Mémoire présenté à l'Université de Bordeaux,

Ecole doctorale Sciences et Environnements

Spécialité Ecologie évolutive, fonctionnelle et des communautés

par

Myriam Heuertz

En vue de l'obtention de l'Habilitation à diriger des recherches

Evolutionary processes in non-model tree species and species complexes: inference from natural populations

Membres du jury

Prof. Pilar Catalán, Professeur des Universités, Universidad de Zaragoza

Dr. Thomas Couvreur, Directeur de recherche, IRD, Montpellier

Dr. Antoine Kremer, Directeur de recherche émérité, Biogeco, INRA-Univ. Bordeaux

Dr. Marta Benito Garzón, Chargée de recherche HDR, Biogeco, INRA-Univ. Bordeaux

Dr. Jérôme Chave, Directeur de recherche, EDB, CNRS, Toulouse

Dr. François Lefèvre, Directeur de recherche, INRA URFM, Avignon

Contents

1. Acknowledgements

My life as a researcher has given me the opportunity to meet and work with fantastic colleagues, from a diversity of places and with a diversity of backgrounds. It is clear that I would not have reached the point of completing this report without the collaboration with, and the support from these colleagues, including undergraduate students, PhD students, postdocs and senior colleagues. So here every colleague and collaborator is thanked, right at the start!

At this stage, my most heartfelt thanks go to my mentors for the trust, confidence and support they have given me, and the important roles they have played in my career choices and career development, especially Xavier Vekemans, my PhD thesis supervisor, and Olivier Hardy, with whom I embarked on the African rainforest adventure as a young postdoc. Santiago González-Martínez also belongs to these mentors: despite never having been my boss, his support was decisive for me to reach a level of increasing maturity in my researcher career after my first postdocs. He has also kindly supervised my progress during the writing of this report, and I express my profound gratitude to him, for his support and continued encouragements.

I would also like to take the opportunity to thank all my students, for all the fruitful interactions, stimulating discussions, disagreements, paper rejections we have been through together. Sometimes students are too fast for me… sometimes progress is slow; everybody is unique and special. All these experiences make you learn and grow, eventually! Special thanks go to my PhD students Guillaume Koffi, Katha Budde, Paloma Torroba-Balmori and Sylvain Schmitt, and to the many MSc and PhD students with whom I collaborated across the years. Thanks also to my first postdoc, Sanna Olsson, for always believing in the *Symphonia* project, despite all the difficulties encountered.

My international researcher career would also not have been possible without the welcoming, hosting, quality of human interactions and support I experienced from colleagues, supervisors, heads of units, in all the research institutions I have been working, distributed across six countries, not counting those places where I did research stays: the CRP-Gabriel Lippmann in Luxembourg, the Université Libre de Bruxelles, Uppsala University, INIA-CIFOR in Madrid, Real Jardín Botánico-CSIC in Madrid, the University of Fribourg and INRA UMR Biogeco. Thanks to all my colleagues in these places, who have contributed to building the environment in which it is possible to develop as a scientist.

I would also like to take the opportunity to thank you, who have accepted to serve as evaluators of my HDR report. I know you are busy professionals, and you have accepted this duty nevertheless. I sincerely thank you for having done so, and hope that we will have the opportunity for a good discussion on this report. Many thanks in advance for all your comments and suggestions.

Thanks also to all my friends, especially those eager to share a few km running or some walls climbing. The moments with you have been precious; a welcome escape, essential for balance in life!

Finally, a huge thank you goes to my fantastic family, for always believing in me and creating the conditions in which it is possible to be both a mom and a professional.

Thanks to you all!

2. Curriculum vitae

Myriam Heuertz, Plant population geneticist

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Biographical sketch and research interests

I am a research scientist (CRCN) at INRA, mixed research unit Biogeco – Biodiversity, Genes and Communities – since December 2014. I hold a PhD in Science from the Université Libre de Bruxelles, Belgium (2003) and have postdoctoral experience in Sweden, Luxembourg, Belgium, Switzerland and Spain, where I held a 5-year 'Ramón y Cajal' Independent Researcher position (2010-2014).

My research focuses on ecological population genetics of plants, mostly trees, from temperate and tropical regions. I use molecular tools including high-throughput sequencing to unveil evolutionary processes within species and in communities of closely related species. I investigate species delimitation and population demographic history and use genetic data in conjunction with phenotypes and microenvironmental data to characterize drivers of adaptive evolution.

I have participated in 37 national and international research and development projects funded through competitive calls, in 10 of them as principal investigator. I have published 55 papers in journals included in the Science Citation Index, 24 of them as first or senior author. My H-index is 28 by Google scholar and my ORCiD ID is orcid.org/0000-0002-6322-3645. I have supervised one Bachelor student, seven Master students, two PhD students and two postdocs and I am currently supervising two PhD students and one Master student. I have contributed to the supervision of ten PhD students, leading to co-authorship in publications. I am associate editor of *Molecular Ecology* and *Plant Ecology and Evolution* and recommender for *Peer Community in Evolutionary Biology*.

Education

Université Libre de Bruxelles   Brussels, Belgium
PhD in Biological Sciences, in collaboration with CRP - Gabriel Lippmann,   2003
Luxembourg

Université Libre de Bruxelles   Brussels, Belgium
Diplôme d'Etudes Approfondies in Science   2001

Université Libre de Bruxelles   Brussels, Belgium
Licence in Biological Sciences   1998

Centre Universitaire Luxembourg   Luxembourg
Certificat d'Etudes Scientifiques   1995

Professional appointments

Institut National de Recherche Agronomique, INRA   Cestas, France
Research Scientist (Chargé de Recherche, Catégorie Normale, CRCN), Biogeco,   Since Dec 2014
INRA – Univ. Bordeaux

Instituto Nacional de Investigación Agraria, INIA-CIFOR   Madrid, Spain
Ramón y Cajal Independent Researcher   2010 – 2014
Biodiversity hotspots: evolution and conservation of forest trees

Université de Fribourg   Fribourg, Switzerland
Senior Marie Curie Intra-European Fellow :   Jul 2013 – Aug 2014
Recent evolution in an African biodiversity hotspot

Real Jardín Botánico-CSIC — Madrid, Spain
Postdoc, JAE Doc Program, Ministry of Science and Innovation — Jun – Dec 2009
Incipient speciation in *Antirrhinum charidemi*

Instituto Nacional de Investigación Agraria, INIA-CIFOR — Madrid, Spain
Postdoc, EU-Project NOVELTREE – Adaptive evolution in *Pinus pinaster* — Apr – May 2009

Estudios y Proyectos LINEA — Valladolid, Spain
Biologist, consultant — Jan – Apr 2009

Université Libre de Bruxelles — Brussels, Belgium
Research associate, Belgian National Fund for Scientific Research, FNRS — Oct 2005 – Jan 2009
Phylogeography of African lowland rainforest tree species

CRP-Gabriel Lippmann — Luxembourg, Luxbg
Scientist – Grapevine genetic diversity in countries bordering the Black Sea — Nov 2004 – Oct 2005

Uppsala University — Uppsala, Sweden
Postdoc, Ministry of Science and Education, Luxembourg — Mar 2003 – Oct 2004
Climatic adaptation in Norway spruce

CRP-Gabriel Lippmann — Luxembourg, Luxbg
Internship – Population genetics of common ash — Feb – Mar 2003

CRP-Gabriel Lippmann — Luxembourg, Luxbg
PhD student, Ministry of Science and Education, Luxembourg — Nov 1999 – Oct 2002
Population genetics of common ash: a focus on southeastern Europe

CRP-Gabriel Lippmann — Luxembourg, Luxbg
Project scientist - Population genetics of broadleaves in southeastern Europe — Nov 1998 – Oct 1999

Scientific visits (1 month or longer), grants and awards

Université Libre de Bruxelles — Brussels, Belgium
Collaborateur scientifique mandate, Faculty of Science — Since 2009

Instituto Nacional de Investigación Agraria, INIA-CIFOR — Madrid, Spain
Visiting scientist, grant of Fonds National de la Recherche, Belgium (9 months) — Jan – Sep 2008

University of Yaoundé — Yaoundé, Cameroon
Visiting scientist, grant of Fonds National de la Recherche, Belgium (1 month) — Jul 2007

Centre de Recherches Médicales — Franceville, Gabon
Visiting scientist, grant of Fonds National de la Recherche, Belgium (1 month) — Jul 2006

Royal Botanic Gardens Kew — London, UK,
Visiting scientist, EU SYNTHESYS grant (2 months) — Jan – Feb 2006

University of British Columbia — Vancouver, Canada
Scientific training grant of the Rotary Club Luxembourg (1 month) — May 2000

Consiglio Nazionale delle Ricerche, IMPF — Florence, Italy,
Oct – Nov 2000

Consiglio Nazionale delle Ricerche, IMPF — Florence, Italy
Sep 1999

Grants for the promotion of studies and research, Documentation Centre for Higher Studies (CEDIES), Luxembourg. 1st cycle: 1996, 2nd cycle: 1998 (2000€), 3rd cycle: 2003 (6000€).

Research projects granted and participation in research projects

37. *RARETREE* – Eco-evolutionary implications of commonness vs. rarity in Amazonian tree congeners. Labex CEBA (ANR [Agence Nationale pour la Recherche], France), annual project. 2019-2020. 15,000€. **PI: Myriam Heuertz**.

36. *G-BIKE – Genomic BIodiversity Knowledge for Resilient Ecosystems*. EU COST Action CA18134, March 2019 – March 2023. PI: Cristiano Vernesi. **Leader of WG3 on Genomics and ecosystem services: Myriam Heuertz.**

35. *LOCOCAP* – Contribution to the development of low-cost sequence capture to study eco-evolutionary processes. 2019. Projet innovant INRA, Dept. EFPA. 9,800 €. **PI: Myriam Heuertz**.

34. *ASH-ADAPT* – Evolutionary potential of natural *Fraxinus excelsior* populations challenged by novel pests and pathogens. 2019-2021. PI: Lene Rostgaard Nielsen, Univ. Copenhagen. 5,000,000 DEK.

33. *RHYVON* - The role of natural hybridization in plant evolution: bridging the gap between theoretical models and empirical data. Plan Nacional I+D+I, Spain, 2018-2021. PI: Gonzalo Nieto Feliner.

32. *LECYTOMICS* - Species boundaries and ecological genomics in the face of global change in the Brazil nut family (Lecythidaceae), GUYAMAZON program for France – Brazil collaborative research and training, 2019 – 2020. 25.000 €, **PI: Myriam Heuertz**.

31. *ADAPTSHARE* – Drought stress in tropical ecosystems: adaptive allele sharing in tree species complexes. Idex Univ. Bordeaux. Sep. 2016 – Aug. 2018. Two-year postdoctoral contract, incl. research funds of 20,000€. PI: Katharina B. Budde, **host: Myriam Heuertz**.

30. *ECOGENPIN* – Ecological genomics of local adaptation in maritime pine, an iconic conifer with large ecological and economic value in south-western Europe. Chaire d'installation Idex Univ. Bordeaux. Oct. 2016 – Oct. 2019. 198,249 €. PI: Santiago González-Martínez.

29. *COLLEVOL* – Testing Collective Evolution in Neotropical Trees. Labex CEBA (ANR [Agence Nationale pour la Recherche], France), annual project. 2016-2017. 20,000€. **PI: Myriam Heuertz**.

28. *NEOTROPHYL* – Inferring the drivers of Neotropical diversification using an integrative macroevolutionary approach. Labex CEBA (ANR, France), strategic project. 2016-2019. 250,000 € (20,000 € to INRA UMR Biogeco). PI: Frédéric Delsuc. Participants: ISEM, EDB, CNRS Guyane, INRA-BioGeCo.

27. *REVOL* – Spatially explicit mechanisms of Recent EVOLution in an African biodiversity hotspot. European Commission, FP7-PEOPLE-2012-IEF, Marie Curie Action. Jul. 2013 – Aug. 2014. 149,546€. **PI: Myriam Heuertz**. Hosted at the University of Fribourg, Switzerland, host: Christian Lexer.

26. *AFFLORA – The assembly of African floras from lineages to populations: effects of historical climate changes and species ecology.* Plan Nacional de I+D+I, Spain. Jan. 2013 – Dec. 2015. 193,050€. **PI: Myriam Heuertz**. Participants: Real Jardín Botánico-CSIC Madrid, INIA-CIFOR, ULB Brussels, INRA, Université de Yaoundé 1, CNARP Madagascar.

25. *TIPTREE – Scenarios for forest biodiversity dynamics under global change in Europe: identifying micro-evolutionary scale tipping points.* ERAnet BiodivERsA-2. 2012-2015. 35,000€ to INIA-CIFOR. PI: Sylvie Oddou-Muratorio. Participants: INRA-URFM Avignon, Akdeniz University Antalya, INIA-CIFOR, European Forest Institute, ONF Fontainebleau, CNRS-UM Montpellier, INRA-BioGeCo Bordeaux, INRA-Ecofog Kourou, University of Marburg, Uppsala University, CNR-IGV Florence, Fed. of Swedish Forest Owners.

