

# Environmental correlates of toad abundance and population genetic diversity

Kim T. Scribner<sup>a,\*</sup>, J.W. Arntzen<sup>b,1</sup>, N. Cruddace<sup>a</sup>, R.S. Oldham<sup>b</sup>,  
T. Burke<sup>a,2</sup>

<sup>a</sup>Department of Zoology, University of Leicester, Leicester LE1 7RH, UK

<sup>b</sup>Department of Biological Sciences, De Montfort University, Leicester LE7 9SU, UK

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## Abstract

Knowledge of factors that affect population demographic and genetic characteristics is of importance to the development of conservation strategies. Using field and remote sensing of microgeographic and landscape-level features of the aquatic and terrestrial environment, we examined the degree to which habitat characteristics were correlated with population demographic and genetic characteristics in the common toad (*Bufo bufo*). Measures of genetic diversity and departures from random mating are described for 20 semi-isolated breeding populations of *B. bufo* from Leicestershire, Great Britain, based on three species-specific single locus variable number of tandem repeat minisatellite loci. Spatial autocorrelation of allele frequencies revealed significant population structuring or lack of independence in allele frequencies over inter-breeding pond intervals of  $\leq 2$  km. Such estimates of “patch size” which are inferred from correlograms, may be useful in defining the extent of effective gene flow and spatial scales over which landscape-level features could plausibly be used to characterize ecological attributes of importance to individuals from specific local breeding populations. Toad presence or absence at amphibian breeding ponds, population size, and population estimates of observed heterozygosity, allelic richness, and Wright’s inbreeding coefficient ( $F$ ) were individually used as dependent variables in analyses with aquatic and terrestrial habitat characteristics. Inter-population variation in inbreeding coefficients was significantly related to presence of additional ponds near each breeding site. Allelic richness, population size, and toad presence/absence were primarily associated with terrestrial habitat variables, including pond density and vegetative characteristics reflecting surrounding land use (e.g. presence of woodlands, hedgerows, extent of area in livestock [sheep] pasture, and anthropogenic development). In the absence of definitive population demographic data, specific habitat characteristics may offer predictive potential for inferring population characteristics and future viability. However, the choice of ecologically and evolutionarily relevant habitat characteristics should be made with care. © 2001 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

Conserving ecologically and evolutionary viable species or populations necessitates an understanding of the

physical habitat and of specific environmental parameters affecting population demographics. Unfortunately, predicting population status and viability is often difficult due to complexities posed by accessibility, life histories and habits, and high dispersal abilities. Often little is known of contemporary or historical demographic characteristics such as breeding population size, or of the potential for dispersal. The paucity of data on which to base conservation decisions is of immediate concern. Habitat alteration, fragmentation, and loss has led to the partitioning of organisms into local populations that are increasingly isolated from one another. Population persistence within these isolated groups hinges both on population size (Goodman, 1987), and on the degree of

\* Corresponding author at present address: Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824-1222, USA. Tel.: +1-517-353-3288; fax: +1-517-432-1699.

E-mail address: scribne3@pilot.msu.edu (K.T. Scribner).

<sup>1</sup> Present address: Centro de Estudos de Ciencia Animal, ICETA — Universidade do Porto, Campus Agrario de Vairao, Rua do Monte, Crasto, Vairao, 4480 Vila do Conde, Portugal.

<sup>2</sup> Present address: Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK.

inter-population connectivity (Hanski, 1999), which is a function of both physical distance and the characteristics of intervening habitat (i.e. habitat permeability; Forman, 1995). Populations of most species which have declined in spatial distribution and number may also be subject to declines in levels of genetic diversity.

Amphibians are particularly susceptible to perturbations because of their complex life histories involving both aquatic and terrestrial stages. Amphibian population declines have been reported in many parts of the world (Alford and Richards, 1999). Environmental alterations have been implicated as a primary factor affecting population numbers and species diversity of some amphibian species [e.g. amphibians inhabiting old-growth forests (Welch, 1990) and species inhabiting wetlands in the southeastern United States (Pechman et al., 1991; Pechman and Wilbur, 1994)]. In the absence of definitive data on population demographic or genetic characteristics, estimates of habitat 'quality' may provide valuable predictive potential in elucidating these parameters. In Great Britain, extensive research on amphibians has been conducted over the past two decades (Beebee, 1973, 1981; Swan and Oldham, 1993). One species for which considerable data exist is the common toad (*Bufo bufo*).

