Ecology, population genetics and conservation of the African violet (*Saintpaulia*, Gesneriaceae)

Johanna Kolehmainen

Department of Biological and Environmental Sciences
Faculty of Biosciences
and
Department of Applied Biology
Faculty of Agriculture and Forestry

University of Helsinki
Finland

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Biosciences of the University of Helsinki, for public criticism in Auditorium 1, Viikki Info Centre, (Viikinkaari 11, Helsinki) on January 18th, 2008, at 12 o'clock noon.

Supervised by

Dr Helena Korpelainen
Department of Applied Biology
University of Helsinki, Finland

and

Dr Pia Mutikainen
Department of Biology
University of Oulu, Finland

Reviewed by

Dr Jouni Aspi
Department of Biology
University of Oulu, Finland

and

Dr Roosa Leimu
Institute of Plant Sciences
Bern, Switzerland

Examined by

Dr Pirkko Siikamäki
Oulanka Biological Station
University of Oulu, Finland

Cover illustration: Habitat of *Saintpaulia* with a view of Lwengera valley, Amani Nature Reserve, East Usambara Mountains, drawn by the author. © Johanna Kolehmainen

ISBN 978-952-92-3248-2 (Paperback)
ISBN 978-952-10-4473-1 (PDF)

Yliopistopaino
Helsinki, Finland 2008

ABSTRACT

The ongoing rapid fragmentation of tropical forests is a major threat to global biodiversity. This is because many of the tropical forests are so-called biodiversity 'hotspots', areas that host exceptional species richness and concentrations of endemic species. Forest fragmentation has negative ecological and genetic consequences for plant survival. Proposed reasons for plant species' loss in forest fragments are, e.g., abiotic edge effects, altered species interactions, increased genetic drift, and inbreeding depression. To be able to conserve plants in forest fragments, the ecological and genetic processes that threaten the species have to be understood. That is possible only after obtaining adequate information on their biology, including taxonomy, life history, reproduction, and spatial and genetic structure of the populations.

In this research, I focused on the African violet (genus *Saintpaulia*), a little-studied conservation flagship from the Eastern Arc Mountains and Coastal Forests hotspot of Tanzania and Kenya. The main objective of the research was to increase understanding of the life history, ecology and population genetics of *Saintpaulia* that is needed for the design of appropriate conservation measures. A further aim was to provide population-level insights into the difficult taxonomy of *Saintpaulia*. Ecological field work was conducted in a relatively little fragmented protected forest in the Amani Nature Reserve in the East Usambara Mountains, in northeastern Tanzania, complemented by population genetic laboratory work and ecological experiments in Helsinki, Finland. All components of the research were conducted with *Saintpaulia ionantha* ssp. *grotei*, which forms a taxonomically controversial population complex in the study area.

My results suggest that *Saintpaulia* has good reproductive performance in forests with low disturbance levels in the East Usambara Mountains. Another important finding was that seed production depends on sufficient pollinator service. The availability of pollinators should thus be considered in the *in situ* management of threatened populations.

Dynamic population stage structures were observed suggesting that the studied populations are demographically viable. High mortality of seedlings and juveniles was observed during the dry season but this was compensated by ample recruitment of new seedlings after the rainy season. Reduced tree canopy closure and substrate quality are likely to exacerbate seedling and juvenile mortality, and, therefore, forest fragmentation and disturbance are serious threats to the regeneration of *Saintpaulia*. Restoration of sufficient shade to enhance seedling establishment is an important conservation measure in populations located in disturbed habitats. Long-term demographic monitoring, which enables the forecasting of a population’s future, is also recommended in disturbed habitats.

High genetic diversities were observed in the populations, which suggest that they possess the variation that is needed for evolutionary responses in a changing environment. Thus, genetic management of the studied populations does not seem necessary as long as the habitats remain favourable for *Saintpaulia*. The observed high levels of inbreeding in some of the populations, and the reduced fitness of the inbred progeny compared to the outbred progeny, as revealed by the hand-pollination experiment, indicate that inbreeding and inbreeding depression are potential mechanisms contributing to the extinction of *Saintpaulia* populations.

The relatively weak genetic divergence of the three different morphotypes of *Saintpaulia ionantha* ssp. *grotei* lend support to the hypothesis that the populations in the Usambara/lowlands region represent a segregating metapopulation (or metapopulations), where subpopulations are adapting to their particular environments. The partial genetic and phenological integrity, and the distinct trailing habit of the morphotype 'grotei' would, however, justify its placement in a taxonomic rank of its own, perhaps in a subspecific rank.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications and manuscripts, which are referred to in the text by their Roman numerals:

I Kolehmainen, J. and Mutikainen, P. 2006. Reproductive ecology of three endangered African violet (*Saintpaulia* H. Wendl.) species in the East Usambara Mountains, Tanzania. – African Journal of Ecology 44: 219–227.

II Kolehmainen, J. and Mutikainen, P. 2006. Population stage structure, survival and recruitment in the endangered East African forest herb *Saintpaulia*. – Plant Ecology 192: 85–95.

III Kolehmainen, J. and Korpelainen, H. Morphotypes, varieties or subspecies? - Genetic diversity and differentiation of four *Saintpaulia* morphotypes from the East Usambara Mountains.– Botanical Journal of the Linnean Society (in press).

IV Kolehmainen, J., Korpelainen, H. and Mutikainen, P. Inbreeding and inbreeding depression in a threatened endemic plant, the African violet (*Saintpaulia ionantha* ssp. *grotei*), of the East Usambara Mountains, Tanzania – Unpublished manuscript.

I have conducted the major part of this doctoral research, but I have also collaborated with several people. The table below shows the contribution of different people to each component of the research:

	I	**II**	**III**	**IV**
Original idea	JK	JK	JK	JK
Collection of data in the field	JK	JK	JK	JK
Genetic laboratory work			JK	JK
Greenhouse experiments and ecological laboratory work	JK	JK		JK
Data analysis	JK, PM	JK	JK, HK	JK, HK
Manuscript preparation	JK, PM	JK, PM	JK, HK	JK, HK, PM

JK=Johanna Kolehmainen, HK=Helena Korpelainen, PM=Pia Mutikainen.

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CONTENTS

1 INTRODUCTION

1.1 Global consequences of forest fragmentation

The natural vegetation of the Earth has undergone dramatic degradation since the beginning of settled agriculture, which was followed by human population growth and the development of technology and economic activity (Houghton 1994, Meyer & Turner 1994). The loss and fragmentation of forest is a direct threat to biodiversity. In addition to its intrinsic value, biodiversity is needed for the functioning of ecosystems (c.f. Loreau et al. 2001) and for the production of numerous goods, current and potential, for human consumption (e.g. Schaberg et al. 1999). Biodiversity is lost through the extinction of local populations of species (Hughes et al. 1997), and there is often a time lag between the process of habitat loss and the eventual collapse of populations, i.e., extinction debt (Tilman et al. 1994).

In addition to the loss of biodiversity, the fragmentation and loss of forest endanger important ecosystem services, such as soil and water conservation (e.g., Fearnside 2005), and the storage of carbon, which contributes to the mitigation of climate change (Glenday 2006).

1.2 Consequences of tropical forest fragmentation for plant survival

Tropical forests are areas of high biodiversity, which are rapidly being destroyed worldwide. Isolated fragments of tropical rain forests usually have lower species richness compared to continuous areas with a similar habitat. Thus, the decline and fragmentation of these forests are major threats to global biodiversity (Turner 1996, Benítez-Malvido & Martínez-Ramos 2003). Empirical evidence of the effects of tropical forest fragmentation is relatively recent, and it mainly comes from studies on woody species, whilst understory herbs have received little attention in the fragmentation context (Benítez-Malvido & Martínez-Ramos 2003, Cuartas-Hernández & Núñez-Farfán 2006).

Ecological consequences

The ecological consequences of forest fragmentation can be classified into abiotic effects, and direct or indirect biological effects (Murcia 1995). Both the abiotic and biological effects of habitat fragmentation will ultimately affect the demographic processes of plants, which, in turn, will affect the growth rate and survival of plant populations (Holsinger 2000).

Abiotic effects involve changes in the microclimate both within and on the edge of forest fragments. Fragment edges have higher air and soil temperatures and temperature fluctuations than does the forest interior, and they are more exposed to winds, which reduce humidity and soil moisture, and increase evaporation and desiccation (Lovejoy et al. 1986, Kapos 1989, Saunders et al. 1991, Murcia 1995). The intensity of the edge effects depends much on the fragment size, shape and location in the landscape. Small fragments have a higher relative proportion of edges than do large fragments, which exposes them to greater changes in microclimatic conditions (Saunders et al. 1991). Changes in the microclimate can have direct biological effects on plant regeneration and population growth by increasing mortality (Ferreira & Laurence 1997, Laurance et al. 1998, Mesquita et al. 1999, Tomimatsu & Ohara 2003, Laurance et al. 2007) or decreasing seed germination (Bruna 2002). In tropical forests, where seasonal droughts increase plant mortality, the effects of fragmentation on plant survival are likely to be more severe (Engelbrecht & Kursar 2003, Engelbrecht et al. 2007).

Indirect biological effects mainly involve alterations in the interactions between organisms (Saunders 1991, Murcia 1995). Pollination is the most studied plant-animal interaction in association with habitat fragmentation. Small and/or isolated populations and patches often suffer from pollinator limitation and reduced seed set (Sih & Baltus 1987, Spears 1987, Jennersten 1988, Aizen & Feinsinger 1994, Olesen & Jain 1994, Ågren 1996, Groom 1998, Fuchs et al. 2003, Quesada et al. 2003, Aguilar & Galetto 2004). A reduced seed set can result in reduced recruitment and, consequently, in a reduced population growth rate. However, the type of effect that fragmentation has on pollination depends on the plant species, breeding system, life history and pollination mechanism (Ghazoul 2005). For example, plant species, which are dependent on pollinators for seed set or are specialized for one species or type of pollinator, seem to be more vulnerable to habitat fragmentation (Rathcke & Jules 1993). On the other hand, the replacement of original pollinators with new pollen vectors on the edge of the forest fragments may mitigate the negative effect of fragmentation on pollen dispersal and plant reproductive success (Dick et al. 2003, Ghazoul 2004).

