

Gene flow in the understory of tropical African rain forest
- from population genetic to phylogeographic pattern –
the example Marantaceae

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Gene flow in the understorey of tropical African rain forest
- from population genetic to phylogeographic pattern –
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*Das nachdenkende, betrachtende, forschende Leben ist eigentlich das
Höchste.*

Alexander Freiherr von Humboldt
(1769 - 1859)
deutscher Naturforscher
Begründer der physischen Geographie

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Appendix 6 Ley, A.C., Hardy, O.J. (2017) Hybridization and asymmetric introgression after secondary contact in two tropical African climber species *Haumania*

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ORGANIZATION OF THE THESIS

The thesis is based on a series of published articles and manuscripts of the author on gene flow in the tropical African forest understorey focusing on intra-specific gene flow, species delimitation and inter-specific gene flow and the inference of historical migration patterns.

The thesis is organized in seven chapters starting with a general introduction outlining the evolutionary importance of gene flow and the principle research questions of this thesis. Here the study of gene flow is used at different spatial and temporal scales to infer the history of species as well as the present state and the future evolutionary potential of species in the tropical African rainforest (chapter 1). This chapter is followed by an introduction of the tropical African rainforest understorey as study system with particular emphasis on the current knowledge on patterns of gene flow and major existing hypotheses on its history to be tested in this thesis (chapter 2). The third chapter is a presentation of the existing and in this thesis refined knowledge about the study taxa and the reasons for their selection (chapter 3). In the next three chapters the results gained during my research are summarized focusing on three issues: 1) the extend of local intra-specific gene flow (chapter 4), 2) the different degrees of inter-specific gene flow and the challenges of delimiting species (chapter 5) and 3) the research into tropical African vegetation history using large scale genetic pattern (= phylogeographic pattern) (chapter 6). Each chapter summarizes the content of one to four original articles of the author - it starts with an introduction to the respective topic and ends with a discussion highlighting the major findings and their contribution to current research in the field. The thesis ends with general concluding remarks and an outlook giving insights into the ongoing research and guidelines for future research activities (chapter 7). Finally, an overall summary of the entire thesis is provided (chapter 9).

The thesis is written as an integral text referring in each chapter to the relevant original articles which are printed in their original version in the appendix.

LIST OF PUBLICATIONS RELEVANT FOR THE THESIS

The publications are ordered thematically according to the chapters of this thesis and are followed by information on the specific contribution to each publication by A.C. Ley.

Chapter 3: The studied plant family Marantaceae: taxonomy, biogeography, ecology and phylogeny

- [1] Al-Gharaibeh, M.M., Borchsenius, F., McKechnie, L., Sanmartin, I., **Ley A.C.** (manuscript) Phylogeny of the pantropically distributed family Marantaceae.

Own contribution: Ideas, collection of specimens in the field and through collaboration partners, large part of laboratory acquisition of sequences, partial writing of the manuscript.

Chapter 4: Gene flow within species: breeding system, clonality and dispersal distance

- [2] **Ley, A.C.**, Hardy, O.J. (2016a) Polymorphic microsatellite loci for *Haumania danckelmaniana* and transferability into *H. liebrechtsiana* (Marantaceae). *Applications in Plant Sciences* 4(3): apps.1500116. doi: 10.3732/apps.1500116

Own contribution: Collection of specimens in the field, laboratory manipulations, statistical analyses, writing of the manuscript.

- [3] **Ley, A.C.**, Hardy, O.J. (2016b) Spatially limited clonality and pollen and seed dispersal in a characteristic climber of Central African rain forests: *Haumania danckelmaniana* (Marantaceae). *Biotropica* 58(5) : 618-627.

Own contribution: Part of the ideas, collection of specimens in the field, part of statistical analyses, writing of the manuscript.

Chapter 5: Species delimitation and and gene flow across species boundaries

- [4] **Ley, A.C.**, Hardy, O.J. (2010) Species delimitation in the Central African herbs *Haumania* (Marantaceae) using georeferenced nuclear and chloroplastic DNA sequences. *Molecular Phylogenetics and Evolution* 57: 859–867.

Own contribution: Ideas, collection of specimens in the field, laboratory manipulations, large part of statistical analyses, writing of the manuscript.

- [5] **Ley, A.C.**, Hardy, O.J. (2013) Improving AFLP analysis of large-scale patterns of genetic variation - a case study with the Central African lianas *Haumania* spp. (Marantaceae) showing interspecific gene flow. *Molecular Ecology* 22(7): 1984-1997.

Own contribution: Part of the ideas, collection of specimens in the field, laboratory manipulations, parts of the statistical analyses, large part of writing of the manuscript.

- [6] **Ley, A.C., Hardy, O.J.** (2014) Contrasting patterns of gene flow between sister plant species in the understorey of African moist forests - the case of sympatric and parapatric Marantaceae species. *Molecular Phylogenetics and Evolution* 77: 264-274.

Own contribution: Ideas, collection of specimens in the field, laboratory manipulations, large part of statistical analyses, writing of the manuscript.

- [7] **Ley, A.C., Hardy, O.J.** (submitted) Hybridization and asymmetric introgression after secondary contact in two tropical African climber species *Haumania danckelmaniana* and *H. liebrechtsiana* (Marantaceae). *International Journal of Plant Sciences*.

Own contribution: Part of the ideas, collection of specimens in the field, large part of laboratory manipulations and statistical analyses, writing of the manuscript.

Chapter 6: Phylogeographic pattern and their concordance with the refuge hypothesis

- [8] **Ley, A.C., Heuertz, M., Hardy, O.J.** (2016) The evolutionary history of central African rain forest plants: phylogeographic insights from sister species of the climber genus *Haumania* (Marantaceae). *Journal of Biogeography*. doi:10.1111/jbi.12902

Own contribution: Large parts of the ideas, collection of specimens in the field, laboratory manipulations, statistical analyses, writing of the manuscript.

- [9] **Ley, A.C., Dauby, G., Köhler, J., Wypior, C., Röser, M., Hardy, O.J.** (2014) Comparative phylogeography of eight herbs and lianas (Marantaceae) in central African rainforests. *Frontiers in Genetics* 5: article 403. doi: 10.3389/fgene.2014.00403

Own contribution: Part of the ideas, collection of specimens in the field, most laboratory manipulations, analyses of chloroplast distribution pattern and genetic diversity, writing of the manuscript.

1 The evolutionary importance of gene flow

The evolution of plant diversity in the different ecosystems of the world is still to date one of the major fields of biological research. New species are described every year (Dunn and Moore 2016), more complex processes of differentiation and speciation are revisited (Damasceno et al. 2014), new techniques for more complex genetic data acquisition and analysis are developed and implemented (next generation sequencing (NGS), Davey et al. 2011, Elshire et al. 2011), our understanding of the importance of the interaction of a multitude of different factors for evolution in space and time becomes more sophisticated (Heuertz et al. 2014) and consequently our disposition and capability grows to merge disciplines that have formerly been regarded rather independently (Cutter 2013).

At the base of evolution is the exchange among individuals of (un)altered information encoded in the genome and its transmission from generation to generation, called gene flow. Gene flow is the spatial movement of alleles, achieved in plants largely by pollen transfer and seed dispersal (Bradshaw 1972, Ennos 1994, Petit et al. 2005, Zeng et al. 2012). It is highly important for species reproduction and evolution (Slatkin 1985, Herrera and Pellmyr 2002) as regular outcrossing (xenogamy) fosters genetic diversity. The latter supports species' survival by presenting a source of variable genetic material which allows overcoming deleterious mutations and presents a pool of preadaptations from which the one yielding highest fitness values under a given environment is selected (Takebayashi and Morrell 2001, Coyne and Orr 2004, Wright et al. 2013). The interruption of gene flow through the isolation of individuals or populations through e.g. restricted dispersal and/or the establishment of barriers (geographic, ecological, genetic, etc.), in contrast, will lead to a reduced genetic diversity rendering the taxa more vulnerable to the potential accumulation of deleterious effects of inbreeding and diversity loss through drift. Depending on the quantity and quality of the remaining genetic diversity in these isolated populations a further lack of gene flow can either lead to extinction or an independent genetic development (Wright 1943), which can potentially lead to speciation.

Based on our knowledge of genetic processes and their resulting phylogeographic patterns (Avice 2000, Knowles and Maddison 2002) information on the current gene flow between individuals, populations and species therefore not only provides information on the current evolutionary status of a taxon under consideration (species delimitation) but might also provide information on the past and the future of the species or clade under study. Additional geographic, ecological, geological and palynological data might thereby

strengthen potential conclusions by reducing the number of potential inferences to be drawn from the same genetic patterns (Cruzan and Tempelton 2000, Werneck et al. 2011, Hardy et al. 2013).

More and more appreciation is given to the fact that processes of gene flow on all levels of differentiation (individuals, populations, species) interact and all together shape the evolutionary history of a given taxon and/or ecosystem (Cutter 2013, Parmentier et al. 2014). Thus data on breeding system, dispersal distance and spatial genetic structure might give additional hints for interpretation of phylogeographic pattern of a species (phylogeography) and the evolution of entire species clades (phylogeny). Consequently, “despite a number of challenges conferred by genomic information, the melding of phylogenetics, phylogeography and population genetics into integrative molecular evolution is poised to improve our understanding of biodiversity at all levels“ (Fig. 1.1, Cutter 2013).

In this thesis such a broad genetic approach across former disciplinary entities is conducted to investigate evolutionary patterns in herbs and climbers of the family Marantaceae in the understory of tropical African rainforests.

The specific questions of this thesis are:

- 1) what are the intraspecific genetic prerequisites of species from the rainforest understorey shaped by breeding system and dispersal,**
- 2) how can current species be delimited and is there interspecific gene flow, and finally**
- 3) how do intra- and interspecific gene flow determine spatial genetic patterns and what conclusions do these large-scale spatial patterns allow for on the history of the species and the tropical African rainforest.**

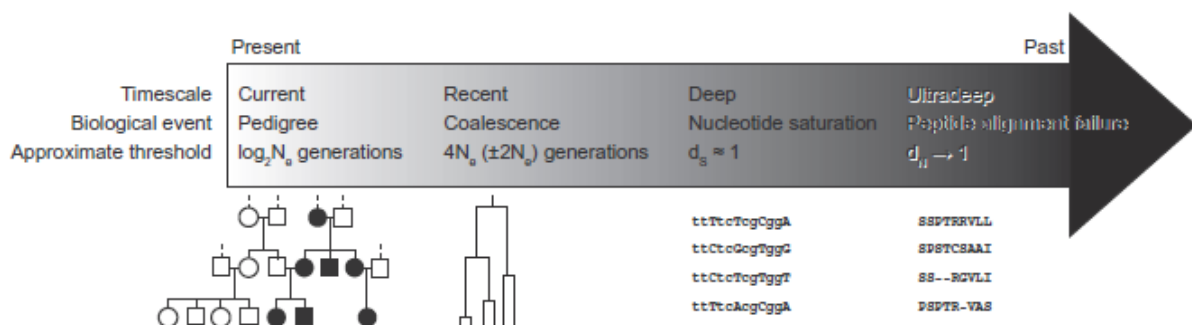


Fig. 1.1: The timescales of evolution. Evolution can be broken down into four broad timescales corresponding to key biological features associated with population genetic and molecular evolutionary processes. N_e = effective population size; d_s = number of synonymous-site substitutions per synonymous site; d_n = number of non-synonymous substitutions per non-synonymous site. Source: Cutter 2013.

2 The history of the tropical African rainforest and its understorey

Most of our planet's plant diversity is harbored in the tropics on a comparably small surface (Myers et al. 2000, Mutke and Barthlott 2005). Here the tropical rainforest organized in different strata ("Stockwerkbau") up to 80m in height (Richards 1954) is characterized by a very high spatial diversity (Hubbell 1997) with an often low individual density per species. How this diversity arose - the history of this vegetation - and how it is maintained is still to date a matter of debate and subject to considerable research (e.g. Plana 2004, Rull 2008, Hardy et al. 2013, Linder 2014).

Considering the African tropics, two major hypotheses of the origin of its high species diversity currently prevail and are tested interdisciplinary combining data from fields such as climatology, palynology, phylogeny and phylogeography:

Allopatric speciation during glacial cycles (Maley 1996) - refugia: Climatic conditions during glacial cycles oscillated between dry-cool and humid-warm conditions forcing rainforest plants during adverse dry-cool conditions to retract into areas of more suitable (stable) humid climatic conditions (= refugia) in swamps, gallery forests and mid-altitude montane regions (Dupont 2001). Through time (i.e. drift) and differential selection these now isolated populations in different refugia developed incompatibilities, eventually forming two species. Localities of refugia are suggested based on palaeo-ecological and floristic data (richness and endemism), especially of poorly dispersing or young taxonomic groups (Fig. 2.1, Maley 1996). Independent evidence for this hypothesis is now researched using phylogeographic data (Hardy et al. 2013, Heuertz et al. 2014, Ley et al. 2014).

Ecological differentiation (Smith 1997): Populations of the same species experience different selection pressures along an ecological gradient leading eventually to parapatric speciation. Thereby gradients could exist between different vegetation types e.g. forest to savanna (Smith 1997), or within the same vegetation type among different microhabitats or along large scale environmental (rainfall, seasonal, temperature etc.) clines (Fig. 2.2, 2.3). Support for this hypothesis was claimed in some zoological (Fieldsa 1994, Freedman et al. 2010) and botanical studies (Holstein and Renner 2011).

There is further evidence for a successional effect of both hypotheses on genetic traces of differentiation within the same species (Duminil et al. 2013) and a call for caution to prevent misinterpretation of phylogeographic data due to the multiple interactive

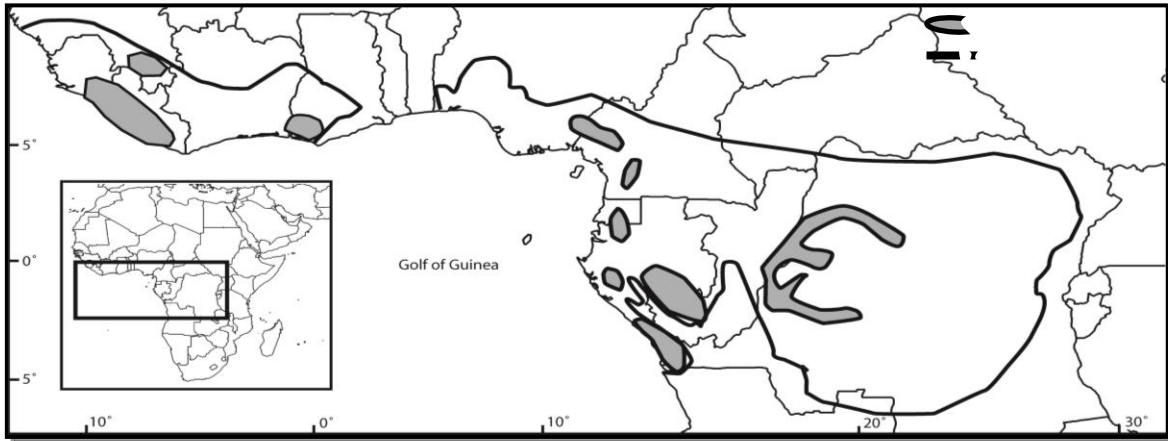


Fig. 2.1: Current extent of tropical rainforest (thick black line) and postulated Pleistocene rainforest refugia in tropical Africa (grey areas, after Maley 1996).

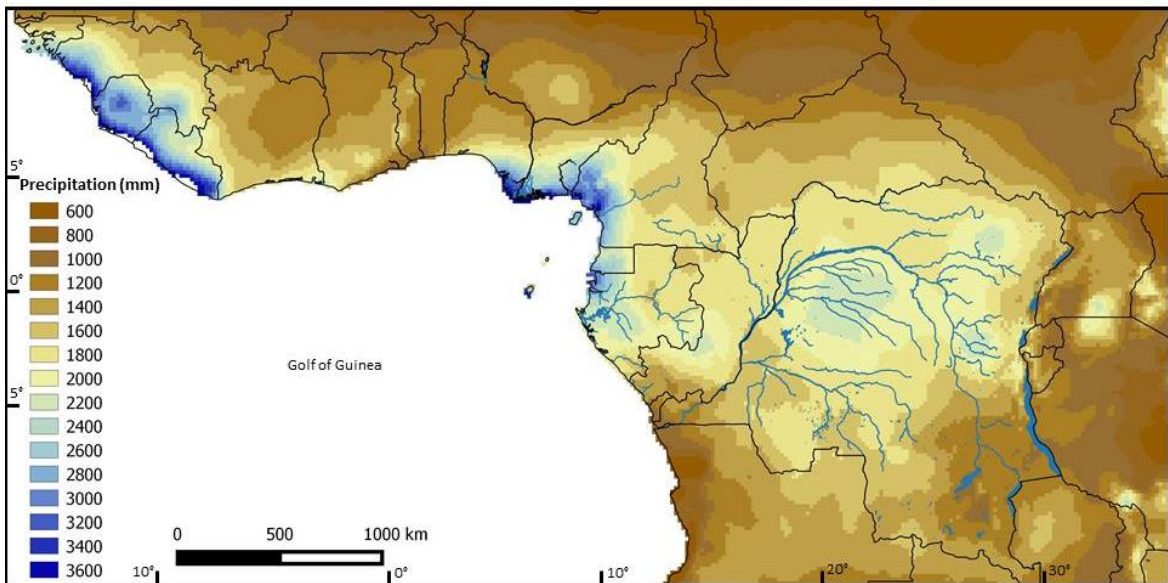


Fig. 2.2: Average annual precipitation in tropical Africa. Data Source: Bioclim: bio_12.

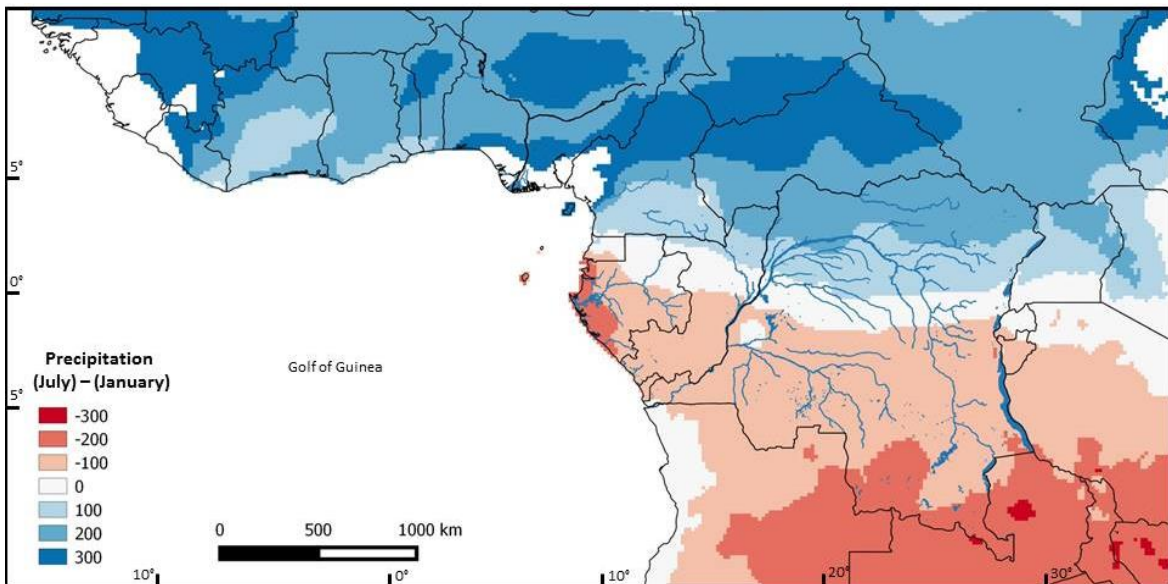


Fig. 2.3: Seasonal inversion: Precipitation July-January in tropical Africa. Data Source: Bioclim: prec_7-prec_1.

impacts of different processes on genetic differentiation and their similar resulting geographic patterns (Heuertz et al. 2013). Compilations of dated phylogenies for tropical regions suggest that repeatedly different events at different moments in time have promoted repeated flushes of speciation in those areas together having led to the high species diversity seen in those areas today (Plana 2004, Rull 2008).

