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Morphotypes, varieties, or subspecies?: genetic diversity and differentiation of four *Saintpaulia* (Gesneriaceae) morphotypes from the East Usambara Mountains, Tanzania

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Saintpaulia (H. Wendl.) is a forest-dependent, endangered plant genus endemic to Tanzania and Kenya. The taxonomy of *Saintpaulia* from the Usambara Mountains and adjacent lowlands in north-eastern Tanzania is problematic because of the morphological similarity of the species and the presence of considerable intraspecific variation. Conventional molecular phylogenetic methods have failed to reveal the genetic structure of this *Saintpaulia* complex. In this study, we assessed the genetic composition of 12 *Saintpaulia* populations, representing four different morphotypes, from the East Usambara Mountains using inter-simple sequence repeat (ISSR) markers. Relatively high genetic diversities were observed within populations (mean $h = 0.320$), indicating their adaptive potential. Little genetic differentiation amongst populations (mean $F_{ST} = 0.063$) and the genetic divergence of the rosulate and trailing morphotypes support the hypothesis of ongoing divergent evolution within the East Usambara metapopulation(s) of *Saintpaulia*. © 2008 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2008, **157**, 347–355.

ADDITIONAL KEYWORDS: African violet – conservation – Eastern Arc Mountains – genetic fingerprinting – Gesneriaceae.

INTRODUCTION

The genus *Saintpaulia* (H. Wendl.), Gesneriaceae, consists of perennial herbs endemic to the Eastern Arc Mountains and coastal lowlands of Kenya and Tanzania. The distribution range of the genus falls within two global biodiversity hotspots: the Eastern Afromontane and the coastal forests of Eastern Africa (Mittermeier *et al*., 2005). *Saintpaulia* species (or African violets) are amongst the most well-known plants of the East African flora. They have been subjected to intensive breeding by the horticultural industry for nearly 100 years, and commercial *Saintpaulia* hybrids are popular ornamental plants in the wealthier parts of the world. In Tanzania and Kenya, the genus has been promoted as a botanical flagship for the conservation of the Eastern Arc Mountains and coastal forests (for example, Eastwood *et al*., 1998). Efforts to make local people benefit from the plant as part of the Eastern Arc ecotourism have also been initiated (Kolehmainen, 2005; Kolehmainen *et al*., 2006).

It is paradoxical that this important genus is critically endangered, most of the remaining populations being small and isolated, especially in the coastal lowlands (Johansson, 1978; Clarke, 1998; Kolehmainen, 2005). The spatial structure of *Saintpaulia* populations has apparently always been rather fragmented because of their specialized ecological niche. However, forest fragmentation, which has continued in the area for centuries and intensified in the 1960s (Hamilton, 1989a), has undoubtedly created further gaps in the distribution of *Saintpaulia* taxa. Twenty *Saintpaulia* taxa were listed in the 1997 World Conservation Union (IUCN) *Red List* (Walter & Gillett, 1998), in which the status of 16 taxa was categorized as 'indeterminate', indicating the lack of scientific

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knowledge of the genus. A more comprehensive review by Eastwood *et al*. (1998) assigned new categories of threat (IUCN, 1994) to 28 *Saintpaulia* taxa. They placed three taxa in the category 'vulnerable' and 16 taxa in the category 'critical', but were unable to assess the status of nine taxa which were categorized as 'data deficient'. A new *Red List* assessment for *Saintpaulia*, incorporating recent field data and the new taxonomic revision of the genus (Darbyshire, 2006), is currently under way by the Missouri Botanical Garden and IUCN (R. Gereau, Missouri Botanical Garden, St. Louis pers. comm.).