24. *FLAG – Génétique écologique des arbres forestiers : interactions entre flux de gènes et variabilité environnementale dans la détermination de l'adaptation locale et du potentiel d'adaptation.* ANR, France. 2012-2015. 306,065€. PI: Ivan Scotti. **Coordinator for INIA-CIFOR: Myriam Heuertz**. Participants: INRA-Ecofog Kourou, INRA-URFM Avignon, INRA-AGPF Orléans, Universidade Federal de Goiás Brazil, INRA-BioGeCo Bordeaux, CNR-IGV Florence.

23. *Ash dieback in Norway – Cause, Impact and Control*. The Research Council of Norway. 2011 – 2014. 5,050,000 NOK, 19,850€ to INIA-CIFOR. PI: Halvor Solheim. **Coordinator for INIA-CIFOR: Myriam Heuertz**. Participants: Norwegian forest and landscape institute, INIA-CIFOR, Swedish University of Agricultural Sciences, Skov & landskap (Denmark).

22. *Evolution of the personate flower: phylogenetic and phylogeographic analyses of the tribe Antirrhineae.* Plan Nacional de I+D+i, Spain. Jan. 2010 – Dec. 2012. 84,700€. PI: Pablo Vargas. Participants: Real Jardín Botánico de Madrid, INIA-CIFOR, John Innes Centre (Edinburg, UK).

21. *FLUGAL – Interaction between gene flow and local adaptation in forest tree species: new analytical and experimental approximations.* Plan Nacional de I+D+i, Spain. Jan. 2010 – Dec. 2012. 207,757€. PI: Juan José Robledo Arnuncio. Participants: INIA-CIFOR.

20. *Biodiversity hotspots: evolution and conservation of forest trees.* Funding: Ministerio de Ciencia e Innovación, Ramón y Cajal project. Jan. 2010 – Dec. 2014. 10,000€. **PI: Myriam Heuertz**. Hosted at INIA-CIFOR.

19. *LINKTREE – Linking genetic variability with ecological responses to environmental changes: forest trees as model systems.* Funding: BiodivERsA-ERANET, Jun. 2009 – Jun. 2012. 1,359,625€. PI: Santiago González-Martínez. Participants: INIA-CIFOR, INRA-Bordeaux, INRA-Avignon, Uppsala University, Universität Marburg.

18. *VAMPIRO – Efectos demográficos y selectivos del fuego en plantas Mediterráneas: variación molecular y pirofitismo*. Ministerio de Ciencia e Innovación, Jan. 2009 – Dec. 2011. 181,379€. PI: Santiago González-Martínez. Participants: INIA-CIFOR, Universidad de Cádiz, CIDE – CSIC, Univ. Libre de Bruxelles.

17. *Understanding and conserving plant biodiversity across tropical forest biomes: a multi-level phylogenetic approach based on DNA sequences*. French National Sequencing Centre Génoscope. 2008 – 2009. 370,000€. PI: Doyle McKey. Participants: Université Libre de Bruxelles, Université Paul Sabatier Toulouse, CNRS Montpellier.

16. *Phylogeography and genetic diversity of African lowland rainforest trees: impact of Pleistocene climatic changes.* Fonds National pour la Recherche Scientifique (FNRS) and Fonds pour la Recherche Fondamentale Collective (FRFC), Belgium. Oct. 2007 – Sep. 2009. 10,500€. **PI: Myriam Heuertz.** Université Libre de Bruxelles.

15. *Développement de la plateforme de génétique moléculaire du Département de Biologie des Organismes: acquisition d'équipements.* Fonds d'Encouragement à la Recherche (FER), Université Libre de Bruxelles, Belgium. 2007 – 2008. 20,595€. **PIs: Serge Aron/Myriam Heuertz**. Université Libre de Bruxelles.

14. *IFORA – Les îles forestières africaines : modèle d'une nouvelle approche de la dynamique de structuration de la biodiversité*. ANR, France. 2006 – 2009. 687.920€. PI: Michel Veuille. Participants: UMR 5202, CNRS-MNHN Paris + EPHE, CBGP Montpellier, Forêts naturelles CIRAD, Univ Montpellier 2-CEFE UMR 5175, IRD – UR 072 Gif, Probabilités UMR 7599 INRA, UMR 1272 CNRS LSCE Gif, Université Libre de Bruxelles, Herbier National Yaoundé (Cameroon), Université Yaoundé 1 (Cameroon), IRET Makokou (Gabon).

13. *Genetic structure, gene flow and scale of local adaptation in tropical trees from Central Africa – What is the relative importance of selection and gene dispersal on genetic diversity organization?* Fonds National de la Recherche Scientifique, Belgium, and Fonds pour la Recherche Fondamentale Collective (FRFC). 2007 – 2009. 100.000€.   PIs: Olivier Hardy/Jean-Louis Doucet. Participants: Université Libre de Bruxelles, Faculté des Sciences Agronomiques de Gembloux (Belgium).

12. *Diversidad y conservación de recursos genéticos de coníferas en la Península Ibérica y Marruecos*. Agencia Española de Cooperación Internacional – PCI. Jan. 2006 – Dec. 2006. 8.400€. PI: Santiago C. González Martínez. Participants: INIA-CIFOR; Université Cadi Ayyad, Morocco.

11. *Phylogéographie et diversité génétique des ligneux des forêts de plaine d'Afrique centrale.* Fonds National de la Recherche Scientifique, Belgium. 2005 – 2007. 30.000€. PI: Olivier Hardy. Participants: Université Libre de Bruxelles (Belgium).

10. *Phylogéographie et diversité génétique des ligneux des forêts de plaine d'Afrique centrale.* Fonds National de la Recherche Scientifique, Belgium. 2005 – 2008. Three year research associate contract plus research funding of 10,000€. **PI: Myriam Heuertz**. Participants: Université Libre de Bruxelles, Belgium.

9. *Plant genetic resources in southeastern Europe III: grapevine genetic resources*: Luxembourg Ministry of Foreign Affairs and International Plant Genetic Resources Institute (IPGRI). 2004-2006. PI: Jean François Hausman. Participants: Institute of Horticulture, Viticulture and Oenology, Georgia; IPGRI, Italy; Università degli studi di Milano, Italy; Institute of Viticulture and Wine-making, Azerbaijan; Magarach Institute for Vine and Wine, Ukraine; National Institute for Viticulture and Oenology, Moldova; CRP-GL, Luxembourg; Armenian Academy of Viticulture; Federal Centre for Breeding Research on Cultivated Plants, Germany; Krymsky Experimental Breeding Station, Russia; Kuban State Agrarian University, Russia.

8. *Genetic basis of budset in Norway spruce.* Carl Tryggers Foundation, Sweden. 2005 – 2006. 378.000 SEK. PI: Niclas Gyllenstrand. Participants: Uppsala University, Swedish University of Agricultural Sciences.

7. *Cooperation INRA-FORMAS (Institut Nacional de la Recherche Agronomique, France - Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning).* Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning. 2004 – 2005. 100,000 SEK. PI: Martin Lascoux. Participants: Uppsala University, Sweden; INRA, France.

6. *Range shift and adaptive response in Scandinavian Picea abies populations*. Philip Sörensen Foundation, Sweden. 2004 – 2006. 150.000 SEK. PI: Martin Lascoux. Participants: Uppsala University.

5. *TREESNIPS – Developing Single Nucleotide Polymorphic (SNP) Markers for Adaptive Variation in Forest Trees.* European Union, 2002 – 2006. QLK3-CT2002-01973. 1,957,622€. PI: Outi Savolainen. Participants: University

of Oulu and Finnish Forest Research Institute, Finland; INRA, France; UNIUD, Italy; INIA, Spain; University of Uppsala, Sweden; SCRI, United Kingdom.

4. *Plant genetic resources in southeastern Europe II: forest genetic resources and grapevine genetic resources.* Luxembourg Ministry of Foreign Affairs and International Plant Genetic Resources Institute (IPGRI). 2001 – 2003. PI: Jean François Hausman. Participants: Forest Research Institute Bulgaria; SICAS, Romania; University of Chisinau, Moldova; Ukrainian Research Institute of Forestry and Forest Imporovement, Ukraine; CRP-GL Luxembourg.

3. *FRENE – Contribution to the conservation of forest genetic resources: study of the genetic diversity of the common ash*. Ministère de l'Education Nationale, Luxembourg. 1999 – 2002. 20,000€. **PI: Myriam Heuertz**. Participants: Université Libre de Bruxelles, Belgium; CRP-Gabriel Lippmann, Luxembourg.

2. *CYTOFOR – Measuring molecular differentiation of European deciduous forests for conservation and management.* European Union, Agriculture and Fisheries (FAIR) specific RTD programme, CT97-3795. 1998 – 2001. PI: Rémy Petit. Participants: INRA, France; University of Edinburgh, United Kingdom; Office National des Forêts Ardon, France; UDESAM Marseille, France; Uppsala University, Sweden; LMU-Muenchen, Germany); CNR Firenze, Italy; CNR Porano, Italy.

1. *Plant genetic resources in southeastern Europe: forest genetic resources.* Luxembourg Ministry of Foreign Affairs and International Plant Genetic Resources Institute (IPGRI). 1998 – 2000. PI: Lucien Hoffmann. Participants: Forest Research Institute Bulgaria; SICAS, Romania; University of Chisinau, Moldova; CRP-GL Luxembourg.

Publications

Publications in journals included in the Science Citation Index

55. Aissi A, Beghami Y, **Heuertz M** (2019) Le chêne faginé (*Quercus faginea*, Fagaceae) en Algérie : potentiel germinatif et variabilité morphologique des glands et des semis. *Plant Ecology and Evolution* (in press).

54. Vargas OM, **Heuertz M**, Smith SA, Dick CW (2019) Target sequence capture in the Brazil nut family (Lecythidaceae): Marker selection and in silico capture from genome skimming data. *Molecular Phylogenetics and Evolution*, 135: 98–104.

53. Goncalves AL, García M, **Heuertz M**, González-Martínez SC (2019) Demographic history and spatial genetic structure in a remnant population of the subtropical tree *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul (Fabaceae). *Annals of Forest Science* 76, 18.

52. Mairal M, Caujapé-Castells J, Pellissier L, Jaén-Molina R, Álvarez N, **Heuertz M**, Sanmartín I (2018) A tale of two forests: ongoing aridification drives population decline and genetic diversity loss at continental scale in Afro-Macaronesian evergreen-forest archipelago endemics. *Annals of Botany* 122, 1005–1017.

51. Torroba-Balmori MP, Budde KB, Heer K, González-Martínez SC, Olsson S, Scotti-Saintagne C, Casalis M, Sonké B, Dick CW, **Heuertz M** (2017) Altitudinal gradients, biogeographic history and microhabitat adaptation affect fine-scale spatial genetic structure in African and Neotropical populations of an ancient tropical tree species. *PLOS ONE* 12(8), e0182515.

50. Nieto Feliner G, Álvarez Fernández I, Fuertes Aguilar J, **Heuertz M**, Marques I, Moharrek F, Piñeiro R, Riina R, Rosselló JA, Soltis P, Villa Machío I (2017) Is homoploid hybrid speciation that rare? An empiricist's view. *Heredity* 118, 513–516.

49. Holliday JA, Aitken SN, Cooke JEK, Fady B, González-Martínez SC, **Heuertz M**, Jaramillo-Correa J-P, Lexer C, Staton M, Whetten RW, Plomion C (2017) Advances in ecological genomics in forest trees and applications to genetic resources conservation and breeding. *Molecular Ecology* 26(3), 706–717.

48. Budde KB, González-Martínez SC, Navascués M, Burgarella C, Hernández-Serrano A, Lorenzo Z, Zabal-Aguirre M, Vendramin GG, Verdú M, Pausas JG, **Heuertz M** (2017) Increased fire frequency promotes stronger spatial genetic structure and natural selection at regional and local scales in *Pinus halepensis* Mill. *Annals of Botany* 119(6), 1061–1072.

47. Tollefsrud MM, Myking T, Sønstebø JH, Hietala A, **Heuertz M** (2016) Genetic structure in the northern range margins of common ash, *Fraxinus excelsior* L. *PLOS ONE* 11, e0167104.

46. Forrest A, Escudero M, **Heuertz M**, Wilson Y, Cano E, Vargas P (2017) Testing the hypothesis of low genetic diversity and population structure in narrow endemic species: the endangered *Antirrhinum charidemi* (Plantaginaceae). *Botanical Journal of the Linnean Society* 183(2), 260–270.

45. Ley A, **Heuertz M**, Hardy OJ (2017) The evolutionary history of central African rain forest plants: phylogeographical insights from sister species in the climber genus Haumania (Marantaceae). *Journal of Biogeography* 44(2), 308–321.