Evidence in plants for the two before mentioned hypotheses has mainly been searched through studies on intra-specific gene flow mostly focused on economically important trees (Ude et al. 2006, Daïnou et al. 2010, Dick 2010, Allal et al. 2011, Sebbenn et al. 2012, Audigeos et al. 2013). Climbers, shrubs and herbs of the tropical understorey that differ remarkably in their life histories are rarely considered. However, the understorey contributes substantially to the overall forest diversity (Gentry and Dodson 1987, Poulsen and Balslev 1991, Poulsen 1996, Chittibabu and Parthasarathy 2000, Hubbell et al. 2005) and plays thus an important role in the maintenance of the ecological stability of this system (but see *Psychotria* in Loiselle et al. 1996, *Polypodiaceae* in Hooper and Haufler 1997, *Piper* in Lasso et al. 2009).

Large trees are generally out-crossed (Bawa 1979, Ward et al. 2005, Devy and Davidar 2006) and exhibit a low population density (Hubbell and Foster 1983, Bawa 1990) requiring long-distance pollen dispersal (Stacy et al. 1996, Lacerda et al. 2008) resulting in extensive gene flow. Further, also fruits of large trees are generally widely dispersed due to their large size and high production making foraging on these species energetically viable for large-bodied dispersers with large home ranges (Carbone et al. 1991).

In contrast, understorey herbs and shrubs generally produce smaller fruits. They are often self-compatible, exhibit mixed-mating breeding systems (Bawa 1990, Richards 1997) and form aggregated populations due to asexual spreading (Myers et al. 2000, Kinsman 1990, Sagers 1993). It is hypothesized that the combination of these traits in understorey plants might much more than in large trees facilitate the diversification of populations in response to local natural selection or genetic drift and may lead to different patterns of diversity (Lasso et al. 2011). Additionally, the shorter generation times and the asexual passing-on of somatic mutations to “offspring” from non-reproductive individuals (Ohta 1993, Kay et al. 2006) might accelerate the rate of molecular evolution in shrubs and herbs.

Climbers might be hypothesized to be somewhat intermediate between large trees on the one hand and herbs on the other hand. Climbers ascend to the forest canopy using other plants as support (Bongers et al. 2002). By flowering high up in the canopy, climbers

might take advantage of the same long-distance dispersing vectors as canopy trees, but their local population structure might be more similar to some clonal understorey herbs because their high rate of clonality and the fact that flowering individuals are mainly restricted to gaps and forest edges with favorable light conditions lead to a rather clumped spatial distribution (Terauchi 1990, Schnitzer et al. 2000, 2012, Nabe-Nielsen and Hall 2002, Ledo and Schnitzer 2014). Overall, clonality in climbers serves primarily to reduce competitors by impeding their recruitment (Penalosa 1982, Stevens 1987, Bongers et al. 2002, Doucet 2003). Thereby, climbers often show a particular vigorous clonal growth after environmental disturbance (i.e., an opening of the closed forest canopy), resulting in a higher climber diversity and abundance in treefall gaps than in non-gap areas (Schnitzer and Carson 2001, Dalling et al. 2012, Ledo and Schnitzer 2014). The mating system has rarely been documented in climbers, but both predominantly outcrossing and selfing has been recorded (see Terauchi 1990, Foster and Sork 1997, Meeus et al. 2011).



Fig. 2.4: Example of a Marantaceae dominated understorey of tropical African rainforest (here: *Sarcophrynium prionogonium*).

Thus I here set up to investigate gene flow in tropical forest understorey climbers and herbs (Fig. 2.4, 2.5) which are bound to the shady underneath of the much more investigated large canopy trees but are potentially characterised by very different evolutionary prerequisites. The challenge will be to reconcile the different findings from different life forms to a coherent picture of the evolutionary history of tropical African rainforest.

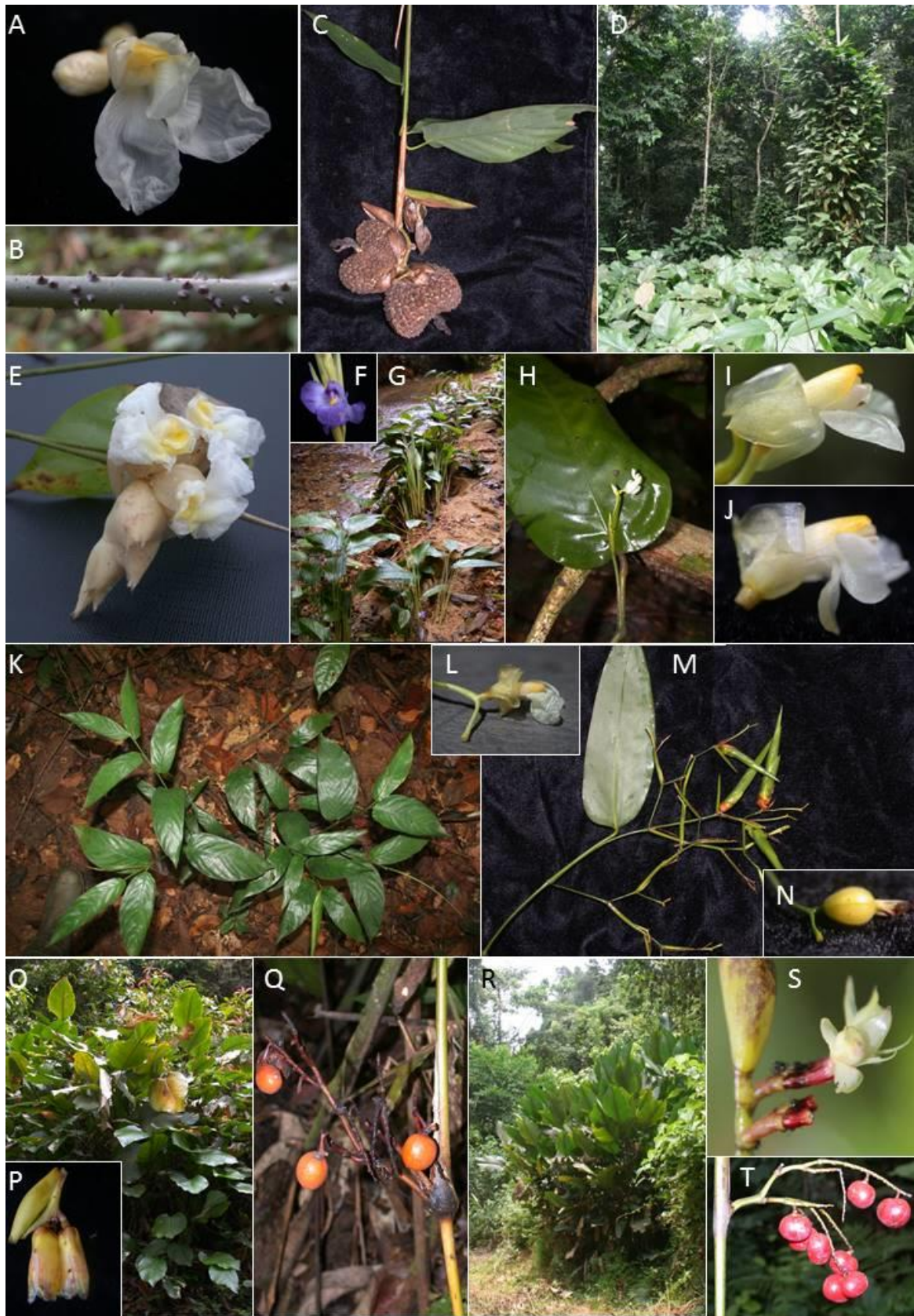


Fig. 2.5: Study species: *Haumania danckelmaniana* (A-D); *H. liebrechtsiana* (E); *Halopegia azurea* (F, G); *Marantochloa monophylla* (H, I); *M. incertifolia* (J); *M. congensis* (K-N); *Megaphrynium trichogynum* (O-Q); *Mega. macrostachyum* (R-T).

3 The studied plant family Marantaceae: taxonomy, biogeography, ecology and phylogeny

Relevant publication (see Appendix 1):

[1] Al-Gharaibeh, M.M., Borchsenius, F., McKechnie, L, Sanmartin, I, Ley A.C. (manuscript) Phylogeny of the pantropically distributed family Marantaceae.

As major research objects I chose taxa from the pantropically distributed plant family Marantaceae due to their well known taxonomy, perennial herb and liana growth form, presence in many different habitats of the tropical rainforest understory and the high diversity of ecological traits found among its species.

The Marantaceae Petersen (32 genera / 530 species, Zingiberales, Andersson 1998) are a pantropically distributed family of perennial herbs and lianas from the understory of tropical rainforests with about 40 species in Africa (Dhetchuvi 1996, Jongkind 2008, Ley 2008, Fig. 2.4, 2.5), 50 species in Asia (Suksathan 2009) and 450 species in America (Andersson 1998). New species are being described continuously (Dhetchuvi 1996, Suksathan and Borchsenius 2003, Braga 2008, Jongkind 2008, Kennedy 2012, 2014, Ley and Claßen-Bockhoff 2012).

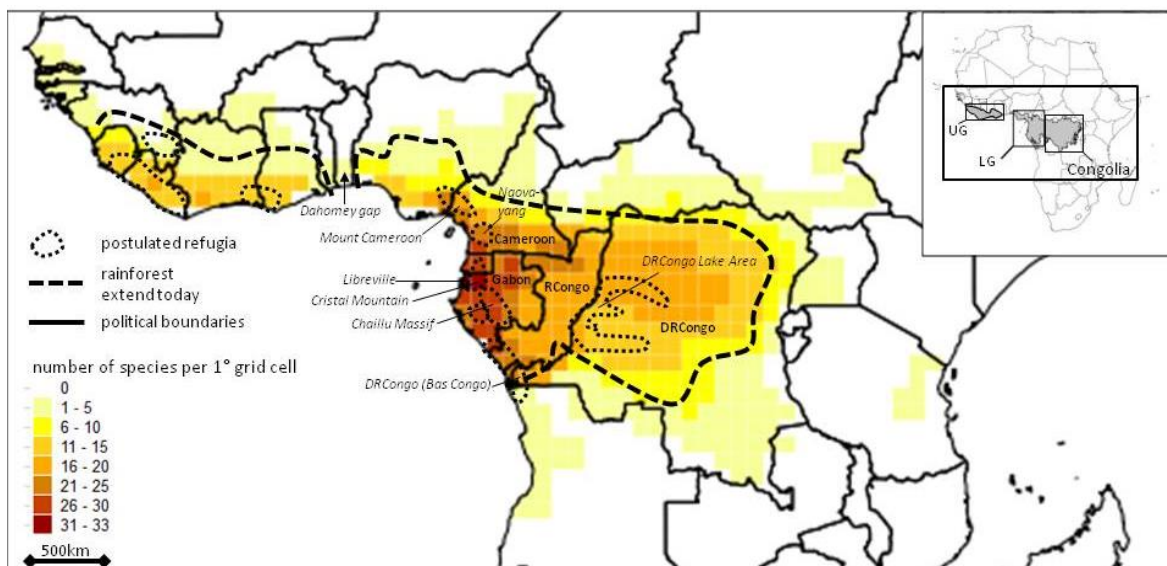


Fig. 3.1: Pattern of species diversity of Marantaceae in tropical Africa based on Schnell (1957), Dhetchuvi (1996), Jongkind (2008) and Ley and Claßen-Bockhoff (2012). Postulated refugia after (Maley 1996); DR Congo, Democratic Republic of Congo; R Congo, Republic of the Congo. Inset shows phytochoria after (White 1979): UG, Upper Guinea; LG, Lower Guinea; C, Congolia (Source: Ley et al. 2014).

In Africa the distribution pattern of the family closely follows the continuous distribution of lowland rainforest from Senegal to Kenya interrupted at the Dahomey gap (Fig. 3.1). One species is reported from Madagascar. The diversity center of the family in Africa lies in Gabon, Equatorial Guinea and Cameroon with a maximum of 33 species per 1° grid cell.

African Marantaceae show almost uniformly typical traits of forest understorey plants such as self-compatibility, clonal growth via rhizomes, adventive shoots and bulbils and animal pollination and dispersal (Ley 2008). They inhabit a diversity of different habitats from swamps, to river and creek banks, to the understorey of primary to secondary forest on terre firme (Dhetchuvi 1996) often forming a dominant layer in the rainforest understorey leading to the introduction of an own forest type (“Marantaceae forest”, Letouzey 1968). Often several species can be encountered in sympatry (pers. observ.).

Floral morphology and respective adaptation to different pollinators are manifold and show patterns of parallel evolution among clades (Ley and Claßen-Bockhoff 2010). Pollinator sharing between congeners is suggested by the finding of identical pollinators in sympatric species (Ley and Claßen-Bockhoff 2009). Further, there is a potential for a high rate of geitonogamy (Ley and Claßen-Bockhoff 2009) and thus increased selfing especially in large clones based on tested self-compatibility and observed pollinator behavior (Ley and Claßen-Bockhoff 2013). Likewise, fruit morphology is very diverse among species suggesting the adaptation to very different dispersal agents (for a summary see Ley 2008).

Phylogenetic analyses at the level of the order Zingiberales suggested a start of the diversification of the family about 64mya (Kress and Specht 2006). Currently, five major clades are recognized which are largely endemic to a single continent each (Prince and Kress 2006a). The African species fall into two different major phylogenetic clades and three isolated genera (Prince and Kress 2006b). The species level resolution of the two larger clades can be found in Ley and Claßen-Bockhoff (2011). The origin of the family is proposed to be in Africa (Prince and Kress 2006b).

However, still the backbone of the currently available phylogenetic tree is not yet resolved leaving the relationships among major clades as an open question. Furthermore, some of the major clades are still lacking a species-resolution. Dating is based on fossils solely from outside of the family Marantaceae rendering the relative ages of specific clades within the family rather imprecise (see Kress and Specht 2006). Thus the sister clades of the African clades and their respective ages are still vague as well as the resolution of the relationships within the African genus *Haumania*.

Therefore, we here conducted a new phylogenetic analysis including more taxa and genetic markers than ever before. The major questions of this study were 1) how are African clades related to each other and to clades from other continents and 2) what is the species-resolution of the genus *Haumania*.

The obtained results from Al-Gharaibeh et al. (in prep., [1]) are now providing a higher resolution and support for the backbone of the Marantaceae phylogeny in comparison to what was achieved in all previous studies. The addition of more molecular data and taxa than in previous studies strengthened the hypothesized topology and suggested generic limits of the relationships within the family Marantaceae (compare to Prince and Kress 2006a, Suksathan et al. 2009, Ley and Claßen-Bockhoff 2011, Borchsenius et al. 2012). Major new findings include highly supported infrasectional topologies of major clades and many subclades that were not achieved in any of the previous studies. Additionally, at least four potentially non-monophyletic genera were identified: *Calathea*, *Ischnosiphon*, *Maranta* and *Schumannianthus*. Only the genus *Haumania* was left with uncertain affinity within the Marantaceae family phylogeny.

The resolution within the African genus *Haumania* suggests a sister relationship of *H. danckelmaniana* from Cameroon and Gabon with *H. liebrechtsiana* from Gabon and the Congos together being sister to *H. leonardiana* from the North of the Democratic Republic of the Congo (Al-Gharaibeh et al. (in prep., [1])). This topology was already used in this thesis to specifically select sister species for analyses on their divergence (see e.g. Ley and Hardy 2010 [4]).

In the future we aim at further reinvestigating the biogeographical pattern and the timing of divergence within in the family Marantaceae using new biogeographical tools (e.g. LAGRANGE, Ree et al. 2013) and fossils within the family Marantaceae to test e.g. whether observed pattern of parallel evolution (see Ley and Claßen-Bockhoff 2010) also evolved at the same time and to which historical (geological, climatic, etc.) events the major diversification events of Marantaceae corellated. Of course sampling of species goes on to get closer to a final tree of the Marantaceae with a complete species sampling. Current major sampling shortcomings are in the highly diverse American genera *Goepertia* and *Calathea* with over 400 species (Borchsenius et al. 2012). Furthermore, it would be desirable to obtain more nuclear markers (biparentally inherited) to confirm the current tree topology as phylogenies based predominantly on markers from the maternally inherited chloroplast genome can sometimes be misleading (see Petit and Excoffier 2009, Ley and Hardy 2010 [4]).

4 Gene flow within species: breeding system, clonality and dispersal distance

Relevant publications (see Appendix 2-3):

- [2] Ley, A.C., Hardy, O.J. (2016a) Polymorphic microsatellite loci for *Haumania danckelmaniana* and transferability into *H. liebrechtsiana* (Marantaceae). *Applications in Plant Sciences* 4(3): apps.1500116. doi: 10.3732/apps.1500116
- [3] Ley, A.C., Hardy, O.J. (2016b) Spatially limited clonality and pollen and seed dispersal in a characteristic climber of Central African rain forests: *Haumania danckelmaniana* (Marantaceae). *Biotropica* 58(5) : 618-627.

Breeding system, reproduction and dispersal are important aspects of plant evolution determining a species ability to spread and migrate and thereby affecting the spatial genetic structure (SGS) and finally the long-term survival of a species. Frequent long-distance gene dispersal (i.e., high gene flow) will ensure relatively high genetic diversity within populations, which is important for the long-term survival of a species, including the preadaptation to a changing environment (Kremer et al. 2012). It may, however, prevent local adaptation and speciation and thereby evolutionary diversification (Ackerman and Ward 1999, Coyne and Orr 2004, but see also Smith et al. 2005). Restricted gene flow instead might allow for local adaptation and speciation (see Galen et al. 1991, Sambatti and Rice 2006, Akerman and Bürger 2014), but also carry along the risk of extinction due to an accumulation of deleterious alleles and/or the lack of appropriate variants preadapted to a changing environment (Couvét 2002). Very little is known of this from species of the tropical rainforest understorey.

Generally, gene dispersal is composed of pollen and seed dispersal whereby seed dispersal goes less far than pollen dispersal at least in the canopy (Dainou et al. 2010, Budde et al. 2013). And while large fruit crops in trees attract more large-bodied dispersers with larger home ranges, therefore increasing dispersal distances (Carbone et al. 1991, Haskell et al. 2002) it might be the contrary with the small fruits produced by many herbaceous understorey plants. Genetic studies in the subtropical understorey already indicated a less efficient long-distance dispersal in comparison to trees (Zeng et al. 2012) but the relative contribution of pollen and seed dispersal to overall gene dispersal in the tropical understorey is still unknown.

The aim of this study (Ley and Hardy 2016b [3]) was to characterize, for the first time, the breeding system and the spatial extent of clonality and gene dispersal of a

frequent Central African rain forest climber species: *Haumania danckelmaniana* (J. Br. & K. Schum.) Milne-Redh. (Marantaceae) (Fig. 2.5 A-D). To this end, the SGS of its populations was assessed at different scales using newly established microsatellite markers to: (1) quantify clonal diversity and growth at a very local scale; (2) assess the breeding system (outcrossing rate) from the population inbreeding coefficient; and (3) estimate indirectly the mean gene dispersal distance using isolation by distance model predictions.

Microsatellites (SSR – Simple Sequence Repeats) are suitable tools to assess intra-specific genetic variation for the determination of breeding system, clonal sizes and dispersal distances due to their high variability, codominance and evolutionary neutrality (Bruford et al. 1998). Formerly restricted to model organisms, next generation sequencing now allows the easy and cost-efficient primer establishment also for non-model organisms (Micheneau et al. 2011).

Here primers for eight polymorphic microsatellites with four to nine alleles per locus were established for the perennial climber *Haumania danckelmanniana* (Ley and Hardy 2016a [2]) using the 454 GS-FLX Titanium platform (454 Life Sciences, a Roche Company, Branford, Connecticut, USA) through a procedure that combined multiplex microsatellite enrichment and pyrosequencing (Malause et al. 2011, Micheneau et al. 2011). Positive transferability with the related species *H. liebrechtsiana* was observed for the same eight markers. Seven of these were used in the subsequent study of clonality, breeding system and gene dispersal distance in *Haumania danckelmanniana* sampled in southeastern Cameroon (Ley and Hardy 2016b [3]) as well as in two further studies on hybridisation (Ley and Hardy submitted [6]) and phylogeographic pattern (Ley et al. 2016 [8]).

