a single subpopulation can be listed under C2a(ii), as this subpopulation would automatically have 100% of the individuals (subject to the rest of Criterion C being met).

The term location is found in Criterion B and Criterion D. In Criterion B the number of locations is included in all three subcriteria. In subcriterion a, a species is considered Threatened if it exists in 10 locations or fewer. A species is also considered Threatened if it shows continuing decline in the number of locations (subcriterion b) and/or extreme fluctuation in the number of subpopulations (subcriterion c). If all subcriteria are fulfilled then species can be listed as B1/B2ab(iv)c(iii). In Criterion D a species with five (or fewer) locations qualifies as Vulnerable, due to its severely restricted area, and can be listed as VU D2 (subject to the species being prone to the effects of human activities or stochastic events within a very short time period).

Severely fragmented species are referred to in Criterion B, subcriterion a, where a statement of whether or not the species is severely fragmented is required. Species considered severely fragmented are listed with the code B1a/B2a. A taxon is considered severely fragmented if most (>50%) of its total area of occupancy is in habitat patches that are (1) smaller than would be required to support a viable population, and (2) separated from other habitat patches by a large distance.

(very small or restricted populations). Even when limited data are available, assessors are still encouraged by IUCN to assign a category based on the available data (IUCN Standards and Petitions Working Group 2008). Box 2 gives further details on the incorporation of the terms of population subdivision in the IUCN Red List.

The key objective of this paper is to evaluate different ways to effectively and consistently estimate population structure, using the IUCN definitions and criteria, through GIS-based analysis of data available for every species in the herbaria around the world. We use a near-complete set of herbarium specimen data available for eleven species of *Delonix sensu lato* from Madagascar to address population structure according to the terms of the IUCN Red List: subpopulations, locations and fragmentation. Due to equivocal phylogenetic interpretations (Du Puy et al. 1995; Haston et al. 2005), the related monotypic genera *Colvillea* and *Lemuropisum* are also included in this study as part of *Delonix s.l.*

Methods

Delonix s.l., a genus from the family Leguminosae, is the taxonomic focus of this study. Leguminosae is the world's third largest angiosperm family and has been identified as a family that can be used as a proxy for evaluating global patterns of angiosperm diversity (Nic Lughadha et al. 2005). The taxonomy of *Delonix s.l.* follows Du Puy et al. (2002) and recognises 11 species endemic to Madagascar: *Delonix boiviniana*, *D. brachycarpa*, *D. decaryi*, *D. floribunda*, *D. leucantha*, *D. pumila*, *D. regia*, *D. tomentosa*, *D. velutina* and the monotypic genera *Colvillea racemosa* and *Lemuropisum edule*. A further two species of *Delonix* found outside Madagascar are not included in this study. *Delonix s.l.* includes both widespread species and narrow endemics (e.g. *D. tomentosa* is known only from a single locality). Georeferenced specimen data used in this study are primarily from herbaria at the Royal Botanic Gardens, Kew (hereafter RBG Kew), the Museum National d'Histoire Naturelle, Paris, and the Missouri Botanical Garden, with additional specimen data from another 11 herbaria. Estimates show that collections of *Delonix s.l.* from RBG Kew, Paris, and Missouri encompass 94% of all available *Delonix s.l.* collections from Madagascar (M. Rivers unpublished data); duplicate specimens of the same collection

Table 1 Summary table of the number of herbarium specimens and georeferenced collections consulted from 14 herbaria: B, BM, BR, C, E, G, K, MO, NY, P, PRE, TAN, TEF and WAG. See Index Herbariorum for full information (Thiers 2009)

	Total number of herbarium specimens	Uniquely georeferenced collection localities
<i>Colvillea racemosa</i>	60	43
<i>Delonix boiviniana</i>	93	82
<i>Delonix brachycarpa</i>	9	9
<i>Delonix decaryi</i>	61	38
<i>Delonix floribunda</i>	72	51
<i>Delonix leucantha</i>	19	19
<i>Delonix pumila</i>	28	24
<i>Delonix regia</i>	39	30
<i>Delonix tomentosa</i>	1	1
<i>Delonix velutina</i>	13	9
<i>Lemuropisum edule</i>	21	18
Total <i>Delonix s.l.</i>	416	324

from different herbaria were identified and excluded from our analyses so that each collection would be used only once. In total 324 herbarium collections from 14 herbaria (B, BM, BR, C, E, G, K, MO, NY, P, PRE, TAN, TEF and WAG; Thiers 2009) were used across the 11 species (Table 1).