Altered post-pollination processes can also lead to the failure of plant recruitment in forest fragments. Changes in the diversity, density, diet, or behavior of the dispersers can affect the efficiency of seed dispersal (Klein 1989, Martinez-Garza & Gonzales-Montagut 1999, Restrepo et al. 1999, Ortiz-Pulido et al. 2000), whereas increased seed predation by rodents (Asquith et al. 1997, Jules & Ratchke 1999) and seedling herbivory (Asquith et al. 1997, Rao et al. 2001, Lopez & Terborgh 2007) can impede the recruitment of the dispersed seeds.

Genetic consequences

Altered abiotic and biotic conditions in forest fragments imply altered selection pressures on plant populations. To be able to respond to changing selection pressures, the populations have to posses sufficient levels of genetic variation (Frankham et al. 2003, p. 46). Theoretically, the reduced size and increased spatial isolation of populations may lead to the erosion of genetic variation through increased random genetic drift and inbreeding, and reduced gene flow between populations (Ellstrand & Elam 1993, Young et al. 1996). Population genetic variation is measured by parameters, such as the allelic richness, gene diversity, proportion of polymorphic loci and level of heterozygosity (Weising et al. 2005, p. 223). Reduced allelic richness may decrease the potential of a species to adapt to an environmental change in the long term. The allelic richness is expected to decrease shortly after the fragmentation because the small remnant populations contain only a small sample of the original gene pool (genetic bottleneck). Thereafter, the loss of alleles may continue due to random genetic drift if the remnant populations remain small and isolated (Barrett & Kohn 1991, Young et al. 1996, Honnay & Jacquemyn 2007). Numerous studies show evidence for the correlation between small population size and reduced genetic variation (e.g. Leimu et al 2006, Honnay & Jacquemyn 2007).

Several studies also show that small populations are more susceptible to biparental inbreeding or inbreeding through self-fertilization, which increase the level of homozygosity within populations (Keller & Waller 2002). Increased homozygosity can cause inbreeding depression, i.e., lack of vigour in the inbred offspring that can affect population performance (e.g., Charlesworth & Charlesworth 1987, Barrett & Kohn 1991, Ellstrand & Elam 1993, Keller & Waller 2002). This is thought to be mainly caused by the expression of deleterious recessive or partly recessive alleles in the homozygous individuals (Charlesworth & Charlesworth 1999). However, small and isolated populations with a long history of inbreeding may have purged most of the deleterious recessive alleles and, as a result, inbreeding depression can be low or may not exist at all (Lande & Schemske 1985, Barrett & Charlesworth 1991).

It is generally assumed that forest fragmentation reduces inter-population gene flow, which is likely to result in the genetic divergence of the populations (Young 1996), but evidence for this assumption is inconsistent. Some studies have reported decreased inter-population gene flow and increased population differentiation as a result of forest fragmentation (Hall et al. 1996, Cuartas-Hernández & Núñez-Farfán 2006), while others have reported extensive long-distance pollen-mediated gene dispersal among fragments (White et al. 2002), or no effect of fragmentation on gene flow (Nason & Hamrick 1997). For example, studies on the temperate tree *Acer saccharum* have even shown increased inter-population gene flow as a consequence of fragmentation (Foré et al. 1992, Young et al. 1993).

Because forest fragmentation decreases population sizes, the effect of stochasticity must also be considered. The smaller the population is, the more probable is its stochastic extinction due to demographic, environmental and genetic factors. Demographic stochasticity arises from random chances of individual plants to survive, die and reproduce, which causes variation in the population growth rate. Environmental stochasticity refers to the temporal variation of habitat conditions, to the populations of competitors, predators, parasites, and diseases, and to unpredictable natural catastrophes. Genetic stochasticity results from random changes in gene frequencies due to founder effect, random fixation of alleles, or inbreeding (e.g., Shaffer 1981 and references therein).

To summarize, forest fragmentation has been shown to reduce the diversity of plant species and, therefore, it should also have negative effects on the viability of populations of forest plants. Fragmentation alters species interactions, causes abiotic edge effects, may decrease migration between fragments, and increases genetic drift and inbreeding depression, which change the demographic and genetic structure of the populations. However, the understanding of fragmented ecosystems is still limited and the consequences of fragmentation are not always consistent or predictable (Turner 1996, Lopez & Terborgh 2007). Generalization of the mechanisms by which fragmentation affects populations is difficult because several factors modify the intensity of the edge effect. These modifiers include the size, shape, and age of the fragment, the management history of the fragment and matrix, the location of the fragment in the landscape, climatic conditions, biological interactions, life-history characteristics of the species, and interactions between abiotic and biotic edge processes (Saunders et al. 1991, Murcia 1995, Harper et al. 2005, Honnay et al. 2005).

1.3 Conservation of taxonomically complex plants

The conservation of endangered plants is difficult without adequate information on their biology. The essential information includes taxonomy, life history, reproductive biology, and spatial and genetic structure of the populations (Given 1994). Moreover, the comparison of rare and common plant taxa can help to identify biological traits that contribute to rarity and that need to be taken into account in conservation measures (e.g., Farnsworth 2007).

Threatened or endemic plants may have biological characteristics that bring extra challenges to their research and conservation. Taxonomically complex groups of plants form such a challenge (Hey et al. 2003, Mace 2004). If the taxonomic status of populations cannot be assigned, appropriate conservation decisions are extremely difficult to make, or if the taxonomic status is not correctly assigned, conservation resources may be wasted on the populations of common species, or hybrids between species, while unrecognized endangered species may be allowed to become extinct (Frankham et al. 2003, p. 366).

Species are generally recognized as a natural taxonomic unit that form the basis for both conservation assessments and management (Mace 2004). However, delimiting species is not always straightforward because of the variety of species concepts (Mayden 1997). The two most important ones, the biological species concept (BSC) and phylogenetic species concept (PSC) have important practical implications for plant conservation. Under the BSC, species are taxa that can overlap geographically because they are reproductively isolated (Mayr 1963). In many cases, especially in plants in which interspecific hybrids are rather common, the BSC is not practical (Donoghue 1985). For example, the endangered genus *Saintpaulia* would be treated as a single species because of the lack of reproductive isolation among the taxa (Arisumi 1964), although the genus consists of geographically isolated entities that differ both genetically (Lindqvist & Albert 1999) and morphologically (Burtt 1958, Darbyshire 2006). The BSC would thus overestimate the geographical range and the number of extant populations of *Saintpaulia*, and place it in a low category of threat.

According to one of the several definitions of the PSC, a phylogenetic species is "the smallest diagnosable cluster of individuals within which there is a parental pattern of ancestry and descent" (Cracraft 1983). Within the PSC, the phylogenetic level that corresponds to a species is not defined, which tends to result in the splitting of taxa (Horvath 1997). The PSC can recognize even 48% more species than does the BSC (Agapow et al. 2004). This increase in the species numbers is referred to as 'taxonomic inflation' (Isaac et al. 2004), and it will result in a higher number of threatened species, because the geographical range and population size of the taxa will decline (Agapow et al. 2004). Taxonomic inflation is also caused by the description of taxa based on inadequate sampling (e.g., Darbyshire 2006, p. 51) or taxonomic exaggeration of charismatic or popular groups (Pillon & Chase 2007). For example, the popularity of orchids in Europe has resulted in the over-splitting of the group. Taxonomic inflation in such groups can result in serious consequences if rare, poorly defined species are prioritized for conservation over taxonomically 'good' species (Pillon & Chase 2007).

The lack of consensus on the concept of species has decreased the usefulness of species as a unit for conservation actions and led to the development of alternative units. Most biologists agree that conservation must focus on preserving evolving populations, in which adaptive diversity and the potential for evolutionary change is maintained (Mace 2004). Therefore, Ryder (1986) introduced the definition of the evolutionarily significant unit (ESU) as a population, or populations, representing significant adaptive variation within a species based on concordance between sets of ecological and genetic data. This concept has been criticized for poor applicability in the real world because it has evolved over time so that the ESUs are now often defined based only on molecular phylogenies that stem from historical legacy rather that adaptive diversity (Crandall et al. 2000). Instead of using the ESU, Crandall et al. (2000) proposed that populations should be classified as management units according to whether they show recent or historical ecological or genetic exchangeability. The idea of ecological exchangeability is that individuals can be moved between populations because they share adaptations, and genetic exchangeability refers to ample gene flow between populations (Crandall et al. 2000).

Apparently, the definitions of management units in conservation continue to evolve, and new approaches need to be tested (c.f. Rader et al. 2005). What seems evident is that whichever approach will be applied, both molecular genetic and ecological research is needed to define appropriate conservation units in taxonomically difficult plant groups. This is likely to increase the cost of conservation in taxonomically complex plants compared to taxonomically well-defined groups.

1.4 Genetics in the practical management of threatened plants

In addition to helping resolve taxonomic uncertainties and delineate management units, genetic research is needed to answer also other questions related to the practical management of threatened taxa. Genetic markers, increasingly frequently taxon-specific and co-dominant microsatellite markers that reveal heterozygosity and allelic diversity, are applied to diagnose the status of a species or population (Frankham et al. 2003). Genetic markers are used to examine the level of genetic variation within populations, genetic divergence among populations (e.g., Li & Xa 2005), and levels of inbreeding (e.g., Kettle et al. 2007). The application of genetics in the conservation of wild populations has largely been limited to the diagnoses of their genetic status, whereas the use of genetic information to plan conservation management is still rare (Frankham et al. 2003). An example of conservation management, where genetic information in needed, is the recovery of small and isolated populations with a low genetic diversity by the introduction of unrelated individuals from other populations (e.g., Heschel & Paige 1995, Bossuyt 2007). When introducing individuals to a population, there is a risk of outbreeding depression, i.e., a reduction in the reproductive fitness due to the crossing of two populations, if the two populations have undergone significant adaptation to their local environments (Waser 1993, Frankham et al. 2003). Moreover, when re-establishing extinct populations, it should be done with individuals that are best adapted to the introduction habitat (Picó & Groenendael 2007).

