Kolehmainen & Mutikainen 2006

Reproductive ecology of three endangered African violet (Saintpaulia H. Wendl.) species in the East Usambara Mountains, Tanzania.

Afr. J. Ecol. 44: 219-227.

REFNO: 3499

KEYWORDS:

Africa, Ecology, Saintpaulia, Tanzania

Reproductive ecology of three endangered African violet (*Saintpaulia* H. Wendl.) species in the East Usambara Mountains, Tanzania

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Abstract

Knowledge of the reproductive biology of endangered plants is essential for their effective conservation. It also provides important information for understanding the evolutionary processes that affect speciation, thus helping the definition of proper units for conservation in endangered plants with problematic taxonomy. We studied the reproductive potential and possibility for hybridization in the endangered genus *Saintpaulia* (Gesneriaceae) by examining flowering phenology, flower and seed production and pollination of three sympatric cross-compatible *Saintpaulia* species in the East Usambara Mts., Tanzania. The synchrony observed in flowering in *S. confusa* and *S. difficilis* may enable hybridization between these two species, whereas partial phenological separation may contribute to the integrity of *S. grotei*. Although the level of flower abortion is high in *S. confusa*, each pollinated flower yields about 1000 seeds. *Saintpaulia confusa* produces fruits following both self- and cross-pollination but spontaneous self-pollination seems not to occur. Thus, seed production depends on sufficient pollinator service. Floral heteromorphy (i.e. enantiostyly) and bee pollination are likely to further enhance cross-pollination, suggesting that the genus predominantly outcrosses. Thus, *Saintpaulia* populations are likely to suffer from negative effects of inbreeding if they become small and isolated.

Key words: flower abortion, fruit set, interspecific hybridization, pollination, reproductive phenology

Résumé

La connaissance de la biologie de la reproduction des plantes en danger est essentielle pour l'efficacité de leur conservation. Elle fournit aussi des informations importantes pour la compréhension des processus évolutifs qui touchent la spéciation et donc pour la définition d'unités adéquates pour la conservation de plantes en danger dont la taxonomie pose des problèmes. Nous avons étudié le potentiel reproductif et la possibilité d'hybridation du genre menacé *Saintpaulia* (Gesneriaceae) en examinant la phénologie de la floraison, la production de fleurs et de semences, et la pollinisation de trois espèces de *Sainpaulia* sympatriques de l'est des Usambara Mountains, en Tanzanie, qui peuvent se croiser. La synchronie de la floraison observée avec *S. confusa* et *S. difficilis* permet peut-être l'hybridation entre ces deux espèces, tandis qu'une séparation phénologique partielle pourrait contribuer à conserver l'intégrité de *S. grotei*. Même si le niveau d'avortement est élevé pour les fleurs de *S. confusa*, chaque fleur fécondée produit environ 1000 semences. *S. confusa* produit des fruits après la pollinisation par sa propre espèce ou par croisement avec une autre espèce, mais il semble qu'il n'y ait pas d'auto-pollinisation spontanée. Donc, la production de semences dépend de l'abondance des pollinisateurs. L'hétéromorphie florale (i.e. énantiomorphisme) et la pollinisation par les abeilles sont susceptibles de stimuler la pollinisation croisée, ce qui suggère que ce genre se reproduit surtout de cette façon. Il est donc probable que les populations de *Saintpaulia* risquent de souffrir des effets négatifs de l' *inbreeding* si elles deviennent trop petites et isolées.

Introduction

African violet (*Saintpaulia* H. Wendl.) is perhaps the best known genus of the family Gesneriaceae which comprises mostly tropical herbs and shrubs, many of which are cultivated as ornamentals (Heywood, 1996). *Saintpaulia* is

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endemic to the Eastern Arc Mountains and the adjacent coastal forests of Kenya and Tanzania that together form one of the 25 global biodiversity hotspots (e.g. Myers, 1988). In the northern countries, the genus has been subjected to intensive breeding for *c.* 100 years, which has resulted in thousands of cultivated varieties that are mass propagated by the horticulture industry (Baatvik, 1993). In their natural environment, the wild species of the African violet are of growing economic potential as a special attraction of nature-based tourism (Chiro, 2005; Kolehmainen, 2005) and as a genetic reservoir for the development of new horticultural varieties (Eastwood *et al.*, 1998).