The collection localities of the specimens were plotted in ArcView 3.1 and ArcGIS 9.2 to allow spatial GIS analyses using the Spatial Analyst extension (ESRI 2006), Hawth's Analysis Tools (Beyer 2004) and RBG Kew's Conservation Assessment Tools extensions (Moat 2007; Moat 2008).

Measures of subpopulations

The subpopulation structure based on analysis of the geographic distribution of collection data was assessed with four methods: grid adjacency (IUCN 1994), circular buffer, Rapoport's mean propinquity (Rapoport 1982) and alpha hull (Burgman and Fox 2003; Edelsbrunner et al. 1983) (Fig. 1). These methods were chosen as they have been used for estimating species range in the context of IUCN conservation assessments. Here we have adopted these methods to fit with the assessment of subpopulation structure.

Grid adjacency method

The number of subpopulations is estimated by overlaying a grid onto mapped collection localities, and contiguous occupied cells are considered to be a single subpopulation (Fig. 1a). The area of each subpopulation is calculated as the area of the contiguous occupied cells. The grid is positioned on the mapped points in a manner that minimises the number of occupied cells (IUCN Standards and Petitions Working Group 2008). To investigate the influence of grid cell size on the structure of subpopulations, grids of 2, 10 km and 1/10th of the maximum distance between any pair of points ("1/10th max") were compared here. "1/10th max" is a sliding scale, based on the interpoint distances between specimen collections, that is species-specific, and so takes into account the fact that widespread species often have a lower collection density across their range than do narrowly distributed species. A 2×2 km² grid is recommended by IUCN for estimating area of occupancy (AOO) (IUCN 2001); a 10×10 km² grid is often used in AOO estimates in conservation assessments from Missouri Botanical Garden (Schatz 2000; Good et al. 2006; Callmander et al. 2007); and a "1/10th max" grid is used in AOO estimates in conservation assessments at RBG Kew (Willis et al. 2003; Moat 2007).

Circular buffer method

Each collection locality is buffered with a circle of a set radius. Overlapping circles are merged to form a single subpopulation, while non-overlapping circles are considered separate subpopulations (Fig. 1b). Radii of 5.64 km (buffer area of 100 km equivalent to a single cell in a 10×10 km² grid), 10 km (equivalent to the minimum distance between two subpopulations in a 10×10 km² grid) and "1/10th max" were used in this study.

Rapoport's mean propinquity method

An extension of the buffer method is Rapoport's mean propinquity method. All points are connected using a minimum spanning tree: a tree connecting all points together by the