1.5 Aims of the research

Of all species of vascular plants, 44% are confined to 25 biodiversity hotspots that comprise only 1.4% of the land surface on the Earth (Myers 2000). Hotspots are areas hosting exceptional concentrations of endemic species and experiencing exceptional loss of habitat (Myers 1988, 1990). Concentrating a large proportion of the limited conservation resources on these areas has been suggested as the most profitable strategy to combat the mass extinction of species that is now underway (Myers 2000). From this standpoint, targeting biological research efforts in the hotspot areas is also well justified. Furthermore, it is justifiable to target research effort on 'flagship taxa' of a hotspot or another biologically valuable area because they attract the attention of the public and conservation funders' and decision makers' interest in the area (Caro & O'Doherty 1999). There is no strict definition of a flagship, but they are often endangered and widely known and/or popular species restricted to a particular ecosystem (Caro & O'Doherty 1999).

The Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya form a global biodiversity hotspot on the African continent. In Tanzania, the Eastern Arc Mountains have the highest known number of plant and animal species of any region in the country and, for many taxa, it is one of the richest areas in all of Africa (Newmark 2002). The genus *Saintpaulia* H. Wendl. (or African violets), the research target of this thesis, has been promoted as a botanical 'panda' symbolizing the decline of the Eastern Arc Mountains' forests (Eastwood et al. 1998). The genus is also being promoted as a tourist attraction in an effort to develop ecotourism in the hotspot (Kolehmainen 2005, Kolehmainen et al. 2005, 2006).

Saintpaulia provides an excellent example of a little studied conservation flagship which is threatened by forest fragmentation. When initiating this research, I found it very surprising that despite the global horticultural popularity of the *Saintpaulia*, there were only few studies published on the ecology of the wild species, although they are known to be severely threatened narrow-range endemics within a biodiversity hotspot. Johansson (1978) described the habitats of some Tanzanian taxa and stated, based on the available literature, that "very little is known about the *Saintpaulia*s in their natural environment". He was able to cite only one study (Haarer 1955), which had presented a short and very general account of the *Saintpaulia*s 'in the wild'. After Johansson (1978), not a single ecological field study was published on the genus, although conservationists' interest in the Eastern Arc Mountains and concern for survival of the *Saintpaulia*s increased in the 1980s in response to the destructive commercial loggings conducted in the area (Anonymous 1985, Mather 1989).

The main objective of this research was to increase understanding of the life history, ecology and population genetics of *Saintpaulia*, which is needed for the design of appropriate conservation measures for the genus. A further aim was to provide ecological insights on the difficult taxonomy of *Saintpaulia*. The study is composed of four interlinked substudies, each of which focuses on a specific question or questions, and which are hereafter referred to by the roman numerals I-IV. All substudies included field work in the south-central part of the

Amani Nature Reserve in the East Usambara Mountains, northeastern Tanzania, complemented by population genetic laboratory work and ecological experiments at the University of Helsinki, Finland. The study populations represent three different species of *Saintpaulia* or one subspecies, depending on which taxonomic classification is followed. The objective of the first substudy was to describe the reproductive phenology and performance, and pollination mode. The second study focused on the viability and seasonal dynamics of the populations by examining plant life-history traits, population stage structures, and the effects of habitat quality on plant performance. In the third substudy, I assessed the genetic structure and viability of the populations, and in the fourth one, I studied the levels of inbreeding in the populations and susceptibility to inbreeding depression by investigating the effect of inbreeding on progeny fitness.

2 MATERIAL AND METHODS

2.1 Genus *Saintpaulia*

African violets (*Saintpaulia* H. Wendl.) belong to the Gesneriaceae, a large plant family (125 genera, about 2000 species) comprising mostly tropical herbs and shrubs, many of which are popular cultivated ornamentals (Heywood 1996). The genus *Saintpaulia* consists of perennial herbs endemic to the Eastern Arc Mountains and coastal lowlands of Kenya and Tanzania (Baatvik 1993). 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*s are among the most well-known plants of the East African flora. They have been subjected to intensive breeding by the horticulture industry for nearly a hundred years, and commercial *Saintpaulia* hybrids are popular ornamental plants in the wealthier parts of the world.

Taxonomy

Until 2006, twenty *Saintpaulia* species and four further varieties were recognized (Burtt 1958, 1964). In Burtt's taxonomy, a narrow species concept was applied due to the lack of knowledge of the morphological variation in the wild populations, and nearly half of the described species were found in the Usambara Mountains and in the adjacent lowlands. Phylogenetic studies based on the nuclear ribosomal DNA internal transcribed spacer (ITS; Möller & Cronck 1997) and 5S nuclear ribosomal DNA non-transcribed spacer (5S-NTS; Lindqvist & Albert 1999, 2001) have later demonstrated only poor resolution among the taxa of Usambara and the lowlands. 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. Crossing experiments which have shown that the majority of the *Saintpaulia* taxa can hybridize and that hybrid offspring are fertile (Clayberg 1961, Arisumi 1964) support the concept of weak genetic divergence of the taxa. Darbyshire (2006) subsequently applied a broader species concept in the updated taxonomic revision, in which the total number of species was reduced to six. According to Darbyshire (2006), all *Saintpaulia* populations in the East Usambara Mountains and the adjacent coastal lowlands belong to a single highly variable species *S. ionantha* consisting of nine subspecies, which have been defined on the grounds of morphology and geographic location.

Ecology

*Saintpaulia*s thrive in moist and shaded conditions under forest canopy from sea level up to about 1,400 m of altitude (Johansson 1978, Baatvik 1993, Eastwood et al. 1998). They are mainly lithophytic growing on rock faces and outcrops, but occasionally also on the forest floor, on decomposing logs and even as epiphytes on living trees (Johansson 1978; Baatvik 1993; J. Kolehmainen, pers. obs.). They reproduce by seeds and vegetatively from leaf cuttings and through the division of old plants (J. Kolehmainen, pers. obs.). Vegetative growth may be an important means of reproduction in the trailing taxa. The flowers are hermaphroditic and the styles are deflected either to the left or right side of the floral axis (i.e., enantiostyly), which is assumed to enhance outcrossing (Jesson et al. 2003). Both left- and right-styled flowers are found in one plant; a condition termed monomorphic enantiostyly (Jesson et al. 2003). The large, bright yellow anthers that contrast with the blue-violet petals are characteristics associated with bee-pollination (Vogel 1978). The anthers do not shed pollen spontaneously because they are fused at the top (e.g. Wilson 1898). The flowers are pollinated by bees of the genus *Amegilla,* which use buzz-pollination to release pollen from the anthers (Martins 2005; V. Heimala, unpublished). In the buzz-pollination, the flowers extrude pollen from their anthers in response to the vibration of the anthers by the flower-visiting bee (Buchmann 1983).

The small size of the seeds (Figure 3; IV) and the spatial structure of populations in different types of habitats (J. Kolehmainen, pers. obs.) suggest that water-transport is the most likely dispersal method in populations located along streams, whereas wind dispersal and passive dispersal based on gravity are the most likely dispersal methods on rocky mountain slopes. Some animals may also disperse the seeds on their feet, such as rock hyraxes (*Procavia capensis*) which dwell in the same habitats as *Saintpaulia* (See illustration on the cover). These mechanisms are likely to disperse the seeds only over relatively short distances.

Conservation

The *Saintpaulia* populations have apparently always been more or less substructured due to the specialized ecological niche of the species, but it is believed that they have also been severely affected by forest fragmentation (Johansson 1978, Eastwood et al. 1998). Twenty *Saintpaulia* taxa were listed in the 1997 IUCN Red List (Walter & Gillett 1998), in which the status of 16 taxa was categorized as 'indeterminate', indicating the lack of scientific 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–The World Conservation Union (R. Gereau, pers. comm.).

2.2 Study taxa

The target populations of this research were assumed to represent three different species, *S. confusa* B.L. Burtt, *S. difficilis* B.L. Burtt and *S. grotei* Engl. (Burtt 1958) when I initiated the research, but they are now included within one subspecies, *Saintpaulia ionantha* H.Wendl. subsp. *grotei* (Engl.) I.Darbysh (Darbyshire 2006). The two first articles of this thesis follow the pre-2006 taxonomy and the third and the fourth follow the revised taxonomy. In the articles III and IV, and throughout this thesis summary, the previously recognized species are treated as morphotypes which are named according to their earlier species names, i.e., 'confusa', 'difficilis' and 'grotei'. The genetic relationship and the taxonomic status of the morphotypes are discussed in substudy III.

The morphotypes 'confusa' and 'difficilis' are rosulate forms, and they are morphologically very similar (Figure 1, p. 10). They 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 leaves, and usually more than two flowers per flower stalk. These two morphotypes 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 sub-erect. 'Confusa' has thin to thickish leaves with crenate-serrate leaf margins and appressed long leaf hairs, which are usually sparser than in 'difficilis'. The species/morphotype 'grotei' is distinct from 'confusa' and 'difficilis' (Figure 2, p. 10). It 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. As a result of the trailing growth habit, it tends to form extensive uniform stands. The 'hybrid' type has characteristics intermediate of '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 had an intermediate number of flowers per flower stalk (usually three). Reference collections were made of all morphotypes, and the specimens are 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.