Saintpaulia species thrive in moist and shaded conditions under forest canopy from sea-level up to an altitude of about 1400 m (Johansson, 1978; Baatvik, 1993; Eastwood *et al*., 1998). The genus is mainly lithophytic, but occasionally grows on the forest floor, on decomposing logs, and even as epiphytes on the bases of living trees (Johansson, 1978; Baatvik, 1993; Kolehmainen & Mutikainen, 2006a). It reproduces by seeds and vegetatively from leaf cuttings and through the division of old plants (J. Kolehmainen, pers. observ.). Reasonable to abundant fruit production has been observed in several *Saintpaulia* populations in the little disturbed habitats of the East Usambara Mountains, Tanzania (Kolehmainen & Mutikainen, 2006a). The genus has a mixed mating strategy: fruits can develop as a result of self- or cross-pollination, but spontaneous self-pollination does not seem to occur (Kolehmainen & Mutikainen, 2006a). The flowers have two different stylar morphs (enantiostyly), and bees have been observed to be the main pollinators (Martins, 2005; V. Heimala, Helsinki, unpubl. data). The flower morphology and pollination biology thus give a strong reason to suspect that the genus largely outbreeds. Crossing experiments have shown that most *Saintpaulia* taxa can hybridize, and that hybrid offspring are fertile (Clayberg, 1961; Arisumi, 1964).

Until 2006, 20 *Saintpaulia* species and four further varieties were recognized (Burtt, 1958, 1964). In Burtt's taxonomy, a narrow species concept was applied because of the lack of knowledge of the morphological variation in wild populations, and nearly one-half of the described species were found in the Usambara Mountains and the adjacent lowlands. Molecular studies based on nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) (Möller & Cronck, 1997) and 5S nrDNA non-transcribed spacer (5S-NTS) (Lindqvist & Albert, 1999, 2001) data later demonstrated only poor resolution amongst the taxa of Usambara and the lowlands. Lindqvist & Albert (1999, 2001) also postulated that the characters used earlier to distinguish *Saintpaulia* taxa, i.e. trichome morphology, pigmentation, and flower colour, are likely to be controlled by very few genes, and that the variation observed in these characters may represent genetic polymorphism within a single entity. Darbyshire (2006) further suggested that the habit, which was used earlier as a distinctive character of some *Saintpaulia* taxa, is likely to be largely phenotypic in origin. Yet, populations in different microenvironments also seem to have begun to adapt genetically to their environments, as the habit can persist in cultivation (Darbyshire, 2006). Lindqvist & Albert (1999, 2001) hypothesized that the Usambara/lowlands clade may not be a species group but a metapopulation, where the relative isolation of the subpopulations has resulted in the observed morphological variability and genetic substructuring within the entity. In accordance with the results from molecular studies, Darbyshire (2006) applied a broader species concept in the updated taxonomic revision, in which the total number of species was reduced to six. Populations in the East Usambara Mountains and coastal lowlands have now been included within a single highly variable species consisting of nine subspecies, which have been defined on the grounds of morphology and geographical location.

There are no published population level studies on the genetic structure of *Saintpaulia* taxa in the Usambara Mountains/lowlands using neutral genetic fingerprinting markers, although they are more capable than the more conserved nrDNA ITS and 5S-NTS sequences of detecting genetic structures. DNA fingerprinting methods are also needed to obtain information about the level of genetic variation within the populations, which helps in the identification of management units for conservation. The level of genetic variation indicates the potential of the species or population for evolutionary responses to environmental changes. An increased level of inbreeding and genetic drift has been predicted to occur in small populations, leading to increased homozygosity and the random loss of alleles, and thus to a decrease in genetic diversity (Endler, 1986; Barrett & Kohn, 1991; Ellstrand & Elam, 1993; Cruzan, 2001).