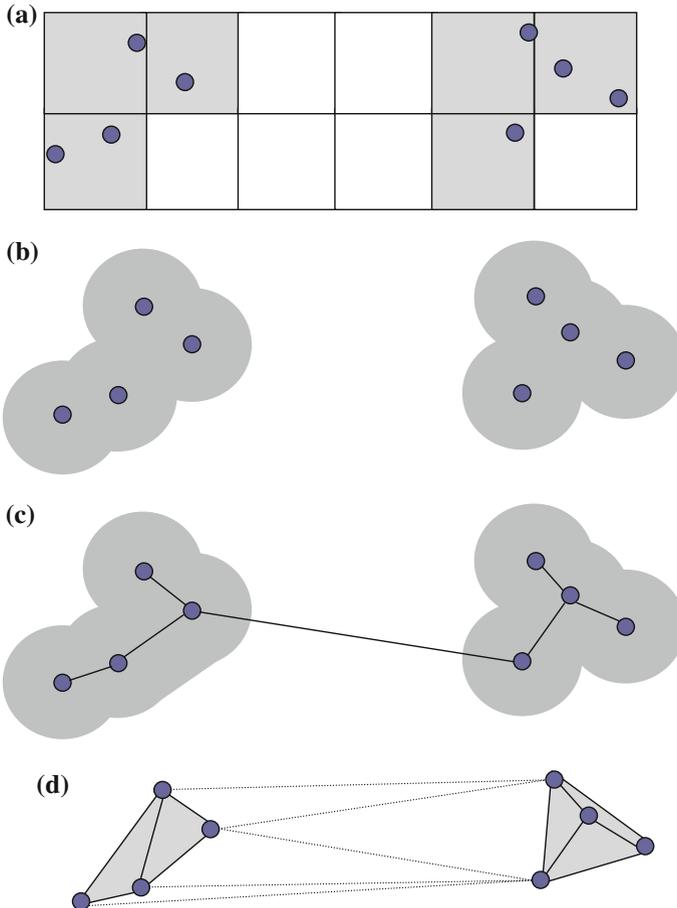


Fig. 1 Diagram of the four methods used to define subpopulations. **a** Grid adjacency method: adjacent occupied cells form a single subpopulation; **b** Circular buffer method: overlapping buffered circles form a single subpopulation; **c** Rapoport's mean propinquity method: the radius of the shaded buffers is equal to the mean branch length of the minimum spanning tree (*black line*); **d** alpha hull method: the lines represent the Delaunay triangulation; when $\alpha \cdot \text{mean side length}$ is shorter than *dashed lines*, two subpopulations are formed

shortest distance (Fig. 1c). The mean branch length (distance between points) of the minimum spanning tree is used as the radius of the buffer around the points and on both sides of the branches (if those branches are shorter than twice the mean). Again, overlapping buffers form a single subpopulation and non-overlapping buffers are considered separate subpopulations. This method was developed by Rapoport (1982) and adapted at RBG Kew (Willis et al. 2003; Moat 2007).

Alpha hull method

Another method based on the distances measured between points is the alpha hull method (Edelsbrunner et al. 1983; Burgman and Fox 2003). All points are connected using a

Delaunay triangulation, where lines are drawn joining all points such that no lines are allowed to intersect, maximizing the minimum angle of all the angles of the triangles in the triangulation (Fig. 1d). The mean length of the sides of every triangle is then calculated. Lines from the Delaunay triangulation are removed if they exceed the size of a multiple (alpha) of the mean length; the smaller the value of alpha, the finer the resolution of the hull. As lines are removed, the range is divided into subpopulations (Fig. 1d). Alpha hulls have been used in range estimation, and for this purpose values of alpha of 2 (IUCN Standards and Petitions Working Group 2008) or 3 (Burgman and Fox 2003) have been recommended. Alpha values of 1 (mean line length), 2 (twice mean line length) and 3 (three times mean line length) were used in this study.

Results

Measures of subpopulations

Estimates of the number of subpopulations from the four methods are presented in Table 2. The grid adjacency method using a 2×2 km² grid gives the highest estimates of the number of subpopulations in all species analysed. Similarly the results from the 10×10 km² grid, the circular buffer with a 5.64 km radius and a 10 km radius also show relatively high estimates of subpopulation numbers. The grid of “1/10th max” and the circular buffer of “1/10th max” show much lower estimates for all species. Rapoport’s mean propinquity method gives subpopulation estimates similar to the “1/10th max” buffer for most species. The alpha hull method with both alpha = 2 and alpha = 3 show the lowest estimates of subpopulation number. However, an intermediate number of subpopulations was predicted when alpha = 1, similar in number to results from Rapoport’s mean propinquity method and both “1/10th max” grid and buffer.

The relationship between number of collection localities and number of subpopulations was tested using linear regression (Table 3). Regression analyses show that the fixed grid adjacency and fixed circular buffer methods have a significant positive relationship between number of collection localities and subpopulations ($P < 0.001$). This indicates that grid-adjacency and circular buffer methods are closely correlated with the number of collection localities at small radii and grid sizes, often with a single collection in each subpopulation. Rapoport’s mean propinquity method and the alpha hull method (alpha = 1) also show a positive correlation between number of collection localities and subpopulations. However, the species-specific methods of “1/10th max” grid, “1/10th max” buffer and alpha hull method where alpha = 2 or 3 show no significant correlation between number of collection localities and number of subpopulations ($P > 0.05$). Table 4 summarises the strengths and weaknesses of all methods investigated. It shows that the circular buffer method with the “1/10th max” sliding scale is the most desirable method, as all other methods show some weaknesses.