Genetics data have increasingly been used to provide an alternative means of resolving questions of species or population viability. Levels of genetic variation within and among populations, as assessed using polymorphisms within particular segments of the genome, have been shown to be influenced by a variety of population characteristics of direct importance to conservation (e.g. inbreeding — Gall, 1987; gene flow — Slatkin, 1985; and effective population size — Lande and Barrowclough, 1987). The importance of preserving levels of genetic variation within populations has long been recognized for influencing short-term population viability as well as for maintaining a population's adaptability to environmental changes (Allendorf and Leary, 1986; Frankham, 1995). Indicators of fitness in anuran populations have been shown to be strongly correlated with population levels of genetic diversity (e.g. larval growth rates in *Bufo calamita*; Rowe et al., 1999).

One class of genetic markers which has received increasing attention for analysis of genetic variation in natural populations are the variable number of tandem repeat (VNTR) minisatellite loci (Burke, 1989; Burke et al., 1991). High rates of mutation (Jeffreys et al., 1988) and concomitant high levels of variation documented for specific minisatellite loci suggest that these genetic markers may be extremely sensitive to changes in population breeding size and structure, and thus may be a powerful tool in addressing conservation issues (Scribner et al., 1994, 1997).

The objectives of this study were: (1) to determine the extent to which populations of *B. bufo* differ in abundance and in levels of genetic diversity and magnitude of

deviation from Hardy-Weinberg genotypic expectations; and (2) to determine if specific attributes of aquatic and terrestrial habitats within and adjacent to breeding ponds could predict *B. bufo* presence, abundance, population genetic characteristics, and inferentially population demography and gene flow. We propose that surveys of gene frequency of different aquatic breeding sites in a region could be used to define genetic "neighborhoods" or distances of effective terrestrial gene flow among breeding ponds. Additionally, habitat features may offer insight into population demographic and genetic characteristics.

## 2. Materials and methods

### 2.1. Study area

Toads were collected from each of 20 ponds over a 300-km<sup>2</sup> area in the northwestern part of Leicestershire, in central Great Britain (Fig. 1). This area is characterized by gently undulating agricultural and pasture land, with an abundance of hedgerows, small plots of woodland, small settlements, and roads. Distances between ponds varied from 0.9 to 17.7 km.

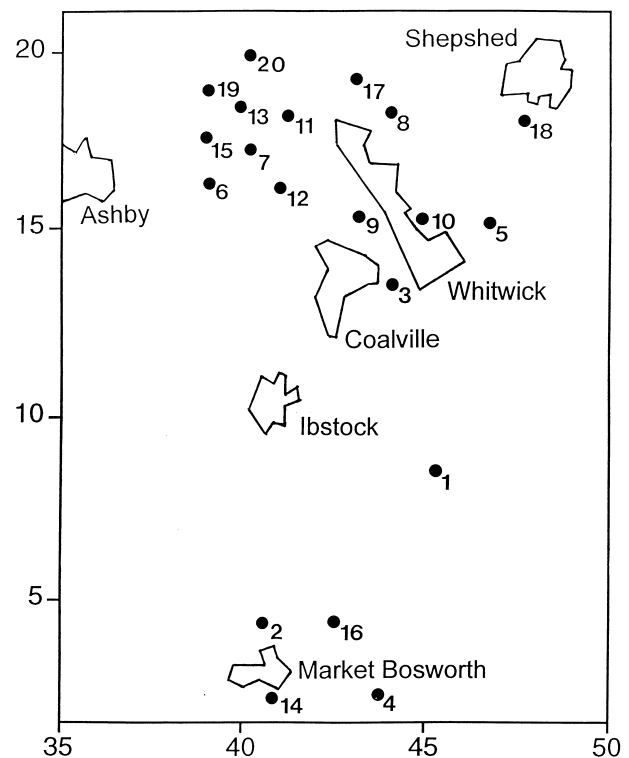


Fig. 1. Map of the distribution of *Bufo bufo* breeding ponds in Leicestershire, in central Great Britain. Pond numbers correspond to those in Table 2. Towns are indicated by name. Grid coordinates correspond to Ordnance Survey maps and provide measures (in km) of spatial dispersion among sites.

## 2.2. Population abundance

Ideally population sizes would be assessed using an intensive capture-mark-recapture (CMR) technique, or by interception of the adult population during spring migration. These two techniques were employed for several locales. However, resources were not sufficient to assess all 20 sites in this manner. At four sites, population sizes were assessed using CMR techniques. At three of these four sites, and for an additional 15 of the study ponds numbers were assessed by head count. At the final site, numbers were assessed by interception. For the four populations where multiple population estimators were employed (CMR or total inception and estimates based on head counts) all estimates were comparable (data not shown) though not all estimates have associated standard errors. At these four sites, night counts represented on average 16% of the CMR estimate of total male population size. Differences in detectability among ponds reflected the ease of finding animals, usually the result of differences in shoreline access and to differences in vegetation density. To account for inter-pond variation in access, counts were adjusted based on the proportion of the pond perimeter which could be surveyed. To account for pond-specific differences in sightability due to differences in vegetation density, head-count data was categorized in relation to vegetation profiles and peak head counts were corrected on the basis of peak count CMR ratio described above.