In this study, inter-simple sequence repeat (ISSR) markers were used as a genetic fingerprinting method to investigate the genetic diversity within populations and the divergence of four different *Saintpaulia* morphotypes in the East Usambara Mountains, Tanzania. ISSRs are arbitrary multiloci markers produced by the polymerase chain reaction (PCR) amplification with an anchored repeat primer. They are highly polymorphic, and no prior genomic information is required for their use (Bornet & Branchard, 2001; Rakoczy-Trojanowska & Bolibok, 2004). Three of the studied morphotypes ('confusa', 'difficilis', and 'grotei') were treated as distinct species in the old classification of the genus (Burtt, 1958), but are now included within *Saintpaulia ionantha* H.Wendl. ssp. *grotei* (Engl.) I.Darbysh. (Darbyshire, 2006). The fourth

morphotype ('confusa × grotei') is an intermediate between 'confusa' and 'grotei', and could have been treated as a hybrid between *S. confusa* B.L. Burtt and *S. grotei* Engl. in the old taxonomic classification.

MATERIAL AND METHODS

Study area

Fieldwork was conducted in the Amani Nature Reserve (5°04′–5°13′S and 38°33′–38°40′E) in the southern part of the East Usambara Mountains, north-eastern Tanzania. The study area (Fig. 1) was about 40 km^2 and located at 900–1000 m above sea-level. In this area, the mean annual rainfall varies from about 2220 mm on the central plateau at Kwamkoro to about 1470 mm on the edge of the west-facing slope at Ndola (Tanzania Meteorological Agency; based on measurements in Kwamkoro in 1998–99 and 2002–03 and in Ndola in 2002–03). There are two rainy seasons: the 'long rains' in March–May and the 'short rains' in October–December. The mean annual temperature at the study sites within the forest is about 19.4 °C (Onset HOBO Pro 8 data loggers), the highest temperatures occurring from December to March (monthly mean of 21.2 °C) and the lowest from June to September (mean of 17.3 °C). The highest measured daytime temperature was 28.3 °C (in March) and the lowest night-time temperature was 11.4 °C (in August). The natural vegetation is submontane evergreen forest with ill-defined strata. The average canopy height is 20–30 m, and the emergent trees reach to 40 m (Hamilton *et al*., 1989). In the study area, natural forest remains on the plateau and the upper sections of the mountain slopes (Fig. 1).

Morphotypes and their habitats

The four studied morphotypes of *Saintpaulia ionantha* ssp. *grotei* are spatially clustered in the study area (Fig. 1). The populations were subjectively defined on the basis of the distribution information from field surveys. In the slope areas, the population boundaries are suggestive because it was not possible to explore the extent of the populations on the steepest slopes. Each of the populations represents a single morphotype, except for one population of 'difficilis' (Ngua) and one of 'confusa' (Arbo), in which a few plants were observed to have characteristics intermediate between these two types. Morphotypes 'confusa' and 'difficilis' have thick and short (usually 0–10 cm) succulent stems, the leaves arranged in an apical rosette, a combination of short and long hairs on the upper surface of the leaves, and usually more than two flowers per flower stalk. These two types differ only in their leaf characteristics. The leaves of 'difficilis' are thickish with rather coarsely serrated margins, deep veins, and the long hairs on the upper surface of the leaves are arced to suberect. 'Confusa' has thinnish or thickish leaves with crenate-serrate leaf margins and appressed long leaf hairs, which are usually sparser than in 'difficilis'. Type 'grotei' has a trailing growth habit (i.e. long internodes) with thin and up to about 100 cm long stems. The leaves of 'grotei' are thin with crenate to dentate margins, only short appressed hairs on the upper surface of the leaves, and one or two flowers per flower stalk. It tends to form extensive uniform stands as a result of the trailing growth habit. The 'hybrid' type has characteristics intermediate between 'grotei' and 'confusa', i.e. subtrailing habit, and thin to thickish leaves with short and sparse long appressed hairs on the upper surface of the leaves. The few observed fertile individuals of the 'hybrid' type have an intermediate number of flowers per flower stalk (usually three). Reference collections were made of all morphotypes, and the specimens were deposited in the Botanical Museum of the Finnish Museum of Natural History. Duplicates of the specimens are in the herbarium of the University of Dar es Salaam, Tanzania.

