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Gene flow in a direct-developing, leaf litter frog between isolated mountains in the Taita Hills, Kenya

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Abstract Amphibians are in decline worldwide, and high altitude tropical areas appear to be the worst affected. This is in stark contrast with current information we have on gene flow in amphibian populations which focus on temperate pond breeding species. Using AFLP markers, we show that a small, direct-developing, leaf litter frog from the Taita Hills in south–west Kenya (*Schoutedenella xenodactyloides*) has extended populations covering large areas (>3.5 km) of fragmented, forest habitat, uncharacteristic of typical amphibian models. Further, we demonstrate high levels of gene flow ($F_{ST} < 0.065$) through unsuitable dry savannah habitat which might otherwise be considered a barrier to dispersal. Landscape genetic analysis demonstrates a strong link between hydrologic features, and further highlights links between sites through specific catchments. We propose a model of passive-active dispersal for the Dwarf Squeaker, *S. xenodactyloides*, which features passive downhill and active uphill movements over large areas, contrasting with limited cross slope movements. Our study highlights the importance of the diverse reproductive strategies of the Amphibia when considering dispersal and gene flow, and hence conservation management.

Keywords Anura · Dispersal · AFLP · Africa · Cloudforest · Leaf-litter

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Introduction

Recent work on global amphibian declines has begun to precise particular scenarios in which population losses are more likely to occur. Some of the most alarming amphibian declines are from high altitude sites (Houlahan et al., 2000; Morrison and Hero, 2003), including the tropics (Lips et al., 2003; Pounds et al., 2006), and also where habitat is lost or fragmented (e.g. Curtis and Taylor, 2003). Amphibian conservation efforts can be greatly aided by an understanding of population structure and of the ability of target species to disperse (Beebee, 2005; Beebee and Griffiths, 2005). Information gained would make a substantial contribution to determining conservation management units and their connectivity. However, we remain ignorant of basic life-history information let alone detailed population dynamics of amphibian species with tropical distributions as most studies are made on temperate species (but see for example Driscoll, 1998; Lampert et al., 2003). Clearly, urgent efforts are needed to compare results from studies on temperate species and assess their relevance for the conservation of species from high altitude tropical locations.

Amphibians are usually described as poor dispersers (Blaustein et al., 1994), their populations in most cases show a strong phylogeographic structuring (Avise,

2000) which is indicative of low dispersal abilities. Thus amphibians have been considered as a crucial group in conservation biology (e.g. Stuart et al., 2004; Beebee, 2005). However, recent evidence for long distance dispersal in ecological studies suggest that at least some species are far from sedentary; instead that regular dispersal by a few individuals may cover several kilometres (see Smith and Green, 2005 for a recent review). Observations on dispersal may be made directly or indirectly through such techniques as mark-recapture (e.g. Lampert et al., 2003; Funk et al., 2005b). Although informative, such methods require huge investment in terms of field-time, are often inefficient at detecting long-distance dispersal, and the movement of individuals cannot be equated with gene flow (Slatkin, 1985). Inferring dispersal as a result of genetic studies has become a popular alternative, especially for evaluating potential barriers to gene flow (e.g. Lampert et al., 2003; Burns et al., 2004; Kraaijeveld-Smit et al., 2005). While many studies continue to demonstrate the generality that amphibians are highly philopatric (e.g. Rowe et al., 2000; Shaffer et al., 2000; Kraaijeveld-Smit et al., 2005), others show high dispersal abilities in an increasing contrast of terrain (e.g. Burns et al., 2004; Funk et al., 2005a).

Dispersal of amphibians is often considered to centre on movement to or from breeding ponds (e.g. Shaffer et al., 2000; Funk et al., 2005a; Mazerolle and Desrochers, 2005), with the high site fidelity of adults giving rise to strongly differentiated and genetically distinct population structure within otherwise homogeneous habitats. This has given rise to ''ponds as patches'' models which propose that most populations of amphibians conform to metapopulation theory (e.g. Marsh and Trenham, 2000), but it has been argued that many examples do not meet the conditions for a true metapopulation structure (Smith and Green, 2005). Subsequently, genetic studies reveal conflicting evidence suggesting that for some species ponds represent discrete populations (Lampert et al., 2003; Kraaijeveld-Smit et al., 2005), whereas for others they do not (Jehle et al., 2005).

Breeding in ponds is typical for amphibians in temperate regions, and external aquatic fertilisation without parental care is the presumed ancestral and most widespread amphibian reproductive strategy (Duellman and Trueb, 1986). However, it is only one of a wide array of reproductive strategies that are displayed by the Amphibia (see Crump, 1995; Wake, 2003). Differences in the way amphibians reproduce might be expected to play an important role in how different species disperse (see Vences et al., 2002) as well as the sizes of their populations (see Crawford, 2003). For example, the genetic relationships between populations of so called ''explosive breeders'' (participating in mass spawnings in ponds on only a few days of the year) may contrast dramatically with a direct-developing species with an extended breeding season.