2.3 Study area

I conducted this research in the Eastern Arc Mountains, which are a group of isolated precambrian basement mountains stretching from southeast Kenya to south-central Tanzania (Figure 3, p. 11). The Eastern Arcs rise to 1,000 - 2,800 m above sea level, and they are often referred to as forested 'islands' surrounded by a 'sea' of dry savanna. The Eastern Arc forests have a high species richness and endemism, which is thought to be caused by their geographic isolation, high precipitation and climatic stability over millions of years due to the proximity to the Indian Ocean (Lovett 1993). Environmental stability has enabled the survival of moist forest taxa during adverse climatic periods and also promoted differentiation of new taxa of restricted distribution (Fjeldså & Lovett 1997). New plant and animal species continue to be found in the Eastern Arc forests every year (e.g., Couvreur et al. 2006, Fjeldså et al. 2006, Pócs 2006, Menegon et al. 2007), which is an indication of insufficient taxonomic knowledge of many groups of organisms in the area. Of the known species occurring within the Eastern Arc forests, 3 - 82%, depending on the group, are endemic to these forests. A great number of the species, especially among the endemics, are critically endangered, endangered or vulnerable (Newmark 2002). Due to the exceptionally high degree of endemism and threats to their biodiversity, the Eastern Arc Mountains have been identified among the global biodiversity hotspots and as one of the most threatened ecosystems worldwide (Myers 2000, Mittermeier et al. 2005).

It is estimated that less than 30% of the natural forest currently remains in the Eastern Arc Mountains (Newmark 1998, 2002). Much of the loss of the forest cover has occurred during the last 200 years due to the dramatic increase in the human population, as well as technological development (Temple 1972). Currently, the predominant cause of deforestation is small-scale agriculture, which expands at a fast rate as a result of rapid population growth. Historically, and to some degree also presently, commercial logging, the establishment of coffee and tea plantations, cardamom cultivation and exotic tree plantations have contributed

Figure 1. Morphotype 'difficilis'. Photo: Finnish Saintpaulia Society.

Figure 2. Morphotype 'grotei'. Photo: Finnish Saintpaulia Society.

to the loss of forest in the Eastern Arcs. The collection of fuel wood and building poles also depletes the Eastern Arc forests, especially in areas of high human density (Newmark 2002). In 2003 and 2004, the Eastern Arcs, especially the Usambara mountains, were also affected by tens of thousands of gold miners, who excavated streams in forest reserves causing extensive damage to the aquatic systems and vegetation (Boy 2004, Burgess et al. 2004).

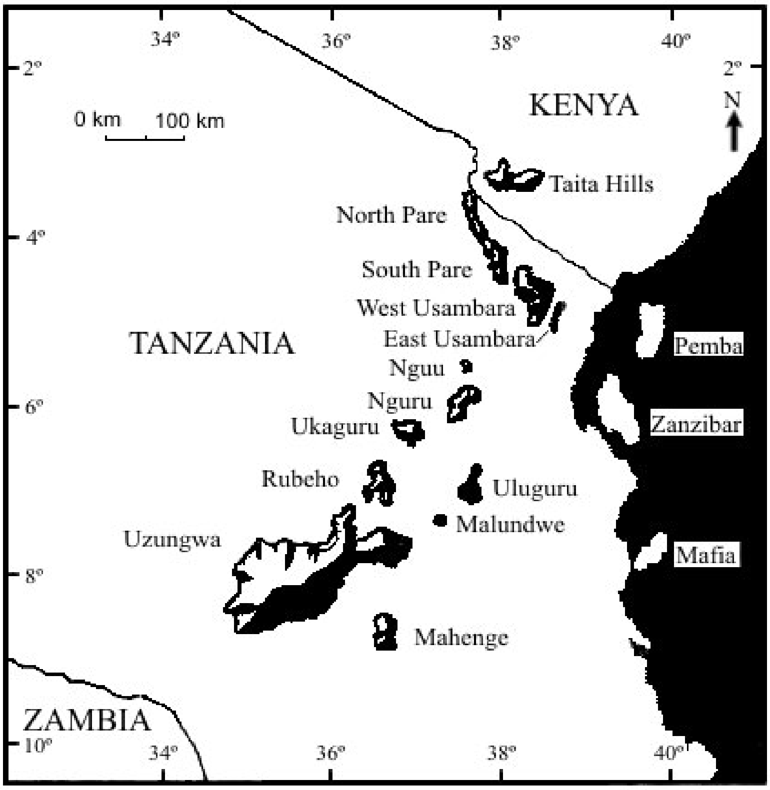

Figure 3. Map showing the Eastern Arc Mountains of Tanzania and Kenya.

Most of the remaining Eastern Arc forest is currently legally protected as government forest reserves that are managed for water catchment. A smaller proportion is protected as a national park, nature reserves, local authority forest reserves or village forest reserves. Despite the large cover of protected area, forest degradation continues both in protected and unprotected forests due to, e.g., illegal timber extraction. Lack of sufficient government funds for the management of the forest reserves is a factor that allows much of the illegal activities to continue (Burgess et al. 2007).

The study area was located in the Amani Nature Reserve (lat. 5º 04'–5º 13' S and long. 38º 33'–38º 40' E) in the East Usambara Mountains, northeastern Tanzania. A total of 621 tree and shrub species have been recorded from the Amani Nature Reserve (ANR). Of them, 19 are endemic to the Usambara Mountains and 49 are near-endemic (Frontier Tanzania 2001). To my knowledge, the diversity of herbaceous plant species has not been systematically surveyed in the ANR or elsewhere in the East Usambara Mountains.

The study area was about 6 km x 8 km covering most of the Amani Nature Reserve, excluding the northern-, eastern- and southernmost parts. The study sites were located in altitudes between 850 and 1,000 m above sea level. In this area, 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). The natural forest remains on the upper sections of the mountain slopes and on the plateau where it is fragmented by large tea plantations, farms, settlements and *Eucalyptus* plantations (Figure 1; I, II, III, IV).

The lush natural forest is maintained by abundant rainfall that, however, is not equally distributed. The precipitation declines on the plateau to the north and west (Hamilton 1989).

At Kwamkoro, in the south-central part of the plateau, the mean annual rainfall is 2,220 mm and on the edge of the west facing slope at Ndola about 1,470 mm (Tanzania Meteorological Agency; based on measurements for Kwamkoro in 1980–98 and measurements for Ndola in 2002–03). There are two rainy seasons, ‘the long rains’ in March–May and ‘the short rains’ in October–December (Figure 2; II). The mean annual temperature at the study sites within the forest is about 19.4°C, the highest temperatures occurring from December to March (monthly mean 21.2°C) and the lowest from June to September (mean 17.3°C; Figure 2; II).

I selected this study area for two reasons. First, it contains three different species (or morphotypes) in a relatively small area, which offered me an opportunity to study the genetic structure of this taxonomically controversial population complex. Second, I was able to accommodate myself in the heart of this area, which allowed me to reach the study sites without major logistical problems. By selecting this area, however, I was unable to directly study the potential effects of forest fragmentation on the viability of *Saintpaulia* populations due to the lack of fragmentation of the forest.

2.4 Populations

The *Saintpaulia* taxa/morphotypes are spatially clustered in the study area (Figure 1; I, II, III, IV). Morphotype 'difficilis' is confined to the west-facing slope and to a stream valley in the middle of the plateau. The habitats of 'difficilis' on the west-facing slope are drier than those in the stream valleys in the central, southern and eastern parts of the plateau, obviously because of lower rainfall (Hamilton 1989), because of a more open canopy (II), and probably also because of stronger and more frequent winds. Morphotype 'confusa' is confined to the southern and south-western slopes and to the stream valleys in the southern parts of the plateau. Morphotype 'grotei' grows in a more continuous forest in the eastern part of the plateau and in the vicinity of the east and southeast-facing slopes (Figure 1; I, II, III, IV).

The populations were subjectively delimited based on the field surveys conducted by the author and field assistants in 1998-1999 (Kolehmainen 2000) and by the author and the Saintpaulia Conservation Project team in 2002-2003 (Kolehmainen et al. 2005). The population boundaries are partly suggestive because it was possible to explore only the accessible parts of the slope areas and because the time available for the population explorations was limited. Therefore, some of the discontinuations in the occurrence of *Saintpaulia* (Figure 1; I, II, III, IV) may not truly exist. Due to this uncertainty in the population delimitation, I sometimes use the term 'locality' or 'site' instead of 'population', especially in contexts where population range is not so relevant or of interest (substudies I, II), or if I talk about a specific locality, e.g., an experimental site, within a population. The terms 'population', 'locality' and 'site' could also be replaced with the term 'subpopulation' on the grounds of the relatively small geographical scale of the study area.

For the ecological substudies (I, II), which required several visits to the sites, the populations were selected mainly based on their accessibility. For the genetic substudies (III, part of IV), all populations were sampled once.

2.5 Ecology

2.5.1 Reproductive phenology and potential (I)

In order to study the possibilities for hybridization between the sympatric *Saintpaulia* morphotypes, reproductive phenology was monitored on a total of 25 permanent 1 m^2 study plots for a period of one year at two sites of morphotype 'confusa', four sites of 'difficilis', and one site of 'grotei'. The plots were subjectively established in accessible places where the study species formed distinct stands. All plots were established on vertical to nearly vertical rock faces by marking the corners of the plot on the rock using white paint. The numbers of flower buds, open flowers, and seedpods per individual plant were recorded for 'confusa' and 'difficilis'. For 'grotei' the total number of reproductive structures was recorded only per study plot due to the clonal growth habit of the morphotype. To relate the phenological patterns with climatic seasonality, data on the yearly pattern of rainfall and temperature were acquired from the local weather stations and by using automatic data loggers placed at the study sites.