Two of the 'difficilis' localities are situated on the west-facing slope (Ngua and Ndola A), one in a stream valley in the immediate vicinity of the slope (Ndola B), and one in a stream valley in the middle of the plateau (Emau). The type 'confusa' is confined to the southern and south-western slopes (Gonja, Kw 12, and a part of Arbo) and to the stream valleys on the plateau (Kw 1, Kw 11, and a part of Arbo). Habitats on the west- and south-west-facing slopes are drier than those in the stream valleys on the plateau, apparently because of lower rainfall, a more open canopy, and stronger and more frequent winds (Kolehmainen & Mutikainen, 2006b). The localities of the trailing type 'grotei' (Kihuhwi and Kimbo) are situated in the moister and more shaded forests in the eastern part of the plateau and in the vicinity of the south-east-facing slopes that receive more rain (Hamilton, 1989b). The locality of the 'hybrid' type is between the sites of 'grotei' and 'confusa' on the edge of the south to south-east-facing slope (Fig. 1).

DNA analysis

The populations were sampled during field trips conducted between December 2002 and January 2004. Leaves were torn up into slices and dried with silica gel in small plastic bags. The dried leaves were kept at room temperature during the field trips and were later stored at –80 °C until the extraction of DNA. DNA isolation was conducted from a minute piece of leaf using a DNA isolation kit (DNeasy Plant Mini Kit,