Recent publications have highlighted the importance of assessing the influence of landscape on conservation genetics (Manel et al., 2003). The powerful combination of digitalised geographic information systems (GIS) and samples from precise localities within a landscape provides the possibility of testing models to determine the influence of landscape features on gene flow (e.g. Arnaud, 2003; Geffen et al., 2004; Sumner et al., 2004). Landscape genetics of amphibians have proposed and tested models for movement in mountainous sites. For example, the study of Spear et al. (2005) found that gene flow follows a straight line topographic route, with river crossings and open shrub habitat reducing gene flow in the blotched tiger salamander, *Ambystoma tigrinum melanostictum*. Funk et al. (2005a) found that high topographic relief acted as an effective barrier for Columbia spotted frogs, *Rana luteiventris*. They proposed a model consisting of three points: (i) high gene flow between low populations (see also Tallmon et al., 2000); (ii) little gene flow between high populations, and (iii) small exchange between high and low populations. These studies highlight the importance of large gene pools in basins which can contribute to the genetic diversity of high altitude sites.

Mountains provide a wide range of climatic zones which can differ significantly from the surrounding area, and have been shown to be effective barriers for amphibian dispersal in temperate areas (Funk et al., 2005a). Janzen (1967) suggested that topographic barriers may be even more effective in the tropics, asserting that from a physiological viewpoint tropical mountain passes are higher and tropical valleys lower. Janzen (1967) argued that in temperate regions ectotherms are acclimated to significant seasonal changes in temperature and are therefore more tolerant of variable temperatures when dispersing at different altitudes. Conversely, in the tropics the reduced fluctuations in seasonal temperature would result in a reduction of thermal tolerance for higher or lower altitudes. Hence we might expect that the efficacy of mountains and valleys as barriers for ectotherms such as amphibians is elevated in the tropics.

The Eastern Arc Mountains of Kenya and Tanzania are topped by lush tropical cloud forests at altitudes three times higher and with precipitation up to seven times greater than the surrounding low, hot and dry savannah (Newmark, 2001). The Eastern Arc

Mountains and coastal forests have been recognised as one of the most important areas for biodiversity worldwide (Myers et al., 2000), as well as being one of the most threatened (Brooks et al., 2002). Of the high altitude sites, the Taita Hills in southeast Kenya have the least remaining natural forest (Newmark, 1998), and have been used as a model for the problems of habitat fragmentation in a tropical forested landscape (Githiru et al., 2002) following in depth analysis of avian population demographics and genetics (Galbusera et al., 2000; Lens et al., 2002; Galbusera et al., 2004). A key feature of the landscape is that natural and plantation forest fragments are not only divided by a recent agricultural patchwork, but also by ancient and deep divisions between mountain blocks which reach down to the Tsavo plain (Fig. 1a), a hot, dry savannah and effective barrier to dispersal movements for some bird species (Galbusera et al., 2000, 2004).

We chose a small, abundant, direct-developing, leaf litter frog, the Dwarf Squeaker *Schoutedenella xenodactyloides*, indigenous to the cloud forests of the Taita Hills. We used 50 amplified fragment length polymorphisms (AFLPs) of 159 adult frogs to study the genetic variation within and between 8 sampling sites in the Taita Hills in order to respond to the following questions: (1) In the absence of breeding ponds, are populations structured by the extent of their forested habitats? (2) Is habitat size related to genetic heterogeneity? (3) Does the hot, dry savannah form a barrier to gene flow?

Materials and methods

Study species

The Dwarf Squeaker *S. xenodactyloides*, is a small (10–22 mm snout-vent length) leaf litter frog with a large but discontinuous distribution in eastern Africa (Barbour and Loveridge, 1928; Channing and Howell, 2005). Although little is known about the natural history of this species, it is known to be direct developing with