Discussion and recommendations

Measures of subpopulations

The use of herbarium specimens to generate IUCN conservation assessments is not new. However, it often relies on range estimates of the extent of occurrence (EEO) or the area of

Table 2 Estimates of numbers of subpopulations for species of *Delonix s.l.* using grid adjacency, circular buffer, Rapoport's mean propinquity and alpha hull methods

	Grid adjacency			Circular buffer			Rapoport's mean propinquity			Alpha hull		
	2 × 2 km ²	10 × 10 km ²	1/10th max	5.64 km	10 km	1/10th max				α = 1	α = 2	α = 3
<i>Colvillea racemosa</i>	34	21	3	23	19	4	5	4	3	3		
<i>Delonix boiviniana</i>	50	32	3	36	31	4	13	7	2	1		
<i>Delonix brachycarpa</i>	9	8	4	9	8	5	3	2	1	1		
<i>Delonix decaryi</i>	19	12	3	15	11	8	9	4	1	1		
<i>Delonix floribunda</i>	32	17	3	21	15	6	7	6	3	1		
<i>Delonix leucantha</i>	15	8	4	11	7	5	3	3	2	1		
<i>Delonix pumila</i>	6	1	6	1	1	5	2	2	1	1		
<i>Delonix regia</i>	19	10	3	12	7	3	4	3	2	2		
<i>Delonix tomentosa</i>	1	1	n/a	1	1	n/a	n/a	n/a	n/a	n/a		
<i>Delonix velutina</i>	4	2	2	2	2	2	2	2	1	1		
<i>Lemurophisum edule</i>	9	3	4	5	3	5	3	3	1	1		

Table 3 Relationship between collection numbers (x) and subpopulation number estimates (y) for *Delonix s.l.* ($n = 11$) using linear regression ($y = ax + b$)

Method	a	b	R^2	P
Grid $2 \times 2 \text{ km}^2$	0.62	-0.35	0.92	<0.001
Grid $10 \times 10 \text{ km}^2$	0.39	-0.91	0.86	<0.001
Grid “1/10th max”	0.00	3.17	0.00	0.59
Circular buffer 5.64 km	0.44	-0.51	0.87	<0.001
Circular buffer 10 km	0.36	-1.16	0.85	<0.001
Circular buffer “1/10th max”	0.03	3.52	0.12	0.29
Rapoport’s mean propinquity	0.14	0.51	0.85	<0.001
Alpha hull ($\alpha = 1$)	0.07	1.18	0.91	<0.001
Alpha hull ($\alpha = 2$)	0.02	1.02	0.36	0.05
Alpha hull ($\alpha = 3$)	0.01	1.12	0.03	0.59

Table 4 Summary of methods to determine subpopulation structure based on GIS analysis of collection data, and how they are affected by the factors listed in the left-hand column

Attribute	Method			
	Grid adjacency	Circular buffer	Rapoport’s mean propinquity	Alpha hull
Collection number dependent	Yes (No “1/10 max”)	Yes (No “1/10 max”)	Yes	No (Yes $\alpha = 1$)
Collection density dependent	No	No	Yes	Yes
Species-specific scale	No (Yes “1/10 max”)	No (Yes “1/10 max”)	Yes	Yes
Grid placement dependent	Yes	No	No	No
Dependent on position of all points in population	Yes	No	Yes	Yes
Time, effort and cost	Low	Low	Low	Low

Desirable traits are shown in bold

occupancy (AOO), while measures of subpopulation number and fragmentation have only occasionally been addressed (Schatz 2000; Willis et al. 2003; Good et al. 2006; Callmänder et al. 2007). Several comparisons exist of the different measures for calculating range using herbarium specimens (Willis et al. 2003; Hernandez et al. 2006; Callmänder et al. 2007). However, there has previously not been a comparative analysis of different measures of the IUCN definitions of subpopulation structure, number of locations or degree of fragmentation. The strengths and weaknesses of all methods examined are summarised in Table 4, with detailed discussion of each below.