44. Olsson S, Seoane-Zonjic P, Bautista R, Claros MG, González-Martínez SC, Scotti I, Scotti-Saintagne C, Hardy OJ, **Heuertz M** (2017) Development of genomic tools in a widespread tropical tree, *Symphonia globulifera* L.f.: a new low-coverage draft genome, SNP and SSR markers. *Molecular Ecology Resources* 17(4), 614–630.

43. Macaya-Sanz D, **Heuertz M**, Lindtke D, Lexer C, González-Martínez SC (2016) Causes and consequences of large clonal assemblies in a poplar hybrid zone. *Molecular Ecology* 25, 5330–5344.

42. Ribeiro P, Souza ML, Muller LAC, Ellis VA, **Heuertz M**, Lemos-Filho JP, Lovato MB (2016) Climatic drivers of leaf traits and genetic divergence in the tree *Annona crassiflora*: a broad spatial survey in the Brazilian savanna. *Global Change Biology* 22(11), 3789–3803.

41. Correa Ribeiro P, Lemos-Filho JP, Buzatti RS, Lovato MB, **Heuertz M** (2016) Species-specific phylogeographic patterns and Pleistocene east-west divergence in Annona (Annonaceae) trees in the Brazilian Cerrado. *Botanical Journal of the Linnean Society* 181, 21–36.

40. Ojeda F, Budde KB, **Heuertz M**, Segarra-Moragues JG, González Martínez SC (2016) Biogeography and evolution of seeder and resprouter forms of *Erica coccinea* (Ericaceae) in the fire-prone Cape fynbos. Plant Ecology 217, 751–761.

39. Unger GM, **Heuertz M**, Vendramin GG, Robledo-Arnuncio JJ. 2016. Assessing early fitness consequences of exotic gene flow in the wild: a field study with Iberian pine relicts. *Evolutionary Applications* 9(2), 367–80.

38. Pokorny L, Riina R, Mairal M, Culshaw V, Meseguer AS, Serrano M, Carbajal R, Ortiz S, **Heuertz M**, Sanmartin I. 2015. Living on the edge: timing of Rand Flora disjunctions congruent with ongoing aridification in Africa. *Frontiers in Genetics* 6, 154.

37. Plomion C, Bastien C, Bogeat-Triboulot M-B, Bouffier L, Déjardin A, Duplessis S, Fady B, **Heuertz M**, Le Gac A-L, Le Provost G, Legué V, Lelu-Walter A-M, Leplé J-C, Maury S, Morel A, Oddou-Muratorio S, Pilate G, Sanchez L, Scotti I, Scotti-Saintagne C, Segura V, Trontin J-F, Vacher C (2016) Forest tree genomics: 10 achievements from the past 10 years and future prospects. *Annals of Forest Science* 73, 77–103.

36. Jaramillo-Correa JP, Grivet D, Lepoittevin C, Sebastiani F, **Heuertz M**, Garnier-Géré P, Alía R, Plomion C, Vendramin GG, González-Martínez SC (2015) Molecular proxies of climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics* 199(3), 793–807.

35. Lexer C, Wüest R, Mangili S, **Heuertz M**, Stölting KN, Pearman P, Forest F, Salamin N, Zimmermann N, Bossolini E (2014) Genomics of the speciation continuum in an African plant biodiversity hotspot I: drivers of population divergence in *Restio capensis* (Restionaceae). *Molecular Ecology* 23, 4373–4386.

34. Källman T, De Mita S, Larsson H, Gyllenstrand N, **Heuertz M**, Parducci L, Suyama Y, Lagercrantz U, Lascoux M (2014) Patterns of nucleotide diversity at photoperiod related genes in Norway spruce [*Picea abies* L. (Karst)], *PLOS ONE* 9, e95306.

33. Dauby G, Duminil J, **Heuertz M**, Koffi KG, Stévart T, Hardy OJ (2014) Congruent phylogeographic patterns of eight tree species in Atlantic Central Africa provide insights on the past dynamics of forest cover. *Molecular Ecology* 23, 2299–2312.

32. Budde KB, **Heuertz M**, Hernández-Serrano A, Pausas JG, Vendramin GG, Verdú M, González-Martínez SC (2014). *In situ* genetic association for fire serotiny, a fire-related trait, in Mediterranean maritime pine (*Pinus pinaster* Aiton). *New Phytologist* 201, 230–241.

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3. Dauby G., Duminil J, **Heuertz M**, Hardy OJ (2010) Chloroplast DNA polymorphism and phylogeography of a Central African tree species widespread in mature rainforests: Greenwayodendron suaveolens (Annonaceae). *Tropical Plant Biology* 3, 4–13.

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Conference contributions and seminars

Participation in conferences

Heuertz M, Jehanne Q, Budde KB, Caron H, Tysklind N, Scotti-Saintagne C, Guichoux E, Molino JF, Sabatier D, El Mujtar V, Franc A, Garnier-Géré P, Mariette S. **Poster**. Evolutionary history of tropical tree species complexes: species delimitation and adaptive genetic variation in the Brazil nut clade (Lecythidaceae). Evolution, II Joint Congress on Evolutionary Biology – Montpellier, August 2018.

Schmitt S, **Heuertz M**. Tysklind N, Hérault B. Ecological genomics of niche exploitation and individual performance in tropical forest trees. **Poster**. European Conference of Tropical Ecology, GTOE18, Paris 26-29 March 2018.

Budde KB, Olsson S, Torroba-Balmori P, González-Martínez SC, Loureiro J, Rakotonandrasana S, Rakotondrafara A, Sanmartín I, **Heuertz M**. **Talk**. Macro- and microevolutionary processes in the genus *Symphonia* (Clusiaceae) in Madagascar: species delimitation, hybridization and species habitat associations. European Conference of Tropical Ecology, GTOE18, Paris 26-29 March 2018.

Heuertz M, Jehanne Q, Budde KB, Caron H, Tysklind N, Scotti-Saintagne C, Guichoux E, Molino JF, Sabatier D, El Mujtar V, Franc A, Garnier-Géré P, Mariette S. **Talk**. Evolutionary history of tropical tree species complexes: species delimitation and adaptive genetic variation in the Brazil nut clade (Lecythidaceae). European Conference of Tropical Ecology, GTOE18, Paris 26-29 March 2018.

Heuertz M, Olsson S, Budde KB, Torroba-Balmori P, González-Martínez SC, Rakotonandrasana, SR, Rakotondrafara A, Sanmartín I. Species delimitation, hybridization and patterns of adaptive genetic variation in the tropical tree genus Symphonia (Clusiaceae) in Madagascar. **Talk**. XXIth AETFAT Congress, Nairobi, May 15-19, 2017.

Palma-Silva C, Leal BSS, Araujo VR, Chaves CJN, Huacre LAP, **Heuertz M.** Phylogeography, population genomics and adaptive variation on species complex Pitcairnia lanuginosa (Bromeliaceae). **Poster**. Evolution 2017, Portland, Oregon, USA, May 2017.

Heuertz M, Jehanne Q, Budde KB, Caron H, El Mujtar V, Franc A, Garnier-Géré P, Guichoux E, Scotti-Saintagne C, Molino JF, Sabatier D, Mariette S. Hybridization and patterns of adaptive genetic variation in tropical trees of the Bertholletia clade (Lecythidaceae) in French Guiana. **Talk**. Meeting of the German Society for Tropical Ecology, GTOE17, Brussels, February 6-10, 2017.

Olsson A, Budde K, Torroba-Balmori MP, Rakotonandrasana S, Rakotondrafara A, Sanmartin S, **Heuertz M**. Poster. Hybridization and species delimitation of Symphonia (Clusiaceaea) in Madagascar. **Poster**. Meeting of the German Society for Tropical Ecology, GTOE17, Brussels, February 6-10, 2017.

Heuertz M, Olsson S, Torroba-Balmori P, Budde K, Rakotonandrasana S, Rakotondrafara A, Caron H, Scotti-Saintagne C, Mariette S, Franc A, Jehanne Q, Sanmartín I. Hybridization and the evolution of tropical tree species complexes. **Talk**. Annual Meeting of the Excellence Laboratory "Centre for the study of biodiversity in Amazonia", Cayenne, French Guiana, Oct 2016.

Heuertz M, Olsson S, Torroba-Balmori P, Rakotonandrasana S, Rakotondrafara A, Béatrix M, Caron H, Scotti-Saintagne C, Mariette S, Franc A, Jehanne Q, Sanmartín I. 2016. **Talk**. Hybridization and the evolution of tropical tree species. ATBC Annual Meeting, Montpellier, June 2016.

Torroba-Balmoria P, Parchman TL, Budde KB, Heer K, Santiago C. González-Martínez SC, Scotti-Saintagne C, Dick CW, **Heuertz M**. 2016. **Talk**. Demography at different scales in the wide-spread tropical species *Symphonia globulifera* (Clusiaceae). IUFRO Genomics and Forest Tree Genetics Conference, Arcachon, France, May 2016.

Lexer C, Bossolini E, Caseys C, **Heuertz M**, Kessler M, Lindtke D, Paris M, Pearman PP, Salamin N, Stölting KN, Wüest RO, Zimmermann NE. 2015. **Talk**. Speciation Genomics in Plants: Divergence Continuum and Beyond. International Plant and Animal Genome Conference XXIII 2015.

Olsson S, Bautista R, Claros MG, Scotti-Saintagne C, Scotti I, Rakotonandrasana RS, Rakotondrafara A, Gonzáelz-Martínez SC, **Heuertz M**. **Talk**. Studying the evolution of tropical tree taxa: opportunities and limitations of genomic approaches. Joint meeting between the British Ecological Society and the Société Française d'Ecologie, Lille, December 9-12, 2014.

Heuertz M. 2014. Phylogeography of tropical trees. **Talk**. Annual Meeting of the Excellence Laboratory "Centre for the study of biodiversity in Amazonia", Toulouse, Oct 2014.

Ley A, **Heuertz M**, Hardy OJ. 2014. The evolutionary history of central African rain forest plants: phylogeographic insights from sister species of the liana Haumania (Marantaceae). **Talk**. iSEQ - Methods and applications of

Next Generation Sequencing in evolutionary research, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, November 13-14, 2014.

González-Martínez SC, Grivet D, Ruiz-Daniels R, Jaramillo-Correa JP, Lorenzo Z, Budde K, Rodríguez-Quilón I, **Heuertz M**, Alía R, Vendramin GG. 2014. **Talk**. Molecular signatures of climate adaptation and range expansions in Mediterranean conifers. IUFRO Forest Tree Breeding Conference, August 25-29, Prague, Czech Republic.

Heuertz M, Budde KB, Heer K, Torroba-Balmori MP, Dick CW, Hardy OJ, Scotti I. 2014. Evolutionary history in the genus Symphonia. **Talk.** XXth AETFAT Congress Stellenbosch, South African Republic. Scripta Botanica Belgica 52, 187.

Lexer C, **Heuertz M**, Mangili S, Bossolini E, Wüest R. ´Next generation´ biogeography: towards understanding the drivers of diversification in radiations of African plants. **Talk.** XXth AETFAT Congress Stellenbosch, South African Republic. Scripta Botanica Belgica 52.

Berthouzoz M, **Heuertz M**, Lexer C. 2014. Microevolutionary drivers of species diversification and persistence in Cape Proteas. **Talk.** XXth AETFAT Congress Stellenbosch, South African Republic. Scripta Botanica Belgica 52.

Dauby G, Duminil J, **Heuertz M**, Koffi G, Stévart T, Hardy OJ. 2014.Congruent phylogeographic patterns of eight tree species in Atlantic Central Africa provide insights on the past dynamics of forest cover. **Talk.** XXth AETFAT Congress Stellenbosch, South African Republic. Scripta Botanica Belgica 52.

Correa Ribeiro P, Carvalho Areal Miller L, Pires de Lemos-Filho J, Lovato MB, **Heuertz M**. 2013. Comparative phylogeography of Annona (Annonaceae) species from the Brazilian Cerrado (savannah). Poster. Workshop: Conservation biology in the 21st century. Fribourg, Switzerland, September 5-6, 2013.

Heuertz M, Budde KB, Heer K, Bautista R, Dick CW, González-Martínez SC, Hardy OJ. 2012. Phylogeography and the geographic scale of adaptation in a widespread rainforest tree, *Symphonia globulifera* (Clusiaceae). 1st Annual meeting of Plant Ecology and Evolution. **Talk**. Meise, Belgium, November 2012.

Budde KB, **Heuertz M**, González-Martínez SC. 2012. Demographic and selective effects of fire in Mediterranean pines: Molecular Variation and Pyrophytism. First Joint Congress on Evolution. Talk. Convention Centre Ottawa, Canada, July 6-10.