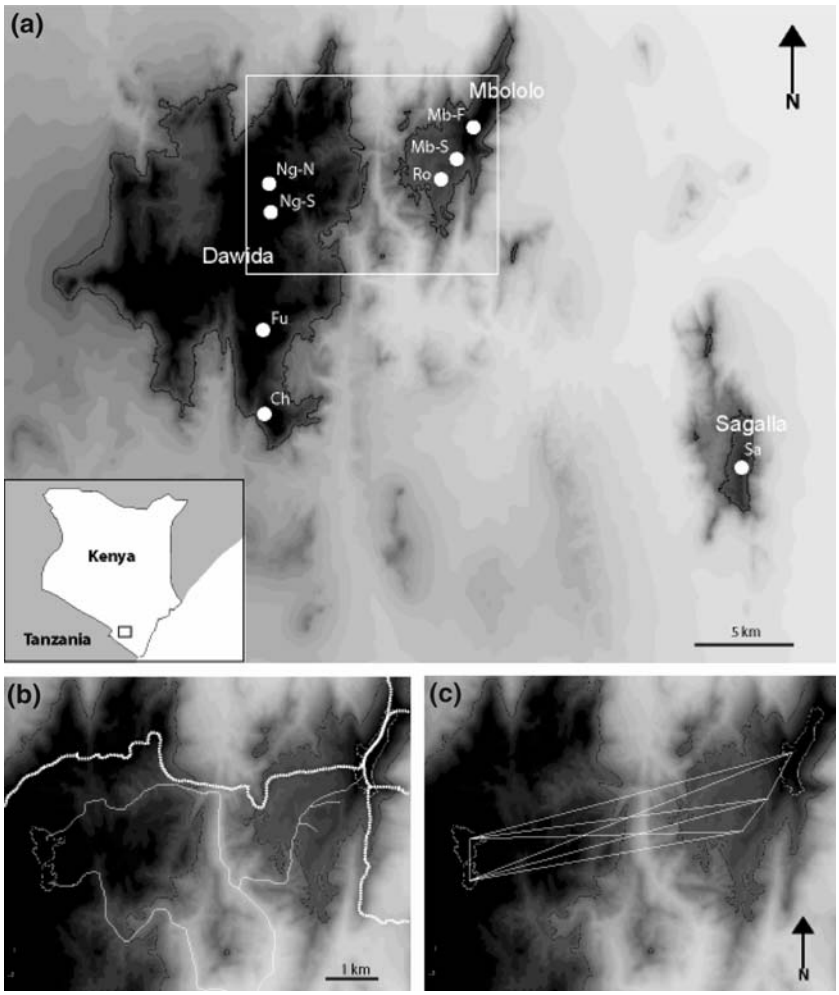

Fig. 1 Distribution of sample sites for the Dwarf Squeaker within the Taita Hills, Kenya shown by (a) topographical relief with collection sites marked as white points, mountain block names are indicated together with the position of the area in Kenya (inset). The white square demarcated in (a) corresponds to parts (b) which shows detail of the Ngangao-Mbololo hydrographical basin (dotted white line) and the distances between the 5 sites (solid white line) with respect to an upstream flow model (implemented in ARCVIEW 9), and (c) straight line distances between sites; used in the Mantel and partial mantel tests (see text, Table 4)

eggs laid in leaf litter (Channing, 2001; Channing and Howell, 2005). The newly hatched juveniles consume mostly collembolans and mites, while adults are general predators of leaf-litter invertebrates with ants dominating prey ingested at one site in Malawi (Blackburn and Moreau, 2006). This species can be found over a wide elevational range (Poynton, 2003), but in the Taita Hills it is found in abundance above 1300 m asl in natural pristine and disturbed forests as well as exotic plantation forests (GJM unpublished data).

Study area

Sites selected (shown in Fig. 1 and Table 1) were in indigenous cloud forest (see Beentje, 1988; Wilder et al., 1998) separated by a mosaic of small-scale agricultural holdings and exotic plantations (see Measey, 2004). Choice of sampling sites was made within the three mountain blocks in the Taita Hills, i.e. Dawida, Mbololo and Sagalla (Fig. 1). Dawida and Mbololo are separated by the Paranga Valley which reaches down to about 900 m asl and where climatic conditions and vegetation are similar to the surrounding Tsavo plain. The addition of a sample from the isolated hill of Sagalla (more than 25 km from any other site, Fig. 1.) was made to compare the possible effects of isolation by distance with Mbololo across the Tsavo plain. Specifically, samples were made in two sites within Ngangao Forest (Dawida) and between adjacent habitats on Mbololo to assess small-scale population structure within and between habitats.

Sampling

Adult Dwarf Squeakers were collected during December 2004 and January 2005 from 8 sites. Collection timing represents a brief dry period between the major bimodal monsoonal rains in East Africa (Newmark, 2001) typical of the Taita Hills (see Malonza and Measey, 2005). Two methods of capture were used. Three sets of drift fencing (0.3 m high) and pit fall traps (0.25 m diameter and 0.4 m deep) were placed in a Y-shaped array (i.e. three fences and four traps) with about 200 m between arrays, at each site for a period of four days. Traps and fences were checked for the presence of frogs each morning and evening. If after three nights less than 15 specimens had been collected, additional search-and-seize procedures were carried out in measured areas of leaf litter. Positions at all sites were obtained with a global positioning system (GPS, Garmin 12XL). Dwarf Squeakers were euthanased with anaesthesia (MS222, Sandoz) with a small amount of thigh muscle removed and stored in 98% ethanol until processing in the laboratory at the University of Antwerp. Specimens are deposited in the collection of the National Museums of Kenya (NMK/A/4537-45).

Sample processing

AFLPs have become a standard DNA fingerprinting technique in botany since their description (Vos et al., 1995), providing a viable alternative to microsatellites (Gaudeul et al., 2004). More recently, this method has become more popular with zoologists as an alternative to the costly development of microsatellite markers (e.g. Jehle and Arntzen, 2002). Previous use of ALFPs on amphibians suggest that this should be a useful technique for population level genetic inference (Curtis and Taylor, 2003), as well as other levels (Riberon et al., 2004; Whitlock et al., 2006). To our knowledge, no microsatellite primers have been developed for the anuran family Arthroleptidae, hence we decided to use AFLPs to investigate dispersal and gene flow for populations of Dwarf Squeakers in the Taita Hills.