The CMR estimates for males were obtained by catching toads at night at the breeding sites (after head counts had been made) at intervals of 1 or 2 days. Animals were marked with a date tag using dye marks and released immediately. Recaptured animals were given an additional mark. The procedure was started at the beginning of the breeding season and repeated up to 10 times. The data were analyzed using Jolly's method (Blower et al., 1981) to provide estimates of the numbers of animals present at the breeding site on each catch night with associated variances, and with estimates of influx and exodus. These were used to determine an estimate of the overall adult population size at the four sites. The standard errors of the estimates of males for individual nights at the peak of the breeding season varied from 2 to 13% across the four sites (mean 9%).

Head counts of male toads were made at night, by torchlight. The entire area surrounding each pond was traversed, and counts of the number of individuals were made. Animals in the water and those within 1 m of the water's edge were included. Approximately seven sites were counted per night on a 3-night rotation. Dates of breeding sometimes varied by several days, even for sites separated by less than 1 km. To ensure that the peak of the breeding season was included, each site was visited on at least four occasions.

Head counts for males and females were considered separately, as females stay at the breeding site for a shorter period than do males and their numbers cannot be estimated easily. However, samples of the population obtained by trapping on land are not sex-biased and the sex ratio was derived from captures taken throughout the spring influx at drift fences or perimeter fences at six sites. The proportion of females ranged from 14 to 19% (mean  $17 \pm 3\%$ ). This was used as a conversion factor to derive a total population estimate.

Determination of toad presence or absence across 259 ponds within the same area (including the 20 ponds with population estimates) was based on site visits during the breeding season and extensive searches for breeding adults, larvae, or eggs. Surveys were conducted by two or three individuals. All sites were visited on at least two occasions in the spring and early summer.

## 2.3. Genetics data collection

Toads ( $n = 891$  or 50 from most locales) were collected as adults, tadpoles, or metamorphs. Adults and metamorphs were captured either at drift fences which surrounded several sites or during intensive searches of aquatic or terrestrial vegetation within or adjacent to the pond. Tadpoles were collected using dip nets. Tissues from adults were obtained by clipping a digit from a hind foot and placing the sample in 100% ethanol. Tadpoles and metamorphs were sacrificed and placed immediately in 100% ethanol. Specific methodologies for extraction of DNA and laboratory protocols for analysis and scoring of the three minisatellite loci used for subsequent analyses (*BbuMS1*, *BbuMS2*, *BbuMS3*) can be found in Scribner et al. (1994, 1997).

## 2.4. Environmental data

For each of 259 randomly chosen ponds a series of habitat variables were recorded in three general categories (aquatic, terrestrial, and "landscape"). Aquatic and terrestrial variables were recorded during site visits, and characterize habitat within ponds and within a 100-m radius around each pond, respectively. Landscape variables were recorded from topographic maps and from an interpreted "Thematic Mapper" (TM) satellite image, for an area with a 1-km radius around each pond. This ecological "neighborhood" (Addicott et al., 1987) was chosen as an approximation of toad home range size (cf. Sinsch, 1988), on the basis of spatial autocorrelation analysis of population gene frequencies (see beyond) which suggested an "effective" distance of terrestrial gene flow among ponds. In the absence of firm a priori knowledge on the environmental variables important to the ecology and population genetics of toads, a large number of ecological parameters were recorded (Table 1).

TM data was recorded on 16 April, 1984. Spatial resolution of the image was 30 m. The selection of training sites was done in ILWIS (1992), using a false color composite image of TM bands 3, 4, and 5, and was aimed at obtaining maximum differentiation in the

vegetation cover. Ground truth data were recorded from recent color aerial photographs in combination with field visits (for natural, semi-natural, and other non-agricultural fields), or extracted from the cropping history files provided by local farmers. Classification

Table 1  
Environmental variables recorded for 259 ponds in the Leicestershire region of central Great Britain