Plants from the forest understorey are frequently reported to be self-compatible, exhibit clonal growth via rhizomes, adventive shoots and/or bulbils and are animal pollinated and dispersed (Bawa 1990, 1992, Sagers 1993, Myers et al. 2000). Strict autogamy has been discussed as a dead end in evolution (Stebbins 1957, Schemske and Lande 1985, Husband and Schemske 1996, Takebayashi and Morrell 2001) and only 20–25% of plant taxa are predominantly selfing (Barrett and Eckert 1990) as genetic diversity can only be ensured through out-crossing between individuals. This is thus the prevalent mechanism in the plant kingdom (Charlesworth and Charlesworth 1987) and many mechanisms have evolved to prevent selfing (Barrett 2002). A certain degree of selfing, however, has been hypothesised as advantageous as a mean of self-assurance in an ecosystem such as the tropical forest understorey of an overall low or variable pollination

frequency (Schoen et al. 1996, Barrett and Harder 1996, Anderson et al. 2003, Rathcke 2003, Ley and Claßen-Bockhoff 2009). Thus, the results of low inbreeding ($F_{IScorr} \leq 0.0001$) in *H. danckelmaniana* (Ley and Hardy 2016b [3]) fits into this picture suggesting predominant outcrossing and supporting earlier findings of self-incompatibility in this species (Ley and Claßen-Bockhoff 2013).

The evolutionary advantage of clonality - vegetative propagation without genetic changes - is said to lie in the establishment of a local population able to persist through times of adverse conditions where sexual reproduction is hindered by a temporal or spatial lack of suitable partners for reproduction (Haddadchi et al. 2014) and/or dispersal agents (Ehrlen and Lehtilä 2002, Honnay and Bossuyt 2005, Honnay et al. 2006, Silvertown 2008). The established clonality of *Haumania danckelmanniana* was found moderate (clonal extend: 15–25 m, clonal diversity 0.4–0.65; compare with Ellstrand and Roose 1987, Vandepitte et al. 2010, Schleuning et al. 2011) indicating the importance of recruitment from seeds at this locality (Ley and Hardy 2016b [3]).

Of course these results will still have to be verified under different disturbance regimes as this is known to be the main factor in determining clonal growth (Vandepitte et al. 2010, Schleuning et al. 2011). Here it would be of particular interest to compare *H. danckelmanniana* to its sister species *H. liebrechtsiana* to confirm personal observations of larger clones in this latter species and test the hypothesis that this higher clonality is correlated with an observed higher invasion potential in *H. liebrechtsiana* (Ley and Hardy 2010 [3]), Ley et al. 2016 [8]) - all of which seems to have profoundly influenced the evolutionary history of the genus and thus the current distribution patterns of the *Haumania* species in the Guinea-Congolian lowland rainforest (see Ley et al. 2016 [8]).

Furhter, a very limited gene dispersal distance was detected in *H. danckelmanniana* ($r_g = 9\text{--}50$ m, neighborhood sizes $N_b = 23\text{--}67$; Ley and Hardy 2016b [3]). This is in accordance with the large, heavy and inconspicuous fruits of *H. danckelmanniana* which are probably only narrowly gravity or rodent dispersed (Ley 2008). It is, however, in disaccordance with its long distance pollinators (Ley and Claßen-Bockhoff 2009). Here we assume that population density has a non-negligible part to play in determining gene dispersal distance restricting pollen transfer distance in densely flowering populations (Ley and Claßen-Bockhoff 2009). As the pollinating large carpenter bees in *Haumania* are known as long distance dispersers (Pasquet et al. 2008) their specific behavior, i.e. their search rules, foraging constancy and foraging range (optimal foraging theory, Pyke 1984, Karron et al. 1995, Degen and Roubik 2004, Jha and Dick 2010) in those massiv flowering

patches might have an important impact. In tropical trees highly variable pollinator ranges are recorded with sometimes considerable gene flow due to insects flying long distances (in the range of 100 m to 14 km see Ward et al. 2005, Hamrick and Murawski 1990, Stacy et al. 1996, Dick and Heuertz 2008). Flight distances of small bees of the tropical understorey are still unknown (but see Bawa 1990) but are hypothesized to be much smaller due to the dense understorey structure.

With its narrow dispersal distance *H. danckelmanniana* is an ideal candidate for phylogeographic analyses (Ley and Hardy 2010 [4], Ley et al. 2016 [8]) as its spatial genetic pattern will be more pronounced and need more generation for its deletion (Maley 1996). Indeed the SGS was marked ($S_p = 0.011\text{--}0.026$) but still lower than in herbs (Vekemans and Hardy 2004, Lasso et al. 2011) and more similar to that reported in tropical trees (Dick et al. 2008). This might be due to the combination of its high outcrossing rate, limited clonality and the limited pollen and seed dispersal being partly reversed by limited drift due to a high population density. The SGS might increase with augmented clonality as in many herbs, especially under more severe disturbance regimes (see Vandepitte et al. 2010, Schleuning et al. 2011).

This is, to our knowledge, only the second study on clonality and gene flow in a tropical African climber (see Foster and Sork 1997) with several genetic measures for a climber reported here for the first time which will be considered when interpreting large scale phylogeographic pattern in this species (see later chapters).

Next it would be interesting to compare the here obtained data to herbal rainforest understorey species and investigate their breeding system, reproduction (clonality) and dispersal distance. In contrast to the current finding of predominant outcrossing in the climber from this study (Ley and Hardy 2016b [3]) it is actually expected to find a lot more selfing. For the species of perennial herbs within the Marantaceae e.g. self-compatibility has already been proven in hand pollination experiments (Ley and Claßen-Bockhoff 2013). These herb species further show a lower floral density and a much lower pollinator frequency. It would be interesting to see which influence this has on the species' SGS given the rather small pollinators visiting those species and the dense structure of the rainforest understorey. Potentially the importance of selfing might increase as means of self-assurance as already hypothesized elsewhere (Barrett and Harder 1996, Schoen et al. 1996, Anderson et al. 2003, Rathcke 2003).

5 Species delimitation and gene flow across species boundaries

Relevant publications (see Appendix 4-7):

- [4] Ley, A.C., Hardy, O.J. (2010) Species delimitation in the Central African herbs *Haumania* (Marantaceae) using georeferenced nuclear and chloroplastic DNA sequences. *Molecular Phylogenetics and Evolution* 57: 859–867.
- [5] Ley, A.C., Hardy, O.J. (2013) Improving AFLP analysis of large-scale patterns of genetic variation - a case study with the Central African lianas *Haumania* spp. (Marantaceae) showing interspecific gene flow. *Molecular Ecology* 22(7): 1984–1997.
- [6] Ley, A.C., Hardy, O.J. (submitted) Hybridization and asymmetric introgression after secondary contact in two tropical African climber species *Haumania danckelmaniana* and *H. liebrechtsiana* (Marantaceae). *International Journal of Plant Sciences*.
- [7] Ley, A.C., Hardy, O.J. (2014) Contrasting patterns of gene flow between sister plant species in the understorey of African moist forests - the case of sympatric and parapatric Marantaceae species. *Molecular Phylogenetics and Evolution* 77: 264–274.

The species is the fundamental unit of biological classification important for any kind of communication among politicians to evolutionists (Agapow et al. 2004). The first classifications were based on simple morphological observations but soon new and more complex concepts of species arose by adding more and more featuring characteristics from ecology to reproduction and genetics (reviewed by Mayden 1997, Harrison 1998, deQueiroz 1999). These concepts are often incompatible in their definitions and thus different in their results (see e.g. Duminil and Di Michele 2009).

The species concept - the theoretical concept of the species - is closely tied to species delimitation, i.e. how to determine the boundaries and numbers of species from empirical data (deQueiroz 2007). Many different methods are proposed here by now (Carstens et al. 2013). One of them is the DNA barcoding initiative trying to establish a universal standard of genetic species identification using a short genetic marker in an organism's DNA (Hebert et al. 2003, CBOL Plant Working Group, 2009).

A major challenge in genetic species delimitation is the ongoing nature of evolution – far from reaching a final steady state of total species resolution but presenting ongoing varying degrees of gene flow within and between populations and species (Cutter 2013). Thus, species delimitation remains a difficult task to date in many groups of organisms

(Ellis et al. 2006), notably in species rich ecosystems like tropical rainforests (Lee 2003, Dayrat 2005, Duminil et al. 2012).

Frequently, interspecific hybridization is observed between closely related plant species with overlapping distribution ranges, especially in zones of secondary contact after isolation in different refugia (Rieseberg 1999, Marchelli and Gallo 2001, Haselhorst and Buerkle 2011). Although well studied in temperate regions (Petit et al. 2002, Fussi et al. 2010), interspecific hybridization is little documented in tropical regions while it may be frequent in these high species diversity areas.

Here we conducted several studies on species delimitation and the degree of sister species differentiation in the Central African rainforest understorey using phylogenetic and phylogeographic approaches and three different marker systems (chloroplast and nuclear DNA sequences, AFLP, microsatellites) covering chloroplast and nuclear DNA.

We started by determining the degree of speciation in the two closely related climber taxa from the tropical African genus *Haumania* J. Leonard (Ley and Hardy 2010 [4]). The two species *H. danckelmaniana* (J. Br & K. Schum.) Milne-Redh. and *H. liebrechtsiana* (De Wild. & Th. Dur.) J. Leonard display distinct vegetative traits (Ley and Hardy 2010 [4], Ley and Hardy submitted [6] – Fig.1) despite a high similarity in reproductive traits (Ley and Claßen-Bockhoff 2009) and a partial overlap in distribution area (Dhetchuvi 1996) which might facilitate gene flow.

Phylogenetic and phylogeographic analyses were combined using 54 nuclear (nr) and 200 chloroplast (cp) DNA sequences in comparison to morphological species descriptions (Ley and Hardy 2010 [4]). The nuclear dataset unambiguously supported the morphological species concept in *Haumania* (Ley and Hardy 2010 [4] – Fig.2). However, the main chloroplastic haplotypes were shared between species (Ley and Hardy 2010 [4] – Fig.3) and, although a geographic analysis of cpDNA diversity (kinship distance curve, Hardy and Vekemans 2002) confirmed that individuals from the same taxon were more related than individuals from distinct taxa, cp-haplotypes displayed correlated geographic distributions between species (Ley and Hardy 2010 [4] – Fig.4). Hybridization was the most plausible reason for this pattern (see Lexer et al. 2006, Acosta and Premoli 2010) supported by the partial overlap in the distribution range between the two taxa, ecological data showing high similarities in habitat preference, flowering time and probably pollinators (Dhetchuvi 1996, Ley 2008, Ley and Claßen-Bockhoff 2009).

The study Ley and Hardy 2010 [4] highlighted the gain of information on the speciation process in *Haumania* by adding georeferenced molecular data to the

morphological characteristics. It also showed that nr and cp sequence data might provide different but complementary information, questioning the reliability of the unique use of chloroplast data for species recognition by DNA barcoding (CBoL Plant Working Group 2009) highly recommending the use of nuclear markers for species delimitation (see also Acosta and Premoli 2010).

To address the lack of coverage of the nuclear genome in the former study (Ley and Hardy 2010 [4]) we conducted a second study (Ley and Hardy 2013 [5]) in which amplified fragment length polymorphisms (AFLP) were used. This improved the study of patterns of population genetic variation and gene flow (Gaudeul et al. 2004, Schönswetter et al. 2004, 2008, Schorr 2009, Oberprieler et al. 2010). AFLP are a valuable addition to cpDNA sequences as they provide a large number of mostly nuclear markers, they can be obtained at a relatively low cost in any taxon without prior genomic knowledge, and recent advances in laboratory methods have become increasingly reliable in producing reproducible AFLP patterns (DeHaan et al. 2003; Odiwuor et al. 2011). Thus, AFLPs have already repeatedly been used to address questions on species history (Gaudeul et al. 2004; Schönswetter et al. 2004, 2008; Schorr 2009) and to identify hybridization events (Oberprieler et al. 2010).

However, the reliability of AFLP scoring is still critical (O'Hanlon and Peakall 2000, Vekemans et al. 2002, Gort et al. 2006, Meudt and Clarke 2007, Bonin et al. 2007). Therefore, first a novel approach based on quantitative genetics to assess band reproducibility and account for it when estimating genetic relatedness was developed (Ley and Hardy 2010 [5] – Fig. 1). This method can be applied after the use of an automated method for AFLP scoring (tinyFLP, Arthofer 2010).

We thus developed 123 new AFLP markers based on two primer pairs that amplified reliably and showed some degree of polymorphism in the pretests (EcoRI AAC - MseI CAC, EcoRI AAC - MseI CAG) and submitted it to the novel approach to select reliable bands from their degree of reproducibility (Ley and Hardy 2013 [5]). Applying Bayesian clustering to the so established AFLP dataset confirmed the recognition of the two *Haumania* species obtained by the single-locus nrDNA (5S) sequence data before (see Ley and Hardy 2010 [4] – Fig.2). These nrDNA (5S) data were, however, incongruent with the results of the cpDNA data set in assigning a given individual to one or the other species and the AFLP data showed a continuous gradient of assignments of individuals to one or the other species (Ley and Hardy 2013 [5] – Fig. 3). Further supported by the decay of the relatedness between individuals of distinct species with geographic distance this

demonstrated that hybridization occurred between the two species and affected also the nuclear genome (see also Hardy and Vekemans 2001, Palma-Silva et al. 2011). This study thus underlined the importance of including the nuclear genome into the interpretation of species history as cpDNA data can occasionally be misleading due to its solely maternal inheritance (see also Duminil et al. 2012). Although the AFLP markers might have been substantially affected by nongenetic factors, their analysis using the new methods developed considerably advanced our understanding of the pattern of gene flow in the two model species.

Next we tried to quantify the degree of hybridization in more detail (Ley and Hardy (submitted) [6]). We concentrated on the area of distributional overlap and included for the first time morphological intermediates.

265 individuals from nine populations in Northern Gabon were investigated using seven microsatellites (from Ley and Hardy 2016a [2]) and the chloroplast (cp) sequence *trnC-petN1r* (from Ley and Hardy 2010 [4]). 13 % and 41 % of intermediates were found based on morphological versus genetic data, respectively, (Ley and Hardy (submitted) [6]) indicating only a partial congruence of both datasets supporting the assumption that intermediate morphological characters are not mandatory for hybridization (see also Riesenbergs 1995). There was an equal amount of backcrosses to both parental species (15 %). Genetic diversity within each species was highest in populations in the center of the study area where both species co-occurred, corroborating the positive effect of hybridization on intraspecific diversity (Andersson 1948, Soltis and Soltis 1999, Shimizu-Inatsugi et al. 2009, Symonds et al. 2010). Intermixed populations showed higher distinction from populations of pure *H. danckelmaniana* than from pure *H. liebrechtsiana*. A comparison at local scale of SSR and cpDNA sequence data revealed a unidirectional introgression from *H. liebrechtsiana* into *H. danckelmaniana*, with *H. danckelmaniana* acting predominantly as mother and *H. liebrechtsiana* as father (=pollen donor) (see also Currat et al. 2008, Excoffier et al. 2009, Li et al. 2015). This is in line with the theoretical expectation of chloroplast capture in the case of an invading species (Petit et al. 2004, Tsitrone et al. 2003, here: *H. liebrechtsiana*) into the range of a local species (here: *H. danckelmaniana*) and the proposed phylogeographic scenario for the genus *Haumania* (described next in Ley et al. 2016 [8]).

We next compared the pattern of gene flow between other sister species with different pattern of distributional overlap (Ley and Hardy 2014 [7]) - here among three species from the herbaceous genus *Marantochloa* Brongn. & Gris. (Marantaceae) (Fig.

2.5). These species are sympatrically distributed in the understorey of the Central tropical African rainforest (Dhetchuvi 1996). Data from the chloroplast and nuclear genomes (DNA sequences and AFLP) were used and compared to the existing data on species delimitation and gene flow in the two parapatric climber sister species from the genus *Haumania* J. Leonard (Ley and Hardy, 2010, 2013).

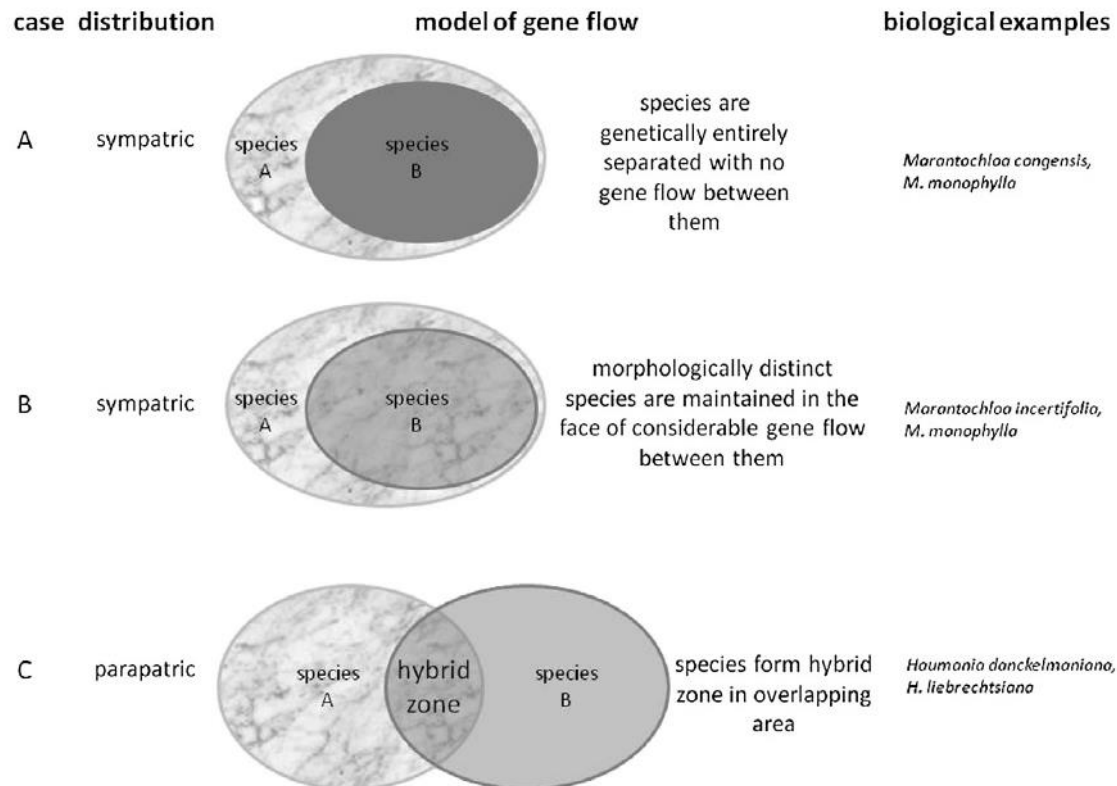


Fig. 5.1: Comparison of the degree of geographic range overlap and gene flow of species pairs. (A) total range overlap with inter-specific incompatibility, (B) total range overlap with considerable inter-specific gene flow in the plastid and nuclear genomes, (C) partial overlap with the formation of a hybrid zone.

Highly contrasting patterns were found (Ley and Hardy 2014 [7] - Fig. 3): while there was no evidence of gene flow between *M. congensis* and *M. monophylla*, species identity between *M. monophylla* and *M. incertifolia* was maintained despite considerable gene flow. We hypothesize that *M. incertifolia* originated from an ancient hybridization event between *M. congensis* and *M. monophylla*, considering the current absence of hybridization between the two assumed parent species, the rare presence of shared haplotypes between all three species and the high percentage of haplotypes shared by *M. incertifolia* with each of the two parent species. Hybridisation among the three species might be facilitated by the overlap in distributional ranges and their high similarities in habitat preference, flowering time and probably pollinators (Dhetchuvi 1996, Ley 2008,

Ley and Claßen-Bockhoff 2009). The current absence of gene flow between *M. congensis* and *M. monophylla* might be based on reinforcement after a relatively ancient event of speciation and long re-contact and sympatry (Butlin 1987, Ortiz-Barrientos et al. 2009).