Heuertz M, Kovacik I, González-Martínez SC, Alía R. 2012. Genetic structure of mountain pines (*Pinus mugo* complex) and risk of genetic introgression from Scots pine in southern marginal populations. **Talk**. 1st AFORGEN (Alpine Forest Genetics group) workshop, Rifugio Collini, Adamello Brenta National Park, June 25-26. Italy.

Heuertz M, Budde KB, Heer K, Hardy OJ. Evolutionary history of *Symphonia globulifera* (Clusiaceae). **Poster**. Symposium: The impact of a major environmental crisis on species, populations and communities: the fragmentation of African forests at the end of the Holocene". Académie des Sciences – Paris, Fondation Simone et Cino del Duca, Paris, March 2012.

Hardy OJ, Budde KB, Daïnou K, Dauby G, DeboutvG, Duminil J, Ewedje EE, **Heuertz M**, Koffi GK, Ley A. 2012. Talk. Phylogeography of guineo-congolian forest plants: a first synthesis. Symposium: The impact of a major environmental crisis on species, populations and communities: the fragmentation of African forests at the end of the Holocene". Académie des Sciences – Paris, Fondation Simone et Cino del Duca, Paris, March 2012.

González-Martínez SC, **Heuertz M**, Grivet D, Voltas J, Jaramillo-Correa JP, Climent J, Vendramin GG, Alía R. 2012. Testing geographical genetic clines and environmental associations in Mediterranean pines. **Talk**. Adaptive Landscape Genomics meeting, Neuchâtel, Switzerland, February 2012.

Heuertz M, Budde KB, Hardy OJ, Dick CW. 2011. Transatlantic species cohesion since the mid-Miocene in the ancient tropical tree *Symphonia globulifera* (Clusiaceae). **Talk**. Evolution 2011, University of Oklahoma, OK, USA, July 2011.

Heuertz M, Forrest A, Cano E, Vargas P. 2011. Microevolution in critically-endangered plants: high genetic diversity in the narrow endemic Antirrhinum charidemi. **Talk**. ECOFLOR meeting, Toledo, Spain, 2-4 February.

Koffi G, **Heuertz M**, Hardy O. Intraspecfic diversity of cpDNA sequences of an African tropical forest tree: Santiria trimera (Burseraceae). **Poster** presented at the IXth AETFAT Congress, Antananarivo, Madagascar, 2010. Scripta Botanica Belgica 46: 245.

Duminil J, **Heuertz M**, Hardy OJ. Species delimitation and phylogeography of a complex of African tropical tree species. **Talk** presented at the IXth AETFAT Congress, Antananarivo, Madagascar, 2010. Scripta Botanica Belgica 46: 157.

Heuertz M, Savolainen V, Budde K, Daïnou K, Duminil J, Koffi GK and Hardy OJ. Can Pleistocene refuge theory explain within-species patterns of genetic diversity in African lowland rainforest trees? **Talk** presented at the IXth AETFAT Congress, Antananarivo, Madagascar, 2010. Scripta Botanica Belgica 46: 212.

Albaladejo RG, González-Martínez SC, **Heuertz M**, Vendramin GG, Aparicio A. Spatiotemporal mating pattern variationin the wind-pollinated Mediterranean shrub *Pistacia lentiscus* L. **Poster** presented at the IX Congreso de la Asociación Española de Ecología Terrestre en Úbeda (Jaén), Spain, 2009.

Heuertz M, Savolainen V, Budde K, Daïnou K, Duminil J, Koffi GK and Hardy OJ. Can Pleistocene refuge theory explain within-species patterns of genetic diversity in African lowland rainforest trees? **Talk** presented at the ATBC-GTÖ Conference, Marburg, Germany, 2009.

Budde K, González-Martínez SC, Dick CW, Hardy OJ, **Heuertz M**. Genetic structure of *Symphonia globulifera* at different geographical scales. **Poster** presented at the ATBC-GTÖ Conference, Marburg, Germany, 2009.

Duminil J, **Heuertz M**, Hardy OJ. Species delimitation and phylogeography of a complex of African tropical tree species. **Poster** presented at the ATBC-GTÖ Conference, Marburg, Germany, 2009.

Parmentier I, Dauby G, Debout G, Duminil J, Ewedje E, **Heuertz M**, Koffi G, Lachenaud O & Hardy OJ. **Talk** at the Syposium of African Botany in Brussels: Phytogeography and spatial organisation of diversity in tropical Africa: uncertainties, progress and perspectives. 2009.

De-Lucas AI, González-Martínez SC, Hidalgo E, Bravo F, **Heuertz M**. Origen del pino negral en la Meseta Castellana: ¿Mezcla de los gene pools atlántico y mediterráneo o resultado de una rápida colonización a partir del este Peninsular? **Talk** presented at the V Congreso Forestal Español, Avila, Spain, 2009

Heuertz M, Koffi G, Savolainen V, Hardy O. Nucleotide diversity and demographic history of African lowland rainforest tree species. **Talk** presented at Conférence Jacques Monod, Génomique Evolutive. Roscoff, France, 2007.

Heuertz M, Koffi G, Savolainen V, Hardy O. Phylogeography of African lowland rainforest trees. **Talk** presented at XVIII AETFAT Congress (Assosciation pour l'Etude de la Flore d'Afrique Tropicale), Yaoundé, Cameroon, 2007.

Heuertz M, Koffi G, Savolainen V, Hardy O. Phylogeography of African lowland rainforest trees. **Talk**, presented at Young Botanists' Day, KUL Leuven, 2006. Astracts of the Young Botanists Day 2006: "Tropical Botany in the Benelux", Belgian Journal of Botany 140:249-261

Koffi G, **Heuertz M**, Hardy O. Intraspecfic diversity of cpDNA sequences of an African tropical forest tree: Santiria trimera (Burseraceae). **Poster** presented at Young Botanists' Day, KUL Leuven, 2006. Abstracts of the Young Botanists Day 2006: "Tropical Botany in the Benelux", Belgian Journal of Botany 140:249-261

Heuertz M, Koffi Kouamé G, Savolainen V, Hardy OJ, 2006. Phylogeography of African lowland rainforest trees: Preliminary results. **Talk** presented at the IUFRO/COST Conference : Population Genetics and Genomics of Forest Trees: From Gene Function to Evolutionary Dynamics and Conservation. Alcalá de Henares, Madrid, Spain. 01.-06.10.2006

Heuertz M, Källman T, Gyllenstrand N, Lagercrantz U, Lascoux M, 2005. Genetic variation in phenology candidate genes from Norway spruce (Picea abies (L.) Karst) populations featuring contrasting histories. **Poster** presented at the ConGen Workshop: Refining the conservation genetics paradigm: genetic sources of extinction, environmental stress, and the maintenance of adaptive diversity, Santiago de Compostela, Spain.

Heuertz, M, S. Gorislavets, V. Risovanaya, L. Hoffmann, J.F. Hausman, 2005. Characterisation of grapevine genetic resources in the Black Sea and Caucasus regions. **Poster** presented at the Xth Congress of the European Society for Evolutionary Biology, Jagellonian University, Krakow, Poland, 15-20.08.2005.

Heuertz, M., T. Källman, N. Gyllenstrand, U. Lagercrantz, and M. Lascoux, 2004. Poster: SNP variation in candidate genes for climatic adaptation in Norway spruce (*Picea abies* L.). IUFRO Symposium on Population, Evolutionary and Ecological Genomics of Forest Trees, Asilomar Conference Grounds, Pacific Grove, California, USA.

Heuertz, M., O. J. Hardy, J.-F. Hausman, M. Palada, and X.Vekemans, 2002. Talk: Estimating pollen versus seed dispersal from spatial genetic structure in the common ash. International Conference DYGEN: Dynamics and conservation of genetic diversity in forest ecosystems, Strasbourg, France.

Heuertz, M., G.G. Vendramin, S. Carnevale, O.J. Hardy, J.-F. Hausman and X, Vekemans, 2002. Talk: Nuclear markers refine diversity patterns derived from chloroplast DNA: case study of common ash (*Fraxinus excelsior* L.) in Europe. IUFRO Symposium on Population and evolutionary genetics of forest trees. Stara Lesna, Slovakia.

Heuertz, M., G.G. Vendramin, J.-F. Hausman, and X. Vekemans, 2002. Talk: Understanding genetic structure among European populations of common ash (*Fraxinus excelsior* L.) International Symposium on Phylogeography, Vairão, Portugal.

Heuertz, M., J.-F. Hausman and X. Vekemans, 2001. Poster: Within-population genetic structure in *Fraxinus excelsior* assessed from microsatellite data. VIII Congress of the European Society for Evolutionary Biology, Aarhus, Denmark.

Heuertz, M., J.-F. Hausman and X.Vekemans, 2001. Talk: Assessment of genetic structure within and among southeast European populations of the common ash (*Fraxinus excelsior* L.). 7th Meeting of PhD Students in Evolutionary Biology, Bernried, Germany.

Heuertz, M., J.-F. Hausman and X.Vekemans, 2000. Poster: Etude de la structure génétique à l'intérieur et entre populations de frêne commun (*Fraxinus excelsior* L.) en Bulgarie. XXIst meeting of the Groupe de Biologie et Génétique des Populations, Dijon, France.

Heuertz, M., J.-F. Hausman and X.Vekemans, 2000. Talk: Microsatellite variation and assessment of genetic structure in the common ash (*Fraxinus excelsior* L.) in Bulgaria. 6th Meeting of PhD Students in Evolutionary Biology, Vaalbeek, Belgium.

Invited talks

Hybridization and the evolution of tropical tree species. **Invited talk.** IUFRO Genomics and Forest Tree Genetics Conference, Arcachon, France, May 2016.

Phylogeography and the evolution of tropical tree species complexes. **Invited Conference Talk**. 5th Porto Alegre Evolutionary Biology Workshop (PABEW), October 2015.

Ancient invasion without speciation: neutral and adaptive processes in a widespread rainforest tree, *Symphonia globulifera* (Clusiaceae). **Invited talk**. Congress Asociación Española de Ecología Terrestre, Pamplona, May 2013.

Genetic structure in plant species: from meters to continents. **Keynote talk.** Biology 2012: Joint meeting of the Swiss Zoological Society, Swiss Botanical Society, Swiss Systematic Society. Fribourg, February 2012.

Can Pleistocene refuge theory explain within-species patterns of genetic diversity in African lowland rainforest trees? **Invited talk.** Fronteras en Ecología & Evolución, Biodiversidad 2010, Seminarios del Instituto de Ecología, Universidad Autónoma de Mexico, September 2010.

Heuertz M, González-Martínez SC, Koffi KG, Hardy O. Demographic history of African lowland rainforest fruit trees and conservation issues. **Keynote talk.** Second Workshop of SAFORGEN (Working Group on Sub-Saharian African Food Tree Species Forest Genetic Resources Network), organized by SAFORGEN (Bioversity International) in collaboration with INIA-CIFOR, Spain. Cotonou, Benin, December 2007.

Heuertz M, Savolainen V, Budde K, Daïnou K, Duminil J, Koffi GK and Hardy OJ. Can Pleistocene refuge theory explain within-species patterns of genetic diversity in African lowland rainforest trees? **Invited talk.** II Jornadas de Conservación de la Diversidad Biológica. La Coruña, Spain, 2009.

Other talks and seminars

Analysing resequencing data for species delimitation in the Lecythidaceae. Tropical tree biodiversity, Ecology and Evolution. Talk. Workshop organized by ULB, Anseremme, Belgium, Nov. 11-13, 2018.

Heuertz M, Jehanne Q, Budde KB, Caron H, Scotti-Saintagne C, Franc A, Mariette S. Species delimitation and introgression in tropical trees: Lecythidaceae in French Guiana. INPA, Manaus, Brazil, July 2017.

Heuertz M, Jehanne Q, Budde KB, Caron H, Scotti-Saintagne C, Franc A, Mariette S. Species delimitation and introgression in tropical trees: Lecythidaceae in French Guiana. Meeting of the INRA EFPA department Genetics group. Orleans. June 22, 2017.

Evolution of tropical tree species complexes. Seminar at the Department of Evolutionary and Systematic Botany. University of Vienna, March 27, 2017.

RAD-Seq in related taxa: marker development vs. direct use for evolutionary studies. Animation of Biogeco Axis 4 on Mathematics and bioinformatics. March 2017.

Phylogeography and the evolution of species complexes in tropical trees. INRA Bordeaux, January 2015.

Evolutionary history of Symphonia (Clusiaceae). Centre national d'application des recherches pharmaceutiques, Antananarivo, Madagascar, October 2014.

Spatially explicit mechanisms of recent evolution in an African biodiversity hotspot. WSL Birmensdorf, Switzerland, December 2013.

Genomics of the speciation continuum: Spatially explicit mechanisms of recent evolution in an African biodiversity hotspot. University of Fribourg, Switzerland. November 2013.

REVOL: Recent evolution in an African biodiversity hotspot. ETH Zürich, Switzerland. November 2013.