Saintpaulia species are habitat specialists that thrive in moist and shaded conditions under dense forest canopy, typically on rocky outcrops along streams and on steep mountain slopes. There has been some human impact on *Saintpaulia* habitats for *c.* 2000 years, but since the 1960s the destruction of these forest habitats has intensified (e.g. Hamilton, 1989). Because of this, a large proportion of *Saintpaulia* habitats have probably been lost and the entire genus has become threatened. Most of the remaining *Saintpaulia* populations are small and isolated, especially in the coastal lowlands (Johansson, 1978; Clarke, 1998; Kolehmainen, 2005). Majority of the species are currently known from just one or a few localities, but additional field surveys could reveal new populations (Eastwood *et al.*, 1998). Effective conservation of the African violets *in* and *ex situ* is hindered by e.g. inadequate knowledge of their taxonomy and ecology and lack of information of species distribution and genetic structure of the populations (e.g. Schulman & Kolehmainen, 2004). There is no information on the reproductive performance of *Saintpaulia* in the wild, although such knowledge is of fundamental importance for the management of the endangered populations. Furthermore, it is not known if sexual regeneration is sufficient to maintain the current sizes of and genetic variability within the populations.

Uncertain taxonomy is a major problem for conservation of *Saintpaulia*. So far, twenty species and four varieties have been classified (Burtt, 1958, 1964). However, in an ongoing revision study, a reduction in the number of species is expected, especially in the Usambara Mountains, Tanzania (S. Simiyu, pers. comm.). The Usambara/lowland clade of *Saintpaulia* is poorly resolved and the taxa in this area are suspected to be in an active state of evolution (Lindqvist & Albert, 1999). Most of the *Saintpaulia* species can hybridize with their congenerics and produce fertile hybrids (Arisumi, 1964). Interspecific hybridization is very likely to occur in the East Usambara Mountains where different *Saintpaulia* species occur sympatrically and where plants exhibiting intermediate morphological characteristics have been found (J. Kolehmainen, pers. obs.). *Amegilla* sp. bees seem to be the main pollinators of many *Saintpaulia* species (V. Heimala, unpubl. data; D. Martins, pers. obs.) and they probably also transfer pollen between *Saintpaulia* species. However, even if the different *Saintpaulia* species share pollinators, pollen transfer between the species is possible only if the flowering times overlap. Interspecific hybridization may have important implications for the conservation of *Saintpaulia* species, in addition to the increasing isolation of the populations. Knowledge of interspecific hybridization is needed to address the taxonomic problems and to define appropriate management units for conservation.

The breeding system of *Saintpaulia* has not been studied in the wild populations. Spontaneous self-pollination has been reported to occur frequently in some of the commercial African violet cultivars through an abnormal mode of flower development in which the stigma grows into the anther (Anonymous, 2002). In general, self fertilization and mating between close relatives in small populations may lead to inbreeding depression that reduces the fitness of the individuals and thereby the survival of the populations (Charlesworth & Charlesworth, 1987). As *Saintpaulia* populations are often small and isolated, they may also suffer from inbreeding depression. However, to our knowledge, there is no data on inbreeding depression from wild populations of any of the *Saintpaulia* species. Knowledge of the breeding system is necessary for understanding hybridization patterns and other population level processes that shape the taxonomy of *Saintpaulia*.

The objective of this study was to investigate the reproductive potential, breeding system and possibility of hybridization in wild *Saintpaulia* populations by examining the following reproductive characteristics: (i) reproductive phenology and yearly fruit production, (ii) flower longevity, flower abortion and fruit set and (iii) the mode of pollination. We focused on three *Saintpaulia* species, *S. confusa* B.L. Burtt and *S. difficilis* B.L. Burtt with rosulate growing habits and the trailing *S. grotei* Engl. These species grow relatively close to each other and could thus potentially hybridize.

Material and methods

Study area

Field work was conducted in the Amani Nature Reserve (ANR), which is located in the southern part of the East Usambara Mountains, NE Tanzania, approximately latitude 5°04′–5°13′S and longitude 38°33′–38°40′E (Fig. 1). The study sites are located 900–1000 m a.s.l., where the mean annual rainfall is about 2000 mm (East Usambara Tea Company and Tanzania Meteorological Agency). There are two rainy seasons, the long rains in March to May and the short rains in October to December. The mean annual temperature at the study sites is about 19.4°C, the highest temperatures occurring from December to March (monthly mean 21.2°C) and the coldest from June to September (mean 17.3°C; Fig. 2). 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 forests are rich in species and have a high number of endemics (Davis, Heywood & Hamilton, 1994).