Thus the observed pattern in *Marantochloa* were in contrast to those found in the two parapatrically distributed species from the genus *Haumania* (Fig. 5.1) forming a hybrid zone restricted to the area of overlap (Ley and Hardy 2010 [4], Ley et al. 2016 [8]). Hybridization in *Haumania* is typical for a recent re-contact of two once allopatric species that are still compatible and can form hybrids in a secondary contact zone (compare to Petit et al. 2002). In the case of *Haumania*, the two species do not seem to have developed prezygotic barriers (see also Coyne and Orr 2004, Wu and Ting 2004) such as observed between *Marantochloa congensis* and *M. monophylla*, and their hybridization has not resulted in the formation of a new species as hypothesized for *M. incertifolia* (Ley and Hardy 2014 [7]). Further, interspecific gene flow in *Haumania* is much more intense for the chloroplast than for the nuclear genome, leading to chloroplast captures (Ley and Hardy 2010 [4], Ley et al. 2016 [8]), whereas gene flow between *M. monophylla* and *M. incertifolia* involved both genomes alike (Ley and Hardy 2014 [7]).

This work (Ley and Hardy 2014 [7]) thus illustrates the diversity of speciation/introgression patterns that can potentially be encountered in the flora of tropical Africa. Further examples of sister species comparisons and their degree of speciation between can be found in Ley et al. 2014 [9]. In the future it would be interesting to illuminate more in detail the relation of genetic differentiation to phylogenetic distance between species, and the degree of overlap in distributional range and ecological niche.

The key conclusions from these four studies on species delimitation and inter-specific gene flow are:

- The reliability of the unique use of chloroplast data for species recognition by DNA barcoding is questioned. Instead the use of nuclear markers for species delimitation is highly recommended (Ley and Hardy 2010 [4]).
- A novel approach for AFLP analyses after automated AFLP scoring was developed based on quantitative genetics to assess band reproducibility and account for it when estimating genetic relatedness (Ley and Hardy 2013 [5]).
- A case of unidirectional introgression was detected in line with hybridization after secondary contact which might be common in Central Africa as the forest flora in

this area has undergone repeated cycles of fragmentation and expansion following past climate changes (Ley and Hardy submitted [6]).

- Different levels of inter-specific differentiation and hybridisation were detected illustrating the diversity of speciation/introgression patterns that can potentially occur in the flora of tropical Africa (Ley and Hardy 2014 [7]).

Based on these findings of frequent hybridization in Central tropical Africa, it is highly recommended for any future genetic study to always include 1) chloroplast and nuclear markers to cover the full genetic information and uncover potential events of hybridization and 2) to include sister species to ensure that detected spatial genetic patterns are not due to hybridisation which would totally change the interpretations of results (see also Ley et al. 2014 [9]).

In the future it would be worthwhile exploring further the different processes that might have led to one or the other degree of species differentiation. As outlined in chapter 2, different mechanisms of speciation are already suggested for tropical Central Africa such as genetic differentiation in allopatry due to climatic cycles (Maley 1996) and/or ecological niche separation along ecological gradients (Smith 1997). Each of them might, however, be accompanied by many additional factors of specific species life-history traits and genetic processes that merit further research. Tools to differentiate between those two major hypotheses are already out there and have been applied to some tropical species complexes (see based on phylogenetic data: Couvreur et al. 2011). However, still the interaction of both processes in the diversification of the tropical African biota should not be underestimated (Smith et al. 2005; see also Mayol et al. 2015).

One such example that might merit further investigation is the differentiation of the here investigated species of the genus *Haumania*. Here allopatric differentiation might have been coupled with ecological differentiation allowing *H. liebrechtsiana* rather than *H. danckelmaniana* to extend its current range and invade the respective other species. More finescale ecological analysis within the secondary contact zone might allow detecting whether hybridisation is correlated with different ecological traits and habitat preferences shaping the invasion by *H. liebrechtsiana* (see Abbott and Brenan 2014, Curry 2015). Here the ecological background of invasion/invasibility would have to be illuminated in more detail. Details on ecological differences between *H. danckelmaniana* and *H. liebrechtsiana* are still unknown.

6 Phylogeographic pattern and concordance with the refuge hypothesis

Relevant publications (see Appendix 8-9):

- [8] Ley, A.C., Heuertz, M., Hardy, O.J. (2016) The evolutionary history of central African rain forest plants: phylogeographic insights from sister species of the climber genus *Haumania* (Marantaceae). *Journal of Biogeography*. doi:10.1111/jbi.12902
- [9] Ley, A.C., Dauby, G., Köhler, J., Wypior, C., Röser, M., Hardy, O.J. (2014) Comparative phylogeography of eight herbs and lianas (Marantaceae) in central African rainforests. *Frontiers in Genetics* 5: article 403. doi: 10.3389/fgene.2014.00403

The origin of the high species diversity in the African lowland rain forest, a diversity hotspot within this continent (Barthlott et al. 2005), is still a matter of debate. The current lowland tropical rain forest in Africa covers two large blocks, from Sierra Leone to western Ghana (Upper Guinea), and from southern Nigeria to the eastern Democratic Republic of the Congo (Lower Guinea and Congolia) being divided into three distinct regions (phytogeographic domains) based on species composition (White 1979). The changing climatic conditions following glacial cycles, i.e. cycles of cooling and drying in tropical Africa (e.g. 10 to 70 Kyr bp, 130 to 190 Kyr bp for the last and penultimate glacial periods, respectively, Petit et al. 1999), have been proposed as an important factor shaping current species distributions in this region. It is hypothesised that these glacial cycles forced rain forest plants to retreat into localized refuge areas of suitable climate, thereby isolating populations, which potentially led to allopatric speciation, essentially during the Pleistocene (Maley 1996). Palynological data document cyclic vegetation changes from forest to grasslands and vice versa during the Quaternary (Dupont et al. 2000, see also Alexandre et al. 1998). However, palynological data are rare and unevenly distributed in tropical Africa (Bonnefille 2007). The locations of postulated refugia during the Pleistocene have thus been proposed essentially from current centres of species diversity and endemism (e.g. Maley 1996, Robbrecht 1996, Sosef 1996, Colyn et al. 1991, see Fig. 1.2). However, this might confound the impact of past forest fragmentation and environmental effects on speciation events (Dauby et al. 2014). To shed more light on the refuge hypothesis and its impact on the phytogeographic domains of tropical Africa, it is desirable to gain further independent evidence, notably from phylogeographic patterns (Hardy et al. 2013, Dauby et al. 2014, Heuertz et al. 2014).

Phylogeographic studies on tropical African trees already showed a partial concordance of geographic patterns of genetic diversity and allelic endemism with postulated Pleistocene refuge areas (e.g., in genera: *Milicia*, Dainou et al. 2010; *Santiria*, Koffi et al. 2011; *Symphonia*, Budde et al. 2013; *Erythrophleum*, Duminil et al. 2013; multispecies patterns in: Hardy et al. 2013, Dauby et al. 2014, Heuertz et al. 2014) suggesting that climatic oscillations have indeed influenced plant species in the hypothesised way. However, these studies dealt only with trees and were often restricted to Lower Guinea (excluding Congolia) or concentrated on the more obvious differentiation between West and Central Africa (or Upper and Lower Guinea, see Plana 2004, Koffi et al. 2011, Cubry et al. 2013, Duminil et al. 2013). Further, to demonstrate the importance of refugia in genetic divergence and speciation, one would have to investigate in-depth the history of phylogenetically close taxa from different refugia.

Here we aim to test the refuge hypothesis in forest understorey plants (herbs and climbers) as a major driver of genetic differentiation to explain the development of distinct phylogeographic domains in the tropical central African rain forest. To address this aim the phylogeographic pattern in several pairs of plant sister species from the family Marantaceae were studied and compared.

A first study concentrated on the sister species *Haumania danckelmaniana* and *H. liebrechtsiana* (Marantaceae) (Ley et al. 2016 [8]). Seven polymorphic microsatellite loci (from Ley and Hardy 2016a [2]) were genotyped in a total of 513 individuals from both species trying to cover the entire distribution area of each species. The microsatellite data supported the recognition of the two morphological species, *H. danckelmaniana* and *H. liebrechtsiana*, as formerly found based on pDNA and AFLP data (Ley and Hardy 2010 [4], 2013 [5]). Using population genetic structure analyses three distinct homogeneous genetic clusters were detected in *H. danckelmaniana* and two in *H. liebrechtsiana*. These clusters corresponded geographically with the patterns found in the plastid DNA sequences (see Ley and Hardy 2010 [4]). Genetic diversity and endemism was unevenly distributed between clusters and in both datasets (pDNA and microsatellites) most clusters exhibited signals of population expansion.

These results indicate a complex history involving allopatric differentiation with each species originating in a different phylogeographic domain - *H. danckelmaniana* originating in Lower Guinea and *H. liebrechtsiana* in the large swamps close to the Congo River in Congolia – divided by a dry corridor which has been and partly still is savanna dominated (Maley 1996, Anhuf et al. 2006). These observed patterns were thus globally

consistent with the refuge hypothesis suggesting that the allopatric division potentially resulted from past forest fragmentation (Maley 1996) and was followed by population expansion which probably occurred during a phase of forest expansion. Most areas proposed as refugia are characterised by a pronounced topographical structure in the case of *H. danckelmaniana* or azonal water in the case of *H. liebrechtsiana* in the Congo Basin swamps that might have provided a suitable microclimate for forest patches where individuals of both species could survive in periods of adverse climate conditions (Leal 2001). In addition to the so far postulated refugia in Lower Guinea the analyses of *H. danckelmaniana* suggests the retraction of the species to even more areas, i.e. SECameroon/NRCongo and NGabon (Ley et al. 2016 [8]). These areas might be recognized as additional refugia as already suggested for several other plant groups (see also Robbrecht 1996, Sosef 1996, Laraque et al. 1998, Budde et al. 2013).

From here the species expanded into their current distribution range at times of favourable climate conditions during warmer and more humid periods following the expansion movements of larger rain forest trees for which equivalent hypotheses of range expansion exist (Hardy et al. 2013, Dauby et al. 2014). Inter-specific introgression - concordant across genetic datasets (Ley and Hardy 2010 [4], 2013 [5], submitted [6]) – was detected probably as a consequence of either a short isolation time and/or a slow genetic divergence, which did not yet lead to incompatibility between the two species (see also Holstein and Renner 2011).

The presented scenario (Ley et al. 2016 [8] – Fig.2) illustrates evolutionary processes that likely have contributed to shaping the African phytogeographic domains. As the forest flora of Central Africa has undergone repeated cycles of fragmentation and expansion following past climate changes (Dupont et al. 2000), we expect that similar scenarios of hybridization after secondary contact may be common.

Thus in a second phylogeographic study to get a bigger picture we added more sister species pairs to our phylogeographic analyses (Ley et al. 2014 [9]). Here the phylogeographic patterns (chloroplast-DNA sequences) within and between eight (sister)species of widespread rainforest herbs and climbers from four genera of Marantaceae (*Halopegia* K. Schum., *Haumania* J. Leonard, *Marantochloa* Brongn. & Gris, *Megaphrynium* Milne-Redh., Fig. 2.5) were investigated, searching for congruent patterns across species that might have been driven by a common vegetation history, and congruence of these patterns with postulated rainforest refugia that might support the importance of these areas for species survival and population differentiation.

Using 1146 plastid DNA sequences (*trnC-petN1r*) sampled across African tropical lowland rainforest, particularly in the Lower Guinean (LG) phytogeographic region, intra- and interspecific patterns of genetic diversity, endemism and distinctiveness were analysed. Intraspecific patterns of haplotype diversity were concordant among most species as well as with the species-level diversity pattern of Marantaceae (Fig. 6.1; Ley et al. 2014 [9] - Fig.4). Highest values were found in the hilly areas of Cameroon and Gabon. The level of genetic diversity at the plastid gene sequenced (7–19 haplotypes per species, see also nucleotide diversity) was similar to that found for the same plastid marker in tree species from Lower Guinea (6–24 haplotypes per species, Dauby et al. 2014). Equally, genetic differentiation between areas (G_{ST}) were comparable to values found in maternally inherited markers in many other angiosperm taxa, including tropical African trees (see Duminil et al. 2007, Dauby et al. 2014). This is in contrast to our expectation of more sub-structuring in (perennial) herbs/climbers than in trees and may indicate rather similar dispersal and population structure in both growth form groups. Within the Marantaceae, G_{ST} values seemed to correlate superficially with dispersal ability (see also Petit et al. 2003).

The spatial distribution of endemic haplotypes, an indicator for refuge areas in general, however, was not congruent across species Ley et al. 2014 [9]. Each proposed refuge (see Maley 1996) exhibited high values of endemism for one or a few species indicating their potential role as area of retraction for the respective species only. Thus, evolutionary histories seem to be diverse across species. In fact, areas of high diversity might have been both refuge and/or crossing zone of recolonization routes i.e., secondary contact zone. We hypothesize that retraction of species into one or the other refuge happened by chance depending on the species' distribution range at the time of climate deterioration. The idiosyncratic patterns found in Marantaceae species are similar to those found among tropical tree species, especially in southern Lower Guinea (Hardy et al. 2013, Dauby et al. 2014, Heuertz et al. 2014).

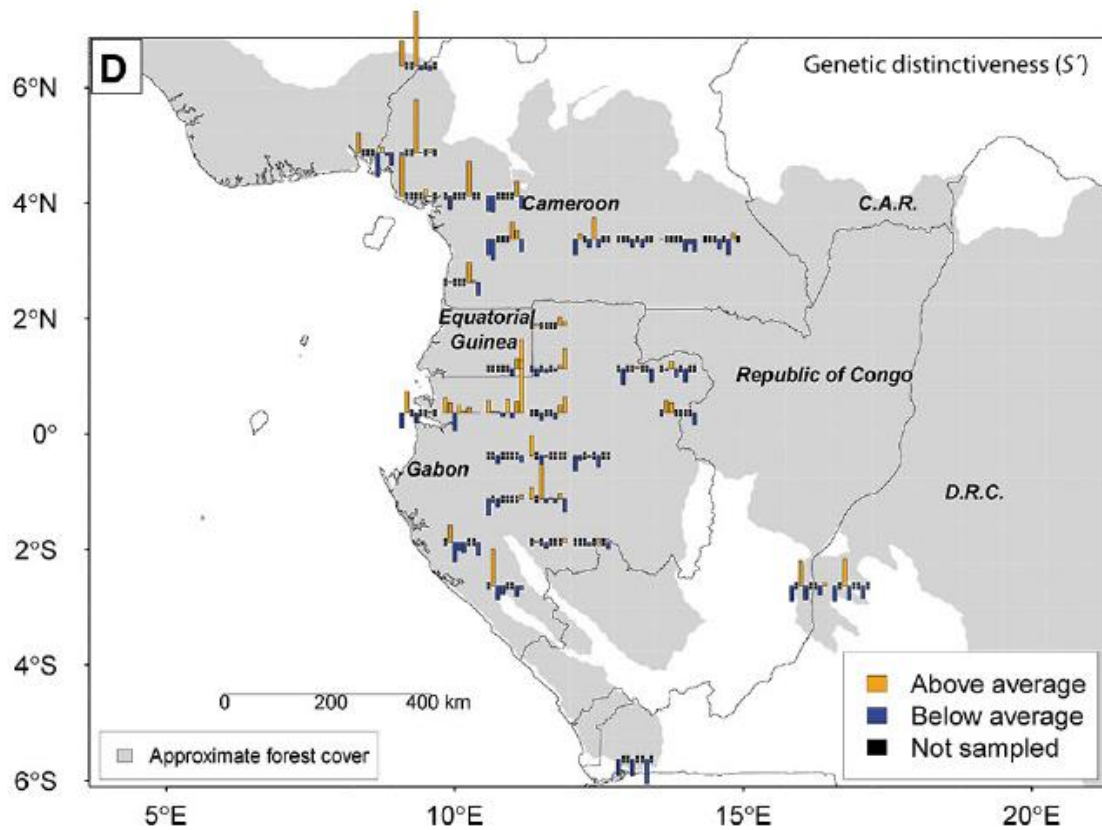


Fig. 6.1: Geographic distribution of standardized (i.e., centered and reduced) genetic diversity and endemism for eight Marantaceae species in Lower Guinea for grid cell size 0.75° . Genetic distinctiveness of each grid cell (S^i). Distinctiveness above or below average is based on standardized pairwise genetic distance (S^{kij} computed for each species) among populations where genetic distance is estimated as the number of mutational steps between two individuals drawn from two populations (v_{ij}). Species along barplots from left to right are: *Halopegia azurea*, *Haumania danckelmaniana*, *H. liebrechtsiana*, *Marantochloa congensis*, *M. incertifolia*, *M. monophylla*, *Megaphrynium macrostachyum* and *Mega. trichogynum*. Source: Ley et al. 2014.

The key conclusions from these studies are:

- Individual pattern of genetic diversity and endemism in species are congruent with formerly proposed Pleistocene refuge areas (Ley et al. 2016 [8], Ley et al. 2014 [9]).
- Based on genetic pattern additional refugia are proposed here: SECameroon/NRCongo and NGabon (Ley et al. 2014 [9]).
- The specific scenario of allopatric speciation in the genus *Haumania* illustrates evolutionary processes that likely have contributed to shaping the African phylogeographic domains (Ley et al. 2016 [8]).
- The idiosyncratic phylogeographic patterns across Marantaceae species indicate diverse evolutionary histories across species, similarly found among tropical tree species (Ley et al. 2014 [9]).

Concerning future directions it is noteworthy that the current studies focused mostly on Central Tropical Africa due to a dense sampling in that particular area (see also Budde et al. 2013). Next it would thus be of interest to extend the phylogeographic work to Upper Guinea and Congolia to estimate the respective role of those areas in the evolution of the investigated taxa. Upper Guinea has been separated repeatedly from Lower Guinea/Congolia at the Dahomey gap (Dupont et al. 2000, Salzmann et al. 2006) and is today carrying a composition of taxa (e.g. mammals, plants, Jongkind 2008, Linder et al. 2012) or genetic populations (Duminil et al. 2015) partly distinct from Lower Guinea/Congolia.

Further unresolved is also the exact process of expansion of *Haumania liebrechtsiana* out of the Congolian refuge at the Congo River into Gabon (Ley et al. 2016 [9]). Here additional sampling would be needed in the Republic of the Congo to investigate in more detail the northwards expansion of the species elucidating the relationship between the two detected genetic *H. liebrechtsiana* clusters from WGabon and DR Congo/R Congo and finding exactly the current southern boarder of the secondary contact zone between *H. danckelmaniana* and *H. liebrechtsiana*.

In the future, it would further be desirable to expand this line of study to other sister species pairs of different growth forms (trees, shrubs) looking for common suture zones between species that might reflect common pattern of forest expansion from refugia after climate change (see for trees Hardy et al. 2013) and find further explanatory support for the observed divisions of the tropical African rainforest into distinct phylogeographic domains.

7 Concluding remarks and outlook

The here presented compilation of publications on gene flow in the tropical Africa rainforest understorey sheds new light on the patterns of intra- and inter-specific gene flow, species delimitation and historical migration of species in this habitat. Due to the wealth of tropical understorey species but the difficulty to access this ecosystem our knowledge on the evolution and functioning of this tropical habitat is still lacking behind. The comparisons of species within this thesis and with other taxa from the literature suggest that there are actually many different patterns that exist in this ecosystem. My results and conclusions thus urge the effort of conducting even more analyses on different species before we will get to an overall picture. Here, the combination of the conducted genetic studies in this thesis illuminates the interest in undertaking in general more overarching genetic studies per species - as pattern of intra- and inter-specific gene flow as well as local and regional gene flow interfere with each other - together shaping a species' evolutionary history and future potential (see also Cutter 2013).

In the future new techniques of data acquisition (e.g. next generation sequencing – with potential to cover larger portions of all parts of the genome) and methods of data evaluation will facilitate and refine these kinds of research efforts. One avenue is certainly the use of coalescence modelling to infer the history of taxa that lead to the phylogeographic pattern seen today. We here already applied methods of coalescence modelling implemented in DIYABC (Cornuet et al. 2010) to the genus *Haumania* yielding a first insight into the advantages and shortcomings of coalescence modelling depending on the available data of the respective study species at hand (Ley et al. 2016 [8]). In an ongoing project of the phylogeographic patterns in the genus *Sarcophrynium* K. Schum. (Marantaceae) using large datasets obtained through genotyping by sequencing (GBS) we are currently applying new spatially-explicit models of coalescence of genetic data which allow analysing more complex evolutionary models and accounting for the limited dispersal ability of species of the tropical understorey (SPLATCHE: Ray et al. 2010, PhyloGeoSim: Dellicour et al. 2014). We combine these analyses with ecological niche modelling based on ever-refined climatic models (Maxent: Phillips et al. 2006, Phillips and Dudík 2008) as further independent source of data to support emerging scenarios of evolutionary history of species and communities.