Variable	Code	Specifier
<i>Aquatic</i>		
Average slope of bank	BAN	<20, 21–40, 41–60, 61–80, >80% (1–5)
Pond depth	DEP	≤20, 20–50, 50–75, 75–100, >100 cm (–5)
Macrofauna	FAU	Sparse, moderate, abundant (1–3)
Emergent vegetation	FEM	0, 1–10, 11–20, 21–50, >50% (1–5)
Floating vegetation	FFL	0, 1–10, 11–20, 21–50, >50% (1–5)
Fish	FIS	Present, absent (1, 0)
Marginal vegetation	FMA	0, 1–10, 11–20, 21–50, >50% (1–5)
Waterfowl	FOW	Present, absent (1, 0)
Submerged vegetation	FSU	0, 1–10, 11–20, 21–50, >50% (1–5)
Inflow and/or outflow	I&O	Present, absent (1, 0)
Pond permanency	PER	Permanent, impermanent (1,0)
Pollution	POL	Not, slight, strong (1–3)
Shade	SHA	0, 1–20, 21–40, 41–60, >60% (1–5)
Pond size	SIZ	ha
Turbidity of water	TUR	Transparent, clear, moderate, opaque (1–4)
Pond type	TYP	Field pond (1), lake (2), marsh (3), quarry (4), other (5)
Pond use	USE	Undetermined, cattle, fowl, fish, multiple (1–5)
<i>Terrestrial (100 m range)</i>		
Arable field	AR	Present, absent (1,0)
Ditch	DI	Present, absent (1,0)
Dry stone wall	DW	Present, absent (1,0)
Garden	GA	Present, absent (1,0)
Hedge	HE	Present, absent (1,0)
Marsh	MA	Present, absent (1,0)
Pasture	PA	Present, absent (1,0)
Pond	PO	Present, absent (1,0)
Relief	RE	Flat, sloping, undulating (1–3)
Road	RO	Present, absent (1,0)
Scrub	SC	Present, absent (1,0)
Stream	ST	Present, absent (1,0)
Urbanization	UR	Present, absent (1,0)
Woodland	WO	Present, absent (1,0)
<i>Satmap (1 km range)</i>		
Altitude	ALTI	m
Arable fields	ARABL	% cover
Canals	CANA	km / km <sup>2</sup>
Ditches	DITC	km / km <sup>2</sup>
Hedges	HEDG	km / km <sup>2</sup>
Lakes	LAKES	% cover
Pasture regular	PASTU	% cover
Ponds	POND	Number/km <sup>2</sup>
Railways (disused)	RAID	km / km <sup>2</sup>
Railways	RAIL	km / km <sup>2</sup>
Relief	RELI	Grid intersections (arbitrary unit)
Rivers	RIVE	km / km <sup>2</sup>
Roads	ROA1	km / km <sup>2</sup>
Roads (secondary)	ROA2	km / km <sup>2</sup>
Rough pasture	ROUGH	% cover
Sheep pasture/parkland	SHEEP	% cover
Soil type	SOIL	drainage capability (1–5)
Streams	STRE	km / km <sup>2</sup>
Urbanization dense	URDEN	% cover
Suburban	URSUB	% cover
Woodland	WOODL	% cover

was carried out on an image derived from six TM bands (i.e. all bands excluding number 6). Principal components were computed with IDRISI (1992) using unstandardized variables, whereby the TM bands were weighted according to their variance. The first three principal components, together explaining 98% of the variance, were retained. Kappa-corrected classification accuracy of the image was 74%. ILWIS was used to register the classified image to the Ordnance Survey map grid.

### 2.5. Statistical analysis

Estimates of observed direct-count heterozygosity ( $H_o$ ) and mean number of alleles per locus (NALL) were obtained using the program BIOSYS (Swofford and Selander, 1981). Estimates of inbreeding coefficients ( $F$ ) were calculated as  $(1-H_o/H_e)$  where  $H_e$  is expected (under Hardy-Weinberg) heterozygosity for each population. The variables  $F$  and  $H_o$  were arcsin transformed before analysis to reduce skewness, as were the landscape land-use cover percentages (see below). Population estimates of NALL may be influenced by historical changes in population size. This measure of genetic diversity can be biased if sample sizes (and thus the likelihood of observation) differs across populations. Estimates of allelic diversity were thus made based on allelic richness ( $r_g$ ; Petit et al., 1998) which standardizes allelic diversities for population differences in sample size. Estimates of inter-population variance ( $F_{st}$ ) were estimated as described by Wright (1965). Significance of  $F_{st}$  was determined as in Workman and Niswander (1970).

Spatial autocorrelation analysis (Sokal and Oden, 1978) was employed to examine the degree of non-random spatial allelic structure among populations at different intervening distances. Evidence of non-independence in allele frequency (i.e. significant autocorrelation) at each distance interval was used to infer distances of effective gene flow and to define plausible landscape scales over which habitat features might profitably be examined. Spatial autocorrelation coefficients (e.g. Moran's  $I$ ; Moran, 1950) indicate whether and to what extent, observed population attributes (i.e. allele frequencies) are influenced by other populations as a function of geographic proximity (Cliff and Ord, 1981). Moran's  $I$  values were estimated for all pair-wise population combinations at each of a series of intervening distances (e.g.  $\leq 2$  km, 2–4 km, etc.). Moran's  $I$  values at each distance level represent tests of the null hypothesis that observations (allele frequencies) are mutually independent (Oden, 1984). Inferences of ecological or evolutionary processes which generate the dispersion of allele frequencies across a landscape may be inferred by examination of all Moran's  $I$  values across all distance classes (i.e. over the entire correlogram; Sokal and Wartenberg, 1981).