Phylogeography and the geographic scale of adaptation in tropical trees of the genus Symphonia (Clusiaceae). V Maratón científico, Real Jardín Botánico, Madrid. October 2012.

Population "genomics" of Symphonia globulifera. *Summer School in Population Genomics and Bioinformatics,* Swiss Institute of Bioinformatics, Adelboden June 17-22, 2012.

Demographic history of the ancient tropical tree *Symphonia globulifera* L.f. (Clusiaceae). 2º Maratón científico INIA-CIFOR, Madrid. July 2012.

Inferring the demographic history of African tree species using population genetics approaches. Seminar, INIA, April 2012.

Evolution of Biodiversity in hotspots. 1er Maratón científico INIA-CIFOR, Madrid. July 2011.

The role of fragmentation in the evolution of biodiversity in hotspots. Seminar, University of Alcalá de Henares, June 2011.

Microevolution in critically-endangered plants: high genetic diversity in the narrow endemic *Antirrhinum charidemi*. Seminar at INIA-CIFOR, March 2011.

Phylogeography of African Rainforest trees. Seminar. Universidad Rey Juan Carlos I. Móstoles, Madrid, Spain, 2008.

Phylogeography of African rainforest trees and perspectives for studying biodiversity evolution in hotspots. Serie de seminarions del INIA-CIFOR, Madrid, Spain, 2008.

Structure génétique et phylogéographie de ligneux des forêts de basse altitude du Bas-Guinéen. Seminar at the Laboratoire de Lutte Biologique et Ecologie Spatiale (LUBIES) and Laboratoire d'Eco-Ethologie Evolutive (EEE), Université Libre de Bruxelles, Belgium, 2007.

Patrons de polymorphisme nucléotidique chez l'épicéa: histoire démographique ou signes de sélection? Semniar at the Laboratoire de Lutte Biologique et Ecologie Spatiale (LUBIES) and Laboratoire d'Eco-Ethologie Evolutive (EEE), Université Libre de Bruxelles, Belgium, 2006.

Phylogeography of African lowland rainforest trees. Centre International de Recherches Médicales de Franceville, Franceville, Gabon. 05.09.2006.

Phylogeography of African lowland rainforest trees. Institut de Recherche en Ecologie Tropicale, Libreville, Gabon. 22.08.2006.

Investigating the phylogeography of African lowland rainforest trees. Jodrell Laboratory, Royal Botanic Gardens, Kew, London, United Kingdom, March 2006.

Phylogeography and genetic structure of forest trees. XIII Ciclo de seminarios sobre Ecología y Evolución de las Plantas (host: Dr. Pedro Jordano), Universidad de Sevilla, Spain, October 2006.

Estimating seed versus pollen dispersal from spatial genetic structure in the common ash. Jodrell Laboratory, Royal Botanic Gardens (host: Dr. Christian Lexer), Kew, London, United Kingdom, February 2006.

Structure génétique de populations d'arbres forestiers en Europe. Société des Naturalistes Luxembourgeois, Musée National d'Histoire Naturelle, Luxembourg, 2005.

Diversité génétique de gènes candidats pour l'adaptation à la phénologie chez l'épicéa. Laboratoire de Génétique et Evolution des Populations, Université de Lille 1 (host : Prof. Xavier Vekemans), France, 2005.

Estimation of population genetics parameters. EU Project FRAXIGEN, University of Göteborg, Sweden, 2004.

Phylogeography of Fraxinus excelsior. Invited talk (host: Dr. Janet Stewart). Oxford University, United Kingdom, June 2002.

Diversitéit a genetisch Sturktur bei natiirliche Populatiounen vu Bëschbeem. Rotary Club, Luxembourg, 2000.

Postgraduate courses followed

Encadrement des doctorants, Université de Bordeaux, January 2018.

Préparer un projet européen H2020: Montage et gestion en réponse aux appels à propositions 2018 & 2019. INRA. Paris, 6 – 7 December 2017.

Egglib – population genetics analysis using Python, INRA Réseach de Génétique, Orléans, June 2017.

Production et analyse de données NGS – Exomes et panels de gènes. Univ. Bordeaux. 20 September 2016.

NGS genome & transcriptome assembly and analysis, CUSO-STAROMICS, Fribourg, June 16-18, 2014.

High-performance computing in the life sciences, Swiss Institute for Bioinformatics, Fribourg, September, 2013.

Conservation Biology in the 21th century, Fribourg, September 2013 (1 ECTS).

Summer School in Population Genomics and Bioinformatics, Swiss Institute of Bioinformatics, Adelboden June 17-22, 2012.

ANDNMO12 course "Analysis of NGS Data from Non-Model Organisms", Instituto Gulbenkian de Ciência, Oeiras, Lisbon, June 4-6, 2012.

Molecular evolution: genetic drift or genetic draft. 2007. Uppsala University, Drs K Thornton, M Schierup y M Lascoux. 3 ECTS.

A historical introduction to fundamental concepts in ecology. 2006. Université Libre de Bruxelles. Belgium. Drs J Chave and O Hardy. 2 ECTS.

Analysis of sequence data II (Advanced). 2005. Uppsala University. Drs. G McVean y M Lascoux. Sweden. 3 ECTS.

Analysis of sequence data I (Advanced). 2004. Uppsala University, Sweden. Drs P Joyce y Dr. M Lascoux. 3 ECTS.

Advanced Training in Forest Genetic Resource Management. 2000. University of British Columbia, Canada. Drs G Namkoong and M Koshy, 15 ECTS.

Teaching and mentoring

Teaching

01.10.00 – 30.09.02	Associate Professor for Botany Practicals ("Certificat d'Etudes Scientifiques" equivalent to 1st year Bachelor's degree in Life Sciences). Centre Universitaire de Luxembourg, Luxemburg (2 ECTS).
01.10.01 – 31.01.02	Associate Professor for Plant Physiolgy. ("Certificat d'Etudes Scientifiques" equivalent to 2nd year Bachelor's degree, Centre Universitaire de Luxembourg, Luxemburg (1 ECTS).
2001 – 2002	Co-supervision of practicals in Population Genetics. Université Libre de Bruxelles, Belgium (1 ECTS).
2004	Co-supervision of practicals in Population Genetics. Uppsala University, Uppsala, Sweden.
2005 – 2007	Teaching assistant for Population Genetics. Université Libre de Bruxelles, Belgium.
March 2008	Lecture in "Demographic history of African lowland rainforest trees and conservation issues". Seminario en el programa de doctorado con Mención de Calidad "Conservación y uso sostenible de los sistemas forestales", Universidad de Valladolid.
March 2009	Teacher in the "Curso de conservación de recursos genéticos forestales, Centro de Formación de la Cooperación Española" in Cartagena de Indias, Colombia.
July 2009	Teaching assistant in the Evoltree Network of Excellence course "DNA Sequence analysis and association genetics", CENEAM, Segovia.
November 2010	Teacher in the V Curso Internacional sobre Conservación y Utilización de Recursos Genéticos Forestales, INIA, Madrid.
June 2013	Teacher in the EvolTree EU Network of Excellence course: "Next-generation sequencing for beginners: applications to population genetics, forest health and tree breeding". Valladolid, June 17-19, 2013.
December 2013	Teacher in the "Advanced topics in ecology and evolution" course, MSc level. Université de Fribourg, Switzerland.

Bachelor students supervision

Daniel Ducry, co-supervision with Christian Lexer, Université de Fribourg, Switzerland, 2014. Phylogeography of two *Protea* species from the Cape Floristic Region of South Africa.

Master students supervision

Thomas Folituu, co-supervision with Olivier Lepais. Univ. Bordeaux. 2019 (ongoing). Development of molecular markers using high-throughput sequencing in Eschweilera (Parvifolia clade, Lecythidaceae).

Janan Gawra, co-supervision with Katharina Budde, Olivier Hardy, ERASMUS MUNDUS TROPIMUNDO, Université Libre de Bruxelles, 2018. An update to the pylogeography of *Symphonia globulifera* in Africa.

Sana Miloudi, co-supervision with Katharina Budde, Université de Bordeaux, 2018. Phylogeography of the hyperdominant *Eschweilera coriacea*. Sana failed her MSc year.

Quentin Jehanne, co-supervision with Stéphanie Mariette, Université de Bordeaux, 2016. Exploration de méthodes pour la détection de SNPs sur données de RAD-Seq et confirmation expérimentale de polymorphismes.

Marjorie Berthouzoz, co-supervision with Christian Lexer, Université de Fribourg, Switzerland, 2015. Microevolutionary drivers of species diversification and persistence in Cape Proteas.

Katharina B. Budde, co-supervision with Santiago C. González-Martínez, Universidad Complutense de Madrid, 2010. Spatial genetic structure in tropical and temperate forest trees.

Miguel Rodríguez Blanco, Universidad de Valladolid, 2009. Filogeografía y estructura morfológica de dos árboles tropicales cercanos filogenéticamente: *Tricoscypha acuminata* y *T. oddonnii* (Anacardiaceae) en la región fitogeográfica de Baja Guinea, África Central.

Guillaume Kouamé Koffi, Université Libre de Bruxelles, 2007. Co-supervision with Olivier Hardy. Phylogeographie d'un arbre tropical en Afrique, *Santiria trimera*, Burseraceae.

PhD students supervision

Sylvain Schmitt, co-supervision with Bruno Hérault, CIRAD. Univ. Bordeaux, 2017-ongoing. Ecological genomics of niche exploitation and individual performance in tropical forest trees.

Paloma Torroba Balmori, co-supervision with Ricardo Alía, INIA-CIFOR Madrid and Universidad de Valladolid, 2013-ongoing. Adaptive evolution in the tropical tree genus *Symphonia* (Clusiaceae). Paloma is a forest engineer for the Spanish state administration.

Katharina B. Budde, co-supervision with Santiago C. González-Martínez, INIA-CIFOR Madrid and Universidad Complutense de Madrid, 2009-2014. Genetic structure of forest trees in biodiversity hotspots at different spatial scales. Katharina Budde was a postdoc at the University of Copenhagen (DK) in 2014-2016, an IdEx postdoc at University of Bordeaux (September 2016 – Auguest 2018) and is currently a postdoc at INRA, UMR Biogeco.

Guillaume Kouamé Koffi, co-supervision with Olivier J. Hardy, Université Libre de Bruxelles, 2006-2010. Title: Genetic structure and ecology of a tropical tree with large morphological variation and a large distribution in Central Africa, *Santiria trimera*, Burseraceae. Guillaume Koffi is a lecturer at the University of Abijan, Côte d'Ivoire.

Contribution to the supervision of PhD students

Abdeljalil Aissi, Université Batna 1 Hadj Lakhdar, Batna, Algeria. 2015 – (ongoing). Main supervisor: Yassine Beghami. Le chêne zéen à petites feuilles (*Quercus faginea* Lamk.) dans l'Aurès: cartographie, écologie et taxinomie.

André Carneiro Muniz, Univ. Minas Gerais, Brazil. Main supervisor: Maria Bernadete Lovato. Population genetics and species boundaries of *Dimorphandra* species (Leguminosae) from the Cerrado and Atlantic Forest: implications for conservation.

Alejandra L. Goncalves, Facultad de Ciencias Naturales y Museo - Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina, 2014-2019. Main supervisors: María Victoria García and Rolando Rivera

Pomar. Estructura genética espacial en paisajes fragmentados: Un estudio en poblaciones naturales de curupay (Leguminosae: *Anadenanthera colubrina* var. *cebil*).

Mario Mairal Pisa, Real Jardín Botánico-CSIC, Madrid and Universidad Autónoma de Madrid, 2015. Main supervisor: Isabel Sanmartín. Macroevolución y microevolución en sistemas insulares: el patrón Rand Flora en el género Canarina.

David Macaya Sanz, Universidad de Valladolid, 2015. Main supervisors: Santiago C González-Martínez and Christian Lexer. Filogeografía, diversidad y hibridación en Populus spp.

Priciane Cristina Ribeiro Correa, University of Minas Gerais, Brazil, 2014. Main supervisor: María Bernadete Lovato. História evolutiva de *Annona crassiflora* Mart. e *A. coriacea* Mart (Annonaceae) e determinantes ambientais da divergencia genética e morfológica de populações no Cerrado.

Gilles Dauby, Université Libre de Bruxelles, 2012. Main supervisors: Olivier J Hardy and Tariq Stévart. Structure spatiale de la diversité intra- et interspécifique en Afrique centrale.

José Carlos Santana Pérez, Universidad de Valladolid, 2011. Main supervisor: Elena Hidalgo. Caracterización genética de variedades de vid en Castilla y León con fines de conservación.

Kasso Daïnou, Université de Liège, Belgium, 2012. Main supervisors: Jean-Louis Doucet and Grégory Mahy. Population genetic structure and conservation of *Milicia excelsa* (Moraceae), a tree species that is locally threatened from extinction in Central Africa.

Ana I De-Lucas, Universidad de Valladolid, 2009. Main supervisors: Santiago C González-Martínez and Elena Hidalgo. Genetic structure in fragmented and continuous populations of maritime pine.