To assess reproductive potential, the floral biology, fruit production, and seed number per seedpod were studied. In one population of 'confusa', a total of 132 pedicels on 16 randomly chosen individuals were marked with small plastic tags to examine the duration of anthesis, timing of fruit abortion, and level of fruit set. The reproductive structures were monitored approximately every two days for a period of one month. Fruit production per 1 m^2 study plot for the entire year was obtained by summing the estimated yearly fruit production of the individual plants on the study plots. However, for 'grotei', the month with the highest number of seedpods was used as an estimate of the yearly fruit production per plot. To examine the average number of seeds per seedpod, seeds were counted from one seedpod of each randomly chosen individual of 'confusa' (n = 11) and 'difficilis' (n = 19).

2.5.2 Pollination mode (I)

The pollination mode of plants may greatly affect their reproductive success in habitat fragments (Ratchke & Jules 1993). I studied the pollination mode of 'confusa' in a hand-pollination experiment where several flower stalks were marked and bagged. Each flower stalk of an individual plant was randomly assigned to one of the following treatments: 1) bagging and cross-pollination with pollen from another individual plant that was located approximately 10 m from the pollen recipient, 2) bagging and self-pollination with pollen from the same flower, 3) bagging and no treatment to test for spontaneous self-pollination, and 4) natural pollination, which did not involve bagging. A total of 138 flowers on 23 individual plants were marked for the experiment. In addition, 36 flowers on a different set of plants (n = 20) were marked as a control for natural pollination to test if bagging interferes with pollinator visitation to the experimental plants. The pollination of a flower was considered successful if a seedpod started to develop.

2.5.3 Population stage structure, survival and recruitment (II)

Climatic seasonality and habitat quality can have significant effects on the performance of plant populations (Poorter & Hayashida-Oliver 2000, Riba et al. 2002, Engelbrecht & Kursar 2003). To study the seasonal variation in the population stage structure, and the effect of habitat quality on the performance of *Saintpaulia*, I collected data for one year on population stage structure, survival and recruitment from the same permanent 1 m^2 study plots, which were used to collect data on reproductive phenology and fruit production (See 2.5.1). Moreover, I examined the presence of a soil seed pool.

Life-cycle stages

Life-cycle stages and population stage structures were examined in 'confusa' and 'difficilis'. An individual plant is composed of one or several stems and each stem may decay from the end as the apical bud continues growth (pers. obs). The plants may develop multiple stems from the early developmental stage (seedling or a rooting leaf propagule) to adulthood (old plants with a long prostrate stem may develop new stems from axillary buds). Stems may become independent due to vegetative reproduction by division. The term 'individual' was used for clearly independent solitary shoots with a single stem and for groups of aggregated shoots, physiologically dependent or independent, which were likely to have originated from one ancestor shoot. The life-cycle stages used in this study are based on plant leaf diameter (mean of the two largest leaves) and the presence of flower stalks:

(1) seedlings: leaf diameter less than 5 mm.
(2) juveniles: leaf diameter between 5 and 15 mm.
(3) sterile adults: leaf diameter > 15 mm and not reproductive.
(4) fertile adults: leaf diameter > 15 mm and reproductive.
(See article II for justification of this life stage classification.)

All plant individuals, excluding seedlings, were marked on the permanent plots and their survival, reproductive status, and size (the number of leaves and leaf diameter measured from the two largest leaves) were monitored monthly for a period of one year. Seedlings in the plots were counted every month and, if they were large enough, they were marked and treated as juveniles. The life-cycle spectrum of each population was calculated as the average frequency of each life-cycle stage per study plot (1 m^2).

Habitat quality

The type of substrate was recorded for all individual 'confusa' and 'difficilis' plants that were marked on the study plots. All substrates were rock surfaces and they were categorized as follows: 1) rock surface with humus cover, 2) rock surface with dense cover of bryophytes, 3) rock surface with sparse cover of bryophytes and 4) bare rock surface. The distinction between dense and sparse bryophyte cover was subjective. The tree canopy cover was measured to all four directions of the compass, both on each study plot and from 10 m distance from the plot, using a spherical densiometer. An average of the eight measurements was used in the analyses. Herbivore damage on each study plant was recorded monthly by counting the number or leaves damaged by herbivores.

Soil seed pool

To study the presence of soil seed pool and the density of viable seeds in the soil, soil samples were collected from two localities of 'confusa', one small and isolated subpopulation with few fertile plants (KW 4, 51 samples) and one relatively large and continuous population with many fertile plants (KW 1, 28 samples). One sampling transect was established in KW1 (5 m in length) and two in KW 4 (6 and 12 m in length). The samples were collected in 2 to 5 rows at 50 or 100 cm intervals along each transect. The area of each soil sample was 10 cm x 10 cm and the maximum depth was 5 cm. Since *Saintpaulia* predominantly grows on rocks, the samples were largely obtained by scraping the bryophytes and organic debris from the rock surface using a small brush and a knife. After collecting, the samples were left to dry in the sun and then stored at room temperature until further study. After nine months, the samples were moisturized and left to grow for 6 months. Emerging *Saintpaulia* seedlings were counted approximately once a month (Baskin & Baskin 2001).

2.6 Genetics

2.6.1 Genetic diversity and divergence of the populations and morphotypes (III)

The maintenance of genetic diversity is a primary objective in the management of threatened species, and understanding the genetic structure of populations is important because the evolutionary potential of a species depends on the degree of population subdivision (Frankham et al. 2003, Allendorf et al. 2007). To investigate the level of genetic diversity and divergence of the populations and morphotypes, twelve populations were sampled during the field trips conducted between December 2002 and January 2004. Leaf samples were collected from 18 to 34 individuals per population by taking care that the distance between the sampled individuals was always more than two meters. The 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 -80ºC until the extraction of DNA. DNA isolation was conducted from a minute portion of leaf using a DNA isolation kit. Thirty-three inter simple sequence repeat (ISSR) primers (UBC primer set no. 9, Biotechnology Laboratory, University of British Columbia) were tested with a subset of samples. Four of them (840, 842, 857 and 873) produced interpretable polymorphic bands, and they were used for the analysis of the whole set of 194 samples. Details of the polymerase chain reaction (PCR) protocol are given in the article III. Amplification products were analyzed on 1.4% agarose gels, stained with ethidium bromide and visualized under UV light. Band sizes were estimated by comparison to a DNA ladder loaded simultaneously with the amplified DNAs.

2.6.2 Inbreeding and inbreeding depression (IV)

The level of inbreeding in populations

The same DNA samples that were used for the study of genetic diversity were used to determine the levels of inbreeding in the populations. Four specific primer pairs (StrepDN110, StrepJH432, StrepCtg16 and StrepD14), which determine microsatellite loci in the related genus *Streptocarpus* and which have been shown to be transferable to *Saintpaulia velutina* (Hughes et al. 2004), were tested with a subset of samples. Only the primer pair determining the locus StrepD14 produced interpretable and polymorphic products in the PCR amplification, and, thereafter, this locus was genotyped in each DNA sample. The details of the PCR and genotyping protocols are presented in article IV.

Enantiostyly

In plants with monomorphic enantiostyly, the amount of inter-floral self-pollination (geitonogamy) depends on the ratio of left- and right-styled flowers on a plant, i.e., the more the ratio deviates from 1:1, the more the condition resembles dimorphic enantiostyly which has been shown to best promote cross-pollination (Jesson & Barrett 2002). To gain a rough understanding of the amount of geitonogamy in the study species, I examined the ratio of the stylar morphs on plants which I grew from seeds that originated from two different populations, Ngua (morphotype 'difficilis') and Arbo (morphotype 'confusa'). I monitored the flowering of 57 plants from Ngua and 46 plants from Arbo (representing 10 and 8 single-mother families, respectively) in the greenhouse for four weeks. During that time, I recorded the number of open flowers in each plant and the deflection of the style in each flower (left, right or straight) at about four-day intervals.

The effect of inbreeding on progeny fitness

If inbreeding occurs in the *Saintpaulia* populations, it may result in inbreeding depression, i.e., reduced fitness of the inbred offspring and, thus, lowered population viability. I conducted an experiment to study the effect of selfing on progeny fitness with a total of 36 plants from one non-isolated and one isolated patch of the morphotype 'confusa' (18 plants per patch). The plants were grown in pots placed in a random pattern in a greenhouse. The following pollination treatments were applied to produce inbred and outbred offspring: i) self pollination with pollen from the same plant individual, ii) cross pollination with pollen from another plant of the same locality (cross 1) and iii) cross pollination with pollen from a plant of the other locality (cross 2). For the cross pollinations, a pollen donor plant (father) was selected randomly for each mother plant and the same father was used for all flowers within a plant assigned for each particular treatment. Each of the three treatments was applied to three flowers per individual plant. Ripe seedpods were collected about three months after the pollination.

The proportions of the mature seeds and the aborted seeds/unfertilized ovules were determined from two seedpods per treatment per individual plant. The average proportion of the mature seeds from two seedpods per treatment per mother plant was used in the data analysis.

To test the germination of the seeds, I randomly chose twenty seeds per seedpod from two seedpods per treatment per plant. The seeds were sterilized and the germination was tested on 1% agar medium with light nutrient solution. The germination levels were checked after 10, 20, 30, 40 and 60 days. The average germination rate of the seeds from two seedpods per treatment per mother plant was used in the data analysis.

Of the seedlings resulting from the germination trials, seven randomly chosen seedlings from one seedpod per treatment per plant were planted in soil in 3 cm growing cells. The seedlings were grown for 30 days, after which the juvenile mortality was checked. From each of the three treatments, two randomly selected juveniles per mother plant were transplanted to pots and grown in growth chambers for eleven weeks. The survival, the number of flower stalks, and the dry mass were determined for each plant at the end of the experiment. The average values of the two replicates per treatment per mother plant were used in the data analysis.

2.7 Data analysis

In the article studying the reproductive ecology (I), the differences between the morphotypes in the timing of the beginning of flowering and in the duration of flowering were tested with Mann-Whitney U tests. Correlations between the tree canopy cover and beginning of flowering, and the tree canopy cover and duration of flowering were tested with a Spearman correlation. Variation in yearly fruit production among the three morphotypes was tested with a one-way analysis of variance (ANOVA), and pairwise comparisons between the morphotypes were conducted with Tukey HSD multiple comparisons tests. Differences between the morphotypes 'difficilis' and 'confusa' in the ovule number and the number of seeds per seedpod were tested with *t*-tests. In the experiment studying the pollination mode, the differences in the level of fruit set among the pollination treatments were tested with one-way ANOVA using ranked values.