DNA was extracted using the DNeasy Tissue kit (Qiagen). Quality and quantity of DNA from extractions was estimated by running all samples on a 1% agarose gel. AFLP analyses were performed using a modified protocol of Vos et al. (1995). AFLP templates were prepared by simultaneous digestion of about

Table 1 Dwarf Squeaker sample site information

Mountain block	Sample site	*N*	Latitude	Longitude	Elevation (m asl)	Forest fragment size (ha)	Hj	SE(*Hj*)
Dawida	Chawia	19	426624	9615596	1,605	93.596	0.24636	0.02563
	Fururu	24	426463	9620811	1,710	8.373	0.32374	0.02347
	Ngangao_S	26	427057	9627713	1,774	135.864	0.29549	0.02500
	Ngangao_N	13	426962	9629264	1,800	135.864	0.32091	0.02228
Mbololo	Mbololo_fo	13	438890	9632531	1,691	178.795	0.31373	0.02364
	Mbololo_sh	16	437890	9630813	1,415	0.005	0.28221	0.02526
	Ronge	13	436945	9629581	1,305	148.003	0.27923	0.02506
Sagalla	Sagalla	17	454790	9612494	1,545		0.22257	0.02496

Map datum ARC1960 was used for UTM coordinates. *N* is the sample size, *Hj* and SE*(Hj)* the expected heterozygosity and standard error from AFLP-SURV

100 ng of DNA with *Eco*RI and *Mse*I. Ligation of the restriction fragments to the adapters was performed in the same step. A 1:10 dilution of the restricted and adapter-ligated DNA was used as a template in the pre-amplification reactions. Pre-amplification products were generated using an *Eco*RI-primer (E-A) in combination with a *Mse*I-primer (M-C). For the final selective amplification, the 1:10 diluted pre-amplified DNA was amplified using a fluorescent labelled *Eco*RI-primer and a *Mse*I-primer each carrying three selective nucleotides (E-ACT or E-ACG with either M-CAC or M-CAG). Pre-selective and selective primer pairs were chosen based on the result of an initial screening for polymorphisms among a limited number of samples. Following amplification, an equal volume of formamide loading dye was added to the PCR products. After denaturation, the products were separated electrophoretically on 5% denaturating polyacrylamide gels, using a Li-Cor 42000 sequencer.

Data analysis

Output images of gels were analysed with Saga^{MX} AFLP® Analysis Software. Only distinct major bands ranging in size from 50 bp to 400 bp were scored for presence (1) or absence (0). We avoided using AFLP markers with low band absence frequencies ($0 < 3$), as recommended by Lynch and Milligan (1994) because their estimator is biased for such markers.

We used a Bayesian clustering approach implemented in STRUCTURE v 2.1 (Pritchard et al., 2000) to estimate the number of populations (K) in the sample and to assign individuals to one or more of these populations from sample sites (k). Hardy–Weinberg equilibrium and linkage equilibrium between loci within populations were assumed. For each K ($K = 1$ to 8) we ran an admix model with a series of 5 independent runs of 10^6 iterations following a burn-in period of 50,000 iterations.

Expected heterozygosity (Hj) and the degree of genetic differentiation among populations (F_{ST}) were calculated using the Lynch and Milligan (1994) method in AFLP-SURV (Vekemans et al., 2002), with data coded following Pritchard et al. (2000). This uses the average expected heterozygosity of the marker loci, or Nei's gene diversity, as a measure of genetic diversity of AFLPs. We assumed Hardy–Weinberg equilibrium to examine Hj at each site sampled to look for evidence of isolated populations using AFLP-SURV (Vekemans et al., 2002). Discrete genotypes are not generated in AFLP band profiles, therefore for analysis each AFLP marker was treated as a single locus with two alleles (Blears et al., 1998). Because AFLP markers are dominant, within-population genetic structure (i.e. F_{IS}) could not be assessed.

Next, we explored the relationship between Hj and the size of forest fragment in which frogs were captured together with other variables (habitat size, latitude, longitude and sample size) in order to identify potentially confounding effects. We used a Gamma statistic (in STATISTICA v 6) as this is preferable to Spearman R when the data contain many tied observations (see Table 1).

Following Funk et al. (2005a), we used two approaches to investigate the effects of landscape features. Firstly a qualitative examination of pairwise F_{ST} given from results of running AFLP-SURV (see above). Secondly, we used Mantel and partial Mantel tests (Smouse et al., 1986; Funk et al., 2005a; Spear et al., 2005) with pairwise F_{ST} values in ZT (Bonnet and Van de Peer, 2002) for correlations with distance. Distance was calculated in three ways: (i) direct distance in a straight line (Null model); (ii) distance in a straight line corrected for contours, i.e. topographical distance and (iii) distance via watercourses. This last distance measurement was calculated using the ''Upstream Flow Length'' as implemented in ARCVIEW 9.0 (ESRI). This calculates the longest upslope distance along the flow path, from each cell to the top of the drainage divide. The point of convergence along the flow paths from a pair of sites was used as the turning point. Lengths of all paths were calculated from a Digital Elevation Model of the Taita Hills (L. Lens and E. Matthysen, unpublished data) using ARCVIEW 9.0. Natural logarithms (ln) of all distance measurements were used to linearise the relationship between distance and F_{ST} (see Funk et al., 2005a). As watercourse distances could only be calculated between sites in the same hydrogeographic basin, Sagalla, Chawia and Fururu are not included in Mantel tests.