Thelma Barbará, Open University London, 2008. Main supervisor: Mike F Fay. Genetic structure of four Bromeliaceae species affected to inselbergs in Brazil.

Postdocs supervised

Katharina B. Budde, postdoc of the Idex excellence program of the Université de Bordeaux, Sept. 2016 – Aug. 2018. Drought stress in tropical ecosystems: adaptive allele sharing in tree species complexes. Katharina is currently a postdoc at INRA, UMR Biogeco.

Sanna Olsson, postdoc in the AFFLORA project "The assembly of African floras from lineages to populations: effects of historical climate changes and species ecology." Jan 2014 – June 2015, INIA-CIFOR Madrid. Sanna was a postdoc Real Jardín Botánico- CSIC Madrid in 2015-2016 and is currently a bioinformatics engineer at INIA-CIFOR, Madrid.

Host for scientific visits (1 month or longer)

André Carneiro Muniz, Univ. Minas Gerais, Brazil. Population genetics and species boundaries of *Dimorphandra* species (Leguminosae) from the Cerrado and Atlantic Forest: implications for conservation. CAPES Sandwich fellowship, September 2018 – September 2019.

Boris B. Demenou, Univ. Libre de Bruxelles. Plastome phylogeography of two rain forest legueme trees in the Dahomey Gap, West Africa. Labex COTE mobility to INRA-Univ. Bordeaux, January – March 2018.

Segbeji Armel Loïc Donkpegan, Université de Liège. Population genomics of the tropical tree genus Afzelia in Central Africa. Labex COTE mobility to INRA-Univ. Bordeaux, February – April 2017.

Bárbara Simoes Santos Leal, São Paulo State University, Labex COTE mobility to INRA-Univ. Bordeaux, October-November 2016, and FAPESP Sandwich Fellowship February – July 2017. Population genomics and adaptive variation of the *Pitcairnia lanuginosa* complex (Bromeliaceae): development of ddRAD-Seq.

Paloma Torroba-Balmori, EU TREES4FUTURE Transnational Access grant to INRA-Bordeaux, September – October 2015. Development and genotyping of transcriptomic SNP markers in Symphonia (Clusiaceae), ADAPMADA.

Alexandra Ley, University of Halle-Wittemberg, Germany. SYHTHESYS Access grant ES-TAF-2746 to visit AFFLORA principal investigators Isabel Sanmartín and Myriam Heuertz at INIA-CIFOR and RJB-CSIC, Madrid, March – April 2013. Diversification in the tropical understorey – from cross-continental speciation pattern on family level to the local demographic history of sister species

Priciane Cristina Ribeiro Correa, University of Belo Horizonte, Brazil, visit to INIA-CIFOR in the framework of CAPES program for PhD students, April – October 2013. Filogeografia e diversidade genética de duas espécies de *Annona* (Annonaceae) do Cerrado brasileiro.

Katrin Heer, University of Ulm (Germany), visit to INIA-CIFOR, Madrid, August - September 2011. Microsatellite analysis of *Symphonia globulifera* samples from Brazil.

Guillaume Kouamé Koffi, Université Libre de Bruxelles, visit to INIA-CIFOR and Real Jardín Botánico – CSIC, Madrid, June-July 2010. Interpretation of double peaks in sequence profiles of *Santiria trimera*.

Other mentoring activities

Participation as a mentor in the mentoring program of the Association for Tropical Biology and Conservation, 2016 conference in Montpellier, mentee Armel Donkpegan.

Participation as invited scientist (videoconference) in the Career Planning Workshop, Iberian Ecology Congress, Lisbon, June 2015.

Training of scientists from East European countries (Moldova, Ukraine) in molecular analysis techniques.

Institutional responsibilities

Member of the team for Scientific and Technical Animation of INRA UMR Biogeco, since January 2019.

Member of the Scientific Advisory Board (Conseil Scientifique) of INRA-UMR BioGeCo, 2016 - 2018.

Member of the Scientific Board of the French Excellence Laboratory CEBA (Center for the study of biodiversity in Amazonia) since 2015, including project evaluation in the annual call for research proposals and evaluation of candidates for PhD funding (2015, 2016).

Responsible for the organization of Department seminars, Nature Conservation and Genetics Department, University of Uppsala, 2003-2004.

Participation in evaluation boards

Member of the evaluation board of the PhD thesis of Franck K. Monthe, Université Libre de Bruxelles, Jan. 2019.

Member of the evaluation board of the PhD thesis of Boris Brice Demenou, Université Libre de Bruxelles, Feb. 2018.

Member of the evaluation board and reporter of the PhD thesis of S Armel L Donkpegan, Université de Liège – Gembloux AgroTech. July 2017.

Evaluation board of Master (M2) internships in the Master programs "Biodiversité et Fonctionnement des Ecosystèmes Terrestres" and "Biodiversité et Suivis Environnementaux". June 2017 and Febreuray 2018.

Member of the evaluation board of the PhD thesis of Adama Faye, Université de Montpellier. December 2015.

Member of the evaluation board and reporter of the PhD thesis of Adja Majiguene Diallo, University of Copenhagen. June 2015.

Member of the evaluation board and reporter of the PhD thesis of Lélia Lagache, Unversité de Bordeaux 1. 2012.

Member of evaluation board of Agencia Nacional de Evaluación y Prospectiva for "Ramón y Cajal" 5-year researcher contracts in Animal and Plant Biology and Ecology (BAVE commission). 2011.

Member of the evaluation board of the PhD thesis of Grazyna Bochenek, University of Göteborg. 2011.

Member of the evaluation board and reporter of the PhD thesis of José C Santana Pérez, Universidad de Valladolid. 2011.

Member of the evaluation board of the PhD thesis of Ana I. de Lucas, Universidad de Valladolid, 2009.

International representation

Representative of Luxembourg in the EUFORGEN (European Forest Genetic Resources Programme) Noble Hardwoods Network, phase I (1997-2000) and II (2001-2004), under the auspices of the "International Plant Genetic Resources Institute, FAO". Attending of 5 international meetings in Austria (1999), Ireland (2001), Bulgaria (2001), Portugal (2002) and Denmark (2005) and contribution to the dissemination of scientific results and the drafting of documents for the conservation of genetic resources of scattered hardwood species.

Scientific reviewing

Manuscript reviewing for the following scientific journals: American Journal of Enology and Viticulture, Anales del Jardín Botánico de Madrid, Annals of Botany, Annals of Forest Science, Australian Journal of Botany, Biology and Environment: Proceedings of the Royal Irish Academy, Botanical Journal of the Linnean Society, Central European Journal of Biology, Conservation Genetics, Forest Ecology and Management, Genetica, Genetics, Heredity, International Journal of Plant Sciences, Journal of Biogeography, Journal of Ecology, Journal of Heredity, Molecular Ecology, Molecular Biology and Evolution, Molecular Phylogenetics and Evolution, New Phytologist, Nordic Journal of Botany, PPEES Perspectives in Plant Ecology Evolution and Sytematics, Plant Ecology and Evolution, Scientific Reports, Silvae Genetica, Tree Genetics and Genomes.

Project reviewing for: Polish National Research Council (1 project 2012, 1 project 2015), Agencia Nacional de Evaluación y Prospectiva (ANEP, 1 project 2010, 3 projects 2011, 1 project 2016, 1 project 2017, 1 project 2018); Biotechnology and Biological Sciences Research Council (BBSRC, United Kingdom, 1 project 2007); Dutch Research Council (NOW, 1 project 2009); Fonds National de la Recherche Scientifique Belgium (4 projects 2010, 1 project 2011, 1 project 2012); National Science Foundation (NSF, USA, 1 project 2007), ANR France (1 project 2015), NERC (1 Fellowship Application, 2016).

Scientific editing

Editor of Plant Ecology and Evolution (IF 2017: 1.14), since March 2010, editorial decicions reached for ca. 30 manuscripts.

Associate Editor of Molecular Ecology, since November 2010 (IF 2017: 6.13), editorial decisions reached for 196 manuscripts.

Recommender for Peer Community in Evolutionary Biology, since March 2019. Just started editorial duties.

Membership in scientific associations

Member of Société Royale Belge de Botanique, Association for Tropical Biology and Conservation - ATBC, Society for the Study of Evolution-SSE (USA) and of the European Society of Evolutionary Biology-ESEB.

Organization of scientific meetings

Organization of 1st and 2nd Science Marathon of INIA-CIFOR, 2011 and 2012.

Participation in the scientific organization board of the IUFRO Genomics and Forest Tree Genetics meeting in Arcachon, France, June 2016.

Outreach activities

Organization of an outreach meeting: The value of our Forests: from scientific knowledge to management. UN year of Forests, INIA-CIFOR 2011.

Organization and presentation of science workshops:

- Daycare centre for 1-3 year olds: Escuela Infantil El Tomillar, Centro Público de Educación Infantil, Torrelodones, Espagne, March 2013.

- Kindergarten, 4 and 5 year olds: Schule Vignettaz B, Fribourg, Switzerland, May 2015

Radio and TV broadcasts:

- Grapevine genetics in the Black Sea region, interview, RTL Tele Luxembourg, 2006.
- Genetic diversity in tropical African rainforests, interview, Radio Fribourg, 2014.

Several news articles for the promotion of research articles.

Main scientific collaborations

Santiago C. González-Martínez, INRA Bordeaux, France
Juan José Robledo Arnuncio, INIA-CIFOR, Madrid
Olivier Hardy, Université Libre de Bruxelles, Belgium
Gonzálo Nieto Feliner, Real Jardín Botánico-CSIC, Madrid, Spain
Pablo Vargas, Real Jardín Botánico-CSIC, Madrid, Spain
Isabel Sanmartín, Real Jardín Botánico-CSIC, Madrid, Spain
Christopher Dick, University of Michigan, USA
Ivan Scotti, INRA, Avignon, France
Caroline Scotti-Saintagne, INRA, Avignon, France
François Lefèvre, INRA, Avignon, France
Pieter van 't Hof, Universidad San Francisco de Quito, Ecuador
Giovanni Vendramin, National Research Council, Florence, Italy
Christian Lexer, University of Vienna, Austria
Mari Mette Tollefsrud, Norwegian Forest and Landscape Institute, Ås, Norway
Gonzalo Claros, University of Malaga, Spain
Bernadete Lovato, Univ. Minas Gerais, Brazil
Clarisse Palma-Silva, Sao Paulo State University Brazil
Niklas Tysklind, INRA, Kourou, French Guiana
Bruno Hérault, CIRAD, France
Cristiano Vernesi, Fondazione Edmund Mach, Italy

3. Introduction

3.1. Biographical sketch and research context

The central theme of my research is the investigation of evolutionary processes in natural populations of non-model tree species and species complexes. These processes include population demographic changes, range dynamics, local-scale gene flow and genetic drift, adaptive evolution, population divergence and speciation, as well as hybridization and introgression. I have examined these processes in a series of woody plant systems, including boreal, temperate and Mediterranean trees and tropical trees from Africa and the Neotropics. My research has led me to develop and use different types of molecular markers, ranging from plastid DNA sequences, microsatellites or candidate genes, to genomic approaches, and to use varied data analysis and inference approaches, frequently involving computer simulations and including complementary data on plant traits or the environment.

After my PhD on population genetics of common ash, *Fraxinus excelsior* L. (Oleaceae), supervised by Dr. Xavier Vekemans at the Université Libre de Bruxelles (1999-2003) (Figure 1), my research has been characterized by a high degree of internationalization. A first postdoc at the University of Uppsala in Sweden (2003-2004) familiarized me with molecular techniques to identify and sequence candidate genes in Norway spruce, *Picea abies* (L.) Karst, and with coalescent demographic modelling. I subsequently worked on germplasm characterization in grapevine at the Public Research Centre-Gabriel Lippmann in Luxembourg (2005), in a project involving Eastern European countries (Moldova, Ukraine, Georgia). As a Research Associate at the Université Libre de Bruxelles (2006-2008) on phylogeography of tropical trees from Africa, I had the opportunity to design and conduct tropical tree sampling missions in Gabon and Cameroon, develop molecular markers, test phylogeographic hypotheses in the tropics and to mentor my first PhD student.

During a visiting scientist period at INIA-CIFOR in Madrid, Spain (2008), and subsequently as a "Ramón y Cajal" researcher (2010-2014), I added a research line on ecological genetics in Mediterranean plants and trees, mostly pines, which allowed me to develop my research while gaining increased expertise in student mentoring. I continued working in tropical trees, investigating the delimitation of closely related taxa and started using genomic approaches. I also matured as a scientist through serving on the editorial boards of two journals, Plant Ecology and Evolution, and Molecular Ecology, since 2010 and ongoing. A sabbatical year as a senior Marie Curie fellow hosted at the University of Fribourg, Switzerland (2013-2014), let me explore a genomic view of biodiversity evolution in the Cape flora of South Africa. I entered my current position as a research scientist on evolutionary processes in Neotropical tree species complexes in December 2014 and have since then obtained research funding and constructed a small research group that is currently composed of a Master student, a PhD student and a visitng PhD student, and has regularly included one or two master students across the past four years. I continue collaborating actively with scientists in several countries, also through regularly hosting students and visiting researchers.