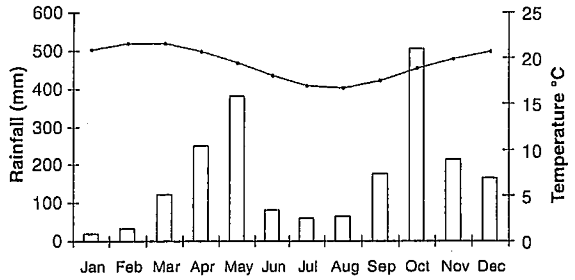

Fig 2 Monthly rainfall (the East Usambara Tea Company, Tanzania Meteorological Agency) and average monthly temperature (Onset HOBO H8 Pro data loggers) in the study area. Based on measurements in 2002–2003

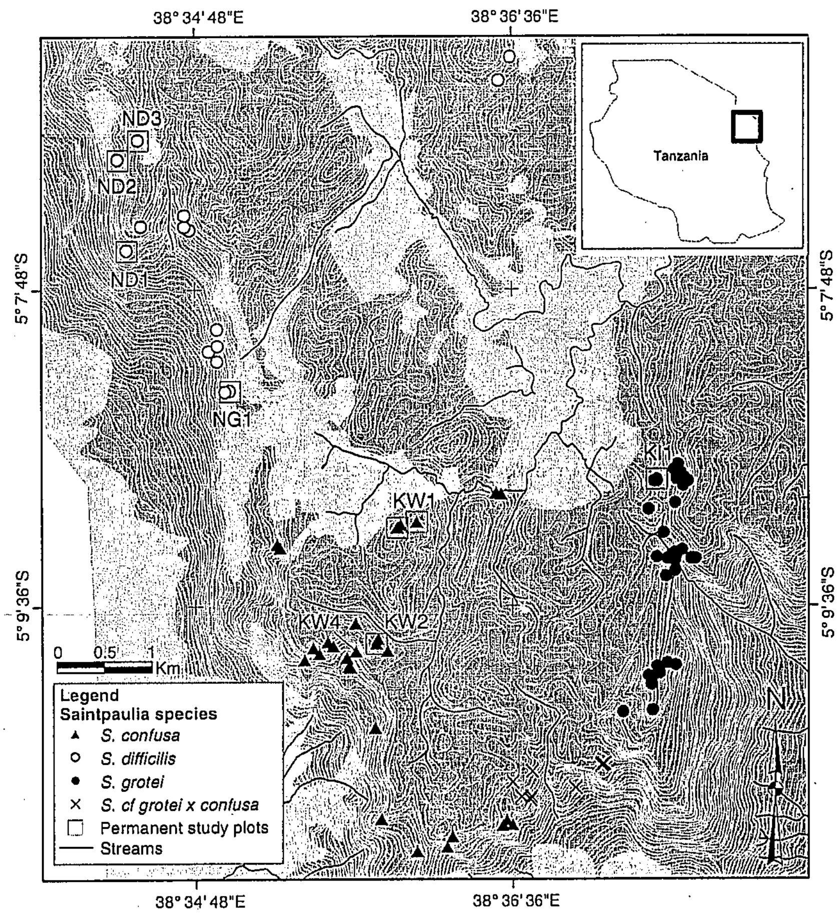

Fig 1 Map of the study area showing the study localities. Darker colour on the background represents forested areas and lighter colour represents open vegetation. Modified from the original base maps of the Finnish Forest and Park Service. Abbreviations of the names of the study sites are indicated