The grid adjacency method is a simple method widely applied to conservation assessments and recommended by IUCN for range calculations (Schatz 2000; IUCN 2001; Good et al. 2006; Callmänder et al. 2007). However, both grid size and grid placement are major determinants in the number of resulting subpopulations (Willis et al. 2003). When the grid size is too small, there may be an overestimate of the number of subpopulations, and when the grid size is too large the result may be an underestimate of subpopulations.

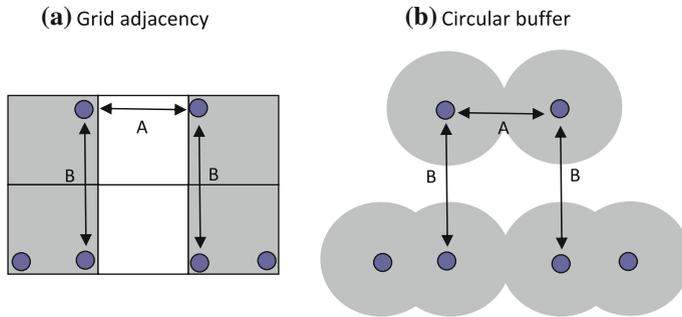


Fig. 2 Hypothetical diagram of collection localities. Despite distance A being shorter than distance B, the grid placement means that the two closer points are put in separate subpopulations in the grid adjacency method (a), though not with the circular buffer method (b)

With a 2×2 km² grid, the estimated number of subpopulations is highly correlated to numbers of collections, and species with a higher number of collections are estimated to have a higher number of subpopulations. The 10×10 km² grid is claimed to correspond better to the average extent of an isolated subpopulation (Callmänder et al. 2007), but this grid size also shows a strong correlation with number of collections. From this we conclude that herbarium data are often too sparse for these grid sizes. Both 2×2 km² and 10×10 km² grids have been recommended in estimating AOO (Schatz 2000; Callmänder et al. 2007; IUCN Standards and Petitions Working Group 2008); however, due to their dependence on collection numbers they are not appropriate for the subpopulation measures used in this study. Fixed grid methods such as these generally lead to an overestimation of numbers of subpopulations for widespread taxa simply because collections are spaced further apart; this is avoided using the “1/10th max” method. The size of the “1/10th max” grid is a species-specific measure that assumes that the geographic extent of a species influences its subpopulation structure. It is not dependent on the number of collections, but instead it takes into account the geographical spread of species and avoids widespread species having an artificially elevated number of subpopulations, as is seen in both the 2×2 km² and 10×10 km² grids. The second major factor affecting the number of subpopulations in the grid adjacency method is the placement of the grid. The grid is placed so as to minimise the number of occupied grid cells (IUCN Standards and Petitions Working Group 2008), and consequently its placement is dependent on the position of all points in the grid. This can lead to points being located at the edges of a grid cell, and two collections may be grouped together even though they are further apart than two collections that are considered to be in separate subpopulations (Fig. 2a).

The dependency on all points for determining the number of subpopulations is avoided using the circular buffer method. The circular shape and placement of the buffer means the problem of grouping more distant collections over more closely situated collections (Fig. 2a) is avoided with the circular buffer method (Fig. 2b). As each point is situated in the middle of its circular buffer, slightly larger estimates of subpopulation numbers are seen when comparing equal-area grids and buffers (buffer of 5.64 km radius and a 10×10 km² grid). As with the grid method, the circular buffer method is dependent on the choice of radius of the buffer; with buffers of small radii there is a high correlation between the number of collections and the number of subpopulations. The species-specific “1/10th max” buffer assumes that the geographic extent of a species influences its

subpopulation structure and avoids widespread species having an artificially elevated number of subpopulations, as is seen with the smaller buffers. If the maximum dispersal distance for a species is known, then this can be used as the radius to mimic biological dispersal capacity.