Lastly, we conducted an a posteriori AMOVA (Excoffier et al., 1992) test in ARLEQUIN v2.001 (Schneider et al., 2000) to determine whether populations were best grouped by their (i) home mountain block (i.e. Dawida, Mbololo or Sagalla), (ii) drainage basin, or (iii) the inferred population grouping from STRUCTURE results (see above). We repeated these tests at a finer scale within a single drainage basin.

Results

AFLP analysis

The AFLP assays were positive for all but 6 individual Dwarf Squeakers, giving a total data-set of 159

individuals from 8 sites (Table 1). No correlation was found between polymorphic AFLP fragment size and frequency ($r = 0.0216$, $P = 0.88$), indicating an absence of size homoplasy (see Vekemans et al., 2002). Band absence for the 50 polymorphic sites ranged from 3 to 145 (mean 76.6, SE 6.49) amongst the 159 individuals.

Population structure

Although STRUCTURE is sensitive to overestimation of values of K we found clear indication from the outputs of the various models that $K = 5$ (Fig. 2). Variability across runs was low, values of α within a run were constant, and values of Ln Pr(X | K) began to plateau at $K = 5$ (see Galbusera et al., 2004). In the a posterori allelic appointments, all sampling sites on Mbololo Mountain block are put into the same population, and Ngangao North and South collapse together. Other sample sites remain as discrete populations. We examined the partitioning for increasing K to determine whether population structure continued to be subdivided into biologically meaningful groups. At $K = 6$, an extra division was made within Ngangao, but not between sample sites; i.e. groups contained a mixture of individuals from Ngangao North and South. At $K = 7$ and 8, no site specific divisions were discernable.

Expected heterozygosity

The overall expected heterozygosity (Hj) was low, with a mean value of 0.29 (Table 1). Two sites, Sagalla and Chawia, were substantially lower than the other sites ($Hj = 0.22$ and 0.24, respectively), indicating a high level of isolation or small populations subject to genetic drift. While this result was expected for the isolated mountain block of Sagalla (minimum distance to other sites of 25 km; Fig. 1), Chawia is within the Dawida block and separated by a relatively short distance from Fururu (5 km). Analysis with the Gamma statistic found no correlations between expected heterozygosity and forest fragment size ($|\gamma| < 0.36$, $P \geq 0.08$; Table 2), nor with sample size, elevation, area searched, latitude or longitude (in all cases $|\gamma| = 0.000$, $P = 1.00$).

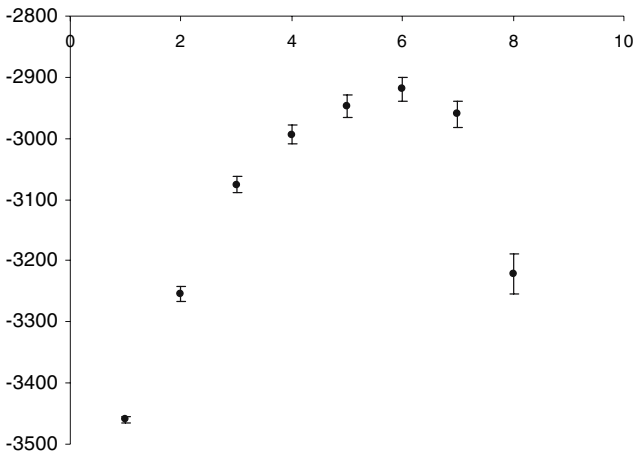

Fig. 2 Inference of K, the number of subpopulations, for Dwarf Squeakers using STRUCTURE v 2 Pritchard et al. (2000). The abscissa follows K as the number of inferred populations, while the ordinate *Ln Pr(X | K)* is the ln probability of the data given K (106 iterations, burn-in 50,000). $K = 5$ was chosen despite the minimum value of *Ln Pr(X | K)* at $K = 6$, see text for explanation

F_{ST} and Mantel tests

There was moderate genetic differentiation among study sites across the Taita Hills ($F_{ST} = 0$ to 0.203). Only one population pair did not a show significant pairwise differentiation: Mbololo forest and Mbololo shamba (Table 3). Genetic differentiation on Mbololo was generally low (Table 3), although the distances between the sites were from 2 km to 3.5 km (Fig. 1c). On Dawida, samples from North and South within Ngangao Forest also show a low pairwise F_{ST} (0.035), although here the distance separating the sites is lower (1.6 km) (Fig. 1c). Hence, examination of pairwise F_{ST}, while broadly confirming STRUCTURE results, suggest that the relationship between sites is more complex than simple isolation by distance, our proposed Null model. There is a significant difference in the pairwise F_{ST} from the three Mbololo sites to Ngangao North (mean $F_{ST} = 0.04$) compared to Ngangao South (mean $F_{ST} = 0.13$, paired t-test; $t_{1,2} = 13.83$, $P = 0.005$; see Table 3). Thus, it is clear that considerable sub-structuring divides the two Ngangao sites. Although all these sites are within the same hydrogeographic basin (Fig. 1a), it should be noted that the two Ngangao sites channel into two different streams whose confluence is after streams coming from Mbololo (Fig. 1b).