Study species

The habitats of *Saintpaulia confusa* range from the moist and shady stream valleys in the southern part of the Amani plateau to the more open and dry habitats on the southern and south-western escarpment (Fig. 1). *Saintpaulia confusa* has not been reported from elsewhere in the East Usambara, but according to Baatvik (1993) it also grows in the West Usambara and Nguru Mountains. As a continuation of the *S. confusa* populations, *S. difficilis* occupies the dryer south-western and western escarpments. *Saintpaulia difficilis* is endemic to the East Usambara where it is only known from the western and northern parts of the ANR. *Saintpaulia confusa* and *S. difficilis* are morphologically very similar and plants with intermediate characteristics have been found in the study area (J. Kolehmainen, pers. obs). *Saintpaulia grotei* is also endemic to the East Usambara, where it has extensive north-south distribution. In the study area, it grows in moist and shady inner forests of the plateau. Plants with hybrid characteristics have been found in the contact zone of *S. grotei* and *S. confusa* (J. Kolehmainen, pers. obs.; Fig. 1). Reference collections were made in all study populations, except in the population of *S. grotei*, and the specimens are deposited in the Botanical Museum of the Finnish Museum of Natural History. Duplicates of the specimens were left in the Herbarium of the University of Dar es Salaam, Tanzania.

Reproductive phenology and yearly fruit production

Reproductive phenology was monitored on a total of 25 permanent 1 m^2 study plots for a period of 1 year from August/September 2002 to August/September 2003 at two sites of *S. confusa* (five and four study plots), four sites of *S. difficilis* (three, three, three and two study plots) and one site of *S. grotei* (five plots). The numbers of flower buds, open flowers, and seedpods per individual plant were recorded for *S. confusa* and *S. difficilis*. For *S. grotei* the total number of reproductive structures was recorded only per study plot because of the clonal growth habit of the species. For the analysis of the phenology, the total number of reproductive structures per study plot per month was calculated for all three species. The phenology is described using the monthly proportion (%) of the number of reproductive structures produced during the whole year. Fruit production per study plot for the entire year was obtained by summing the estimated yearly fruit production of the individual plants on the plot. However, for *S. grotei*, the month with the highest number of seedpods was used as an estimate of the yearly fruit production per plot.

Data on the yearly pattern of rainfall and temperature were acquired from the local weather stations (the East Usambara Tea Company and Tanzania Meteorological Agency), and by using automatic data loggers (Onset HOBO H8 Pro Series) placed at the study sites.

Floral and seed biology

On sixteen randomly chosen individuals of *S. confusa* a total of 132 pedicels were marked with small plastic tags to examine the duration of anthesis, timing of fruit abortion, and level of fruit set. The phenological state and condition of the above-mentioned reproductive structures were recorded approximately every 2 days for a period of 1 month. To examine the average number of seeds per seedpod, seeds were counted from one seedpod of each randomly chosen individual of *S. confusa* ($n = 11$) and *S. difficilis* ($n = 19$).

Pollination experiment

The breeding system of *S. confusa* was studied 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: (i) bagging and cross-pollination with pollen from another individual plant that was located approximately 10 m from the pollen recipient, (ii) bagging and self-pollination with pollen from the same flower, (iii) bagging and no treatment to test for spontaneous self-pollination and (iv) 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. Pollination of a flower was considered successful if a seedpod started to develop.

Results

Phenology and yearly fruit production

A clear seasonal pattern of flowering and fruiting was observed for all three species (Fig. 3), although on some