Based on the here obtained genetic data on the current state and past history of taxa a line of research to be reinforced in the future will be the estimation of the plant's genetic

potentials for the future. In another ongoing project (www.caballiance.org) we are thus going beyond the investigation of plant responses to past climatic changes looking into the potential reactions of plants under future climate change. In common garden experiments we check the existing degree of phenotypic variability of a given study plant and identify fitness traits under selection (Ley et al. in prep.). Thereby the already detected centers of genetic diversity will play an important role as they are the most probable areas - if any - which may carry the necessary preadaptations to survive changing climatic conditions in the future (see Thomassen et al. 2010, 2011). Furthermore, new genetic techniques facilitate here now also in non-model plants the use of coding genetic markers for the identification of the specific trait under selection and the geographic localities where genetic adaptation has taken place (see Jaramillo-Correa et al. 2015, Mayol et al. 2015). For the tropics current models predict that rainforest plants are already operating near a high temperature threshold and climate change might be too fast for evolutionary adaptation to keep up with (Clark 2004, Douthy and Goulden 2008). However, very little has so far actually been tested on tropical plants (but see Ensslin and Fischer 2015). These first results again indicate very different potentials depending on plant species.

The presented research lines thereby go far beyond fundamental research having great importance for current conservation efforts in helping to define the most promising areas and their characteristics for the protection of this highly diverse tropical ecosystem in the future.

8 References

- Abbott, R.J., Brennan, A.C. 2014. Altitudinal gradients, plant hybrid zones and evolutionary novelty. *Philosophical Transactions of the Royal Society B* 369(1648): 20130346.
- Ackerman, J. D., Ward, S. 1999. Genetic variation in a widespread, epiphytic orchid: Where is the evolutionary potential? *Systematic Botany* 24: 282–291.
- Acosta, M.C., Premoli, A.C. 2010. Evidence of chloroplast capture in South American *Nothofagus* (subgenus *Nothofagus*, Nothofagaceae). *Molecular Phylogenetics and Evolution* 54: 235–242.
- Agapow, P.M., Bininda-Emonds, O.R., Crandall, K.A., et al. 2004. The impact of species concept on biodiversity studies. *The Quarterly Review of Biology* 79: 161–179.
- Akerman, A., R. Bürger. 2014. The consequences of gene flow for local adaptation and differentiation: A two-locus two-deme model. *Journal of Mathematical Biology* 68: 1135–1198. doi:10.1007/s00285-013-0660-z.
- Alexandre, A., Meunier, J.D., Lezine, A.M., et al. 1998. Phytoliths: indicators of grassland dynamics during the late Holocene in intertropical Africa. *Palaeogeography, Palaeoclimatology, Palaeoecology* 136: 213–229.
- Allal, F., Sanou, H., Millet, L., et al. 2011. Past climate changes explain the phylogeography of *Vitellaria paradoxa* over Africa. *Heredity* 107: 174–186.
- Anderson, E. 1948. Hybridization of the habitat. *Evolution* 2: 1–9.
- Andersson, L. 1998. Marantaceae. In: Kubitzki, K. (Ed.), *The families and genera of vascular plants. IV: Flowering plants. Monocotyledons. Alismatanae and Commelinanae (except Gramineae)*. Berlin, Springer Verlag. 278–474.
- Anderson, B., Midgley, J., Stewart, B.A. 2003. Facilitated selfing offers reproductive assurance: a mutualism between a hemipteran and carnivorous plant. *American Journal of Botany* 90(7): 1009–1015.
- Anhuf, D., Ledru, M.P., Behling, H., et al. 2006. Paleo-environmental change in Amazonian and African rainforest during the LGM. *Palaeogeography, Palaeoclimatology, Palaeoecology* 239: 510–527.
- Arthofer, W. 2010. tinyFLP and tinyCAT: software for automatic peak selection and scoring of AFLP data tables. *Molecular Ecology Resources* 10: 385–388.
- Audigeos, D., Brousseau, L., Traissac, S., et al. 2013. Molecular divergence in tropical tree populations occupying environmental mosaics. *Journal of Evolutionary Biology* 26(3): 529–544 DOI: 10.1111/jeb.12069
- Avise, J.C. 2000. *Phylogeography: the history and formation of species*. Harvard University Press.
- Barrett, S.C.H. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* 3: 274–284.
- Barrett, S.C.H., Harder, L.D. 1996. Ecology and evolution of plant mating. *Tree* 11(2): 73–79.
- Barrett, S.C.H., Eckert, C.G. 1990. Variation and evolution of mating systems in seed plants. In: Kawano, S. (Ed.), *Biological Approaches and Evolutionary Trends in Plants*. Academic Press, Tokyo. 229–254.
- Bawa, K.S. 1992. Mating systems, genetic differentiation and speciation in tropical rain forest plants. *Biotropica* 24: 250–255.
- Barthlott, W., Mutke, J., Rafiqpoor, M.D., et al. 2005. Global centres of vascular plant diversity. *Nova Acta Leopoldina* 92: 61–83.
- Bawa, K.S. 1979. Breeding systems of trees in a tropical lowland wet forest. *New Zealand Journal of Botany (special issue)* 17: 521–524.
- Bawa, K.S. 1990. Plant–pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics* 21: 399–422.
- Bawa, K.S. 1992. MaBongers, F.J., Schnitzer, S.A., Traore, D. 2002. The importance of lianas and consequences for forest management in West Africa. *Bio-Terre, Revue Internationale de la Science de la Vie et de la Terre, Special edition 2002*: 59–70.
- Bonin, A., Bellemain, E., Bronken Eidesen, P., et al. 2004. How to track and assess genotyping errors in population genetics studies. *Molecular Ecology* 13: 3261–3273.
- Bonin, A., Ehrich, D., Manel, S. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology* 16: 3737–3758.
- Bonnefille, R. 2007. Rain forest responses to past climatic changes in tropical Africa. In: Bush, M.B., Flenley, J.R., Gosling, W.D. (Ed.), *Tropical rain forest responses to climate change*. Praxis Publishing, Chichester. 117–170.
- Borchsenius, F., Suárez, L. S. S., Prince, L.M. 2012. Molecular phylogeny and redefined generic limits of *Calathea* (Marantaceae). *Systematic Botany*. 37(3): 620–635.
- Bradshaw, A.D. 1972. Some of the evolutionary consequences of being a plant. *Evolutionary Biology* 5: 25–47.

- Braga, J.M.A. 2008. Two new species of *Calathea* (Marantaceae) from South-eastern Brazil. *Kew Bulletin* 63(2): 309-314.
- Bruford, M.W., Ciofi, C., Funk, S. M. 1998. Characteristics of microsatellites. In: *Molecular Tools for Screening Biodiversity*, Springer Netherlands. 202-205.
- Budde, K.B., González-Martínez, S.C., Hardy, O.J. et al. 2013. The ancient tropical rain forest tree *Symphonia globulifera* L. f. (Clusiaceae) was not restricted to postulated Pleistocene refugia in Atlantic Equatorial Africa. *Heredity* 111: 66-76.
- Butlin, R., 1987. Speciation by reinforcement. *TREE* 2: 8-13.
- Carbone, C., Mace, G. M., Roberts, S.C., Macdonald, D.W. 1999. Energetic constraints on the diet of terrestrial carnivores. *Nature* 402: 286-288.
- Carstens, B.C., Pelletier, T.A., Reid, N. M., et al. 2013. How to fail at species delimitation. *Molecular Ecology* 22(17): 4369-4383.
- CBOL Plant Working Group. 2009. A DNA barcode for land plants. *PNAS* 106: 12794-12797.
- Charlesworth, D., Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 237-268.
- Chittibabu, C.V., Parthasarathy, N. 2000. Understorey plant diversity in a tropical evergreen forest in the Kolli hills, eastern Ghats, India. *Ecotropica* 6: 129-140.
- Clark, D.A. 2004. Sources or sinks? The responses of tropical forests to current and future climate and atmospheric composition. *Philosophical Transaction of the Royal Society B* 359 : 477-491
- Colyn, M., Gauthier-Hion, A., Verheyen, W. 1991. A re-appraisal of palaeoenvironmental history in Central Africa: evidence of a major fluvial refuge in the Zaire basin. *Journal of Biogeography* 18: 403-407.
- Cornuet, J.M., Ravigné, V., Estoup, A. 2010. Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1. 0). *BMC Bioinformatics* 11(1): 401.
- Couvet, D. 2002. Deleterious effects of restricted gene flow in fragmented populations. *Conservation Biology* 16: 369-376.
- Couvreur, T.L., Porter-Morgan, H., Wieringa, J.J., Chatrou, L. W. 2011. Little ecological divergence associated with speciation in two African rain forest tree genera. *BMC Evolutionary Biology* 11(1): 1.
- Coyne, J.A., Orr, H.A. 1997. Patterns of speciation in *Drosophila* revisited. *Evolution* 51: 295-303.
- Coyne, J.A., Orr, H.A. 2004. *Speciation*. Sinauer, Sunderland, MA.
- Cruzan, M.B., Templeton, A.R. 2000. Paleogeography and coalescence: phylogeographic analysis of hypotheses from the fossil record. *Trends in Ecology and Evolution* 15(12): 491-496.
- Cubry, P., de Bellis, F., Pot, D., et al. 2013. Global analysis of *Coffea canephora* Pierre ex Froehner (Rubiaceae) from the Guineo-Congolese region reveals impacts from climatic refuges and migration effects. *Genetic Resources and Crop Evolution* 60: 483-501.
- Currat, M., Ruedi, M., Petit, R.J., et al. 2008. The hidden side of invasions: massive introgression by local genes. *Evolution* 62: 1908-1920.
- Curry, C.M. 2015. An integrated framework for hybrid zone models. *Evolutionary Biology* 42(3): 359-365.
- Cutter, A.D. 2013. Integrating phylogenetics, phylogeography and population genetics through genomes and evolutionary theory. *Molecular Phylogenetics and Evolution* 69(3): 1172-1185.
- Daïnou, K., Bizoux, J.P., Doucet, J.L., et al. 2010. Forest refugia revisited: SSRs and cpDNA sequences support historical isolation in a wide-spread African tress with high colonization capacity, *Milicia excelsia* (Moraceae). *Molecular Ecology* 19: 4462-4477.
- Dalling, J. W., Schnitzer, S. A., Baldeck, C., et al. 2012. Resource based habitat associations in a neotropical liana community. *Journal of Ecology* 100: 1174-1182.
- Damasceno, R., Strangas, M. L., Carnaval, et al. 2014. Revisiting the vanishing refuge model of diversification. *Frontiers in genetics* 5: article 353.
- Dauby, G., Duminil, J., Heuertz, M., et al. 2014. Congruent phylogeographic patterns of eight tree species in Atlantic Central Africa provide insights into the past dynamics of forest cover. *Molecular Ecology* 23: 2299-2312.
- Davey, J.W., Hohenlohe, P.A., Etter, P.D., et al. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics* 12(7): 499-510.
- Dayrat, B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society*. 85: 407-415.
- Degen, B., Roubik, D.W. 2004. Effects of animal pollination on pollen dispersal, selfing and effective population size of tropical trees: a simulation study. *Biotropica* 36(2): 165-179.
- Dellicour, S., Fearnley, S., Lombal, A., Heidl, S., Dahlhoff, E. P., Rank, N. E., Mardulyn, P. 2014. Inferring the past and present connectivity across the range of a North American leaf beetle: combining ecological niche modeling and a geographically explicit model of coalescence. *Evolution* 68(8): 2371-2385.

- De Queiroz, K. 1999. The General Lineage Concept of species and the defining properties of the species. In: Wilson, R.A. (Ed.), *Species: New Interdisciplinary Essays*, MIT Press, Cambridge, MA. 49-89.
- De Queiroz, K. 2007. Species concepts and species delimitation. *Systematic Biology* 56(6): 879-886.
- Devy, M.S., Davidar, P. 2006. Breeding systems and pollination modes of understory shrubs in a medium elevation wet evergreen forest, southern Western Ghats, India. *Current Sciences* 90: 838-842.
- Dhetchuvi, J.B. 1996. Taxonomie et phytogéographie des Marantaceae et des Zingiberaceae de l'Afrique Centrale (Gabon, Congo, Zaire, Rwanda et Brundi). PhD Thesis, Université Libre de Bruxelles, Belgique.
- Dick, C.W. 2010. Phylogeography and population structure of tropical trees. *Tropical Plant Biology* 3: 1-3.
- Dick, C.W., Heuertz, M. 2008. The complex biogeographic history of a widespread tropical tree species. *Evolution* 62(11): 2760-2774.
- Dick, C.W., Hardy, O.J., Jones, F.A., et al. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. *Tropical Plant Biology* 1: 20-33.
- Doucet, J.L. 2003. L'alliance délicate de la gestion forestière et de la biodiversité dans les forêts du centre du Gabon. PhD dissertation, Faculté Universitaire des Sciences agronomiques, Gembloux, Belgium.
- Doughty, C.E., Goulden, M.L. 2008. Are tropical forests near a high temperature threshold? *Journal of Geophysical Resources* 113: DOI: 10.1029/2007JG000632
- Duminil, J., Fineschi, S., Hampe, A., et al. 2007. Can population genetic structure be predicted from life-history traits? *American Naturalist* 169: 662-672. doi:10.1086/513490
- Duminil, J., Di Michele, M. 2009. Plant species delimitation: a comparison of morphological and molecular markers. *Plant Biosystems* 143(3): 528-542.
- Duminil, J., Kenfack, D., Viscosi, V., et al. 2012. Testing species delimitation in sympatric species complexes: the case of an African tropical tree, *Carapa* spp. (Meliaceae). *Molecular Phylogenetics and Evolution* 62(1): 275-285.
- Duminil, J., Brown, R.P., Ewédjè, E.E. et al. 2013. Large-scale pattern of genetic differentiation within African rainforest trees: insights on the roles of ecological gradients and past climate changes on the evolution of *Erythrophleum* spp. (Fabaceae). *BMC Evolutionary Biology* 13(1): 1.
- Duminil, J., Brown, R.P., Eben-Ezer, B.K.E., et al. 2013. Large-scale pattern of genetic differentiation within African rain forest trees: insights on the roles of ecological gradients and past climate changes on the evolution of *Erythrophleum* spp. (Fabaceae). *BMC Evolutionary Biology* 13: 195.
- Duminil, J., Mona, S., Mardulyn, P., et al. 2015. Late Pleistocene molecular dating of past population fragmentation and demographic changes in African rain forest tree species supports the forest refuge hypothesis. *Journal of Biogeography* 42(8): 1443-1454.
- Dunn, C.B., Moore, K.B. 2016. ESF Lists Top 10 New Species for 2016 - New to science: a species on humans' family tree, a brilliant red fish and a dainty damselfly with a racy name. ESF communications. <http://www.esf.edu/communications/view.asp?newsID=5282>
- Dupont, L.M., Jahns, S., Marret, F., et al. 2000. Vegetation change in equatorial West Africa: time-slices for the last 150ka. *Palaeogeography and Palaeoclimatology* 155: 95-122. doi:10.1016/S0031-0182(99)00095-4
- Ehrlén, J., Lehtilä, K. 2002. How perennial are perennial plants? *Oikos* 98: 308-322.
- Ellis, J.S., Knight, M.E., Carvell, C., et al. 2006. Cryptic species identification: a simple diagnostic tool for discriminating between two problematic bumblebee species. *Molecular Ecology Notes* 6: 540-542.
- Ellstrand, N.C., Roose, M.R. 1987. Patterns of genotypic diversity in clonal plant species. *American Journal of Botany* 74: 123-131.
- Elshire, R.J., Glaubitz, J.C., Sun, Q., et al. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS one* 6(5): e19379.
- Ennos, R.A. 1994. Estimating the relative rates of pollen and seed migration among plant populations. *Heredity* 72: 250-259.
- Ensslin, A., Fischer, M. 2015. Variation in life-history traits and their plasticities to elevational transplantation among seed families suggests potential for adaptive evolution of 15 tropical plant species to climate change. *American Journal of Botany* 102(8): 1371-1379.
- Excoffier, L., Foll, M., Petit, R.J. 2009. Genetic consequences of range expansions. *Annual Review of Ecology, Evolution and Systematics* 40: 481-501.
- Fjeldså, J. 1994. Geographical patterns for relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodiversity and Conservation* 3(3): 207-226.
- Foster, P., Sork, V. 1997. Population and genetic structure of the West African rain forest liana *Ancistrocladus korupensis* (Ancistrocladaceae). *American Journal of Botany* 84: 1078-1078.
- Freedman, A.H., Thomassen, H.A., Buermann, W., Smith T.B. 2010. Genomic signals of diversification along ecological gradients in a tropical lizard. *Molecular Ecology* 19: 3773-3788.

- Fussi, B., Lexer, C., Heinze, B. 2010. Phylogeography of *Populus alba* (L.) and *Populus tremula* (L.) in Central Europe: secondary contact and hybridisation during recolonisation from disconnected refugia. *Tree Genetics and Genomes* 6(3): 439-450.
- Galen, C., Shore J.S., Deyoe, H. 1991. Ecotypic divergence in alpine *Polemonium viscosum*: Genetic structure, quantitative variation, and local adaptation. *Evolution* 45: 1218-1228.
- Gaudeul, M., Till-Bottraud, I., Barjon, F., et al. 2004. Genetic diversity and differentiation in *Eryngium alpinum* L. (Apiaceae): comparison of AFLP and microsatellite markers. *Heredity* 92: 508-518.
- Gentry, A.H., Dodson, C.H. 1987. Diversity and biogeography of neotropical vascular epiphytes. *Annual Missouri Botanical Garden* 74: 205-233.
- Gort, G., Koopman, W.J.M., Stein, A. 2006. Fragment length distributions and collision probabilities for AFLP markers. *Biometrics* 62: 1107-1115.
- Haddadchi, A., Fatemi, M., Gross, C. L. 2014. Clonal growth is enhanced in the absence of a mating morph: A comparative study of fertile stylar polymorphic and sterile monomorphic populations of *Nymphoides montana* (Menyanthaceae). *Annals of Botany* 113(3): 523-532.
- Hamrick, J.L., Murawski, D.A. 1990. The breeding structure of tropical tree populations. *Plant Species Biology* 5:157-165.
- Hardy, O.J. 2003. Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. *Molecular Ecology* 12: 1577-1588.
- Hardy, O.J., Vekemans, X. 2002 SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* 2: 618-620.
- Hardy, O.J., Vekemans, X. 2001 Patterns of allozyme variation in diploid and tetraploid *Centaurea jacea* at different spatial scales. *Evolution* 55: 943-954.
- Hardy, O.J., Born, C., Budde, K., et al. 2013. Comparative phylogeography of African rain forest trees: a review of genetic signatures of vegetation history in the Guineo-Congolian region. *Comptes Rendus Geoscience* 345(7): 284-296.
- Harrison, R.G. 1998. Linking evolutionary pattern and process. In: Howard D.J., Berlocher S.H. (Eds.). *Endless forms: Species and speciation* Oxford University Press, New York. 19-31.
- Haselhorst, M.S., Buerkle, C.A. 2011. Detection of hybrids in natural populations of *Picea glauca* and *Picea engelmannii*. University of Wyoming National Park Service Research Center Annual Report 33(1): 119-123.
- Haskell, J.P., Ritchie, M.E., Olff, H. 2002. Fractal geometry predicts varying body size scaling relationships for mammal and bird home ranges. *Nature* 418: 527-530.
- Hebert, P.D.N., Cywinska, A., Ball, S.L., et al. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B* 270: 313-321. doi:10.1098/rspb.2002.2218.
- Herrera, C.M., Pellmyr, O. (eds.) 2002. *Plant-Animal Interactions: An Evolutionary Approach*. Blackwell Science, Oxford.
- Heuertz, M., Duminil, J., Dauby, G., et al. 2014. Comparative phylogeography in rainforest trees from Lower Guinea, Africa. *PLoS One* 9: e84307. doi:10.1371/journal.pone.0084307
- Holstein, N., Renner, S.S. 2011. A dated phylogeny and collection records reveal repeated biome shifts in the African genus *Coccinia* (Cucurbitaceae). *BMC Evolutionary Biology* 11: 28.
- Honnay, O., Bossuyt, B. 2005. Prolonged clonal growth: Escape route or route to extinction? *Oikos* 108: 427-432.
- Honnay, O., Jacquemyn, H., Rold_An-Ruiz, I., et al. 2006. Consequences of prolonged clonal growth on local and regional genetic structure and fruiting success of the forest perennial *Maianthemum bifolium*. *Oikos* 112: 21-30.
- Hooper, E.A. Hafler, C.H. 1997. Genetic diversity and breeding system in a group of neotropical epiphytic ferns (*Pleopeltis*; Polypodiaceae). *American Journal of Botany* 84(12): 1664-1674.
- Hubbell, S.P. 1997. A unified theory of biogeography and relative species abundance and its application to tropical rain forests and coral reefs. *Coral Reefs* 16 (Suppl.): S9-S21.
- Hubbell, S.P., Foster, R. B. 1983. Diversity of canopy trees in a neotropical forest and implications for conservation. Special publications series of the British Ecological Society.
- Hubbell, S.P., Condit, R. Foster, R.B. 2005. Barro Colorado Forest census plot data. Available at: <https://ctfs.arnarb.harvard.edu/webatlas/datasets/bci>.
- Husband, B. C., Schemske, D. W. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50(1): 54-70.
- Jaramillo-Correa, J. P., Rodríguez-Quilón, I., Grivet, D., et al. 2015. Molecular proxies for climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics* 199(3): 793-807.
- Jha, S., Dick, C.W. 2010. Native bees mediate long-distance pollen dispersal in a shade coffee landscape mosaic. *Proceedings of the National Academy of Science USA* 107: 13760-13764.
- Jongkind, C.C.H. 2008. Two new species of *Hypselodelphys* (Marantaceae) from West Africa. *Adansonia*, ser. 3, 30(1): 57-62.