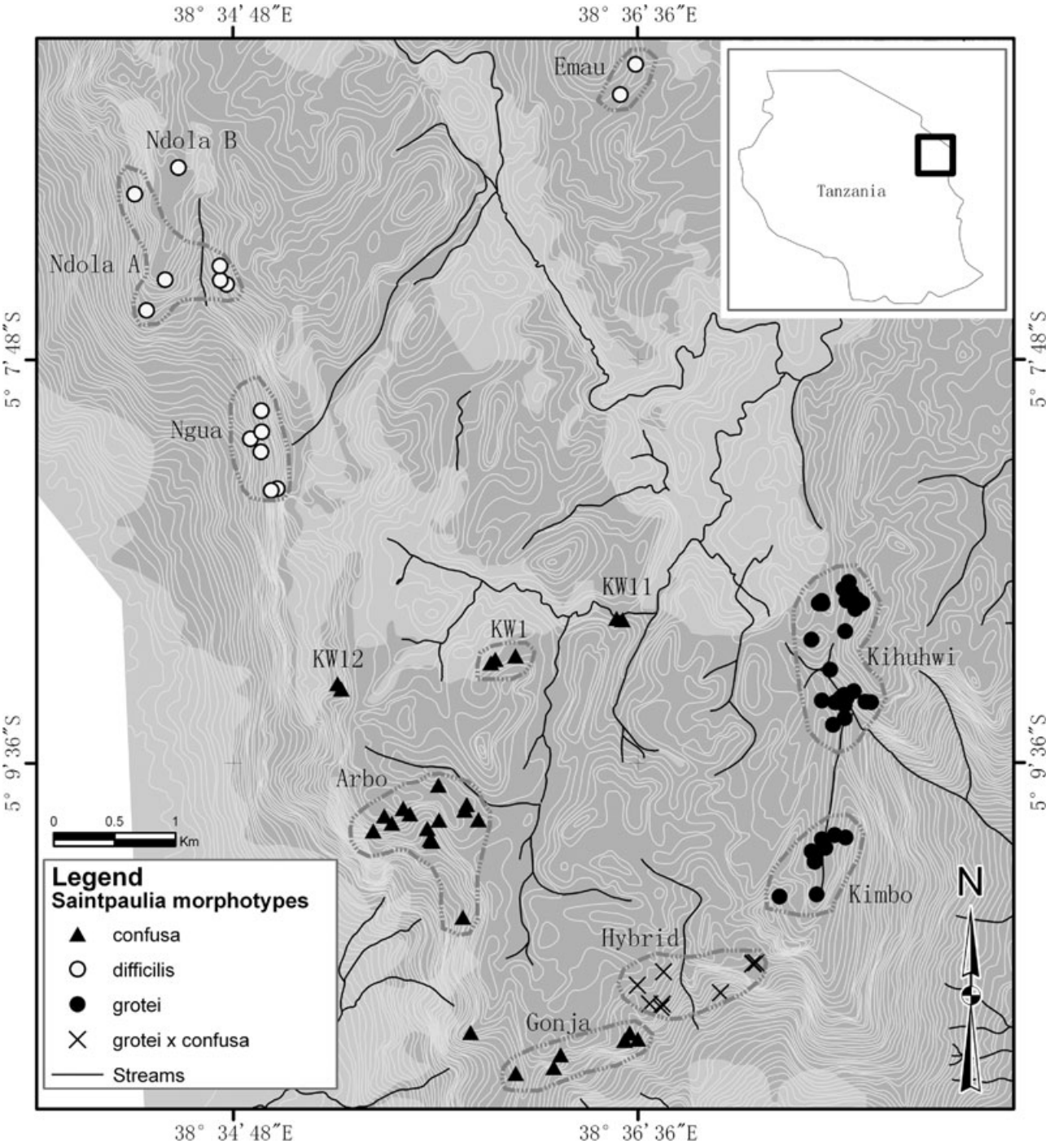

Figure 1. Map of the study area showing the distribution of the morphotypes and the studied populations. Darker colour on the background represents forested areas and lighter colour represents open vegetation.

Qiagen). Thirty-three primers (UBC primer set no. 9, Biotechnology Laboratory, University of British Columbia, Canada) were tested with a subset of samples. Four (840, 842, 857, and 873) produced interpretable polymorphic bands and were used for the analysis of the whole set of 194 samples. The PCRs were conducted in a volume of 20 μL. The reaction mixture contained 2 μL (about 10–20 ng) of extracted DNA, 1.2 units of DyNAzyme II DNA polymerase (Finnzymes), 1 × PCR buffer, 0.4 μL of 10 mM dNTP mix, and 1 μL of a single 5 μM primer. The thermocyler (MJ Research Inc., model PTC-200) was programmed

Table 1. Sample sizes and genetic diversities of the studied populations of *Saintpaulia ionantha* ssp. *grotei* based on inter-simple sequence repeat (ISSR) data

Morphotype	Population	Sample size	Genetic diversity ($h \pm$ SD)
Difficilis	Emau	16	0.329 ± 0.172
	Ndola A	16	0.286 ± 0.152
	Ndola B	17	0.322 ± 0.168
	Ngua	16	0.345 ± 0.180
Confusa	Kw 1	18	0.283 ± 0.148
	Kw 11	15	0.318 ± 0.168
	Kw 12	16	0.350 ± 0.183
	Arbo	16	0.290 ± 0.153
	Gonja	17	0.333 ± 0.174
Confusa × grotei	Hybrid	15	0.339 ± 0.178
Grotei	Kihuhwi	16	0.347 ± 0.181
	Kimbo	16	0.294 ± 0.155
Mean			0.320 ± 0.168

SD, standard deviation.

for 4 min denaturation at 94 °C, followed by 45 cycles of denaturation at 94 °C for 45 s, annealing at 48–55 °C (depending on the primer) for 45 s, and elongation at 72 °C for 90 s, and a final 8 min extension at 72 °C. Amplification products were analysed on 1.4% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. The band size was estimated by comparison with a DNA ladder loaded simultaneously with the amplified DNAs.