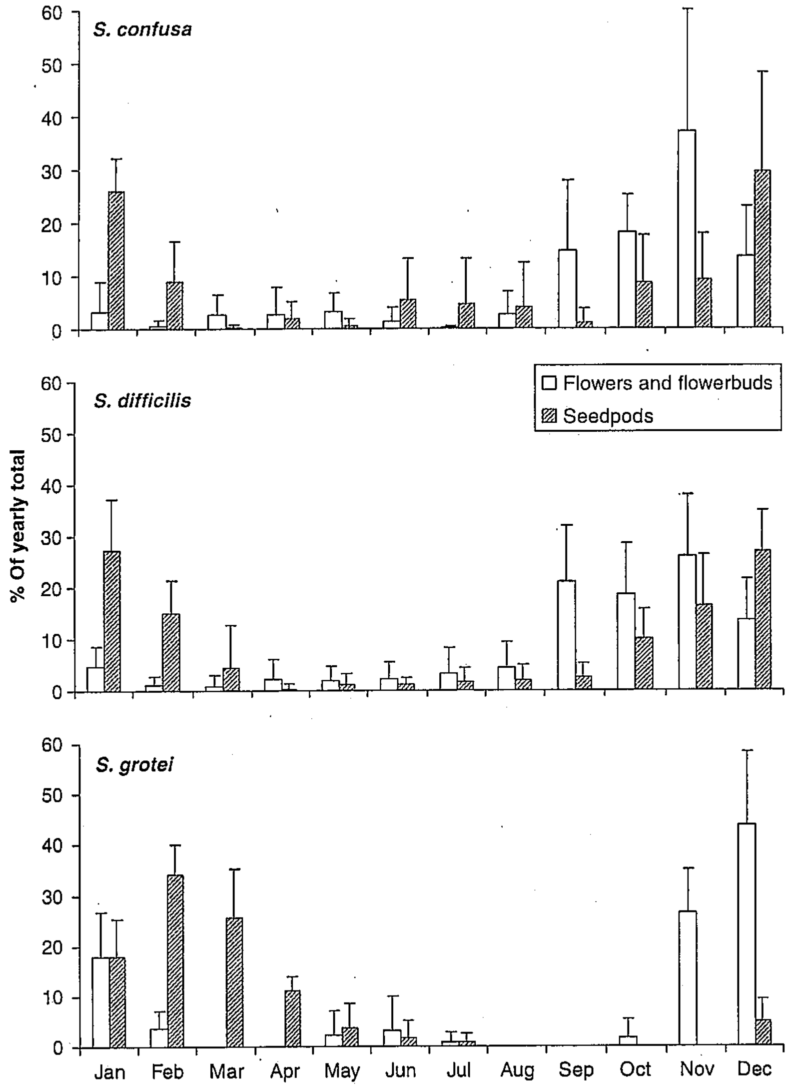

Fig 3 Reproductive phenology of three *Saintpaulia* species (*S. confusa*; nine plots, *S. difficilis*; eleven plots, *S. grotei*; five plots). Vertical bars represent standard deviations

study plots few flowers and fruits were observed throughout the year. Flower production in *S. confusa* and *S. difficilis* increases from August to November, and decreases to a low in January. Fruiting of these two species peaks in December and January. There were no statistical differences between *S. difficilis* and *S. confusa* in the timing of the beginning of flowering (Mann–Whitney $U = 32.5$; $P \geq 0.05$) or in the duration of the flowering season (Mann–Whitney $U = 32.5$; $P \geq 0.05$). The timing of the beginning of flowering of *S. grotei* differed significantly from *S. difficilis* and *S. confusa* ($P = 0.001$ and $P = 0.008$, respectively). The phenology of *S. grotei* lags behind the other two species by about 1 month. The flower production of *S. grotei* peaks in December and is followed by a peak of fruiting in February (Fig. 3). The duration of the flowering season of *S. grotei* did not differ statistically from *S. confusa* ($P = 0.946$) or *S. difficilis* ($P = 0.96$).

The tree canopy cover and beginning of the flowering season was positively correlated for both *S. confusa* and *S. difficilis* (Spearman $r_s = 0.829$; $n = 9$; $P = 0.006$ and $r_s = 0.684$; $n = 11$; $P = 0.02$, respectively; Fig. 4); 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 *S. difficilis* (Spearman $r_s = -0.695$, $P = 0.018$; Fig. 5); i.e. the more closed the canopy, the shorter the

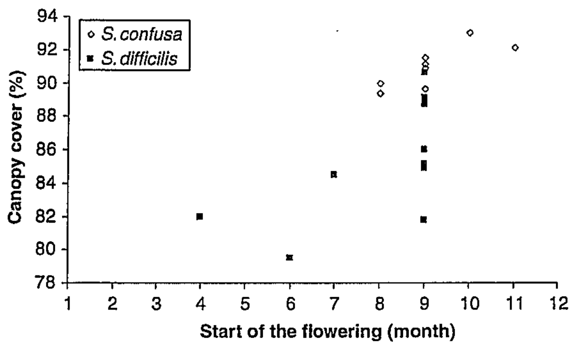

Fig 4 Relationship between the tree canopy cover and start of the flowering season (month). Each symbol represents a single study plot. The initiation of flowering season is indicated as the first month during which at least 5% of the total number of flowers recorded for the year are in flower