- Karron, J.D., Thumser, N.N., Tucker, R., et al. 1995. The influence of population density on outcrossing rates in *Mimulus ringens*. *Heredity* 75: 175–180.
- Kay, K., Whittall, J.A., Hodges, S.A. 2006. A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. *BMC Evolutionary Biology* 6: 1471–2148.
- Kennedy, H. 2012. *Calathea carolineae* (Marantaceae), a new species endemic to Honduras. *Journal of the Botanical Research Institute of Texas*: 55-57.
- Kennedy, H. 2014. *Calathea gordonii* (Marantaceae), A new endemic Panamanian species. *Journal of the Botanical Research Institute of Texas* 8(1).
- Kinsman, S. 1990. Regeneration by fragmentation in tropical montane forest shrubs. *American Journal of Botany* 77: 1626–1633
- Knowles, L.L., Maddison, W.P. 2002. Statistical phylogeography. *Molecular Ecology* 11(12): 2623-2635.
- Koffi, K.G., Hardy, O.J., Doumenge, C., et al. 2011. Diversity gradients and phylogeographic Patterns in *Santiria trimera* (Burseraceae), a widespread African tree typical of mature rain forests. *American Journal of Botany* 98: 254-264.
- Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., et al. 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters* 15: 378–392.
- Kress, W.J., Specht, C.D. 2006. The evolutionary and biogeographic origin and diversification of the tropical monocot order Zingiberales. *Aliso*. 22(1): 619-630.
- Lacerda, de A.E.B., Kanashiro, M. Sebbenn, A.M. 2008. Long-pollen movement and deviation of random mating in a low-density continuous population of a tropical tree *Hymenaea courbaril* in the Brazilian Amazon. *Biotropica* 40: 462–470.
- Laraque, A., Pouyaud, B., Rocchia, R., et al. 1998. Origin and function of a closed depression in equatorial humid zones: the Lake Tele in North Congo. *Journal of Hydrology* 207: 236–253.
- Lasso, E., Engelbrecht, B.M.J., Dalling, J.W. 2009. When sex is not enough: ecological correlates of asexual reproductive ability in eight co-occurring *Piper* species in Panama. *Oecologia* 161: 43–56.
- Lasso, E., Dalling, J.W., Bermingham, E. 2011. Strong spatial genetic structure in five tropical *Piper* species: should the Baker–Fedorov hypothesis be revived for tropical shrubs? *Ecology and Evolution* doi: 10.1002/ece3.40
- Leal, M.E. 2001. Microrefugia, small scale ice age forest remnants. *Systematics and Geography of Plants* 71: 1073-1077.
- Ledo, A., Schnitzer, S. A. 2014. Disturbance and clonal reproduction determine liana distribution and maintain liana diversity in a tropical forest. *Ecology* 95: 2169–2178.
- Lee, M.S.Y. 2003 Species concepts and species reality: salvaging a Linnaean rank. *Journal of Evolutionary Biology* 16: 179–188.
- Letouzey, R. 1968. Étude phytogéographique du Cameroun. Paris, Editions Paul Lechevalier, no. 69.
- Lexer, C., Kremer, A., Petit, R.J. 2006. COMMENT: shared alleles in sympatric oaks: recurrent gene flow is a more parsimonious explanation than ancestral polymorphism. *Molecular Ecology* 15(7): 2007-2012.
- Ley, A.C. 2008. Evolution in African Marantaceae - evidence from floral morphology, ecology and phylogeny. PhD thesis. Institut für Spezielle Botanik, University of Mainz, Germany.
- Ley, A.C., Claßen-Bockhoff, R. 2009. Pollination syndromes in African Marantaceae. *Annals of Botany* 104: 41-56.
- Ley, A.C., Claßen-Bockhoff, R. 2010. Parallel evolution in plant-pollinator interaction in African Marantaceae. In: van der Burgt, X., van der Maesen, J., Onana, J.-M. (Eds.), *Systematics and Conservation of African Plants*, Royal Botanic Gardens, Kew. 847-854.
- Ley, A.C., Claßen-Bockhoff, R. 2012. Five new species of Marantaceae from Gabon. *Adansonia* 34(1): 37-52.
- Ley, A.C., Claßen-Bockhoff, R. 2013. Breeding system and fruit set in African Marantaceae. *Flora* 208: 532-537.
- Ley, A.C., Dauby, G., Köhler, J., et al. 2014. Comparative phylogeography of eight herbs and lianas (Marantaceae) in central African rainforests. *Frontiers in Genetics* 5: article 403. doi: 10.3389/fgene.2014.00403
- Ley, A.C., Hardy, O.J. 2010. Species delimitation in the Central African herbs *Haumania* (Marantaceae) using georeferenced nuclear and chloroplastic DNA sequences. *Molecular Phylogenetics and Evolution*. 57(2): 859-867.
- Ley, A.C., Claßen-Bockhoff, R. 2011. Evolution in African Marantaceae-evidence from phylogenetic, ecological and morphological studies. *Systematic Botany*. 36(2): 277-290.
- Ley, A.C., Hardy, O.J. 2013. Improving AFLP analysis of large-scale patterns of genetic variation - a case study with the Central African lianas *Haumania* spp (Marantaceae) showing interspecific gene flow. *Molecular Ecology* 22(7): 1984-1997.

- Ley, A.C., Hardy, O.J. 2014. Contrasting patterns of gene flow between sister plant species in the understorey of African moist forests - the case of sympatric and parapatric Marantaceae species. *Molecular Phylogenetics and Evolution* 77: 264-274.
- Ley, A.C., Dauby, G., Köhler, J., et al. 2014. Comparative phylogeography of eight herbs and lianas (Marantaceae) in central African rainforests. *Frontiers in Genetics* 5: article 403. doi: 10.3389/fgene.2014.00403
- Ley, A.C., Hardy, O.J. 2016a. Polymorphic microsatellite loci for *Haumania danckelmaniana* and transferability into *H. liebrechtsiana* (Marantaceae). *Applications in Plant Sciences* 4(3): 1500116.
- Ley, A.C., Hardy, O.J. 2016b. Spatially limited clonality and pollen and seed dispersal in a characteristic climber of Central African rain forests: *Haumania danckelmaniana* (Marantaceae). *Biotropica* 58(5): 618-627.
- Ley, A.C., Heuertz, M., Hardy, O.J. 2016. The evolutionary history of central African rain forest plants: phylogeographic insights from sister species of the climber genus *Haumania* (Marantaceae). *Journal of Biogeography*. doi:10.1111/jbi.12902
- Ley, A.C., Hardy, O.J. (submitted) Hybridization and asymmetric introgression after secondary contact in two tropical African climber species *Haumania danckelmaniana* and *H. liebrechtsiana* (Marantaceae). *International Journal of Plant Sciences*.
- Ley, A.C., K. Gonder et al. (in prep.) Phenotypic plasticity in a tropical African understorey herb along a climatic gradient – *Sarcophrynium prionogonium* (Marantaceae).
- Al-Gharaibeh, M.M., Borchsenius, F., McKechnie, L., Sanmartin, I., Ley A.C. (manuscript) Phylogeny of the pantropically distributed family Marantaceae.
- Li, Y., Itoi, T., Takahashi, H., et al. 2015. Morphological and genetic variation in populations in a hybrid zone between *Leucosceptrum japonicum* and *L. stellipilum* (Lamiaceae) in the central Japanese mainland. *Plant Systematics and Evolution* 301(3): 1029-1041.
- Linder, H.P., de Klerk, H.M., Born, J., et al. 2012. The partitioning of Africa: statistically defined biogeographical regions in sub-Saharan Africa. *Journal of Biogeography* 39(7): 1189-1205.
- Linder, H.P. 2014. The evolution of African plant diversity. *Frontiers in Ecology and Evolution* 2: 38.
- Loiselle, B.A., Sork, V.L., Nason J., et al. 1996. Spatial genetic structure of a tropical understorey shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82(11): 1420-1425.
- Maley, J. 1996. The African rainforest: main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proceeding of the Royal Society Edinburgh B* 104B: 31–73.
- Malausa, T., Gilles, A., Megléc, E., et al. 2011. High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Molecular Ecology Resources* 11: 638–644.
- Maley, J. 1996. The African rainforest: main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. *Proceeding of the Royal Society Edinburgh B* 104B: 31–73.
- Marchelli, P., Gallo, L.A. 2001. Genetic diversity and differentiation in a southern beech subjected to introgressive hybridization. *Heredity* 87(3): 284-293.
- Mayden, R.L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge, M.F., Dawah, H.A., Wilson, M.R. (Eds.), *Species: The units of diversity*. Chapman and Hall 381–423.
- Mayol, M., Riba, M., González-Martínez, S.C., Bagnoli, F., et al. 2015. Adapting through glacial cycles: insights from a long-lived tree (*Taxus baccata*). *New Phytologist* 208(3): 973-986.
- Meeus, S., Jacquemyn, H., Honnay, O., et al. 2011. Self-incompatibility and pollen limitation in the rare tristylous endemic *Hugonia serrata* on La Reunion Island. *Plant Systematics and Evolution* 292: 143-151.
- Meudt, H.M., Clarke, A.C. 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Sciences* 12: 106–117.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., et al. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.
- Micheneau, C., Dauby, G., Bourland, N., et al. 2011. Development and characterization of microsatellite loci in *Pericopsis elata* (Fabaceae) using a cost-efficient approach. *American Journal of Botany* 98: e268 – e270.
- Mutke, J., Barthlott, W. 2005. Patterns of vascular plant diversity at continental to global scales. In: Friis, I., Balslev, H. (Eds.), *Plant diversity and Complexity patterns – Local, Regional and Global Dimensions*. The Royal Danish Academy of Sciences and Letters, Copenhagen, *Biologiske Skrifter* 55: 521-537.
- Nabe-Nielsen, J., Hall, P. 2002. Environmentally induced clonal reproduction and life history traits of the liana *Machaerium cuspidatum* in an Amazonian rain forest, Ecuador. *Plant Ecology*. 162: 215-226.
- Oberprieler, Ch., Barth, A., Schwarz, S., et al. 2010. Morphological and phytochemical variation, genetic structure, and phenology in an introgressive hybrid swarm of *Senecio hercynicus* and *S. ovatus* (Compositae, Senecioneae). *Plant Systematics and Evolution* 286: 153–166.

- O'Hanlon, P.C., Peakall, R. 2000. A simple method for the detection of size homoplasy among amplified fragment length polymorphism fragments. *Molecular Ecology* 9: 815–816.
- Ohta, T. 1993. An examination of generation time effect on molecular evolution. *Proceedings of the National Academy of Science* 90: 10676–10680.
- Ortiz-Barrientos, D., Greal, A., Nosil, P. 2009. The genetics and ecology of reinforcement: implications for the evolution of prezygotic isolation in sympatry and beyond. *Annals of the New York Academy of Science* 1168: 156–182.
- Palma-Silva, C., Wendt, T., Pinheiro, F. et al. 2011. Sympatric bromeliad species (*Pitcairnia* spp.) facilitate tests of mechanisms involved in species cohesion and reproductive isolation in Neotropical inselbergs. *Molecular Ecology* 20: 3185–3201.
- Parmentier, I., Réjou-Méchain, M., Chave, J., et al. 2014. Prevalence of phylogenetic clustering at multiple scales in an African rain forest tree community. *Journal of Ecology* 102(4): 1008–1016.
- Pasquet, R.S., Peltier, A., Hufford, M.B., et al. 2008. Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *Proceedings of the National Academy of Sciences* 105(36): 13456–13461.
- Penalosa, J. 1982. Morphological specialization and attachment success in two twining lianas. *American Journal of Botany* 69: 1043–1045.
- Petit, J.R., Jouzel, J., Raynaud, D., et al. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature* 399: 429–436.
- Petit, R. J., Csaikl, U. M., Bordács, S., et al. 2002. Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management* 156(1): 5–26.
- Petit, R.J., Aguinagalde, I., deBeaulieu, J.-L., et al. 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* 300: 1563–1565. doi:10.1126/science.1083264
- Petit, R.J., Bodénès, C., Ducouso, A., et al. 2004. Hybridization as a mechanism of invasion in oaks. *New Phytologist* 161(1): 151–164.
- Petit, R.J., Duminil, J., Fineschi, S., et al. 2005. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology* 14: 689–701.
- Petit, R.J., Excoffier, L., 2009. Gene flow and species delimitation. *Trends Ecology and Evolution*. 24: 386–393.
- Phillips, S.J., Dudík, M. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* 31: 161–175.
- Phillips, S.J., Anderson, R.P., Schapire, R.E. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190: 231–259.
- Plana, V. 2004. Mechanisms and tempo of evolution in the African Guineo-Congolian rain forest. *Philosophical Transactions of the Royal Society B*. 359: 1585–1594.
- Plana, V. 2004. Mechanisms and tempo of evolution in the African Guineo-Congolian rainforest. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359(1450): 1585–1594.
- Poulsen, A.D., Balslev, H. 1991. Abundance and cover of ground herbs in an Amazonian rain forest. *Journal of Vegetation Science* 2: 315–322.
- Poulsen, A.D. 1996. Species-richness and density of ground herbs within a plot of lowland rainforest in north-west Borneo. *Journal of Tropical Ecology* 12: 843–851.
- Prince, L.M., Kress, W.J. 2006a. Phylogenetic relationships and classification in Marantaceae: insights from plastid DNA sequence data. *Taxon* 55(2): 281–296.
- Prince, L.M., Kress, W.J. 2006b. Phylogeny and biogeography of the prayer plant family: getting to the root problem in Marantaceae. *Aliso*. 22(1): 643–657.
- Pyke, G.H. 1984. Optimal foraging theory: a critical review. *Annual Review of Ecology and Systematics* 15: 523–575.
- Rathcke, B.J. 2003. Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). *American Journal of Botany* 90(9): 1328–1332.
- Ray, N., Currat, M., Foll, M., Excoffier, L. 2010. SPLATCHE2: a spatially explicit simulation framework for complex demography, genetic admixture and recombination. *Bioinformatics* 26(23): 2993–2994.
- Ree, R.H. 2013. Lagrange configurator version 20130526 (beta). <http://www.reelab.net/lagrange/configurator/index>
- Richards, P.W. 1954. *The Tropical Rain Forest*. Cambridge University Press, Cambridge.
- Richards, A.J. 1997. *Plant Breeding Systems*, 2nd edition. Chapman and Hall, London, UK.
- Rieseberg, L.H. 1995. The role of hybridization in evolution: old wine in new skins. *American Journal of Botany* 82(7): 944–953.
- Rieseberg, L.H., Whitton, J., Gardner, K. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics* 152: 713–727.

- Robbrecht, E. 1996. Geography of African Rubiaceae with reference to glacial rain forest refuges. The Biodiversity of African Plants - Proceedings of the 14th AETFAT Congress (ed. by L.J.G. van der Maesen, X.M. van der Burgt, J.M. van Medenbach de Rooy), pp. 564–580. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Rull, V. 2008. Speciation timing and neotropical biodiversity: the Tertiary–Quaternary debate in the light of molecular phylogenetic evidence. *Molecular Ecology* 17(11): 2722–2729.
- Sagers, C.L. 1993. Reproduction in neotropical shrubs: the occurrence and some mechanisms of asexuality. *Ecology* 74: 615–618.
- Salzmann, U., Hoelzmann, P., Waller, M.P. 2006. The termination of the Holocene humid period in west Africa: abrupt climatic changes, local fluctuations and regional trends. *Geophysical Research Abstracts* 8: 2304.
- Sambatti, J.B.M., Rice, K.J. 2006. Local adaptation, patterns of selection, and gene flow in the Californian serpentine sunflower (*Helianthus exilis*). *Evolution* 60: 696–710.
- Schemske, D.W., Lande, R. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39(1): 41–52.
- Schleuning, M., Becker, T., Vadillo, G. P., et al. 2011. River dynamics shape clonal diversity and genetic structure of an Amazonian understory herb. *Journal of Ecology* 99: 373–382.
- Schnitzer, S.A., Dalling, J.W., Carson, W.P. 2000. The impact of lianas on tree regeneration in tropical forest canopy gaps: Evidence for an alternative pathway of gap-phase regeneration. *Journal of Ecology* 88: 655–666.
- Schnitzer, S.A., Mangan, S.A., Dalling, J.W., et al. 2012. Liana abundance, diversity, and distribution on Barro Colorado Island, Panama. *PLoS ONE* 7: e52114. doi:10.1371/journal.pone.0052114.
- Schoen, D.J., Morgan, M.T., Bataillon, T. 1996. How does self-pollination evolve? Inferences from floral ecology and molecular genetic variation. *Philosophical Transactions of the Royal Society London B* 351: 1281–1290.
- Sebbenn, A.M., Licona, J.C., Mostacedo, B., et al. 2012. Gene flow in an overexploited population of *Swietenia macrophylla* King (Meliaceae) in the Bolivian Amazon. *Silvae Genetica* (4–5): 212–220.
- Schnell, R. 1957. Clef empirique des principales Marantacees ouestafricaines (Guinée, Côte d'Ivoire et territoires limitrophes). *Bulletin de l'Institut Fondamental d'Afrique Noire* 4: 1124–1134.
- Schnitzer, S.A., Carson, W.P. 2001. Treefall gaps and the maintenance of species diversity in a tropical forest. *Ecology* 82: 913–919.
- Schönswetter, P., Tribsch, A., Stehlik, I., et al. 2004. Glacial history of high alpine *Ranunculus glacialis* (Ranunculaceae) in the European Alps in a comparative phylogeographical context. *Biological Journal of the Linnean Society* 81: 183–195.
- Schönswetter, P., Elven, R., Brochmann, C. 2008. Trans-Atlantic dispersal and large-scale lack of genetic structure in the circumpolar, arctic-alpine sedge *Carex bigelowii* s. lat. (Cyperaceae). *American Journal of Botany* 95: 1006–1014.
- Schorr, G. 2009. Rekonstruktion der eiszeitlichen Verbreitung und Artbildung vier alpiner Primeln durch Artenverbreitungsmodelle und Phylogeographie. PhD Dissertation, Institut für Spezielle Botanik und Botanischer Garten, Johannes Gutenberg – University, Mainz, Germany.
- Shimizu-Inatsugi, R., Lihova, J., Iwanaga, H., et al. 2009. The allopolyploid *Arabidopsis kamchatica* originated from multiple individuals of *Arabidopsis lyrata* and *Arabidopsis halleri*. *Molecular Ecology* 18: 4024–4048.
- Silvertown, J. 2008. The evolutionary maintenance of sexual reproduction: Evidence from the ecological distribution of asexual reproduction in clonal plants. *International Journal of Plant Sciences* 169: 157–168.
- Slatkin, M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16: 393–430.
- Smith, T.B., Wayne, R.K., Girman, D.J., et al. 1997. A role for ecotones in generating rainforest biodiversity. *Science* 276(5320), 1855–1857.
- Smith, T. B., Calsbeek, R., Wayne, R., et al. 2005. Testing alternative mechanisms of evolutionary divergence in an African rain forest passerine bird. *Journal of Evolutionary Biology* 18: 257–268.
- Soltis, D.E., Soltis, P.S. 1999. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology and Evolution* 14: 348–352.
- Sosef, M.S. 1996. Begonias and African rain forest refuges: general aspects and recent progress. In: van der Maesen, L.J.G., van der Burgt, X.M., van Medenbach de Rooy, J.M., (Eds.), *The Biodiversity of African Plants - Proceedings of the 14th AETFAT Congress* Kluwer Academic Publishers, Dordrecht, The Netherlands. 602–611.
- Stacy, E.A., Hamrick, J.L., Nason, J.D., et al. 1996. Pollen dispersal in low-density populations of three Neotropical tree species. *American Naturalist* 148(2): 275–298.