Mantel and partial Mantel tests explored the relationships between these two populations within the Mbololo–Ngangao hydrographic basin. Results show that the best correlation for F_{ST} is with distances calculated using the path from ''Upstream Flow Length'' between sampling sites (Fig. 1b), and this was the only significant result (Table 4). In other Mantel tests, neither the Null straight line model nor topographic distance gave significant results ($P = 0.117$ for both), and no partial Mantel tests were found to be significant ($P > 0.15$ in each case). We therefore did not need to invoke the Akaike information criterion in order to select the best model (see Spear et al., 2005).

Table 2 Results for Gamma correlations of *Hj* with forest fragment area and other landscape variables

Pair of variables	Valid *N*	Gamma	Z	*P* level
Hj and *n*	8	–0.040000	–0.13093	0.895830
Hj and elevation	8	0.50000	1.73205	0.083265
Hj and forest fragment	7	0.00000	0.00000	1.00000
Hj and area	8	–0.142857	–0.49487	0.620691
Hj and Latitude	8	–0.357143	–1.23718	0.216021
Hj and Longitude	8	0.285714	0.98974	0.322300

Table 3 Pairwise F_{ST} values for Dwarf Squeakers from 8 locations in the Taita Hills, Kenya (below diagonal)

	Chawia	Fururu	Mbololo forest	Mbololoshamba	Ngangao South	Ngangao North	Ronge
Chawia	–	***	***	***	***	***	***
Fururu	0.1305	–	***	***	***	***	***
Mbololo fo	0.1674	0.1769	–	ns	***	***	***
Mbololo sh	0.1833	0.2138	0.0000	–	***	***	***
Ngangao S	0.0943	0.1224	0.1230	0.1497	–	***	***
Ngangao N	0.1204	0.1622	0.0386	0.0623	0.0349	–	**
Ronge	0.1940	0.1802	0.0243	0.0369	0.1209	0.0151	–
Sagalla	0.1378	0.1856	0.1559	0.2034	0.1103	0.1433	0.1732

Above the diagonal, the probability that allelic distributions are different between sampling sites

Table 4 Results of Mantel and partial mantel tests for models for distance and F_{ST} within the Mbololo–Ngangao shared basin using ZT with 10,000 randomisations

Test	Variable	*r*	P
Mantel	$F_{ST} \times \ln$(distance)	0.579	0.117
	$F_{ST} \times \ln$(topo distance)	0.581	0.117
	$F_{ST} \times \ln$(water distance)	0.719	0.033
Partial Mantel	($F_{ST} \times \ln$(topo distance)) · ln(water distance)	–0.151	0.375
	($F_{ST} \times \ln$(water distance)) · ln(topo distance)	0.536	0.167
	($F_{ST} \times \ln$(water distance)) · ln(distance)	0.539	0.167

AMOVA

The a posteriori AMOVA tests showed that the population grouping resulting from STRUCTURE explained 19.2% of the variation between groups, whereas grouping sites by mountain block (12.9%) or drainage basin (10.8%) both resulted in loss of information from the model (Table 5). However, at a finer scale, slightly more variation was explained by grouping Ngangao North with the Mbololo sites, as suggested by the ''Upstream Flow Length'' (see Fig. 1b) and results from the Mantel tests (Table 4), than using the STRUCTURE predicted separation of Ngangao and Mbololo (14.9 and 13.4%, respectively).

Discussion

Genetic structure

A high degree of population differentiation is consistent with amphibian species which have low vagility and high site fidelity (Shaffer et al., 2000). Such patterns are known for many species with high genetic divergence (based on F_{ST}) between neighbouring breeding sites. Although it is incorrect to directly compare F_{ST} values calculated from different molecular markers, illustrations of this generality are helpful. For example, Spear et al. (2005) found generally high F_{ST} values (microsatellite mean 0.24) for populations of long toed salamanders as little as 1 km apart, and this appears to be typical of studies on salamanders (e.g. Tallmon et al., 2000; Curtis and Taylor, 2003). Similarly, many pond breeding anurans show this same pattern (Rowe et al., 2000; Lampert et al., 2003; Burns et al., 2004); Kraaijeveld-Smit et al. (2005) found high F_{ST} values (microsatellite 0.12–0.53) for Mallorcan midwife toads between ponds under 1 km apart. Lastly, Crawford (2003) used nuclear and mitochondrial sequence data of Central American, direct-developing dirt frogs to generate F_{ST} values. He found that populations 10.5 km apart had no sequence divergence at all and on this basis considered that frogs from these localities represented a single population. Indeed, for an amphibian not reliant on ponds (or hydrologic features such as streams or even tree holes)