Data analysis

The amplified DNA products were scored as present (1) or absent (0) to form a binary matrix, which was used as an input file for Arlequin 3.01 (Excoffier, Laval & Schneider, 2005). Nei's gene diversity over loci (h) was used to quantify the amount of genetic variation in each population (Nei, 1987). Analysis of molecular variance (AMOVA) was conducted to calculate the partitioning of the variation amongst morphotypes, amongst populations within morphotypes, and within populations. To measure the population and morphotype differentiation, pairwise genetic distances (F_{ST}) were computed. The Mantel test was used to determine whether there was a correlation between the genetic and spatial distances of the populations. To evaluate the genetic relationship between the morphotypes, a principal component analysis was conducted using SAS 9.1 software (SAS Institute Inc.).

RESULTS

For the 194 samples, the four ISSR primers produced 59 scorable bands, 58 of which were polymorphic (size range, 370–1000 bp). Considerable amounts of intrapopulation genetic variation were observed, and the levels of variation (h) were very similar across all populations and morphotypes (minimum, 0.283; maximum, 0.347; mean, 0.320 ± 0.168; Table 1).

AMOVA showed that, of the total molecular variance, 2.84% was attributed to morphotype divergence, 3.47% to populational differences within morphotypes, and 93.69% to individual differences within populations (Table 2). When the data of the rosulate types 'confusa' and 'difficilis' were combined and the 'hybrid' type was excluded from the analysis, the divergence between the rosulate and trailing types was slightly higher, 5.45% of the total molecular variance, and the within-population variance was lower, 90.72%. When just rosulate types were included in the analysis, only 0.94% of the total variance was explained by morphotype divergence.

As evident from the results of AMOVA, the genetic divergence of the populations was low, the pairwise F_{ST} values of the populations ranging from 0.001 to 0.156 (mean, 0.063; Table 3). A weak but significant divergence of the morphotypes was detected from the pairwise F_{ST} values calculated for the morphotypes (populations within morphotypes pooled). The rosulate types 'difficilis' and 'confusa' were the most similar ($F_{ST} = 0.017$, $P = 0.000$), and the greatest distance was observed between the trailing 'grotei' and the two rosulate types ('difficilis': $F_{ST} = 0.076$, $P = 0.000$; 'confusa': $F_{ST} = 0.064$, $P = 0.000$). The hybrid type 'confusa × grotei' had an intermediate genetic distance to the other morphotypes ('difficilis':

Table 2. Results from the hierarchical analysis of molecular variance (AMOVA) of *Saintpaulia ionantha* ssp. *grotei* morphotypes based on inter-simple sequence repeat (ISSR) data

Source of variation	d.f.	Variance component	Percentage of total variance	*P*
Analysis with four morphotypes: 'confusa', 'difficilis', 'hybrid', and 'grotei'				
Amongst morphotypes	3	0.29	2.84	0.000
Amongst populations within morphotypes	8	0.34	3.47	0.000
Within populations	182	9.17	93.69	0.000
Analysis with two morphotypes: 'confusa + difficilis' and 'grotei'*				
Amongst morphotypes	1	0.55	5.45	0.000
Amongst populations within morphotypes	9	0.39	3.83	0.000
Within populations	168	9.13	90.72	0.022
Analysis with two morphotypes: 'confusa' and 'difficilis'				
Amongst morphotypes	1	0.09	0.94	0.000
Amongst populations within morphotypes	2	0.33	3.49	0.000
Within populations	138	9.10	95.57	0.004

d.f., degree of freedom.
*Rosulate types 'confusa' and 'difficilis' combined as one morphotype.

$F_{ST} = 0.033$; 'confusa': $F_{ST} = 0.034$; 'grotei': $F_{ST} = 0.035$; all values $P = 0.000$).

The principal component analysis conducted with two components explained only 5.5% of the variability, and the plants belonging to each morphotype were poorly grouped. Yet, the graph shows that individuals of the trailing 'grotei' form are partly separated from the individuals of the rosulate types 'confusa' and 'difficilis' (Fig. 2). The Mantel test showed the absence of a correlation between the genetic and geographical distances amongst the populations ($r = 0.051$, $P = 0.355$).