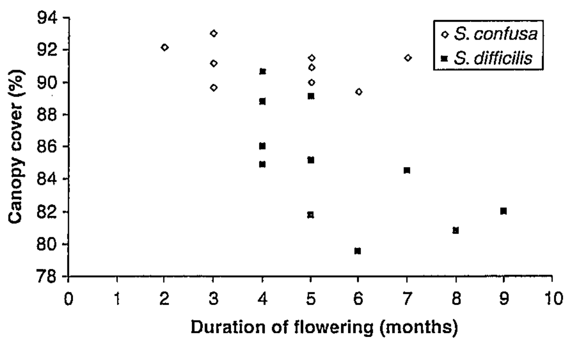

Fig 5 Relationship between the tree canopy cover and duration of flowering season. Each symbol represents a single study plot. The duration of flowering season is indicated as the number of months during which at least 5% of the total number of flowers recorded for the year are in flower

flowering period. Correlation between canopy cover and duration of the flowering season was not statistically significant in *S. confusa* (Spearman $r_s = -0.372$, $P = 0.324$).

Saintpaulia difficilis produced on average 99.1 (SD = 109.3, $n = 11$), *S. confusa* 23.4 (SD = 18.3, $n = 9$) and *S. grotei* 9.2 (SD = 5.5, $n = 5$) seedpods per 1 m^2 study plot. There was significant variation in yearly fruit production among the three species (ANOVA; $F_{2,22} = 3.671$, $P = 0.042$). *Saintpaulia difficilis* differed nearly significantly from both *S. confusa* and *S. grotei* (Tukey HSD multiple comparisons tests; $P = 0.084$ and $P = 0.087$). There was no significant difference in fruit production between *S. confusa* and *S. grotei* (Tukey HSD; $P = 0.938$). In contrast with this, an ANOVA with the study sites nested within the species and with only *S. confusa* and *S. difficilis* included, detected no significant difference in fruit production between the species (ANOVA; $F = 0.31$, $P = 0.605$). Instead, there were significant differences among the study sites within the species ($F = 4.31$, $P = 0.028$).

Floral and seed biology

On average, the flowers of *S. confusa* stayed open for 5 days (minimum = 3, maximum = 8, SD = 0.53, fourteen plants, 31 flowers). On average, 25.8% of the flower buds developed into seedpods (SD = 26.3%; $n = 16$). Thus, a considerable proportion of the flowers were aborted at some developmental stage. Over half of the abortions (63.2% ± 30.9%) had occurred already at the bud stage, 27.9% ± 28.2% at the flowering stage and 9.0% ± 16.1% at the fruiting stage.

The mean number of ovules per flower was 1317 ± 385 ($n = 19$) for *S. difficilis* and 1334 ± 356 ($n = 11$) for *S. confusa*. The number of fully developed seeds per seedpod was 976 ± 526 for *S. difficilis* and 1043 ± 365 for *S. confusa* (68.9% ± 24.5% and 76.8% ± 13.5% of the ovules, respectively). Differences between the two species in the ovule number or the number of seeds per seedpod were not statistically significant (*t*-test; $t = -0.115$, d.f. = 28, $P = 0.909$ and $t = -0.957$, d.f. = 28, $P = 0.347$, respectively).

Breeding system

The results of the pollination experiment suggest that *S. confusa* is able to produce fruits equally well following cross and self-pollination (fruit sets 100% ± 0% and 98.9% ± 5.3%, respectively; Fig. 6). When pollinators were excluded by bagging the flowers, no fruits were produced, which indicates that spontaneous self-pollination did not occur. Almost 60% of the naturally pollinated flowers produced fruits, which is clearly lower than in both of the hand pollination treatments (Fig. 6). Differences in the level of fruit set among the treatments were statistically significant (ANOVA, ranked values; $F_{3,78} = 100.952$, $P < 0.0001$).

Discussion

Reproductive phenology

The overlapping flowering times observed here enable interspecific pollen transfer and hybridization in the wild

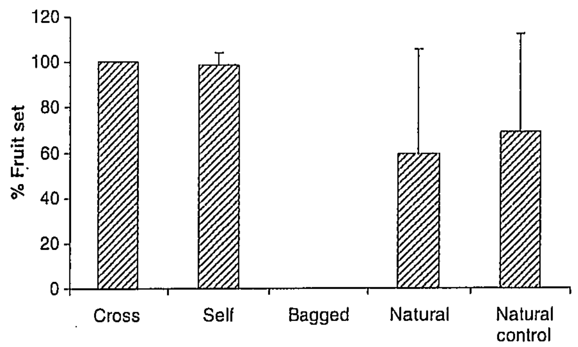

Fig 6 Fruit set (with standard deviations) from different treatments of the pollination experiment. 'Bagged' = bagging of the flowers without a pollination treatment to test for spontaneous selfing. Number of replicates in each treatment: 23 (cross), 22 (self), 21 (bagged), 16 (natural) and 20 (natural control)