Table 5 Results from analysis of molecular variance (AMOVA) with 8 sample sites grouped by (1) mountain blocks, (2) drainage basins, and (3) populations inferred from STRUCTURE results (see text)

In the second group only 5 sites in Ngangao and Mbololo are used to contrast inferred populations with fine scale drainage basins. See text for details

Groups	No. groups	Variance components	Percentage of variation	*P*-value
Mountain Blocks (Dw)(Mb)(Sa)	3	Among groups	12.90	0.004
		Among sites	13.16	<0.001
		Within sites	73.94	<0.001
Drainage basins (Ch–Fu)(Ng–Mb)(Sa)	3	Among groups	10.84	0.020
		Among sites	14.68	<0.001
		Within sites	74.48	<0.001
Inferred populations	5	Among groups	19.17	<0.001
		Among sites	5.00	<0.001
		Within sites	75.82	<0.001
Ngangao–Mbololo drainage basin (5 sites)				
Mountain blocks (Ng) (Mb)—inferred	2	Among groups	13.36	<0.001
		Among sites	5.23	<0.001
		Within sites	81.41	<0.001
Drainage separated (Ng S) (Mb + Ng N)	2	Among groups	14.91	<0.001
		Among sites	5.71	<0.001
		Within sites	79.37	<0.001

for its reproduction we might expect that populations of direct-developing frogs are structured rather differently. Here we consider three possible ways: first, that individuals are faithful to specific sites within a habitat, producing a similar result to pond breeding species, second, that populations are confined to and panmictic within appropriate habitats, or third that a population can span several habitats.

Sample sites 1.6 km apart within the continuous habitat of Ngangao forest had a low F_{ST} of 0.03, and hence there is no evidence that many separate populations occur within the same habitat. Further, on Mbololo genetic differentiation was even lower, with an F_{ST} value <0.0001 for animals sampled 2.0 km apart within different habitat patches. The three sampling sites on Mbololo were found to belong to the same population using STRUCTURE (in accordance with pairwise F_{ST} and AMOVA results), despite being sampled from three discrete habitats. We therefore conclude that, for this direct developing species, populations are not spatially separated in the same way that has been shown for pond breeding species (see above), nor are they confined within habitat boundaries. Instead, populations appear to be continuous within habitats, and even span fragmented habitat patches. If populations are not restricted to habitat patches, this would explain the lack of correlation between forest fragment size and *Hj* (Table 2).

Habitat fragmentation and lack of appropriate dispersal corridors has been linked with causes for amphibian decline (e.g. Blaustein et al., 1994; Beebee and Griffiths, 2005; Funk et al., 2005b). Given our results which show that single populations can span across several discrete habitats, it would be tempting to conclude that habitat fragmentation has no consequences for the Dwarf Squeaker. However, both expected heterozygosity (Table 1) and structural results (Table 3) suggest that Chawia is an isolated population, despite it being in the same mountain block as Ngangao and the same hydrologic basin as Fururu (Fig. 1). This genetic isolation may indeed be the result of habitat fragmentation, or it may relate to other landscape features associated with this site.

Mountain blocks versus basins

The Eastern Arc Mountains, estimated to have arisen between 290 Mya and 180 Mya, are presumed to have been almost entirely forested until recent anthropogenic disturbance (Newmark, 1998, 2001). In the Taita Hills, the current distribution of natural forest is almost entirely on mountain ridges (Wilder et al., 1998), the same geographic features which were interpreted as barriers to the Columbia spotted frog in Montana and Idaho, USA (Funk et al., 2005a). With forest covered mountain ridges and inhospitable dry valleys, the Taita Hills could be thought of as the inverse of the scenario studied by Funk et al. (2005a). So does their 'valley–mountain' model still apply? Here we compare divisions by hydrological basins which are demarcated by ridges, conforming to the 'valley–mountain' model, with divisions made by the Savannah habitat between mountain blocks, an inverse model.

Our prediction that mountain blocks would divide populations was broadly consistent with the F_{ST} values (Fig. 2) and AMOVA results (Table 3), but the inferred population groupings from STRUCTURE, which divided populations within Dawida, provided the best

AMOVA model explaining nearly 20% of the variation between groups. Although structuring by basins received the lowest between groupings support (<11%), when we tested for hydrological groupings within the Ngangao–Mbololo basin, information content was increased by considering the populations in hydrological terms (see Fig. 1b) instead of inferred populations from STRUCTURE (Table 5). This fine scale relationship is further demonstrated by the results of Mantel tests which show the hydrological distance (derived from upstream flow length) to be the only significant variable and to explain 72% of the variation in F_{ST} data (Table 4). Consequently, our hypothesis that smaller mountain blocks (Mbololo and Sagalla) would contain isolated populations with a measurable reduction in expected heterozygosity (*Hj*), was found to be consistent with Sagalla (Table 1), but not with Mbololo.