For each category of environmental variables (aquatic, terrestrial, landscape) a stepwise multiple logistic regression (for analysis of toad presence/absence using habitat data from 259 populations) and a stepwise multiple regression (for toad abundance and genetic variables  $r_g$ ,  $H_o$ , and  $F$ ;  $N=20$  populations) was performed using, in turn, each of the three genetic characters and two demographic characters as dependent variables. Analyses were initially conducted with lenient entry and removal probabilities of  $P<0.15$  (Bendel and Afifi, 1977). Since there were more habitat variables than ponds, only those variables within each category that were identified as explaining a significant proportion of the inter-population variance in the genetic variables were retained for further analysis. The reduced set of variables was subsequently combined and reanalyzed (Sokal and Rohlf, 1981) using a nominal alpha level of  $P<0.05$  to identify the variables that were significant predictors for each dependent variable. Overall regression equations are also described. Statistical analyses were conducted using Systat 5.1 (Wilkinson, 1989) and StatView 1.03 (Feldman et al., 1988).

## 3. Results

### 3.1. Genetic characteristics

Breeding populations of *B. bufo* in the Leicestershire region exhibited considerable spatial genetic structure. Significant differences in allele frequency were observed at each of the three minisatellite loci examined ( $F_{st}$  for *BbuMS1*, *BbuMS2*, *BbuMS3* were: 0.029,  $P<0.01$ ; 0.029,  $P<0.01$ ; 0.077,  $P<0.01$ , respectively; mean  $F_{st}=0.045$ ,  $P<0.01$ ), suggesting low gene flow across the study area. This relationship was further supported by estimates of significant spatial autocorrelation over inter-pond distances of  $\leq 2$  km (Fig. 2). Thus, non-independence in allele frequency, and thus distance of effective gene flow, appears to be restricted to ponds in close proximity.

Estimates of genetic characteristics varied greatly among the 20 toad populations ( $H_o$  — range 0.57–0.76; NALL — range 4.0–11.0;  $r_g$  — range 2.90 — 6.69;  $F$  — range 0.003–0.186; Table 2). Populations which exhibited low levels of heterozygosity (e.g., Holly Hayes;  $H_o=0.586$ , Table 2) were also characterized by lower mean number of alleles per locus and allelic richness ( $r_g=2.90$ ) relative to other populations (Table 2). Measures of genetic diversity were inter-correlated.

However, the significant negative correlation between  $H_o$  and  $F$  (Spearman's  $r=-0.85$ , 19 d.f.,  $P<0.01$ ) was unexpected, and suggests that individuals from populations with low genetic diversities may also be mating in a non-random manner (i.e. may exhibit some level of inbreeding). Alternatively, populations with low heterozygosity and

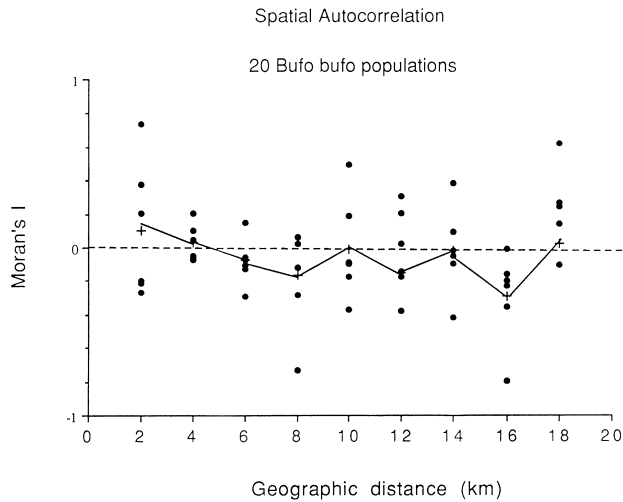


Fig. 2. Spatial autocorrelation for two most common alleles from each of the three minisatellite loci and mean Moran's  $I$  value across all alleles. Allele frequency data were obtained from 20 breeding populations of *Bufo bufo* from central Great Britain. Distance values along the x-axis represent inter-pond geographic distance.

allelic richness may also exhibit a high degree of genetic relatedness (i.e. high coancestry), and thus high estimates of  $F$  due to high reproductive variance and low effective numbers of breeding adults (Scribner et al., 1993). Indices of population size were not correlated to population measures of genetic diversity ( $P > 0.05$ ). However, significant spatial autocorrelation of allele frequencies among breeding ponds  $\leq 2$  km apart suggests that the effective size of many populations may encompass several breeding ponds.