In the study of population stage structure, survival and recruitment (II), the effects of species, habitat type, tree canopy cover and substrate type on population stage structure were tested with chi-square tests. Logistic regression was used to study the effects of life-cycle stage, substrate, herbivore damage, tree canopy cover, plant density, habitat type, and their interactions on plant survival.

In the study of the population genetic structure (III), 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 et al. 2005). Nei's gene diversity over loci (*h*) was used to quantify the amount of genetic variation in each population (Nei 1987). The analysis of molecular variance (AMOVA) was conducted to calculate the partitioning of the variation among morphotypes, among 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 test if there is 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.

In the study of inbreeding depression (IV), a two-way ANOVA was used to analyse the effect of pollination treatment and patch on seed production, seed germination, juvenile survival, and the number of flower stalks and plant dry mass. The Tukey HSD and Mann-Whitney U test were used for pairwise comparisons among treatments within patches. The magnitude of inbreeding depression for each performance parameter was assessed by using the equation $\delta = 1 - (W_S/W_O)$, where W_S is the mean performance of the inbred progeny and W_O is the mean performance of the outbred progeny. If the fitness of inbred offspring was higher than that of outbred offspring ($W_O < W_S$), I calculated inbreeding depression as $\delta = (W_O/W_S) - 1$. Thus, the measure of inbreeding depression is bound between -1 and 1. Positive values indicate that the outbred progeny outperformed inbred progeny, and negative values indicate that inbred progeny outperformed outbred progeny (Ågren & Schemske 1993, Carr et al. 1997). Inbreeding depression was calculated for three different treatment comparisons, i) self vs. cross 1, ii) self vs. cross 2 and iii) cross 1 vs. cross 2. The multiplicative inbreeding depression was calculated using the product of inbreeding values of seed maturation, percentage germination and dry weight for the offspring of each mother plant, and then the mean multiplicative inbreeding depression was calculated for each treatment comparison in both patches by averaging the individual multiplicative inbreeding depression values. For each patch and treatment comparison, a one-sample *t*-test was used to determine if the inbreeding values (δ) deviated significantly from zero. Differences between patches in the multiplicative inbreeding depression values were tested using *t*-test. The software Arlequin ver 3.01 (Excoffier et al. 2005) was used to determine the fixation index (*F*) in the study populations. The fixation indices were calculated using the formula $F = 1 - H_o/H_e$, where H_o is the observed heterozygosity and H_e the expected heterozygosity under random mating (Hardy-Weinberg equilibrium) (Wright 1951). The significance of the deviations of the fixation index from zero was tested using a chi-square test (Li & Horwitz 1953).

All statistical analyses were performed using either SPSS for Windows (versions 12.0.1. and 13.0) or the SAS 9.1 software.

3 RESULTS AND DISCUSSION

3.1 Ecology

3.1.1 Flowering and fruiting phenology (I)

A clear seasonal pattern of flowering and fruiting was observed for all three morphotypes (Figure 3; I). The flower production of 'confusa' and 'difficilis' increases from August to November, and decreases to a low level in January. The fruiting of these two morphotypes peaks in December and January. The phenology of 'grotei' lags behind the other two morphotypes by about one month. The flower production of 'grotei' peaks in December and is followed by a peak in fruiting in February (Figure 3; I).

The overlapping flowering times observed here enable pollen transfer and hybridization among the studied morphotypes. Full synchrony of 'confusa' and 'difficilis' flowering probably allows pollen transfer between these two morphotypes, whereas the partial separation of flowering phenology of 'grotei' from 'confusa' and 'difficilis' may contribute to maintaining the integrity of 'grotei' (See article III for the genetic relationship of the morphotypes).

The observed seasonality in the flowering of *Saintpaulia* creates a floral display that is likely to attract pollinators (Stephenson 1979, Augsburger 1980, Bawa 1983). Seasonal flowering is especially important to ensure pollination in plants that are rare in time or in space, such as *Saintpaulia*. Massive blooming may also be beneficial in facilitating partial escape from bud and floral herbivores (Bawa 1983). Seasonality in the abundance of pollinators may also select for seasonality in flowering. Bees of the genus *Amegilla*, the pollinators of *Saintpaulia*, are not active throughout the year (D. Martins, pers. comm.), which suggests that pollinator abundance and activity also contribute to the evolution of flowering phenology of *Saintpaulia*.

Several warm, calm, and sunny days are needed during the flowering season for successful pollination, because the bees do not forage in bad weather conditions (Roubik 1992). Since the flowering of *Saintpaulia* coincides with the short rains, adverse weather conditions are likely to prevail for at least part of the flowering season. Thus, prolonged flowering may have been selected for in order to maximise the chances of good weather conditions for pollination.

The tree canopy cover and the beginning of the flowering season were positively correlated in both 'confusa' and 'difficilis', i.e., the more closed the canopy, the later the start of the flowering. Furthermore, there was a significant negative correlation between the canopy cover and duration of the flowering season in 'difficilis', i.e., the more closed the canopy, the shorter the flowering period. These results seem to suggest that forest disturbance can increase the flowering in *Saintpaulia* and, therefore, also increase seed production provided that there is sufficient pollinator service (See 3.1.3). However, increased seed production in open habitats would be of little benefit to the populations because of the predicted high seedling mortality caused by increased canopy openness (See 3.1.6).

3.1.2 Floral biology and fruit production (I)

On average, the flowers of 'confusa' stayed open for five days. Only 25.8% of the flower buds developed into seedpods, and over half of the abortions (63.2%) occurred already at the bud stage. Some of the flowers seemed to be predated; insect larvae were frequently observed browsing the floral structures. The high level of abortions already at the bud stage may also indicate resource limitation, and consequent allocation of resources to the developing fruits (e.g., Stephenson 1981). The production of surplus flowers that are then aborted contributes to the floral display that attracts pollinators and thus enhances overall reproductive success. Furthermore, the aborted flowers may also have donated pollen and, thereby, contribute to the plant’s male fitness (Sutherland 1987).

'Difficilis' produced on average 99, 'confusa' 23 and 'grotei' 9 seedpods per 1 m^2 study plot per year. The mean number of ovules per flower was 1326, of which an average of 72% developed into mature seeds. The high number of seeds per fruit contributes to the sexual regeneration of the populations. In habitats that are favourable for seedling establishment, the level of sexual regeneration observed is likely to be enough to ensure population viability.

3.1.3 Pollination mode (I)

The results of the pollination experiment suggest that 'confusa' is able to produce fruits equally well following cross- and self-pollination (fruit sets 100%, and 98.9%, respectively; Figure 6; I). When pollinators were excluded by bagging the flowers, no fruits were produced, which indicates that spontaneous self-pollination does not occur. Almost 60% of the naturally pollinated flowers produced fruits, which is clearly less than the values in both hand pollination treatments.

Since both cross- and self-pollination conducted by hand yielded better fruit sets than did natural pollination, my results suggest that the fruit set of 'confusa' is limited by pollination. Furthermore, because the exclusion of pollinators resulted in zero fruit sets, pollinators seem to be necessary for the sexual regeneration of *Saintpaulia*. Since many of the *Saintpaulia* populations occur in fragmented habitats and are isolated, it is possible that some of them suffer from limited pollinator service. Thus, more data is needed on pollinator abundance and on the reproductive success of small and isolated, versus large and less-isolated, populations of *Saintpaulia*. Since self fertilization produced a fruit set nearly equal to outcrossing, self-incompatibility mechanisms do not seem to operate in *Saintpaulia*. However, selfing may result in inbreeding depression, which will be expressed as loss of vigour in the selfed compared to outcrossed progeny (Charlesworth & Charlesworth 1987). The effects of inbreeding on seed production and progeny fitness are dealt with in the article IV.

3.1.4 Population stage structures (II)

All four life stages were present in the study sites during the research period. The number of adult plants remained rather constant, whereas the number of seedlings fluctuated considerably (Figure 3; II). The relative frequencies of the life-cycle stages differed among the morphotypes. There was a lower frequency of seedlings (57.9 %) and a higher frequency of juvenile (13.3 %) and adult plants (28.8 %) in 'confusa' than in 'difficilis' (74 %, 10.7 % and 15.3 %, respectively). The relative frequencies of the life-cycle stages differed also between the stream valleys and slopes, and between the open (≤90 %) and closed (>90 % cover) canopies. Moreover, there were significant differences in the relative frequencies of the life-history stages between the substrates. On the humus substrate, the adult plants were more frequent and juvenile plants less frequent than expected. On substrates with dense bryophyte cover, juveniles and fertile adults were less frequent and sterile adults more frequent than expected. On substrates with sparse bryophyte cover and on bare rock surfaces, adult plants were less and juveniles more frequent than expected.

The studied populations appear to be viable in terms of population stage structure. The observed high, although fluctuating, number of seedlings indicates that the populations are of the dynamic type (Oostermeijer et al. 1994). Since the two morphotypes occur in distinct habitats (i.e., west-facing slope versus the stream valleys), the differences observed in the population stage structures between the two morphotypes may also reflect environmental differences between the habitat types, in addition to inherent differences in population structure between the morphotypes *per se*. The lower proportion of fertile plants and seedlings in the stream valleys compared to the slope could at least partly be explained by the lower light intensity in the stream valleys. In an experiment conducted with a commercial *S. ionantha* hybrid, low light intensities inhibited floral initiation (Stinson & Laurie 1954). The higher frequency of seedlings in the 'difficilis' populations on the slope is a logical consequence of the high fruit production (I), which, in turn, may suggest that pollinators are more active or more abundant on the slope than in the stream valleys. The observed association between the humus substrate and fertile adult plants is most likely explained by the tendency of large plants to 'collect' organic debris on top of them (J. Kolehmainen, pers. obs.), which will further enhance their survival and reproduction.