Although STRUCTURE results suggest that populations in Dawida and Mbololo are separate, examination of F_{ST} together with AMOVA and Mantel tests (Tables 3, 4 and 6) all suggest that there is a significant amount of gene flow between these populations. The finding that the Paranga Valley, dividing Dawida and Mbololo, is not a barrier for movement of Dwarf Squeakers is important. Firstly, it follows the predictions of Ghalambor et al. (2006), that valleys are not physiological barriers to high altitude ectotherms, over those of Janzen (1967) who suggested that both high mountain passes and low valleys are important physiological barriers in the tropics. Secondly, our finding also has several implications for our understanding of dispersal in direct-developing, leaf-litter amphibians. Although small, these frogs appear to be capable of long distance dispersal and movement through an area of unsuitable dry savannah which does not support populations. Further, although downward movement may be explained in passive displacement with water flow, these findings implicate a significant active movement of at least 4 km up a 10% slope to the nearest suitable habitat. Lastly, our data suggest that this type of dispersal is not uncommon. Here we attempt to explain our results by presenting a simple dispersal model for this amphibian based on a combination of passive downhill dispersal and active uphill movements. This model may be important for any amphibian dependent on high altitude habitats.

Passive-active dispersal

That these small frogs are moved passively downhill through streams and rivers is entirely plausible as the Taita Hills are subject to two relatively intense seasonal monsoons (see Malonza and Measey, 2005), including heavy rains capable of delivering 42 mm in 3 h at Ngangao (GJM unpublished data). Rains are not necessarily confined to mountain tops, so that the Savannah between these catchments may also be temporarily wet, with cloud cover that can last for several days giving temporal respite from high temperatures and dry conditions which may normally form a dispersal barrier. Following such downpours, individuals must actively move uphill to reach suitable habitats at higher altitudes. Riparian corridors may be of great importance to such dispersal.

Surprisingly, there is good support in the literature that such long distance uphill dispersal occurs. Sztatecsny and Schabetsberger (2005) tracked adult toads (*Bufo bufo*) in the Austrian Alps, finding that they migrated a horizontal distance of 1 km and up nearly 400 m altitude. More remarkably, Funk et al. (2005b) demonstrated that juvenile Columbia spotted frogs (approximately 25 mm in total length) migrated >4 km horizontally gaining >700 m of elevation in Montana, USA.

Active downhill migrations are not congruent with our results from Mantel tests (Table 4) as they would presumably not differentiate Ngangao North and South. An alternative scenario, in which individuals actively disperse down streams, seems unlikely as most of these streams only flow in heavy rain showers. It should also be noted that successful migrants probably only represent a minority of those dispersing (Trenham et al., 2001; Smith and Green, 2005), and of these only some may be implicated in successful reproduction (Slatkin, 1985). We imagine a similar scenario here, with the majority Dwarf Squeakers being lost to hostile Savannah conditions as well as predators, and mechanical damage.

Interestingly, implementing our dispersal model in the scenario presented by Funk et al. (2005a) could give rise to the same results. Their strong connection between low altitude valley sites can also be interpreted to be a result of increased movement of water within the valley, which might also explain dispersal from high to low sites. Similarly, links from low to high sites could result from the automatic upward orientation of displaced individuals, especially those recently metamorphosed (see Funk et al., 2005a).

Conclusions

Our study demonstrates that a direct-developing amphibian species, not dependent on migration to

ponds, has extended populations that span habitats over large areas of several kilometres. We interpret this departure from the general amphibian model (Beebee, 2005) as a consequence of the divergent life history characteristics on dispersal and gene flow in these amphibians. There is an urgent need to assess gene flow for a wide range of life-histories of model species in order to provide predictions for increasing numbers of endangered amphibians, which are not always pond breeders (e.g. Pounds et al., 2006). In addition we show that potential anthropogenic and natural barriers (Fig. 1) can be overcome to allow substantial gene flow between populations (Tables 3 and 6). However there are indications (in results of *Hj* and F_{ST} from Chawia and Sagalla, Tables 1 and 3) that there may be limits to gene flow across such barriers. Importantly, our results are not consistent with the conclusion of Funk et al. (2005b) that continuous habitat is necessary for dispersal of amphibians. Brief temporal changes in climatic conditions may be sufficient to allow considerable gene flow between appropriate habitats.

Moreover, our study once again underlines the utility of landscape genetics in the interpretation of gene flow between populations (Manel et al., 2003), and specifically its application to amphibians (Funk et al., 2005a; Spear et al., 2005). Surprisingly, the dispersal model proposed by Funk et al. (2005a) receives support with respect to populations being linked with hydrological basins, despite the contrasting features of both study site and life-history characteristics of study species. We conclude therefore that the importance of landscape hydrological dynamics in amphibian gene flow is pivotal for species with mountain distributions. Further, we suggest that conservation of high altitude amphibian species will depend on a landscape approach with attention to hydrological basins and to both passive and active dispersal mechanisms.

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