### 3.2. Correlations of genetic and habitat variables

Habitat variables have significant predictive potential for toad presence or absence and for resolving differences in toad abundance and genetic characteristics (Table 3). Genetic characteristics were related to both aquatic variables within the breeding ponds and to landscape variables while toad presence and population size were related exclusively to the landscape and terrestrial variables. Terrestrial variables measured immediately adjacent to the pond appeared to have little predictive value for *B. bufo*.

Stepwise regression analysis revealed significant relationships between allelic richness and presence of gardens (a measure of human residential presence), roads and the percentage of the immediate area in livestock (sheep) pasture. Presence of additional ponds immediately adjacent to a study pond was a significant predictor of population inbreeding coefficients ( $F$ ; Table 3). Presence of ponds was indicative of lower inbreeding coefficients. The proportion of inter-population variance in allelic richness explained by habitat was high ( $R^2 = 0.64$ ), while the predictive potential for  $F$  was considerably lower ( $R^2 = 0.21$ ). Habitat variables were not significant predictors of population heterozygosity.

Landscape terrestrial variables had significant predictive potential to resolve population abundance and toad presence or absence ( $R^2 = 0.50$  and  $\rho^2 = 0.52$ , respectively; Table 3). High toad breeding population numbers were typically found associated with woodland habitats but with few ponds immediately adjoining to the study ponds. Toad presence or absence was significantly

Table 2  
Genetic and demographic characteristics of *Bufo bufo* from 20 breeding populations in the Leicestershire region of central Great Britain

Population	Sample size	Number of alleles per locus (NALL)	Allelic richness (ALLELE)	Direct count heterozygosity ( $H$ )	Inbreeding coefficient ( $F$ )	Estimated population size ( $N$ )
(1) Bagworth Park	50	8.0	5.25	0.578	0.161	2300
(2) Bosworth Mill	50	7.0	4.91	0.604	0.148	200
(3) Botshollow	50	6.3	4.37	0.627	0.094	2800
(4) Cadeby Quarry	50	8.7	5.82	0.673	0.039	2400
(5) Charnwood Colony	40	7.7	5.39	0.612	0.176	4100
(6) Farm Town	18	6.3	5.33	0.696	0.056	600
(7) Fish Pond	50	7.0	4.99	0.759	0.003	5100
(8) Grace Dieu	50	8.0	4.96	0.669	0.052	3200
(9) Hermitage	20	6.0	4.88	0.677	0.086	1300
(10) Holly Hayes	50	4.0	2.90	0.586	0.111	700
(11) Kidger	50	7.3	4.81	0.643	0.101	3100
(12) Limby Hall	50	6.7	4.75	0.640	0.165	3500
(13) Lount Abbey	50	11.0	6.69	0.606	0.210	1500
(14) Market Bosworth	50	7.6	5.09	0.731	0.038	5500
(15) NCB	41	7.7	5.52	0.664	0.111	1000
(16) Osbaston	50	6.7	4.85	0.607	0.186	4800
(17) Osgathorpe	50	7.3	4.95	0.691	0.048	2500
(18) Shepshed	50	7.3	4.87	0.574	0.183	2100
(19) Springwood	22	7.7	6.15	0.621	0.141	12,000
(20) Worthington	50	7.7	4.78	0.640	0.112	3700

Table 3

Results of stepwise multiple regression analysis, revealing relationships between three population measures of genetic diversity (heterozygosity, allele richness, and Wright's inbreeding coefficient) and population size ( $N$ ) for the common toad (*Bufo bufo*) and a series of habitat variables recorded during on-site visits ('aquatic' and 'terrestrial') and from satellite image analysis ('satmap')<sup>a</sup>

Dependent variable	Habitat variables		
	'Aquatic' pond data	'Terrestrial' field data (100 m radius)	'Landscape' (1 km radius)
Allele richness	None	GA (9.53)	ROA2 (16.7) SHEEP (6.30)
Heterozygosity	Predictive equation: $ALLELE = 6.554 - 0.793(GA) - 0.318(ROA2) - 0.243(SHEEP)$ , $R^2 = 0.64$		
Inbreeding coefficient	None	None	None
Population size	None	PO (4.91)	None
	Predictive equation: $F = 0.10 + 0.075PO$ , $R^2 = 0.21$		
Toad presence	None	None	POND (6.99) WOODL (12.0)
	Predictive equation: $N = 2240 - 311POND + 261WOODL$ , $R^2 = 0.50$		
	FIS (42.8)	WO (5.52)	ALTI (6.22) CAN (8.61) HEDG (4.49) RELI (17.0) RIVE (12.8) ROUGH (8.30) SOIL (8.23)
	Predictive equation: $P = (1 / (1 + \exp(1.12 - 0.021ALTI + 1.76CAN - 2.92FIS + 0.02HEDG + 0.19RELI + 6.68RIVE - 0.44ROUGH + 0.73SOIL - 1.12WO)))$ , $\rho^2 = 0.52$		