Demographic monitoring over several years and the use of matrix population models would be needed to predict the long term viability of the populations (Caswell 2001). The resources available for this study did not permit such long-term monitoring, but the study provided information on the seasonal dynamics in the populations, which is needed for planning of long-term demographic studies. An important aspect to be investigated in a long-term demographic study is the effect of forest disturbance level on the growth and survival of *Saintpaulia* populations (c.f. Sletvold & Rydgren 2007).

3.1.5 Recruitment and soil seed pool (II)

The number of seedlings began to increase from the end of the March–May rainy season onwards, and recruitment continued up to August in many populations (Figures 2 and 3; II). The average number of transitions from seedling to juvenile stage per 1 m^2 plot during the 12 months was 14 in 'confusa' and 16 in 'difficilis'.

On average, four seedlings per sample emerged from the samples collected from the large and dense population (KW 1), whereas only two seedlings per sample emerged from those collected from the isolated patch (KW 4) within the population 'Arbo'.

The abundant recruitment of seedlings and the presence of viable seeds in the habitat indicate the potential for successful sexual regeneration in the *Saintpaulia* populations studied here. The abrupt increase in the number of seedlings from the end of the March–May rainy season onwards could suggest that a large proportion of seeds produced in the December–February fruiting season (I) germinate in the following rainy season. Similarly, in a seasonal tropical forest in Panama, Garwood (1983) observed that 75% of species (n=185) germinated with the arrival of the next rainy reason following seed dispersal. Because various factors affect seed viability and germination in the field (e.g., Silvertown & Lovett Doust 1993, p. 82), studies on the longevity of *Saintpaulia* seeds in the field are necessary when assessing the role of the seedbank in the dynamics, survival and potential management of *Saintpaulia* populations.

3.1.6 Survival (II)

The number of individuals per study plot decreased from December to March (Figure 3; II). The number of plants per plot was lowest in April after the dry and hot season of January/February. Mortality (number of deaths observed) and rainfall of the previous month correlated negatively in 'confusa', i.e., the lower the rainfall, the higher the mortality (Fig. 5a; II). On average, over 30% of the marked plants died during the research period of 12 months.

When the data on survival was analyzed across morphotypes, the life-cycle stage was the most important factor that explained the probability of survival ($P < 0.0001$; Table 1; II). Survival was lowest in juvenile plants, higher in sterile adult plants, and highest in fertile adult plants (Figure 6a; II). The second most important factor that significantly explained survival was the interaction between tree canopy cover and plant density ($P < 0.0001$). In sites with open canopy, survival was higher in high-density plots, whereas in sites with closed canopy, plant density did not affect survival (Figure 6b; II). On the average, survival was 8.9% higher in closed canopy sites than in open canopy sites. The interaction between substrate and habitat type was the third most important factor explaining survival ($P = 0.035$). In both stream valleys and slopes, survival was highest in plants growing on humus and lowest on bare rock surfaces (Figure 6c; II).

The seasonal alteration of dry and rainy periods appears to be the main factor causing fluctuation in survival in both 'confusa' and 'difficilis'. Seedlings and juvenile plants are more likely to die during the dry season, because they cannot resist drought as well as can the adult plants that are able to store water especially in their thick succulent stems. On the other hand, the degree of tree canopy closure is a major habitat factor affecting survival. Density of the forest canopy largely determines the temperature, relative humidity and wind conditions inside the forest, which, in turn, affect the rate of transpiration (Richards 1996). The lower survival of *Saintpaulia* observed on the slope, where the canopy is more open, could be attributable to the higher rate of transpiration, especially during the dry season. In sites with a more open canopy, the plants can, perhaps, reduce transpiration by growing in dense stands that may retain moisture from the morning mist for a longer time than would sparsely growing individuals. Very bright light can also induce direct physiological damage to the plants; water droplets left on the leaves from morning dew or a rain shower work as a magnifier that can burn the leaves (c.f. Hill & Goodship 1999). If such damage is severe, it could eventually lead to the plant’s death.

High survival on substrate with humus is best explained by the better preservation of moisture on the substrate during the dry period. The bryophyte cover on the rock face probably also buffers against desiccation, and thus enhances the survival of *Saintpaulia*. The lowest survival was observed on bare rock surfaces, where the conditions are the most severe during spells of dry weather. Local climate characteristics affect the quality of the substrates and the formation of humus is probably reduced on the west-facing slope because lower humidity reduces litter decomposition (Williams & Gray 1974). Lower rainfall and humidity are also likely to reduce the abundance of bryophytes that require water for sexual reproduction (Gurevitch et al. 2002). Previously, Johansson (1978) has observed that tree canopy cover and substrate quality affected the performance of *Saintpaulia intermedia* in the Sigi River gorge on the nearby coastal lowland. The plants growing on microsites with a buildup of organic debris in a reasonable shade of trees were larger, in terms of leaf length and width, than were plants growing on bare rock faces that were exposed to direct sunlight for short periods every day.

3.2 Genetics

3.2.1 Genetic diversity and divergence of the populations and morphotypes (III)

Considerable amounts of intrapopulation genetic variation were observed, and the levels of variation (h) were very similar across all populations and morphotypes (min = 0.28, max = 0.35, mean = 0.32; Table 1; III). These relatively high intrapopulation genetic diversities indicate the adaptive potential of the populations. Similar levels of genetic diversity have been reported in other investigations for outcrossing plant species (e.g. Nybom 2004).

Of the total molecular variance, 93.7% was attributed to variation within populations, 3.5% to populational differences within morphotypes, and only 2.8% to morphotype divergence (Table 2; III). The genetic divergence of the populations was low, with a mean pairwise F_{ST} value 0.06, which is much less than what has been reported for other outcrossing species (Nybom 2004; mean $G_{\mathrm{ST}} = 0.22$, 38 studies). This was not surprising because outcrossing species generally have high levels of within-population variability and low interpopulational differentiation (Schoen & Brown 1991). A low degree of interpopulation differentiation was presumed also from the short geographic distances between the investigated populations. There was no correlation between the genetic and geographic distances among the populations.

When conducting AMOVA with a data set in which the rosulate types 'confusa' and 'difficilis' were combined and the 'hybrid' type were excluded, the divergence between the rosulate and trailing types was higher, 5.5% of the total molecular variance. When just rosulate types were included in the analysis, only 0.9% of the total variance was explained by morphotype divergence. A weak but significant divergence of the morphotypes was detected also from the pairwise F_{ST} values calculated for the morphotypes (populations within morphotypes pooled). The rosulate types 'difficilis' and 'confusa' were the most similar while the greatest genetic distances were between the trailing 'grotei' and the two rosulate types. These results indicate 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 suggests that these two growth forms have begun to diverge genetically through adaptation to different environments. Spatial variation in the precipitation, topography, and the pattern of forest fragmentation, are likely to be important factors driving the evolutionary divergence of the populations. Thicker and more hairy leaves characteristic to 'difficilis' are presumed to be an adaptation to more open and dry habitats in the W and N part of the study area, whereas in the more shaded and rainy 'grotei' and some 'confusa' habitats (E and S parts of the study area) thin and smooth leaves are probably selected for. Variable availability of pollinators is also likely to play a role in the evolutionary divergence of the studied rosulate and trailing morphotypes. The rosulate types that produce numerous flowers but invest little in vegetative growth mainly grow on the more open and drier slope habitats, where pollinators are probably more abundant than in the shaded and moist 'grotei' (and some of 'confusa') habitats. In habitats with few pollinators, an increased investment in clonal growth should be an advantage.

3.2.2 Inbreeding and inbreeding depression (IV)

Inbreeding levels in the populations

It was expected that inbreeding levels within populations would be relatively low because the observed ISSR-based genetic diversities within populations were relatively high. However, the level of inbreeding based on the StrepD14 microsatellite locus varied considerably between the populations, from high inbreeding (F = 0.58) in Ndola B to surplus heterozygosity (F = -0.29) in KW 12 (Table 1; IV). The mean fixation index (F) of the study populations equalled 0.21. The observed variation in the level of inbreeding among populations is most likely explained by differences among the populations in the degree of subdivision and in plant density, because inbreeding can be caused by population subdivision (Keller & Waller 2002), and because flower density of *Saintpaulia ionantha* ssp. *grotei* has been observed to correlate with patch visitation rate of the pollinating bees in the study area (V. Heimala, unpublished data). Furthermore, if the ratio of the right- and left-styled flowers on plants varies among the populations, it is likely to affect the inbreeding rates (c.f. Jesson & Barrett 2002). Because the estimates of the inbreeding rate were based on a single microsatellite locus, they are suggestive and should not be used as a basis for genetic management decisions without prior verification using additional loci.

The occurrence of both high genetic diversities (III, IV) and high fixation indices (IV) in many of the studied populations may be explained by within-patch inbreeding. This is because inbreeding within individual patches of a substructured population may result in low heterozygosity at the population level, but the diversity of alleles may still be high as different alleles can be present in different patches (Frankham et al. 2003).

Enantiostyly

There were almost equal proportions of left- and right-styled flowers in both populations studied (49.8 vs. 43.0% and 47.4 vs. 51.2%, respectively) with a very small proportion of straight-styled flowers (7.2 and 1.4%). The mean ratio of the left (L), right (R) and straight-styled (S) flowers per individual plant was L 49.0 : R 43.2 : S 7.8% in Ngua and L 47.9 : R 50.2 : S 1.9% in Arbo (Figure 2; IV). Monomorphic enantiostyly, which both enhances outcrossing and enables some level of self fertilization, is probably an optimal mating strategy for *Saintpaulia* for two reasons. Firstly, selfing enhances the purging of deleterious recessive alleles that accumulate in the heterozygous individuals in outcrossing (Lande & Schemske 1985). Secondly, in plants that depend on animal pollinators, such as *Saintpaulia*, the intermediate selfing rate is expected to be evolutionarily stable (Lande & Schemske 1985, Aide 1986, Barrett & Eckert 1990, Johnson 1998). *Saintpaulia* populations are usually substructured or small and isolated, thus a situation where only a single or a few individuals are simultaneously in flower in a patch is likely to be quite common. With this population structure, a mixed-mating system should provide better sexual reproductive success than obligate outcrossing.