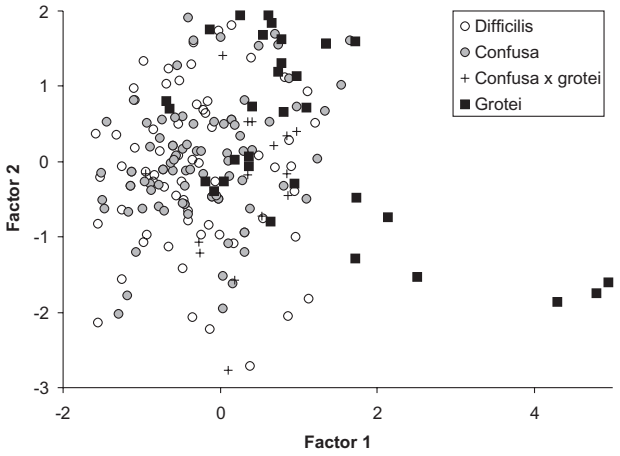

Figure 2. Spatial distribution of individuals using principal component analysis based on inter-simple sequence repeat (ISSR) data.

DISCUSSION

The relatively high intrapopulation genetic diversities (mean $h = 0.32$) suggest that the studied populations possess variation that is needed for evolutionary responses in a changing environment. The observed levels of genetic diversity are similar to those reported in other investigations for outcrossing plant species. A review of 38 studies on outcrossing plant species reported a mean within-population genetic diversity of 0.27 derived from random amplification of polymorphic DNA (RAPD) markers (Nybom, 2004). In the same review, a much higher level of among-population differentiation was reported for outcrossing species (mean $G_{ST} = 0.22$, 38 studies) than that observed in the studied *Saintpaulia* populations (mean $F_{ST} = 0.063$). Taking into account the breeding system of *Saintpaulia*, the result that a major proportion of the total genetic variance is within populations was expected. Low interpopulation differentiation was also presumed from the short geographical distances between the investigated populations. In general, outcrossing species have high levels of within-population variability and low inter-populational differentiation (Schoen & Brown, 1991).

The lack of correlation between the genetic and geographical distances of the populations was not surprising because the geomorphology of the study area may have a greater effect on the amount of gene flow than does the geographical distance. The bees that carry the pollen of *Saintpaulia* may prefer to

Table 3. Pairwise F_{ST} values illustrating differences between the populations of the four *Saintpaulia ionantha* ssp. *grotei* morphotypes

	'Difficilis'				'Confusa'					'Confusa × grotei'	'Grotei'	
	Emau	Ndola A	Ndola B	Ngua	Kw 1	Kw 11	Kw 12	Arbo	Gonja	Hybrid	Kihuhwi	Kimbo
'Difficilis'												
Emau	0.000											
Ndola A	0.000	0.000										
Ndola B	0.002	0.011	0.000									
Ngua	0.010	0.030*	0.008	0.000								
'Confusa'												
Kw 1	0.042*	0.062*	0.032*	0.033*	0.000							
Kw 11	0.064*	**0.110***	0.066*	0.085*	**0.128***	0.000						
Kw 12	0.029	0.030*	0.030*	0.008	0.086*	0.042*	0.000					
Arbo	0.011	0.072*	0.027*	0.027*	0.068*	0.025	0.027	0.000				
Gonja	0.008	0.050*	0.048*	0.024*	0.062*	0.059*	0.028*	0.001	0.000			
'Confusa × grotei'												
Hybrid	0.019	0.069*	0.041*	0.011	0.081*	0.081*	0.063*	0.026	0.019	0.000		
'Grotei'												
Kihuhwi	0.044*	**0.104***	**0.109***	0.053*	**0.122***	**0.156***	0.068*	0.076*	0.020	0.035*	0.000	
Kimbo	0.074*	**0.111***	**0.126***	0.094*	**0.148***	**0.138***	0.090*	0.094*	0.038*	0.058*	0.044*	0.000

F_{ST} values > 0.1 are shown in bold.
*Significant values ($P < 0.05$).

forage horizontally on the slopes rather than vertically. There may thus be more gene flow between the slope populations than between the slope and plateau populations.