While eco-evolutionary questions have always been central in my work, my research approach has gradually evolved from a data-driven analysis and interpretation approach to a more hypothesis-driven research approach. This reflects the global trend, but in my case, I think the evolution is largely attributable to my work as a scientific editor. In this chapter, I give brief a introduction on the peculiarities of the tree growth habit relating to evolutionary processes, and then on the coalescent, a statistical framework we use to infer evolutionary processes from genetic polymorphism data. These topics are of general relevance for my research. In chapter 4, I will summarize my most important contributions in eco-evolutionary research in tree species and species complexes, highlighting the mentoring experience I gained as well as my leadership initiatives. In chapter 5, I will give an overview of my current research and research perspectives on eco-evolutionary processes in trees.
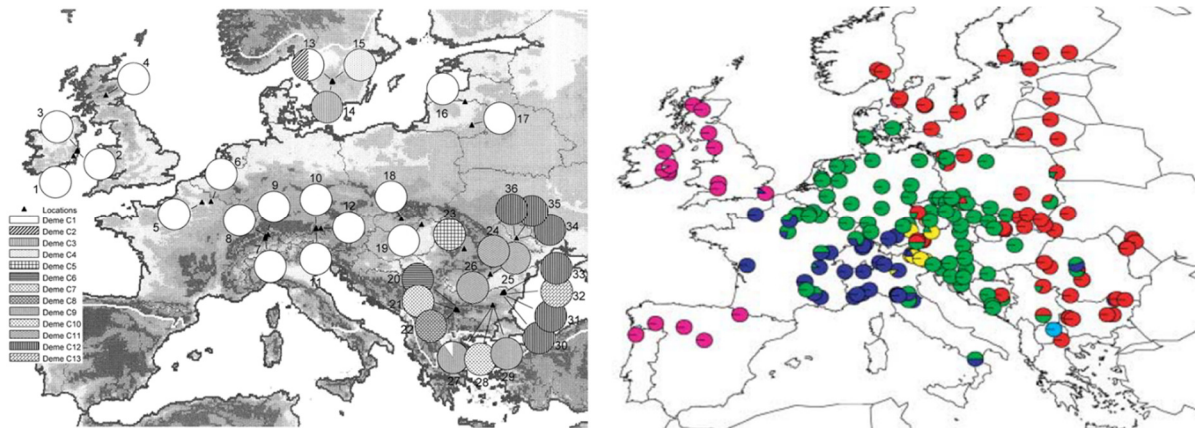
Figure 1 Population genetic structure in Fraxinus excelsior *L. Left: genepools inferred from nuclear SSR markers; right: plastid DNA lineages. From Heuertz, Hausman, et al. (2004); Heuertz, Fineschi, et al. (2004).*

3.2. Evolutionary processes in forest trees

Forests are among the most important repositories of terrestrial biological diversity. Tropical, temperate and boreal forests offer very diverse habitats for plants, animals and micro-organisms. From the human point of view, this biodiversity is the basis for a wide array of goods and services provided by forests, including timber and non-wood forest products, contribution to soil and water conservation, contribution to nutrient cycling and climate regulation including the storage of CO_2, as well as social and cultural benefits (Nelson *et al.* 2009; Isbell *et al.* 2011).

3.2.1. Large effective population sizes (*Ne*)

The tree growth habit has evolved many times in unrelated plant lineages, and this growth habit is associated with some evolutionary peculiarities (Petit & Hampe 2006). Most tree species maintain a high level of genetic diversity but populations are weakly differentiated (Hamrick *et al.* 1992; Nybom 2004). Rates of climate niche evolution are slower for woody plant lineages than for herbaceous lineages (Smith & Beaulieu 2009), and speciation rates in tree clades are often slow (Petit & Hampe 2006). These observations on evolutionary parameters – diversity, differentiation, speciation rates – reflect the large effective population sizes, *Ne*, typically characteristic of tree species (e.g. Brown et al. 2004; Heuertz et al. 2006). *Ne* is tightly connected with genetic diversity as it represents the number of reproducers of an idealised model population that has the same level of genetic variation as the observed population. An example of such an idealised population is the Wright-Fischer population (named after Sewall Wright and Ronald Fisher, founders of the discipline of population genetics), a random mating population of constant size with discrete generations. The connection of observed genetic data with theoretical expectations under a precise model is important to study the evolutionary processes that affect a population or species (see also next section on coalescent theory and modelling). Large *Ne* in trees is a partial consequence of tree attributes such as great size, longevity, overlapping generations and high reproductive output, as well as generally outcrossing mating systems and high levels of gene flow in large, random-mating populations (Hamrick *et al.* 1992; Nybom 2004; Petit & Hampe 2006; Plomion *et al.* 2018). These attributes, especially wide-ranging gene flow, longevity and overlapping generations, can contribute to protecting tree populations from loosing genetic diversity as a consequence of adverse events affecting population census size, for instance following habitat fragmentation (Kramer *et al.* 2008; Lowe *et al.* 2015).

3.2.2. Local adaptation

An important experimental effort in the form of common garden experiments has shown that there is substantial evidence for local adaptation in tree species (Savolainen *et al.* 2007, 2013; Kremer *et al.* 2012; Alberto *et al.* 2013). Local adapation can be defined as the native provenance performing best, or having the highest fitness at its native site (Kawecki & Ebert 2004; Leimu & Fischer 2008). Local adaptation can result when differential selection pressures affect a species across heterogeneous environments. A straightforward illustration of local adaptation in northern hemisphere trees is the clinal variation of phenotypic traits with latitude (Figure 2) (Savolainen *et al.* 2007). A recent literature survey suggests that most tree traits generally exhibit considerable heritability, i.e., trait variation in a population that is due to genetic variation, and that the underlying quantitative genetic variation (Q_{ST}) displays a stronger structure across populations than neutral genetic variation (F_{ST}) in 69% of comparisons across the literature (Lind *et al.* 2017).
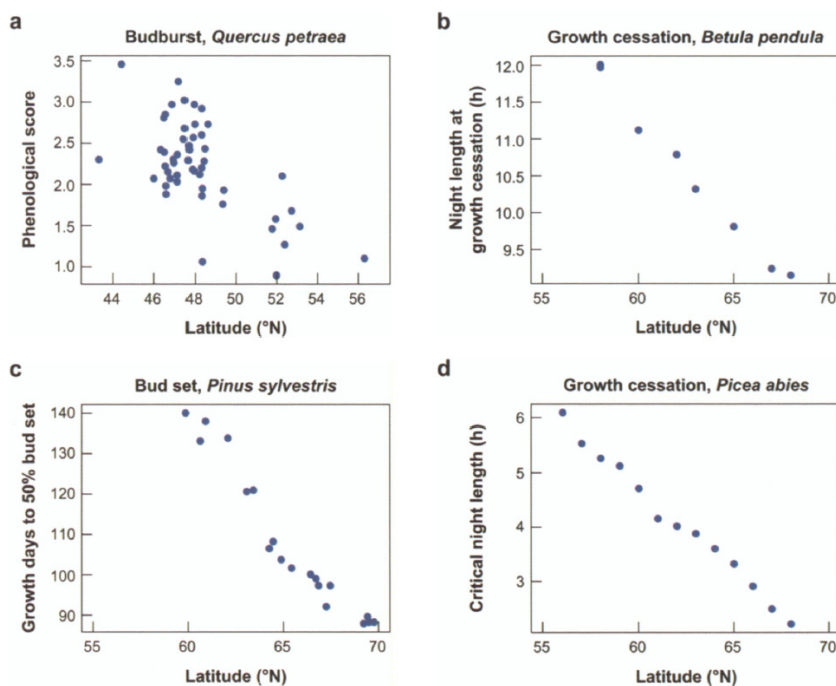
Figure 2 Clinal variation of phenotypic traits in northern hemisphere trees, illustrating local adaptation (from Savolainen et al. 2007).

High fecundity and strong selection in early life stages enable pronounced and rapid local adaptation in trees (Fallour-Rubio *et al.* 2009; Kremer *et al.* 2012), which results in divergent phenotypes for heritable traits and an expected genetic signature of adaptation at the loci underlying these traits (Howe et al. 2003). It is thus expected that neutral and adaptive processes may act on very different time scales in trees: neutral processes maintain diversity over a long time, ensuring species cohesion through high gene flow and large effective population sizes; whereas adaptive processes can lead to rapid phenotypic divergence.

3.2.3. The genomic basis of local adaptation

Local adaptation can leave a range of genomic signatures, including additive, dominance and epistatic effects and their complex interactions. Thus, decoding the genomic architecture of local adaptation remains very challenging in natural populations of non-model species (Anderson *et al.* 2013; Lind *et al.* 2018). There are two broad types of approaches to assess adaptive evolution, departing either from

the phenotype, or from the genotype (Flood & Hancock 2017). When an adaptive phenotype is known and can readily be scored in a large number of individuals, trait mapping can be conducted. When adaptive phenotypes are not known or are difficult to score, genomic scans can identify loci displaying genetic signatures of selection.

Quantitative Trait Locus (QTL) Mapping is based on a mapping population derived from a cross between a known father and mother carrying different phenotypes for a trait of interest. The trait is phenotyped and hundreds or thousands of markers (such as single nucleotide polymorghisms, SNPs) are genotyped in the mapping population. QTLs are then mapped onto the chromosomes, or linkage groups, by identifying the molecular markers that correlate with the trait. This is often an early step in identifying and sequencing the actual genes that cause the trait variation. In forest trees, QTL mapping has been conducted in numerous genera of the Pinaceae (pines, spruces), Fagaceae (oaks), Myrtaceae (eucalypts) and Salicaceae (willows, poplars), and has revealed that phenotypic traits such as those associated to growth, wood properties or stress tolerance are generally controlled by numerous loci (Neale & Kremer 2011). Major drawbacks of the QTL approach are (1) the identified QTLs are instable across the genomic backgrounds and environments, (2) major effect loci are detected preferentially, and thus the number of loci controlling the trait is commonly underestimated, (3) statistical caveats leading to false positives, and (4) low genomic resolution for the positioning of QTLs (González-Martínez *et al.* 2006b). Positional cloning approaches to identify causative variation have seldome been conducted in tree species because they are very challenging due to the probable multi-locus nature of adaptation and, in the case of conifers, because their genomes are very large (Grotkopp *et al.* 2004; González-Martínez *et al.* 2006b).

Association mapping is a trait mapping approach where a population is phenotyped and densely genotyped, or sequenced, and candidate markers under selection are identified through statistical association with the trait of interest. Phenotype-genotype association studies examining single-locus associations to traits of interest have been conducted in numerous tree species, mostly belonging to the same families as those examined in QTL mapping: Pinaceae, Myrtaceae, Salicaceae and Fagaceae (Lind *et al.* 2018) and have identified significant associations, for instance SNPs located in candidate genes carefully selected in meaningful pathways for wood traits or carbon isotope discrimination (González-Martínez *et al.* 2007, 2008). Linkage disequilibrium (LD) decays rapidly in trees (Heuertz *et al.* 2006b; González-Martínez *et al.* 2006b), which increases the success of identifying the relevant nucleotides responsible for the phenotype within a candidate gene (Neale & Savolainen 2004; Neale & Kremer 2011). Larger marker pannels have progressively been used (Holliday *et al.* 2010; Ingvarsson & Street 2011), and in recent years genome-wide variation is being assessed in genome-wide association studies (GWAS)(Uchiyama *et al.* 2013; Porth *et al.* 2013; McKown *et al.* 2014; Minamikawa *et al.* 2018). GWAS provides a great resolution because it relies on the recombination history of the population over a long evolutionary time, however, the multitude of associations that must be tested result in limited statistical power (Visscher *et al.* 2014). Another major challenge is that population structure can confound causative variation (Price *et al.* 2010). Integrating linkage and association mapping approaches can reduce false positive associations (Brachi *et al.* 2010; Du *et al.* 2016), and power can be gained through combining GWAS approaches in a meta-analysis of several populations (Müller *et al.* 2019).

Genomic scans for selection have the purpose of detecting loci that display a genetic signature of selection. The detection of loci under selection rests on the assumption that most new mutations in a genome are neutral, i.e., unaffected by selection (Kimura 1968), or slightly deleterious (Ohta 1992). Putative adaptive loci are distinguished from neutral loci by comparison of their genetic parameter estimates to those of background genomic variation, or to those obtained through simulations under plausible demographic scenarios (Flood & Hancock 2017).