<sup>a</sup>  $F$ -values associated with all significant independent variables in the final model are given in parentheses. Results of multiple logistic regression analysis are presented describing the probability ( $P$ ) of a pond to be inhabited by common toads on the basis of a series of habitat variables. Nomenclature and definitions of habitat variables are provided in Table 1 and in Section 2 of the text.

related to a large number of variables including undulating relief and low overall altitude, poorly drained soils, amount of hedgerows, canals, rivers and the proportion of surrounding landscapes composed rough pasture.

## 4. Discussion

### 4.1. Conceptual background and species biology

Understanding the consequences of habitat alteration, and fragmentation on levels of biological diversity has become a matter of increasing concern. However, in the absence of definitive background data on population demography or genetics, the success of conservation measures may be problematic. Thus, the recognition of habitat components which are important to species ecological needs becomes increasingly important. If certain habitat variables prove predictive of population abundance and of levels of genetic diversity, habitat "quality" may be used as an indirect barometer of current and historical population status and future viability. Our analyses seek to use features of the habitat to elucidate aspects of population demographic and genetic diversity which are of general conservation importance and

which conceptually may be extrapolated to other geographic contexts and species.

Data obtained from our ecological genetic studies of *B. bufo* reveal several important results which have direct relevance to conservation strategies for *Bufo*. Populations differed greatly in measures of genetic variability and in the magnitude of deviation from Hardy-Weinberg genotypic proportions. Differences are most plausibly explained by pond-to-pond variation in breeding effective population sizes (Scribner et al., 1997) which are in part related to characteristics of the aquatic breeding habitat. Secondly, breeding ponds vary greatly with regard to pond aquatic characteristics, surrounding land use, terrestrial habitat characteristics, and proximity to other breeding sites. Several habitat variables have a degree of predictive potential in elucidating toad presence or absence, adult breeding population size, population measures of genetic diversity, and departures from random mating. Finally, different habitat components were found to be correlated with demographic and with genetic dependent variables, suggesting the differential importance of terrestrial and aquatic environments to different life history stages and seasonal activities.

It is of importance to have some criteria for determining spatial scales over which to collect habitat data relevant to the year-round life history and fitness of

individuals within a breeding population. Inter-pond correlations in gene frequency were used to provide the spatial context by which habitat features could be quantified for individual breeding ponds. Significant spatial autocorrelation in gene frequencies were found over inter-pond distance  $\leq 2$  km while populations separated by greater intervening distance were genetically independent.

Once appropriate spatial scales have been identified, interpretation of populations-specific genetic and surrounding environmental relationships necessitates an understanding of *Bufo* population biology. Common toads are a widely distributed boreal species. Toads occupy terrestrial habitats during most of the year. Thus, toad presence and population numbers are intimately tied to terrestrial habitats that provide forage, cover, and hibernation sites as well as determining direction and extent of movements. Aquatic habitats are used solely for breeding. Adults of reproductive age are highly philopatric to aquatic breeding sites. In any year 79–96% of adults return to their pond of origin (Reading et al., 1991). Movements among ponds do occur, but are likely to be restricted beyond inter-pond distances of  $> 1$  km (Sinsch, 1988). Similar estimates of effective gene flow distance have been indirectly inferred based on the spatial variance in single-locus minisatellite gene frequencies (i.e. “patch sizes” of  $\leq 2$  km based on spatial autocorrelation analysis; Fig. 2). Landscape-level habitat features are thus likely to affect both levels of gene flow (i.e., habitat permeability) and survival (i.e. as a fitness function; Forman, 1995; Halley et al., 1996).

Populations differ greatly in all genetic variables measured (Table 2). However, inter-population variance in these measures of genetic diversity are not equally well explained by the habitat data collected. Sixty four percent of the inter-population variance in allelic richness was explained by landscape variables. Conversely, the number of variables and predictive potential is small or absent for inbreeding coefficients and heterozygosity, respectively (Table 3). These results are due in part to the properties of the genetic variables. In the absence of factors that act to maintain variation (e.g. immigration, mutation, heterosis) the expected loss in genetic variation is a direct function of effective population size (Wright, 1969). Only a fraction of the genetic variation will be lost on average in any one generation of random mating because only rare alleles, which contribute little to overall heterozygosity or to heritable variation in quantitative traits will be lost (Lande, 1988). The NALL has been found to be a more sensitive indicator of population differences in genetic diversity than is multi-locus heterozygosity (Leberg, 1992). Leberg (1992) found that weak correlations between population heterozygosity and the number and composition of population founders resulted from an increase in the variance of heterozygosity due to drift in allele frequencies. As estimates of population

NALL are highly dependent on sample size, allelic richness was estimated as a standardized measure of allelic diversity. In previous work we have shown that census population size confers little predictive value for inferences of effective breeding population size (effective breeding population sizes  $[N_b]$  for three populations surveyed in the study were 38, 16 and 60 yielding  $N_b/N$  ratios of 0.003–0.015; Scribner et al., 1997; also see non-significant correlations between population genetic and demographic characteristics cited above).