- Stebbins, G.L. 1957. Self fertilization and population variability in higher plants. *American Naturalist* 91: 337–354.
- Stevens, G.C. 1987. Lianas as structural parasites: The *Bursera simarouba* example. *Ecology* 68: 77–81, doi:10.2307/1938806.
- Suksathan, P., Borchsenius, F. 2003. Two new species of *Stachyphrynium* (Marantaceae) from SE Asia. – *Willdenowia* 33: 403-408.
- Suksathan, P., Gustafsson, M.H., Borchsenius, F. 2009. Phylogeny and generic delimitation of Asian Marantaceae. *Botanical Journal of the Linnean Society* 159(3): 381-395.
- Symonds, V.V., Soltis, P.S., Soltis, D.E. 2010. Dynamics of polyploid formation in *Tragopogon* (Asteraceae): recurrent formation, gene flow, and population structure. *Evolution* 64: 1984–2003.
- Takebayashi, N. Morrell, P.L. 2001. Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. *American Journal of Botany* 88(7): 1143-1150.
- Thomassen, H.A., Buermann, W., Milá, B., et al. 2010. Modeling environmentally associated morphological and genetic variation in a rainforest bird, and its application for conservation prioritization. *Evolutionary Applications* 3: 1-16.
- Thomassen, H.A., Fuller, T., Buermann, W., et al. 2011. Mapping evolutionary process: A multi-taxa approach to conservation prioritization. *Evolutionary Applications* 4: 397-413.
- Terauchi, R. 1990. Genetic diversity and population structure of *Dioscorea tokoro* Makino, a dioecious climber. *Plant Species Biology* 5: 243–253.
- Tsitrone, A., Kirkpatrick, M., Levin, D.A. 2003. A model for chloroplast capture. *Evolution* 57(8): 1776-1782.
- Ude, G.N., Dimkpa, C.O., Anegbeh, P.O., et al. 2006. Analysis of genetic diversity in accessions of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. *African Journal of Biotechnology* 5(3): 219-223.
- Vandepitte, K., Honnay, O., De Meyer, T., et al. 2010. Patterns of sex ratio variation and genetic diversity in the dioecious forest perennial *Mercurialis perennis*. *Plant Ecology* 206: 105– 114.
- Vekemans, X., Hardy, O.J. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* 13: 921–935.
- Ward, M., Dick, C.W., Gribel, R., et al. 2005. To self, or not to self. A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* 95: 246–254.
- Werneck, F.P., Costa, G.C., Colli, G.R., et al. 2011. Revisiting the historical distribution of Seasonally Dry Tropical Forests: new insights based on palaeodistribution modelling and palynological evidence. *Global Ecology and Biogeography* 20(2): 272-288.
- White, F. 1979. The Guineo-Congolian Region and its relationship to other phytochoria. *Bulletin du Jardin Botanic National Belgique* 49: 11-55.
- Wright, S.I., Kalisz, S. Slotte, T. 2013. Evolutionary consequences of self-fertilization in plants. *Proceeding of the Royal Society B* 280: 20130-20133.
- Wright, S. 1943. Isolation by distance. *Genetics* 28(2): 114-38.
- Wu, C.-I., Ting, C.-T. 2004. Genes and speciation. *Nature Reviews Genetics* 5: 114–122.
- Zeng, X., Michalski, S.G., Fischer, M., et al. 2012. Species diversity and population density affect genetic structure and gene dispersal in a subtropical understory shrub. *Journal of Plant Ecology* 5(3): 270–278.

9 Summary

The thesis summarizes a set of studies on gene flow in the tropical Africa forest understorey focusing on i) intra-specific gene flow considering breeding system and the spatial extent of clonality and gene dispersal, ii) species delimitation and different levels of inter-specific gene flow (hybridization) and iii) historical migration scenarios of species inferred from phylogeographic pattern in the family Marantaceae. Furthermore, reciprocal interactions of gene flow from all three investigated genetic-geographic levels are considered.

The first comparative investigation of the spatial genetic structure in a climber species against data from the literature on tropical trees and herbs revealed an intermediate level of spatial genetic structure in the latter explained by a combination of rather species specific traits such as moderate clonality, narrow seed dispersal and high population density with a presumably important impact on pollinator behaviour. In the future, these results should be compared under different disturbance regimes and to further taxa with contrasting life forms and pollination and dispersal systems to gain a more general picture of the pattern and determinants of spatial genetic structure in understory plants of tropical African rainforest in contrast to trees.

In the analysis of genetic species boundaries different levels of inter-specific gene flow were detected from total absence to frequent gene flow between species correlated with phylogenetic distance and geographic range overlap. Hybridisation was probably facilitated by cross-compatibility (low phylogenetic divergence), sympatry (incl. same habitat) and pollinator sharing. A case of asymmetric hybridization was attributed to the effect of unidirectional invasion of one species into the range of the other species. The ecological prerequisites of the respective species for becoming either the invaded or the invasive species still need further investigation. The different marker systems (AFLP, cp & nrDNA, SSR) used in this study yielded complementary information questioning the reliability of the unique use of chloroplast data for species recognition by current DNA barcoding initiatives.

Comparing phylogeographic pattern across eight herb and climber species in Central Africa revealed commonly high levels of diversity in Lower Guinea (Cameroon, Equatorial Guinea, Gabon) versus low levels in Congolia suggesting a frequent origin of species in Lower Guinea. Within Lower Guinea patterns were little congruent indicating an idiosyncratic history of species with random retraction of species to different refugia

during incidents of adverse climate conditions, i.e. glacial cycles. The contrasting patterns of one species congruent with a Congolian origin revealed a potential mechanism for the establishment of phytochoria in Central Africa. Next, the expansion of data to Upper Guinea to identify the role of Upper Guinea in tropical African species evolution and the application of ecological niche-modeling and spatially explicit coalescence modeling are envisioned to account for more complex models of evolution.

Phylogeographic pattern do not only reveal past events of species persistence and migration but also help to identify current centers of diversity important for responses to future climatic changes. In an ongoing effort centers of genetic diversity and distinction and signs of local adaptation to different climate regimes are researched in different plant taxa yielding insights into species' resources for potential responses to future climatic changes with important implications for conservation actions.

The here presented thesis thus sheds new light on the patterns of gene flow in the tropical Africa rainforest understorey contributing to our understanding of the vegetation history of tropical Africa and its currently ongoing evolutionary processes and forms the basis for an estimation of the future potential of those tropical plants in a changing world.

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- **International collaborators:**

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11 Curriculum Vitae

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Education, professional experience

- since 2011 **Post-doctoral researcher** Institute of Geobotany and Botanical Garden, University Halle-Wittenberg, Halle (Saale), Germany
- 2008 - 2011 **Post-doctoral researcher** Institute of Evolutionary Biology and Ecology, Free University Brussels (ULB), Belgium in the lab of Dr. Olivier Hardy, financed by the German Exchange Service (DAAD) and subsequently the German Research Foundation (DFG)
Project: “Phylogeographic pattern and gene flow in African Marantaceae” with repeated field trips to the tropical rainforest in Cameroun and Democratic Republic of Congo
- 2004 – 2008 **PhD student** Institut of “Spezielle Botanik”, Johannes Gutenberg-University, Mainz, Germany financed by the German Research Foundation (DFG)
Project: “Evolutionary tendencies in African Marantaceae – evidence from floral morphology, ecology and phylogeny” with three field trips of three to four months to the tropical rainforest in Gabon (“summa cum laude”) Supervisor: Prof. R. Claßen-Bockhoff.
- 2003 **Research assistant** (6 months) at the Fundacion Amigos de la Naturaleza (FAN), Bolivia
Project: “Mapping of biodiversity for a conservation vision for the biodiversity of the Amboro Madidi Corridor (CAM)”

1997 – 2003 **Study of Biology** at the University of Bonn, Germany

2002/03: **Diploma thesis** “Diversity and Endemism of the Tribus Epidendreae (Orchidaceae) in Bolivia” conducted at the Fundacion Amigos de la Naturaleza (FAN) in Bolivia (Note: sehr gut).

Supervisor: Prof. P. Ibisch.

2000: **Exchange student** (2 semesters) La Trobe University, Melbourne, Australia

Practical work experience

since 2013 **Associated Editor** for “Plant Ecology and Evolution” (www.plecevo.eu)

05/2010 **Botanical expert** on the multidisciplinary 2-month expedition “Congo 2010” along the Congo river in DR Congo funded by the Belgian government

2005-2008 **Visitor Guide** for various subjects in the Botanical Garden of the Johannes Gutenberg-University Mainz (selected subjects: succulent plants, plants of the bible, general tour of the garden, evolution of land plants, agriculturally used plants, carnivorous plants...)

1997-2003 **Internships** at Cape Tribulation, Tropical Research Station and at Darwin, mist-netting in the mangroves, Australia; “Fundación Jatun Sacha”, Ecuador; Vegetation plot survey, Dunedin, New Zealand

Further training

02/2016 “Programming for Evolutionary Biology”, Bioinformatics Leipzig, Germany

09/2015 “NGS in phylogeny”, Black Forest Summer School, Germany

2013-2015 “Hochschuldidaktikzertifikat”, University Halle-Wittenberg, Germany

05/2013 “Aktivierende Lehre”, University Halle, Germany

08/2011 “Scientific Teaching” by Prof. D. Ebert-May, Porto, Portugal

05/2007 “Molecular marker analysis of plant population structure and processes”, University of Copenhagen, Denmark

08/2001 “Tropical Ecology” international course in Uganda, Tropical Biology Association (TBA), <http://www.tropical-biology.org/>
Project: “Ant-plant interaction between *Psydrax subcordata* (Rubiaceae) and *Crematogaster*”

Teaching experience

- 2016 **B.Sc. International Field Course** on “Biodiversity and Conservation” in Gabon (www.caballiance.org)
- 2015 **B. Sc. course** ”Conservation Genetics”, at the Professional Development Workshop, USTM, Franceville (Gabon)
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 - B. Sc. excursion** “Ruderal and segetal vegetation at the Brandberge, Halle”
 - B. Sc. lecture** “Plant systematics”
 - M. Sc. course** “Introduction to phylogeography”
 - M. Sc. course** “Introduction to next generation sequencing methods(NGS)”
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Peer reviewing experience

Adansonia, African Journal of Biotechnology, American Journal of Botany, Annals of Botany, ANR - the French National Research Agency, Biotropica, Blumea, Conservation Genetics, FLORA, Forest Ecology and Management, International Journal of Molecular Sciences, Journal of Tropical Ecology, Nordic Journal of Botany, Organisms Diversity and Evolution (ODE), Plant Ecology and Evolution, Plant Systematics and Evolution, PLoSOne, Scientia Horticulturae, Systematic Botany.

Award received

Award of the University of Mainz for Excellent Dissertations 2008

Languages

Fluent in speaking and writing in German, English and French and speaking in Spanish.

Memberships

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Gesellschaft für Tropenökologie (GTÖ);
Botanical Society of America (BSA)

Halle, 5th October 2016

Dr. Alexandra C. Ley

12 List of all publications produced by the author

- Ley, A.C., K. Gonder et al. (in prep.)** Phenotypic plasticity in a tropical African understorey herb along a climatic gradient – *Sarcophrynium prionogonium* (Marantaceae).
- Ley, A.C. & O.J. Hardy (submitted)** Hybridization and asymmetric introgression after secondary contact in two tropical African climber species *Haumania danckelmaniana* and *H. liebrechtsiana* (Marantaceae). *International Journal of Plant Sciences*.
- Dauby, G., R. Zaiss, A. Blach-Overgaard, L. Catarino, T. Damen, V. Deblauwe, S. Dessein, J. Dransfield, V. Droissart, M.C. Duarte, H. Engledow, G. Fadeur, R. Figueira, R.E. Gereau, O.J. Hardy, D.J. Harris, J. de Heij, S. Janssens, Y. Klomberg, A.C. Ley, B.A. Mackinder, P. Meerts, J.L. van de Poel, B. Sonké, M.S.M. Sosef, T. Stévar, P. Stoffelen, J.-C. Svenning, P. Sepulchre, X. van der Burgt, J.J. Wieringa & T.L.P. Couvreur (2016)** RAINBIO: a mega-database of tropical African vascular plants distributions. *PhytoKeys* 74: 1–18. doi: 10.3897/phytokeys.74.9723
- Dhetchuvi, J.-B. & A.C. Ley (accepted)** Marantaceae. In: Sosef, M.S.M. (Ed.) *Flore d’Afrique Centrale (Republique Démocratique du Congo- Rwanda – Burundi)*. Jardin Botanique Meise, Brussels.
- Ley A.C., M. Heuertz, O.J. Hardy (2016)** Phylogeographic patterns in *Haumania* (Marantaceae) suggest allopatric speciation in spatial and temporal concordance with the rainforest refuge hypothesis in Central Africa. *Journal of Biogeography*. doi:10.1111/jbi.12902
- Ley, A.C. & O.J. Hardy (2016a)** Polymorphic microsatellite loci for *Haumania danckelmaniana* and transferability into *H. liebrechtsiana* (Marantaceae). *Applications in Plant Sciences*. 4(3) doi: <http://dx.doi.org/10.3732/apps.1500116>
- Ley, A.C. & O.J. Hardy (2016b)** Clonality and gene flow in a characteristic climber of Central African tropical rain forests: *Haumania danckelmaniana* (Marantaceae). *Biotropica* 58(5) : 618-627.
- Ewédjè E.B.K., Ahanchédé A., Hardy O.J. & A.C. Ley (2015)** Reproductive biology of *Pentadesma butyracea* Sabine (Clusiaceae) in Benin. *Plant Ecology and Evolution*. 148 (2): 213-228.
- Ley, A.C., G. Dauby, J. Köhler, C. Wypior, M. Röser and O.J. Hardy (2014)** Comparative phylogeography of eight tropical herbs and lianas (Marantaceae) in central African rainforests. *Frontiers in Genetics* 5: article 403. doi: 10.3389/fgene.2014.00403
- Cabezas, F., Velayos, M. & A. C. Ley (2014)** Marantaceae. In: Velayos, M., Aedo, C., Cabezas, F., de la Estrella, M., Barberá, P. & Fero, M. (Eds.) *Flora de Guinea Ecuatorial. Vol. XI Bromelianae - Juncanae*. CSIC, Real Jardin Botánico, Madrid. 31-46.

- Ley, A.C. & O.J. Hardy (2014)** Contrasting patterns of gene flow between sister plant species in the understorey of African moist forests – the case of sympatric and parapatric Marantaceae species. *Molecular Phylogenetics and Evolution* 77: 264-274.
- Ley, A.C. & D.J. Harris (2014)** Flower morphological diversity in *Aframomum* (Zingiberaceae) from Africa - the importance of mechanical isolation through pollinator shifts for speciation? *Plant Ecology and Evolution* 147: 33-48.
- Ley, A.C. & R. Claßen-Bockhoff (2013)** Breeding system and fruit set in African Marantaceae. *Flora*. 208: 532-537.
- Ley, A.C. & O.J. Hardy (2013)** Improving AFLP analysis of large-scale patterns of genetic variation - a case study with the Central African lianas *Haumania* spp (Marantaceae) showing interspecific gene flow. *Molecular Ecology* 22, 7:1984-1997.
- Ley, A.C. & R. Claßen-Bockhoff (2012)** Diversity in floral synorganisation affects pollen deposition and breeding system in Marantaceae. *Botanical Journal of the Linnean Society*. 168, 3: 300-322.
- Ley, A.C. & R. Claßen-Bockhoff (2012)** Five new species of Marantaceae from Gabon. *Adansonia*. 34, 1: 37-52.
- Anthony, N.M., Mickala, P., Abernethy, K.A., Atteke, C., Bissiengou, P., Bruford, M.W., Dallmeier, F., Decaëns, T., Dudu, A., Freedman, A., Gonder, M.K., Hardy, O., Hart, J., Jeffery, K., Johnson, M., Koumba Pambo, F., Ley, A., Korte L., Lahm S.A., Lee M., Lowenstein, J., Mboumba, J.-F., Ndiade Bourobou, D., Ngomanda, A., Ntie, S., Sebag, D., Sullivan, J., Vanthomme, H., Vergnes, V., Verheyen, E. & B. Zimkus (2011)** Biodiversity and conservation genetics research in central Africa: new approaches and avenues for international collaboration. *Conservation Genetics Resources*. (published online: DOI 10.1007/s12686-011-9554-9).
- Ley, A. C. & R. Claßen-Bockhoff (2011)** Ontogenetic and phylogenetic diversification in Marantaceae - a review. In Wanntorp, L. & Ronse de Craene, L (eds.) *Flowers on the Tree of Life*, pp. 239-255. Cambridge Univ. Press.
- Ley, A.C. & R. Claßen-Bockhoff (2011)** Evolution in African Marantaceae - Evidence from Phylogenetic, Ecological and Morphological Studies. *Systematic Botany* 36, 2: 277-290.
- Ley, A.C. & O.J. Hardy (2010)** Species delimitation in the Central African herbs *Haumania* (Marantaceae) using georeferenced nuclear and chloroplastic DNA sequences. *Molecular Phylogenetics and Evolution* 57: 859–867.
- Ley, A.C. & R. Claßen-Bockhoff (2010)**. Parallel evolution in plant-pollinator interaction in African Marantaceae. In: X. van der Burgt, J. van der Maesen & J.-M. Onana (eds.) *Systematics and Conservation of African Plants*, pp. 847-854. Royal Botanic Gardens, Kew.
- Pischtschan, E., A.C. Ley & R. Claßen-Bockhoff (2010)** Diversity and systematic value of the hooded staminode in Marantaceae. *Plant Systematics and Evolution* 286: 91-102.