Rapoport's mean propinquity method is an extension of the "1/10th max" buffer method, also based on species-specific buffers whose size is determined by inter-point distances. It is not surprising, therefore, that the estimates of numbers of subpopulations for both methods are similar. In both methods the placement of the buffer is unambiguous; however, the radius of the buffer in Rapoport's mean propinquity method is not based on maximum geographic range but rather on collection density. If collection density is high, the distances between points are small and therefore the radius of the buffer is small, leading to a greater number of subpopulations. Equally, when collection density is low, the distances between points are large, and consequently the buffer radius is large and the estimated number of subpopulations is small. The sensitivity of Rapoport's mean propinquity method to collection density means that poorly collected species will have fewer subpopulations than well-collected species (of the same geographic distribution). Also, species with a high density of collections from a single area may have artificially inflated number of subpopulations. For Rapoport's mean propinquity method to work best a sufficient collection density needs to be achieved.

As with Rapoport's mean propinquity method the alpha hull method determines subpopulation structure based on the distances between collection points. However, it takes into account not only the minimum distance between all points, but all connections in the Delaunay triangulation. It is therefore less sensitive to collection density than is Rapoport's mean propinquity method. Alpha hulls have been used to estimate species ranges, but have not previously been used to estimate the number of subpopulations. In range-size estimates alpha hulls are less affected by biases due to the shape of species' ranges, errors in recording locations and sampling effort than are other measures (Burgman and Fox 2003). For the purpose of subpopulation structure, both $\alpha = 2$ and $\alpha = 3$ (as recommended for range studies) predict a very low degree of population subdivision, and $\alpha = 1$ seems to be more appropriate for population subdivision. The number of subpopulations found with $\alpha = 1$ correspond closely to those found by grid "1/10th max", buffer "1/10th max", and Rapoport's mean propinquity measures. The high similarities between these methods indicate that geographic extent and collection density may have a similar effect on subpopulation numbers.

Recommendations

For species known primarily from herbarium specimens, where little or no data is available with regard to reproductive isolation, the degree of genetic exchange or dispersal ability, we recommend using a circular buffer method. This recommendation is also true for more well-studied species where more data may be available, however, the subpopulation structure may still not be implicit and spatial analysis tools are still of importance. The circular buffer method avoids the problem of point dependency of the grid adjacency method; it also allows points to be added, subtracted or moved without significantly altering the buffer placement. The most appropriate radius of the buffer is a matter of debate: an ideal size might be the maximum dispersal distance of the species. However, when dispersal characteristics are not known, as is the case for most plant species, using a sliding scale is more suitable. The sliding scale of "1/10th max" is both independent of the number of collections and also species-specific and therefore allows an appropriate spatial

scale to be used for each species. The IUCN Guidelines recognise the need for species-specific analyses and state that “methods for determining the number of subpopulations may vary according to the taxon” (IUCN Standards and Petitions Working Group 2008). A sliding scale such as the one used here therefore allows a species-specific scale to be applied. Although only one sliding scale, “1/10th max”, was investigated in this study, it was tested in both the grid adjacency and circular buffer method, where it consistently performed well. For all methods, the biological relevance of purely spatial analyses needs to be investigated, for example through population genetic analysis.

Further application of GIS methods to IUCN terms of population structure—location and fragmentation

Measures of the number of subpopulations can also be used to assess number of locations and degree of fragmentation, the other two terms regarding population structure used in IUCN Red List assessments.

Locations

The terms location and subpopulation are often seen together, which leads to confusion as they have independent definitions in IUCN terminology (Box 1). The terms are seen together in Criterion B, for example, where continuing decline and extreme fluctuations can be observed in numbers of either subpopulations or locations to fulfil subcriterion b and subcriterion c (Box 2). The definition of location requires a threatening event and the number of locations is based on the area covered by this threat and the species. As a location is a distinct geographic area (IUCN Standards and Petitions Working Group 2008), distribution-based geographical methods could therefore be used to estimate population subdivision in terms of number and position of locations. Furthermore, IUCN guidelines state that when parts of a species distribution are not affected by any threats, then one alternative is to set the number of locations in the unaffected areas to the number of subpopulations in those areas (IUCN Standards and Petitions Working Group 2008). This implies that, despite different definitions, numbers of subpopulations and locations are potentially closely linked, and hence we argue that when dealing with species where information is sparse and when threats are affecting the entire species range, estimates of number of locations could be made from a similar analysis of point locality data to that described for subpopulations above. As with subpopulations, it is important to document the method used to estimate the number of locations.