populations. Full synchrony of *S. confusa* and *S. difficilis* flowering probably allows pollen transfer between these two species, whereas the partial separation of flowering phenology of *S. grotei* from *S. confusa* and *S. difficilis* may contribute to maintaining the integrity of *S. grotei*. To further study the hybridization of these three species in the wild, we are using genetic markers to estimate their genetic distances and conducting controlled hybridization to study the viability of hybrid offspring. Moreover, monitoring of pollinator movement would produce further information on the spatial scale of putative interspecific pollen transfer.

The reproductive phenology of *Saintpaulia* is affected both by biotic and abiotic factors, such as pollinators and weather, respectively. The observed seasonality in 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 proposed 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). As 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 maximize the chances of good weather conditions for pollination.

Flower and seed biology

A large proportion of the *Saintpaulia* flowers do not develop into fruits in the wild. Some of the flowers seem to be predated; insect larvae were frequently observed browsing the floral structures of *S. confusa*. Resource limitation, which has been reported to induce flower and fruit abortion in many species (Stephenson, 1981), may also play a role. The high level of abortions already at the bud stage may indicate resource limitation and consequent allocation of resources for 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).

Although a high proportion of flowers is aborted, a single flower produces many seeds after successful pollination. The high number of seeds per fruit contributes to the sexual regeneration of the populations. However, this is beneficial only if conditions for seed germination and seedling survival are favourable. In habitats where forest is not dense enough, the delicate *Saintpaulia* seedlings are likely to die when exposed to sun, especially during the dry season (Kolehmainen and Mutikainen, unpubl. data). In habitats that are favourable for seedling establishment, the level of sexual regeneration observed might be enough to ensure population viability.

Breeding system

As both cross- and self-pollination conducted by hand yielded better fruit sets than natural pollination, our results suggest that the fruit set of *S. confusa* is limited by pollination. In the naturally pollinated control plants the fruit set was only 9.4% higher than in the naturally pollinated experimental plants, which indicates that bagging did not significantly interfere with natural pollination. This higher fruit set of the control plants compared with the naturally pollinated experimental plants may also be an indication of resource allocation from the naturally pollinated to the hand pollinated flowers (Stephenson, 1981).

Furthermore, because exclusion of pollinators resulted in zero fruit set, pollinators seem to be necessary for the sexual regeneration of *Saintpaulia*. As many of the *Saintpaulia* populations occur in fragmented habitats and are isolated, it is likely that at least some of them suffer from limited pollinator service. We thus need more data on pollinator abundance and reproductive success of small and isolated versus large and less-isolated populations of *Saintpaulia*.

As 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 of the selfed compared with outcrossed progeny (Charlesworth & Charlesworth, 1987). *Saintpaulia* is likely to predominantly outcross, because it is pollinated by flying insects and because the flowers have two different stylar morphs (i.e. enantiostyly), which has been shown to promote cross pollination (Jensson & Barrett, 2002). Furthermore, we did not observe spontaneous self fertilization in *S. confusa*. Outbreeding species and populations are generally expected to show a higher frequency of recessive deleterious alleles that are hidden in heterozygous individuals. Therefore the fitness in outbreeding populations should decrease more with self fertilization than the fitness in habitually inbreeding populations that have purged their genetic load during many generations of inbreeding (e.g. Charlesworth & Charlesworth, 1987). We are currently examining the level of biparental inbreeding depression and using genetic markers to assess the outcrossing rates of *Saintpaulia* populations of different size and degree of isolation.

Acknowledgements

We thank Johari Mtango and Fred Tarimo for help in field research, Ville Hahkala for help in data processing and Juhana Nieminen for map production. Valuable help and constructive comments on the manuscript were given by Dr Helena Korpelainen and Dr Valerie Pence. We also thank Leonard Mwasumbi and Frank Mbago (University of Dar es Salaam) and Raymond Killenga (Tanga Catchment Forest Office) for assistance in practical arrangements. Tanzania Commission for Science and Technology is acknowledged for permission to conduct research in Tanzania and the Finnish Cultural Foundation for financial support.

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(Manuscript accepted 25 January 2006)