Inbreeding depression

Fitness measurements of the inbred and outbred progeny showed that *Saintpaulia* is susceptible to inbreeding depression if reproduction occurs by selfing or between close relatives. Inbreeding depression was observed to be high during seed maturation ($\delta = 0.28$) (mean across all comparisons and populations), in the number of flower stalks (mean $\delta = 0.30$) and in the dry mass (mean $\delta = 0.22$) (Table 4; IV). There was little or no inbreeding depression in fitness in the intervening stages, i.e., the seed germination (mean $\delta = 0.01$) or the juvenile survival (mean $\delta = 0.03$). The average multiplicative inbreeding depression was higher in the non-isolated patch ($\delta = 0.49$) (mean across all comparisons) than in the isolated patch ($\delta = 0.22$), which may suggest that inbreeding has been more intense in the isolated patch, purging much of the genetic load. Outbreeding depression was not observed in the crossing experiment as the fitness of the progeny from the between-patch crosses was similar to or higher than the fitness of the progeny from the within-patch crosses. Therefore, translocation of plants between populations as a management measure (recovery of small and isolated populations with low genetic diversity) is not likely to disrupt local adaptation of populations, at least if conducted in geographical scales similar to this study.

4 CONCLUSIONS

Key findings and their implications

In this thesis, I have examined some important aspects of the reproductive ecology, seasonal dynamics and genetics of threatened *Saintpaulia* populations that form a taxonomically controversial group in a relatively little fragmented protected forest.

The results of the studies on reproductive ecology (I, II) suggest that *Saintpaulia* has a good reproductive performance in forests with low disturbance level in the East Usambara Mountains. This is probably related to the availability of pollinators and to the mixed mating system which enables seed production by both self- and cross-fertilization. Another important finding was that seed production depends on sufficient pollinator service. Thus, the availability of pollinators is an important factor to be taken into account in the *in situ* management of threatened populations.

The observed dynamic population stage structures (II) indicate that the studied populations are also demographically viable. Although there is high mortality of seedlings and juveniles in the dry season, sufficient shade and moisture enable the establishment of a large number of young plants. Decreased habitat quality associated with forest fragmentation (i.e., reduced tree canopy closure and substrate quality) is likely to exacerbate seedling and juvenile mortality and therefore forest fragmentation and disturbance are serious threats to the regeneration of *Saintpaulia*. Restoration of sufficient shade to enhance seedling establishment is the most important conservation measure in populations located in disturbed forests, in small forest fragments and on forest edges. Long-term demographic monitoring is recommended in disturbed habitats, although it is costly and laborious to carry out, and even dangerous in certain habitats.

The high genetic diversity observed in the populations (III) suggests that populations in forests of low fragmentation levels in the East Usambara Mountains possess the variation that is needed for evolutionary responses in a changing environment. Thus, genetic management of the studied populations does not seem necessary as long as the habitats remain suitable for *Saintpaulia*. However, inbreeding depression is a potential threat (IV), especially if large outcrossing populations that have accumulated deleterious recessive and partly recessive alleles in heterozygous individuals suddenly become fragmented. Inherent substructuring of the populations, which is typical in *Saintpaulia* due to the dependence on rock substrate, probably counteracts the inbreeding depression through purging of deleterious alleles in small inbreeding population fragments (e.g., Barrett & Charlesworth 1991).

The distinct morphotypes with distinct geographic occurrence and relatively weak genetic 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 adapting to their particular environments. Partial separation of the flowering phenology of the trailing 'grotei' from the rosulate types (I) probably reduces gene flow between these two growth forms, and is therefore likely to play a role in their evolutionary divergence. The partial genetic integrity (III) and the distinct trailing habit of the, 'grotei', would justify its placement into a taxonomic rank of its own, perhaps into a subspecific rank.

Recommended future research

Future population ecological and genetic research on *Saintpaulia* should focus on small and isolated populations in small forest fragments, especially in the coastal lowland where periodical droughts and high temperatures pose a threat to the regeneration of *Saintpaulia*. Specifically, studies on the availability of pollinators, levels of seed set and juvenile establishment, and seed longevity, are recommended. Demographic studies combined with studies of genetic diversity and inbreeding depression, and studies that compare small and isolated versus large populations would help to assess whether the viability of *Saintpaulia* populations depends more on demographic or genetic factors (c.f. Richards 2000). Genetic fingerprinting methods (preferably taxon-specific methods, such as microsatellite markers) should be used to solve the genetic structure of the Usambaras/lowlands metapopulations of *Saintpaulia.* Knowledge of the genetic structure of these metapopulations is needed not only for the definition of management units for *in situ* and *ex situ* conservation, but also for the verification of the genetic basis of the recently defined taxonomic entities (Darbyshire 2006).

This thesis research in the overall context of Saintpaulia conservation

This thesis helped to fill some gaps in the knowledge of the ecology and population genetics of *Saintpaulia*. Unfortunately, biological research alone can only solve a small fraction of the problems of plant conservation, especially in a country like Tanzania where a high population growth rate results in the over-exploitation of forest resources, and where environmental conservation tends to receive less attention than does the pursuit of economic growth. Because conservation of the *Saintpaulia* habitats conflicts with the needs of the adjacent human communities, the success of conservation efforts essentially depends on the availability of, and the will to use, the limited conservation resources for combating illegal tree felling, for the training of communities in forest conservation and for the generation of livelihoods that utilize forest in a sustainable way. The current trend in conservation that seeks integration of conservation and social development (e.g., Hughes & Flintan 2001, Berkes 2004) is hence a desired approach also in the conservation *Saintpaulia*.

ACKNOWLEDGEMENTS

First of all, I am grateful to my supervisors Dr Pia Mutikainen and Dr Helena Korpelainen, whose experience and continuous support helped me to make the right decisions and solve numerous problems in the course of the research.

I am most grateful to Dr Roosa Leimu for excellent comments on the earlier version of this thesis and the unpublished manuscript. Her careful review helped me to improve the work considerably. Moreover, I want to thank Dr Jouni Aspi for the reviewing work, and Prof. Heikki Hänninen for his critical comments on the thesis, and also for assistance in the practicalities of completing the work. I also thank Kent Tankersley for checking the English language.

I owe special gratitude to Leo Junikka and Dr Leif Schulman, who suggested to me a Master's thesis study on *Saintpaulia* ten years ago. Without them, this doctoral research would have never been initiated and my life would probably not be as interesting as it is now. Thank you Leo for exposing me to Tanzania for the first time. Leif also deserves thanks for arranging for me working space at the University of Helsinki Botanical Garden at the beginning of the thesis project.

I very much appreciate the help from my Tanzanian colleagues Mzee Leonard Mwasumbi, Frank Mbago and William Hamisy, who helped me to acquire the needed permits from the Tanzanian authorities.

I thank all staff of the Amani Nature Reserve for various help during the months I stayed in Amani, especially the Conservator, Corodius Sawe, who facilitated the practical arrangements, and Johari Mtango and Albert Ntemi who assisted in the collection of field data. I also thank Fred Tarimo of the Tanzania Forestry Research Institute, who participated in the data collection.

Moreover, I wish to thank the staff of the Tanga Catchment Forest Office, especially my *kaka* and *shemeji* Raymond Killenga who helped me to start the field research by taking me around Tanga to buy supplies which I needed up in the mountains. He also facilitated many other things, shared the same spirit in so many occasions, and volunteered in things which would bear fruit later. Moreover, I owe gratitude to my fellow Finns in the same office, Dr Veli Pohjonen and Taina Veltheim, who helped with logistics and offered me a place to stay on some of my visits to Tanga.

I thank Dr Matti Nummelin for assistance in some practical things in the initial stages of my research in Tanzania, for the reference letters which helped me to get research funding, and many years later, for offering me a lunch together with important advice: to complete the PhD thesis as soon as possible.

I also thank Prof. Ilkka Hanski and Dr Gerard Hertel for the reference letters to funders. Dr Hertel encouraged me continuously and helped create links with people who have supported my work.

I am grateful to Dr Valerie Pence of the Cincinnati Zoo & Botanical Garden for comments on the first article, and for many discussions on *Saintpaulia* conservation. I also very much appreciate her assistance for my field conservation projects in Tanzania.

I thank all researchers and technicians from the Department of Applied Biology and the Department of Biological and Environmental Sciences who gave me methodological advice and helped in the set-up of the experiments.

I thank the cheerful members of our research group, Hanna, Kirsi, the two Marias, Sakina and Tea, for friendship and encouragement at different steps of my research.

I am also grateful to all members of the Finnish Saintpaulia Society for sharing the 'Saintpaulia enthusiasm', and for the field team of the Saintpaulia Conservation Project 2003 for participation in the collection of genetic samples. Juhana Nieminen deserves special thanks for the production of high quality maps of the study area.

I thank all the villagers from the East Usambara Mountains, with whom I could practice Kiswahili and who showed me their way of life that was different from what I was used to.

I wish to acknowledge the Finnish Cultural Foundation, the Societas pro Fauna et Flora Fennica, the Finnish Biological Society Vanamo, Colin Watkins's 'Saintpaulia Projects', and the University of Helsinki for financial support, and the Tanzania Commission for Science and Technology for allowing me to conduct research in Tanzania.

Finally, I express my warmest thanks to my husband, parents, relatives and friends who were so supportive from the beginning to the end, and did not allow me to give up at any stage.

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