Fragmentation

“Severely fragmented” is the third term in the IUCN Red List that deals with population subdivision (Box 1). It aims to highlight species that may go locally extinct with a reduced probability of recolonisation. Although the IUCN Red List only needs a binary answer to whether a species is severely fragmented or not, this is not always straightforward. The IUCN definition of a severely fragmented species has two parts; it requires that the majority of a species range consists of both small and isolated subpopulations (see Boxes 1 and 2).

The IUCN Guidelines state that isolated subpopulations are “isolated by distances several times greater than the (long-term) average dispersal distance of the taxon” (IUCN

Standards and Petitions Working Group 2008), while definition of a subpopulation already takes into account isolation as having “less than one successful migrant individual or gamete per year” (IUCN Standards and Petitions Working Group 2008). For the majority of plant species, annual and long-term average dispersal distances are not known; thus it is difficult to distinguish between “subpopulations” and “isolated subpopulations”. The separation of subpopulations alone is not sufficient to qualify as fragmented: in addition, the subpopulations need to be small. If the minimum viable area for a successfully breeding subpopulation is known, then the proportion of subpopulations of a viable size can be calculated from a spatial analysis of the number of subpopulations such as that presented here. This measure can then be used with a measure of the number of isolated subpopulations to determine whether a species is considered severely fragmented. However, the minimum viable area is not known for the majority of plant species.

Geographical analysis of population structure can therefore be useful in providing information on the number of subpopulations, their size and their isolation, for use in estimating severe fragmentation. However, geographical analysis alone cannot be used to assess fragmentation. Further information on minimum viable area, dispersal distance and density are essential in order to correctly follow the rules of the IUCN Red List (Box 2). If these factors are known, geographical analysis can aid in estimating subpopulations, both number and degree of isolation. As more species are being analysed with regard to their genetic diversity across their range, this new information ought to somehow be incorporated into conservation assessments to indicate the degree of genetic fragmentation of a species. Although the IUCN Red List do not directly use such information at present to establish number of genetic subpopulations or degree of fragmentation, it is likely that data of this kind will be increasingly available and increasingly useful in the future.

Conclusions

A species’ range can be divided according to a continuum of different thresholds, the largest being no subdivision of the species, and the smallest being every individual constituting a separate subpopulation; the reality is likely to be found somewhere in between. Based on the limited knowledge we have of many plant species, especially those in the tropics, the use of herbarium collections and GIS techniques for estimating the degree of population subdivision often gives the best available estimates of population structure in these species to use in an IUCN conservation assessment of extinction risk.

Since different methods of analysis can result in widely divergent results, it is crucially important to document the procedures followed in any given case and, wherever possible, to make the underlying dataset available for subsequent re-analysis as new data or techniques become available, and ideally, over time, for procedures to become standardised. In going from pattern-based measures of population isolation and fragmentation to a fuller understanding of the process of extinction, details on habitat availability, dispersal ability, biotic interactions and breeding systems are needed (Hartley and Kunin 2003). In addition, genetic diversity analysis can be of crucial importance for understanding some of these factors. In the present paper, we have focused on spatial models of species’ subpopulation structure and their application for IUCN conservation assessments. Our recommendation for assessing subpopulation structure is to use the circular buffer method with a species-specific sliding scale. The next stage is to determine the relationship between these spatial models and the biologically functional subpopulations, and further to address the question of how patterns of fragmentation translate into functional isolation between subpopulations

along a species' potential path to extinction. Ideally, genetic analysis would be undertaken for all threatened species to help fill in these gaps in species knowledge. Such population genetic analyses for *Delonix s.l.* are currently under way and will add a functional dimension to the spatial results considered here that can help determine which of the geographical subdivision methods outlined above best correspond to the subdivision revealed by genetic analysis.

Acknowledgments We would like to thank Justin Moat (GIS Unit, Royal Botanic Gardens, Kew) for technical help and advice. We are also grateful for the material made available for this study from the herbaria B, BM, BR, C, E, G, K, MO, NY, P, PRE, TAN, TEF and WAG. This work was supported by the Natural Environment Research Council NER/S/A/2006/14303.

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