#### 4.2. Environmental variables as predictors of population characteristics

Multiple regression analysis suggests that habitat characteristics could affect population characteristics in several ways. Some ponds may be more optimal breeding sites than others. Proximity of other ponds and characteristics of the landscape in the vicinity ( $\leq 2$  km) of breeding ponds, appear most important in explaining inter-population variance in genetic-dependent variables. Roads and sheep pastures which are typically heavily grazed may hinder dispersal and increase risk of predation because of lack of shelter and direct mortality (i.e. road kills). These habitat components may determine breeding effective population size through effects on adult juvenile survival and effects on the geographic extent of genetic neighborhoods. Ecological factors (particularly the proportion of the female population which successfully produces progeny) can be critical in determining the magnitude and partitioning of gene correlations within and among groups, and to the genotypic composition of offspring produced during each year (Scribner et al., 1993).

Characteristics of the terrestrial environment surrounding each breeding pond (e.g. land use practices; Table 3) may affect forage availability, sources of hibernation sites, and metamorph and adult survival, which directly affects toad abundance. Toad abundance is positively correlated with the proportion of surrounding area in “rough” pasture. Habitat characteristics can also affect the rate of dispersal among semi-isolated breeding sites. *Bufo* which breed in discrete ponds in Great Britain are essentially structured as sets of local populations within a metapopulation. Rates of gene flow among populations are in large part determined by the degree of isolation which is a function of the physical distance between breeding sites (Reading et al., 1991) and presumably on the characteristics (or permeability) of intervening habitat (Gilpin, 1991; Forman, 1995). Habitat components that are a hindrance to dispersal may increase the degree of population isolation and increase the rate of drift and inbreeding. The generality of these findings may vary across species, as different species will utilize terrestrial habitats adjacent to wetlands to different degrees (Semlitsch, 1998).



#### 4.3. Utility of VNTR loci for quantifying genetic diversity

VNTR loci offer a number of unique advantages relative to traditional (i.e. allozyme) markers used to measure nuclear genetic diversity. Mini- and micro-satellite loci reveal levels of variation at the DNA level. Further, these markers are located in non-coding regions of the genome, and thus are not under selection, as may be the case for certain enzyme polymorphisms (Karl and Avise, 1992). Microgram quantities of DNA may be obtained from small samples (e.g. toe clips) and thus a large variety of non-destructive sources of material may be used without requiring immediate freezing or refrigeration. This is particularly true for microsatellites which are based on polymerase chain reaction methodologies (Bruford and Wayne, 1993; Scribner and Pearce, 2000). Perhaps the greatest advantage lies in the large number of alleles routinely resolved at each locus, due in part to the rate and mechanism of mutation (Jeffreys et al., 1988).

#### 5. Conclusions

Agricultural landscapes disrupt natural areas by fragmenting important natural ecological linkages (Carroll, 1987). Pond numbers in Great Britain probably reached a peak about 100 years ago (Rackham, 1986). Since then, and especially after the Second World War, there has been a decline, which means that remnant breeding habitats are becoming increasingly isolated. Populations may be increasingly susceptible to extinction events as local declines in population number, due to declining terrestrial habitat quality, and concomitantly declining genetic diversity, is not likely to be balanced by recolonization and gene flow. Precise data of population dynamics and of genetic characteristics are often unavailable to assess population status. Analyses conducted as part of the present study suggest that habitat data may provide valuable information for assessment of population demographic and genetic characteristics, and inferentially, on expectations for future population viability and recolonization potential.

Quantification of the importance of ecological variables which affect population demographic and genetic characteristics and of spatial scales over which such relationships apply is of value for *B. bufo* and other species of limited vagility which inhabit highly developed landscapes (Beebe et al., 1996; Hitchings and Beebe, 1996). Conceptually, in the absence of definitive population demographic data, specific habitat characteristics may offer predictive potential for inferring population characteristics and future viability. However, complexities posed by population sampling and historical changes in landscape features dictate that choice of ecologically

and evolutionarily relevant habitat characteristics be chosen with care.

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