This study indicates that there is no genetic basis for the taxonomic distinction of the rosulate morphotypes 'confusa' and 'difficilis'. The trailing 'grotei', however, is genetically distinct from the rosulate types, which supports the idea that these two growth forms have begun to diverge genetically through adaptation to different environments. Moreover, the flowering phenology of 'grotei' is partially separated from that of the two rosulate types (Kolehmainen & Mutikainen, 2006a), which should reduce gene flow amongst these two forms. When also considering the strikingly different habit of the trailing 'grotei', we would suggest a subspecific status within the species *Saintpaulia ionantha*.

It is tempting to postulate that pollination biology plays a significant role in the ongoing evolutionary divergence of the studied rosulate and trailing morphotypes of *Saintpaulia*. The rosulate types that produce numerous flowers but invest less on vegetative growth should have a selective advantage in the more open slope habitats where pollinators appear to be more abundant or more active, as indicated by the high fruit production in 'difficilis' (Kolehmainen & Mutikainen, 2006a). In the shaded and moist 'grotei' habitats, where pollinators are probably less abundant, vegetative reproduction should be an advantage, and an investment on many flowers should be selected against. In addition, in such environments, the establishment of vegetative propagules is assumed to be easier than in the more open and dry slope habitats. Differences in the leaf thickness and indumentum amongst the morphotypes are also likely to be a result of adaptation to different environments. Thicker and more hairy leaves characteristic of 'difficilis' are presumed to be an adaptation to more open and dry habitats in the western and northern parts of the study area, whereas, in the more shaded and rainy 'grotei' and some 'confusa' habitats (eastern and southern parts of the study area), thin and smooth leaves are probably selected for.

The observed low levels of population and morphotype divergence lend support to the hypothesis of Lindqvist & Albert (1999) that the populations in the Usambara/lowlands region represent a segregating metapopulation, or, more likely, several metapopulations, where subpopulations are evolving genetically to adapt to their microenvironments. It seems that the genetic divergence of the studied populations is low because of frequent genetic exchange amongst the populations. Recent fragmentation of the entity could result in a similar pattern even without gene flow, but that is highly unlikely, because there is no evidence of population fragmentation in the study area, the forest habitat also having a low level of fragmentation (Fig. 1). As intrapopulation genetic variability appears to be high, targeting conservation efforts to one or two viable populations of each morphotype or evolutionary unit should be a reasoned approach in the East Usambara Mountains. A totally different conservation approach may need to be applied to the lowland areas in which the populations are very small and fragmented because of habitat degradation (Clarke, 1998; Kolehmainen, 2005). We recommend that DNA fingerprinting methods (ISSRs or preferably microsatellite markers) should be used to solve the genetic structure of the Usambara Mountains/lowlands metapopulations of *Saintpaulia*. This is needed not only for the sampling of a representative fraction of the genetic variation for *in situ* and *ex situ* conservation programmes, but also for the verification of the genetic basis of the recently defined taxonomic entities (Darbyshire, 2006).

Lindqvist & Albert (1999) have suggested that the Nguru Mountains should have a high priority in the conservation of *Saintpaulia*, based on the genus having its phylogenetic origin there. We propose an equally high conservation priority for the East Usambara Mountains, where the extensive remaining natural forests provide a large 'playground' for *Saintpaulia* evolution. New *Saintpaulia* taxa are likely to evolve there provided that the habitats remain suitable. This further increases the scientific, conservational, and educational value of the East Usambara Mountains amongst the biological hotspots of the world.

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