- Ley, A.C. & R. Claßen-Bockhoff (2009)** Pollination syndromes in African Marantaceae. *Annals of Botany* 104: 41-56.
- Ley, A.C. (2008)** *Evolution in African Marantaceae - evidence from floral morphology, ecology and phylogeny*. PhD thesis. Institut für Spezielle Botanik, University of Mainz, Germany.
- Ley, A.C., C. Nowicki, W. Barthlott & P.L. Ibsch (2004)** Biogeography and spatial diversity. In: Vasquez, Ch. R. & P.L. Ibsch (Ed.) *Orchids of Bolivia - Diversity and conservation status Vol.2 Laeliinae, Polystachinae, Sobraliinae with updates of the Pleurothallidinae*. Editorial FAN, Santa Cruz de la Sierra, Bolivia. 492-571.
- Ley, A.C., C. Nowicki & P.L. Ibsch (2004)** Distribution maps of the Pleurothallidinae. In: Vasquez, Ch. R. & P.L. Ibsch (Ed.) *Orchids of Bolivia - Diversity and conservation status Vol.2 Laeliinae, Polystachinae, Sobraliinae with updates of the Pleurothallidinae*. Editorial FAN, Santa Cruz de la Sierra, Bolivia. 402-487.
- Nowicki, C., A.C. Ley, R. Caballero, J.H. Sommer, W. Barthlott & P.L. Ibsch (2004)** *Extrapolating distribution ranges – BIOM 1.1, a computerized bio-climatic model for the extrapolation of species ranges and diversity patterns*. In: Vasquez, Ch. R. & P.L. Ibsch (Ed.) *Orchids of Bolivia - Diversity and conservation status Vol.2 Laeliinae, Polystachinae, Sobraliinae with updates of the Pleurothallidinae*. Editorial FAN, Santa Cruz de la Sierra, Bolivia. 39-68.
- Posters:*
- Ley, A.C., M. Heuertz & O.J. Hardy (2014)** The evolutionary history of central African rain forest plants: phylogeographic insights from sister species of the liana *Haumania* (Marantaceae). iSEQ - Methods and applications of Next Generation Sequencing in evolutionary research, Leipzig, 14 November
- Ley, A.C. & O.J. Hardy (2013)** Phylogeographic studies reveal diverse speciation patterns in central African tropical herbs (Marantaceae). ESEB, Lisbon, 19 - 24 August
- Ley, A.C. & O.J. Hardy (2010)** Species delimitation in Central African herbs combining phylogenetic and phylogeographic approaches – the case of *Haumania* J.Leonard (Marantaceae). Projet IFORA; Colloque final de restitution, Montpellier, 21 - 22 June
- Ley, A.C. & R. Claßen-Bockhoff (2008)** Mechanisms of autogamy in Marantaceae. 10th Annual Meeting of the GfBS and the 18th International Symposium "Biodiversity and Evolutionary Biology" of the DBG, Göttingen, 7 – 11 April
- Ley, A.C., E. Pischtschan & R. Claßen-Bockhoff (2007)** Diversity and ontogeny in the hooded staminode of Marantaceae. Botanikertagung, Hamburg, 3 – 7 September
- Ley, A.C. & R. Claßen-Bockhoff (2006)** Floral ecology and pollination of African Marantaceae. 19th Annual Meeting of the Society for Tropical Ecology, Kaiserslautern, 21 – 24 February

Ley, A.C. & R. Claßen-Bockhoff (2005) Floral diversity of African Marantaceae – XVII. International Botanical Congress, Austria Centre Vienna, 17 - 23 July

Talks:

Ley, A.C. , A. Enang, C. Mbella, R. Ambahe, P. Herzog, S. Lachmuth, M. Mitchell, P. Sesink Clee, K. Gonder (2016) Plants in the face of climate change. CABAlliance Research Symposium, Yaounde, Cameroun, June.

Ley, A.C. & O.J. Hardy (2016) Spatially limited pollen and seed dispersal and clonality in a climber of Central African rain forests: *Haumania danckelmaniana* (Marantaceae) *Society for Tropical Ecology / Gesellschaft fuer Tropenoekologie (GTO)*, Göttingen, Germany, February.

Ley, A.C., G. Dauby, J. Köhler, C. Wypior, M. Röser, & O.J. Hardy (2014) Comparative phylogeography of eight tropical herbs and lianas (Marantaceae) in central African rainforests. *International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)*, Dresden, Germany, March.

Ley, A.C., J. Köhler, C. Wypior, M. Röser & O.J. Hardy (2014) Comparative phylogeography of eight tropical herbs and lianas (Marantaceae) in central African rainforests. *20th congress of AETFAT*, Capetown, Southafrica, January.

Ley, A.C. & O.J. Hardy (2012) Phylogeographic studies reveal diverse speciation patterns in central African tropical herbs (Marantaceae). *21st International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)*, Mainz, Germany, September.

Ley, A.C. & D. Harris (2011) Flower morphological diversity in *Aframomum* - potential for adaptations to different pollinators as driving speciation agents? *25th Meeting of the Scandinavian Association for Pollination Ecologists (SCAPE)*. Vingstedcentret, Denmark, October

Ley, A.C. & O. J. Hardy (2010-2011) The impact of Pleistocene climate change on speciation in Central Africa – the case of *Haumania* (Marantaceae).

- *19th International Symposium "Biodiversity and Evolutionary Biology" of the German Botanical Society (DBG)*, Vienna, Austria, September 2010.
- *Annual meeting "Future of Tropical Biodiversity" of the Society for Tropical Ecology / Gesellschaft fuer Tropenoekologie (GTO)*, Frankfurt, Germany, February 2011.

Ley, A.C. & R. Claßen-Bockhoff (2009) Ontogenetic and phylogenetic diversification of the flower in Marantaceae. *Systematics 2009*, Leiden, Netherlands. August.

Ley, A.C. & R. Claßen-Bockhoff (2006 – 2008) Parallel evolution in plant-pollinator interactions in African Marantaceae.

- Botany 2006, Chico, USA. August 2006
- 17 th International Symposium Biodiversity and Evolutionary Biology, Bonn, Germany, October 2006

- 18th AETFAT Congress in Yaounde, Cameroon. February 2007
- Monocots 2008, Copenhagen, Denmark, August 2008

Ley, A.C., C. Nowicki, W. Barthlott & P.L. Ibisch (2004) Modelling and mapping diversity and endemism patterns of plant species: the example of the orchids of Bolivia. 17 th annual meeting of the Society for Tropical Ecology in Bayreuth, Germany. February

Statement

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Halle, 5. Oktober 2016

Erklärung zur vorgelegten schriftlichen Leistung

Hiermit erkläre ich an Eides statt,

dass ich die vorliegende Habilitationsschrift selbständig und ohne fremde Hilfe verfasst, andere als die angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Unterschrift:

(Alexandra Ley)

Appendix

Appendix 1 Al-Gharaibeh, M.M., Borchsenius, F., McKechnie, L, Sanmartin, I., Ley A.C. (manuscript) Phylogeny of the pantropically distributed family Marantaceae.

Phylogenetic resolution of problematic taxonomic groups can be improved and strengthened by increasing the amount of molecular data and the sampling of ingroup taxa. Here we reassess the phylogeny of the pantropically distributed family Marantaceae compiling a complete genera sampling and using both chloroplast and nuclear markers. Phylogenetic analyses were conducted on a set of four genetic markers (chloroplast markers: *trnL*, *matK*, *rps16* and nuclear marker: ITS) for 187 ingroup taxa representing all 29 Marantaceae genera under Maximum Likelihood (ML), Maximum parsimony (MP) criteria and Bayesian Inference (BI). The resulting tree topology focusing on the resolution of major clades was mostly congruent among applied methods and with preexisting family phylogenies. A few relationships within genera or clades were newly resolved here. A genus, *Monophyllanthe*, added to the phylogeny here for the first time appeared within the *Stachyphrynium* clade as sister to the genus *Marantochloa*. Only the affinity of the genus *Haumania* to one of the other major clades still remained uncertain. Four genera, *Calathea*, *Ischnosiphon*, *Maranta* and *Schumannianthus* were identified as being non-monophyletic. Such a robust phylogeny based on multiple molecular markers from both genomes and a complete sampling of Marantaceae genera will be a solid base to investigate in the future the timing of speciation and the migration events leading to the currently observed biogeographical patterns in this family.

Appendix 2 Ley, A.C., Hardy, O.J. (2016a) Polymorphic microsatellite loci for *Haumania danckelmaniana* and transferability into *H. liebrechtsiana* (Marantaceae). *Applications in Plant Sciences* 4(3): apps.1500116. doi: 10.3732/apps.1500116

- *Premise of the study:* Microsatellite markers were developed for the species *Haumania danckelmaniana* (Marantaceae) from central tropical Africa.
- *Methods and Results:* Microsatellite isolation was performed simultaneously on three different species of Marantaceae through a procedure that combines multiplex microsatellite enrichment and next-generation sequencing. From 80 primers selected for initial screening, 20 markers positively amplified in *H. danckelmaniana*, of which 10 presented unambiguous amplification products within the expected size range and eight were polymorphic with four to nine alleles per locus. Positive transferability with the related species *H. liebrechtsiana* was observed for the same 10 markers.
- *Conclusions:* The polymorphic microsatellite markers are suitable for studies in genetic diversity and structure, mating system, and gene flow in *H. danckelmaniana* and the closely related species *H. liebrechtsiana*.

Appendix 3 Ley, A.C., Hardy, O.J. (2016b) Spatially limited clonality and pollen and seed dispersal in a characteristic climber of Central African rain forests: *Haumania danckelmaniana* (Marantaceae). *Biotropica* 58(5) : 618-627.

Gene dispersal and clonality are important aspects of plant evolution affecting the spatial genetic structure (SGS) and the long-term survival of species. In the tropics these parameters have mostly been investigated in trees and some herbs, but rarely in climbers which frequently: (1) show clonal growth leading to a patchy distribution pattern similar to that of understory herbs; and (2) flower in the canopy where they may have access to long-distance dispersal like canopy trees. We thus hypothesize for climbers an intermediate genetic structure between herbs and trees. The study aims at assessing breeding system and spatial extent of clonality and gene dispersal in *Haumania danckelmaniana* (Marantaceae), a common climber in the tropical rain forests from western Central Africa. In eastern Cameroon, 330 ramets were sampled at three spatial scales and genotyped at seven microsatellite loci. Clonality was moderate (clonal extend: 15–25 m, clonal diversity 0.4–0.65) indicating the importance of recruitment from seeds at this locality. The low inbreeding (FIS) suggested predominant outcrossing. The rate of decay of the relatedness between individuals with

distance indicated limited gene dispersal distance ($rg = 9\text{--}50$ m, neighborhood sizes $N_b = 23\text{--}67$) in accordance with narrowly gravity dispersed seeds and restricted pollen transfer distance in densely flowering populations. The marked SGS ($S_p = 0.011\text{--}0.026$) was similar to that reported in tropical trees, but might increase with augmented clonality as in many herbs, especially under more severe disturbance regimes.

Appendix 4 Ley, A.C., Hardy, O.J. (2010) Species delimitation in the Central African herbs *Haumania* (Marantaceae) using georeferenced nuclear and chloroplastic DNA sequences. *Molecular Phylogenetics and Evolution* 57: 859–867.

Species delimitation is a fundamental biological concept which is frequently discussed and altered to integrate new insights. These revealed that speciation is not a one step phenomenon but an ongoing process and morphological characters alone are not sufficient anymore to properly describe the results of this process. Here we want to assess the degree of speciation in two closely related lianescent taxa from the tropical African genus *Haumania* which display distinct vegetative traits despite a high similarity in reproductive traits and a partial overlap in distribution area which might facilitate gene flow. To this end, we combined phylogenetic and phylogeographic analyses using nuclear (nr) and chloroplast (cp) DNA sequences in comparison to morphological species descriptions. The nuclear dataset unambiguously supports the morphological species concept in *Haumania*. However, the main chloroplastic haplotypes are shared between species and, although a geographic analysis of cpDNA diversity confirms that individuals from the same taxon are more related than individuals from distinct taxa, cp-haplotypes display correlated geographic distributions between species. Hybridization is the most plausible reason for this pattern. A scenario involving speciation in geographic isolation followed by range expansion is outlined. The study highlights the gain of information on the speciation process in *Haumania* by adding georeferenced molecular data to the morphological characteristics. It also shows that nr and cp sequence data might provide different but complementary information, questioning the reliability of the unique use of chloroplast data for species recognition by DNA barcoding.

Appendix 5 Ley, A.C., Hardy, O.J. (2013) Improving AFLP analysis of large-scale patterns of genetic variation - a case study with the Central African lianas *Haumania* spp. (Marantaceae) showing interspecific gene flow. *Molecular Ecology* 22(7): 1984-1997.

AFLP markers are often used to study patterns of population genetic variation and gene flow because they offer a good coverage of the nuclear genome, but the reliability of AFLP scoring is critical. To assess interspecific gene flow in two African rainforest liana species (*Haumania danckelmaniana*, *H. liebrechtsiana*) where previous evidence of chloroplast captures questioned the importance of hybridization and species boundaries, we developed new AFLP markers and a novel approach to select reliable bands from their degree of reproducibility. The latter is based on the estimation of the broad-sense heritability of AFLP phenotypes, an improvement over classical scoring error rates, which showed that the polymorphism of most AFLP bands was affected by a substantial nongenetic component. Therefore, using a quantitative genetics framework, we also modified an existing estimator of pairwise kinship coefficient between individuals correcting for the limited heritability of markers. Bayesian clustering confirms the recognition of the two *Haumania* species. Nevertheless, the decay of the relatedness between individuals of distinct species with geographic distance demonstrates that hybridization affects the nuclear genome. In conclusion, although we showed that AFLP markers might be substantially affected by nongenetic factors, their analysis using the new methods developed considerably advanced our understanding of the pattern of gene flow in our model species.

Appendix 6 Ley, A.C., Hardy, O.J. (2017) Hybridization and asymmetric introgression after secondary contact in two tropical African climber species *Haumania danckelmaniana* and *H. liebrechtsiana* (Marantaceae). *International Journal of Plant Sciences* 178(6): 421–430.

Premise of research. Interspecific hybridization is frequently observed between closely related plant species with overlapping distribution ranges, especially in zones of secondary contact after isolation in different refugia. Although well studied in temperate regions, interspecific hybridization is little documented in tropical regions, where it may be frequent in areas with high species diversity. To bridge this gap, we investigate hybridization in two closely related climber species from the genus *Haumania* (*Haumania danckelmaniana* and *Haumania liebrechtsiana*) originating from Gabon/Cameroon and the Democratic Republic of Congo, respectively, and co-occurring in Northern Gabon. The aim of this study was to genetically quantify and qualify the degree of hybridization between these species in their area of distributional overlap by including for the first time morphological intermediates.

Methodology. We investigated 265 individuals from nine sites (seven sites containing both species) by genotyping seven microsatellites and sequencing one chloroplastic region (trnC-petN1r). Bayesian clustering analysis and simulated genotypes allowed the assignment of sampled genotypes to one or the other species or their intermediates (first-generation hybrid or backcrosses).

Pivotal results. We found 13% and 41% of intermediates on the basis of morphological versus genetic data, respectively, indicating only a partial congruence. There was an equal amount of backcrosses to both parental species (15%). However, a comparison at the local scale of microsatellites and cpDNA sequence data revealed a unidirectional introgression from *H. liebrechtsiana* into *H. danckelmaniana*. At the scale of the nine sites, genetic differentiation was much lower among *H. liebrechtsiana* than among *H. danckelmaniana* populations.

Conclusions. These results are in line with the previously proposed phylogeographic scenario for the genus *Haumania* and the theoretical expectation of chloroplast capture when an expanding species (here *H. liebrechtsiana*) invades the range of a local species (here *H. danckelmaniana*). Because the forest flora of Central Africa has undergone repeated cycles of fragmentation and expansion following past climate changes, we expect that similar scenarios of hybridization after secondary contact may be common.

Appendix 7 Ley, A.C., Hardy, O.J. (2014) Contrasting patterns of gene flow between sister plant species in the understorey of African moist forests - the case of sympatric and parapatric Marantaceae species. *Molecular Phylogenetics and Evolution* 77: 264-274.

Gene flow within and between species is a fundamental process shaping the evolutionary history of taxa. However, the extent of hybridization and reinforcement is little documented in the tropics. Here we explore the pattern of gene flow between three sister species from the herbaceous genus *Marantochloa* (Marantaceae), sympatrically distributed in the understorey of the African rainforest, using data from the chloroplast and nuclear genomes (DNA sequences and AFLP). We found highly contrasting patterns: while there was no evidence of gene flow between *M. congensis* and *M. monophylla*, species identity between *M. monophylla* and *M. incertifolia* was maintained despite considerable gene flow. We hypothesize that *M. incertifolia* originated from an ancient hybridization event between *M. congensis* and *M. monophylla*, considering the current absence of hybridization between the two assumed parent species, the rare presence of shared haplotypes between all three species and the high percentage of haplotypes shared by *M. incertifolia* with each of the two parent species. This example is contrasted with two parapatrically distributed species from the same family in the genus *Haumania* forming a hybrid zone restricted to the area of overlap. This work illustrates the diversity of speciation/introgression patterns that can potentially occur in the flora of tropical Africa.

Appendix 8 Ley, A.C., Heuertz, M., Hardy, O.J. (2016) The evolutionary history of central African rain forest plants: phylogeographic insights from sister species of the climber genus *Haumania* (Marantaceae). *Journal of Biogeography*. doi:10.1111/jbi.12902

Aim We sought to test the refuge hypothesis as a major driver of genetic differentiation to explain the development of distinct phylogeographical domains in tropical Central African rain forest. To address this aim, we studied the phylogeographical pattern of a pair of sister plant species, *Haumania danckelmaniana* and *H. liebrechtsiana* (Marantaceae), distributed across the two phylogeographical domains: Lower Guinea and Congolia.

Location Tropical Central Africa including the phylogeographical domains Lower Guinea and Congolia.

Methods Seven polymorphic microsatellite loci were genotyped in a total of 513 individuals from both species. The dataset was analysed for population genetic structure and demographic change and the results compared to the geographical pattern of plastid DNA sequences from a previous publication and other sympatric species.

Results Based on the microsatellite dataset, three distinct homogeneous genetic clusters were detected in *H. danckelmaniana* and two in *H. liebrechtsiana*. These clusters corresponded geographically with the patterns found in the plastid DNA sequences. Genetic diversity and endemism were unevenly distributed between clusters in the two species. Most clusters exhibited signals of population expansion.

Main conclusions The phylogeographical data based on microsatellites and plastid DNA revealed a complex history involving (1) allopatric differentiation with each species originating in a different phylogeographical domain, potentially resulting from past forest fragmentation, followed by (2) population expansion and interspecific introgression, which probably occurred during a phase of forest expansion. Moreover, phylogeographical patterns within each species provided evidence of (3) past population fragmentation followed by partial population admixture in *H. danckelmaniana*, and deep differentiation between Lower Guinea and Congolia in *H. liebrechtsiana*. The observed patterns were globally consistent with the refuge hypothesis and the presented scenario illustrates evolutionary processes that likely have contributed to shaping the African phylogeographical domains.

Appendix 9 Ley, A.C., Dauby, G., Köhler, J., Wypior, C., Röser, M., Hardy, O.J. (2014) Comparative phylogeography of eight herbs and lianas (Marantaceae) in central African rainforests. *Frontiers in Genetics* 5: article 403. doi: 10.3389/fgene.2014.00403

Vegetation history in tropical Africa is still to date hardly known and the drivers of population differentiation and speciation processes are little documented. It has often been postulated that population fragmentations following climate changes have played a key role in shaping the geographic distribution patterns of genetic diversity and in driving speciation. Here we analyzed phylogeographic patterns (chloroplast-DNA sequences) within and between eight (sister) species of widespread rainforest herbs and lianas from four genera of Marantaceae (*Halopogon*, *Haumania*, *Marantochloa*, *Megaphrynium*), searching for concordant patterns across species and concordance with the Pleistocene refuge hypothesis. Using 1146 plastid DNA sequences sampled across African tropical lowland rainforest, particularly in the Lower Guinean (LG) phylogeographic region, we analyzed intra- and interspecific patterns of genetic diversity, endemism and distinctiveness. Intraspecific patterns of haplotype diversity were concordant among most species as well as with the species-level diversity pattern of Marantaceae. Highest values were found in the hilly areas of Cameroon and Gabon. However, the spatial distribution of endemic haplotypes, an indicator for refuge areas in general, was not congruent across species. Each proposed refuge exhibited high values of endemism for one or a few species indicating their potential role as area of retraction for the respective species only. Thus, evolutionary histories seem to be diverse across species. In fact, areas of high diversity might have been both refuge and/or crossing zone of recolonization routes i.e., secondary contact zone. We hypothesize that retraction of species into

one or the other refuge happened by chance depending on the species' distribution range at the time of climate deterioration. The idiosyncratic patterns found in Marantaceae species are similar to those found among tropical tree species, especially in southern LG.