A hard selective sweep is a typical signature of strong positive selection: a new mutation has a strong advantageous effect on the phenotype and is rapidly driven to fixation in the population. As the

mutation increases in frequency over few generations, variation in linked neutral sites in the vicinity is reduced, or "sweeped" out (Maynard-Smith & Haigh 1974; Nielsen 2005). A selective sweep can be detected based on a pattern of extended linkage disequilibrium, perturbations of the allele frequency spectrum (Figure 3) (Tajima 1989; Fay & Wu 2000), or strong genetic differentiation, for example (Beaumont & Nichols 1996; Foll & Gaggiotti 2008). Amino-acid changes can lead to a changed phenotype, therefore high rates of non-synonymous to synonymous mutations ($dN/dS > 1$) also represent signatures of positive selection (Tiffin & Hahn 2002; Yang & Nielsen 2002; Barrier *et al.* 2003). In trees, early studies examined nucleotide variation at candidate genes from selected physiological pathways to detect genetic signatures of positive selection (Kado *et al.* 2003; Garcia-Gil *et al.* 2003). The detection of clear genetic signatures of positive selection has been infrequent in trees (Palmé *et al.* 2008, 2009). It has also become clear that robust inference of selection signatures requires accounting for population demographic history (Heuertz *et al.* 2006b; Källman *et al.* 2014).
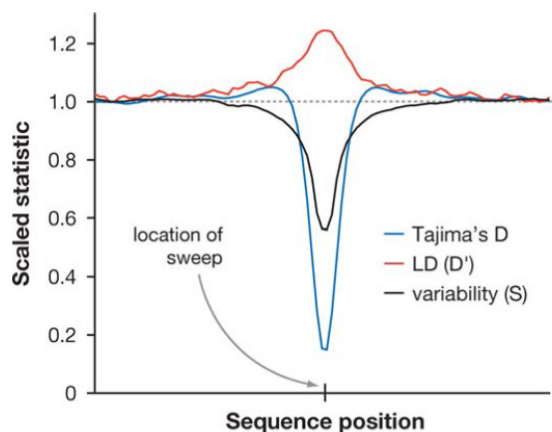
Figure 3 The effect of a selective sweep on genetic variation. The figure is based on averaging over 100 simulations of a strong selective sweep. It illustrates how the number of variable sites (variability) is reduced, LD is increased, and the frequency spectrum as measured by Tajima's D, is skewed, in the region around the selective sweep. All statistics are calculated in a sliding window along the sequence right after the advantageous allele has reached frequency 1 in the population. All statistics are also scaled so that the expected value under neutrality equals one. From Nielsen (2005).

Theoretical and empirical work has shown that adaptive processes in nature are often complex and cannot be adequately captured by the hard selective sweep model (Pritchard *et al.* 2010; Hancock *et al.* 2011; Le Corre & Kremer 2012b). In tree populations, selection can act on numerous genetic variants simultaneously because of high standing genetic variation. In a *soft sweep*, for example, multiple haplotypes that carry the same advantageous variant are driven to higher frequencies simultaneously (Hermisson & Pennings 2005), a situation that is difficult to distinguish from background genomic variation (Schoville *et al.* 2012; Garud *et al.* 2015). Natural selection can also result in *polygenic adaptation*, increasing the frequency of variants at different genes simultaneously (Pritchard *et al.* 2010). This type of adaptation is characterized by subtle to moderate shifts in allele frequencies and may be frequent in traits that are controlled by many loci of small effect, have standing genetic variation and are highly heritable (Pritchard *et al.* 2010; Lind *et al.* 2017). Methods based on genetic variation only do not perform well to discover loci evolving under soft sweep or polygenic adaptation. Power can be gained by assessing correlation of allelic variation with variation in environmental gradients in the absence of phenotype scores (Berg & Coop 2014; Whitlock & Lotterhos 2015; de Villemereuil & Gaggiotti 2015; Hoban *et al.* 2016).

3.2.4. Diversification and hybridization

The large effective population sizes of tree species imply slow lineage sorting in diversification processes in tree clades. Closely related species which belong to the same genus can thus commonly

share ancestral polymorphisms. As a consequence, phylogenetic resolution is often poor in tree clades (Willyard *et al.* 2009; Grivet *et al.* 2013; Daïnou *et al.* 2014).

Hybridization, which is common in plants (Soltis & Soltis 2009; Whitney *et al.* 2010; Abbott *et al.* 2013), can also contribute to the sharing of polymorphisms. Because slow lineage sorting and hybridization can operate simultaneously in closely related species, disentagling their evolutionary history is often challenging. Recently developed genomic tools offer improved power to address evolutionary processes in closely related species, for example allowing to infer periods of isolation and periods of gene flow, and to detect genes that have crossed species boundaries vs. genes impermeable to gene flow (Gao *et al.* 2012; Leroy *et al.* 2017). The genomic pattern of differentiation between diverging sister species corresponds frequently to a mosaic of loci with different levels of permeability to introgression (Guichoux *et al.* 2013; Eaton *et al.* 2015). The correct inference of evolutionary history from such patterns requires using models that incorporate key evolutionary processes such as the dynamic nature of gene flow, selection acting in hybrid populations and recombination rate variation (Payseur & Rieseberg 2016). Interspecific hybridization can be an important evolutionary force by introducing novel genetic variation on which selection can act, thus providing opportunity for adaptive introgression (Flood & Hancock 2017; Suarez-Gonzalez *et al.* 2018). Adaptive introgression is particularly important in rapidly changing environments, when standing genetic variation and mutation alone may only offer limited potential for adaptation (Suarez-Gonzalez *et al.* 2018).

3.3. A statistical framework for evolutionary inference: the coalescent

The genetic polymorphism we observe in a sample of sequences from a population, i.e., differences in nucleotide sequences, represents the outcome of a single implementation of a complex evolutionary history. Our sample contains only the alleles that have been transmitted successfully across generations, and contains no trace of those that have been lost at any previous generation, for example because their carriers did not leave offspring. Indeed, mating patterns, mutations and recombination are largely random events, and a high degree of stochasticity therefore underlies any genetic polymorphism pattern.

To infer the evolutionary history of a population or a species based on the limited genetic information provided by a sample of sequences is thus a challenging task. While descriptive approaches have largely been used for early interpretations of genetic data within species, or between closely related species, notably in phylogeography – the study of the historical processes underlying the contemporary geographic distributions of diversity – , the use of more robust, statistical inference based on modelling approaches has gained wide popularity for population genetic inference in the last 10-15 years (Knowles 2003; Petit 2008; Nielsen & Beaumont 2009; Beaumont *et al.* 2010).

Statistical inference in population genetics frequently relies on the use of simulation approaches to evaluate how likely an observed diversity pattern is. These approaches can be divided broadly into three categories (Yuan *et al.* 2012): 1. *Simulations forward in time*, where an initial population is left to reproduce and evolve over several generations to track its evolution under various genetic models; genetic parameter estimates from the final generation are compared to observed data. These simulations are computationally intensive. 2. *Simulations backward in time, or coalescent simulations*, which simulate the evolutionary history of a sample of the size of interest under several evolutionary scenarios. This simulation technique is the approach of choice when possible; it is efficient as it only models the sample of interest. 3. *Resampling approaches*, where sampling of subsets of the data is performed to assess the robustness of statistics derived from the data.

I here give an overview of the coalescent, a mathematical description of the genealogical process in populations which incorporates the stochasticity of evolutionary processes. The mathematical theory of the coalescent represents an extension of classical population genetics theory and models, as I will

illustrate (reviews in Rosenberg and Nordborg 2002; Nordborg 2000; McVean 2001). The coalescent is used as a statistical framework for modelling the evolutionary history of populations. The genealogical process is first modelled from a current sample backwards in time to obtain a gene genealogy. In a second step, mutations are added onto the genealogy. The basic properties of the coalescent derive from probabilities of descent and mutation.

The coalescent was first described by Kingman (Kingman 1982) but was also independently discovered by Hudson (Hudson 1983) and by Tajima (Tajima 1983). It was first introduced in a Wright-Fisher model of neutral evolution, that is, in a constant-size population of size *N* diploid individuals (*2N* alleles) where inividuals mate randomly, in non-overlapping generations, without migration or selection. Further developments considered, for example, the inclusion of recombination (Hudson 1983; McVean and Cardin 2005; Wang et al. 2014), selection (Krone & Neuhauser 1997), population structure (Wakeley 2004; Wakeley *et al.* 2012), divergence and migration (Hey & Nielsen 2004; Wang & Hey 2010) and spatial implementations of the coalescent (Leblois *et al.* 2003; Currat *et al.* 2004; Ray *et al.* 2010) in increasingly complex models, also increasingly suitable for use with genomic data.

3.3.1. The coalescent process

If we consider two sequences in a diploid Wright-Fisher population of effective size N individuals, or *2N* sequences (Figure 4A, second row from the top), the probability that the two considered sequences coalesce in the previous generation, i.e., that they descend from the same parent sequence, or chromosome, is

$$P_c = \frac{1}{2N} \ .$$

The probability that they descend from different chromosomes in the previous generation is 1-1/(2*N*). At the next generation back in time, the situation is the same: probability of coalescence 1/(2*N*), probability of not coalescing 1-1/(2*N*). Thus the probability of coalescing *t* generations ago is the probability of not having coalesced for *t*-1 generations, multiplied by the probability of coalescing at the given generation,

$$P_c(t) = \left(1 - \frac{1}{2N}\right)^{t-1} \frac{1}{2N} \ .$$

This probability distribution, for non-overlapping generations, is geometrically distributed. Assuming a large population size and continuous time (which allows for overlapping generations), it can be approximated by the exponential distribution,

$$P_c(t) = \frac{1}{2N} e^{-\frac{t-1}{2N}} \ .$$

This exponential distribution is mathematically interesting as both its expected value and its standard deviation are equal to *2N*, thus the expected time of coalescence, or the expected time to the most recent common ancestor (MRCA) for two sequences is (Figure 4C)

$$E(T_2) = 2N \ .$$

A

B

Most recent common ancestor (MRCA)

C

$$E(T_n) = \frac{4N}{n(n-1)}$$

time

Present day →
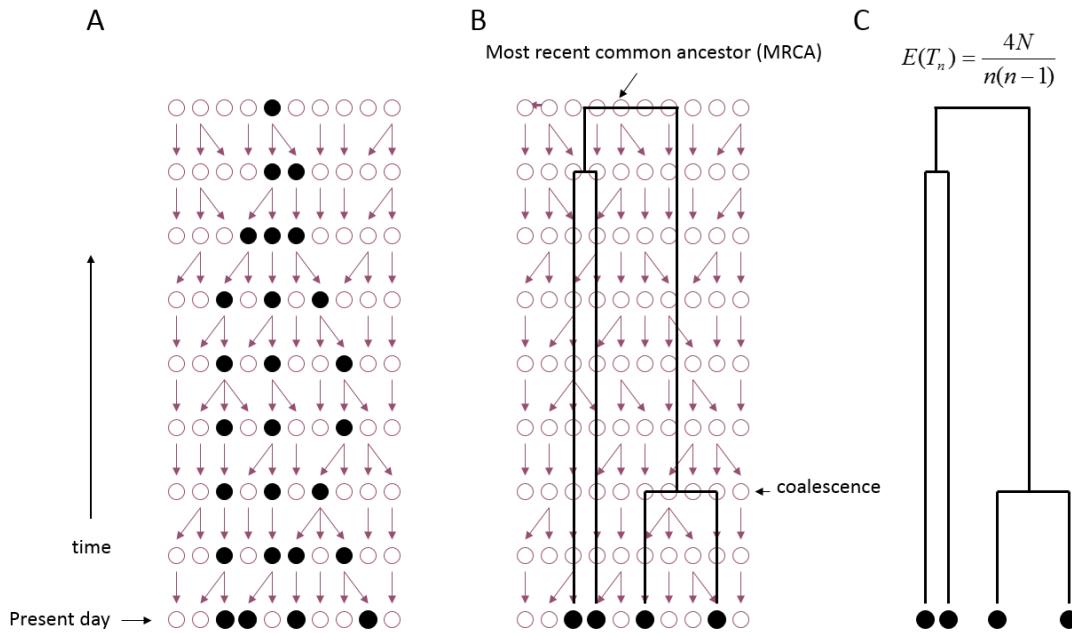
← coalescence

Figure 4 The coalescent process (modified from McVean, 2001). A. Genealogical relationships in a population of size 2N sequences (purple), where each row represents a generation, and genealogy of a sample of n=4 sequences (black). B. Genealogy of the sample, schematic illustration. C. Expected times to coalescence of linages in the sample.

On average, the time to the most recent common ancestor for two sequences sampled in a population of size *2N* sequences is thus equal to the population size, expressed in number of generations (Figure 4C; Figure 5). For example, in a population of 100 diploid individuals, the most recent common ancestor for 2 random sequences is expected 200 generations back in time. The exponential distribution of coalescence times has a large variance. It is characterized by having many short events – 63% of all coalescent events are expected to be shorter than the average – and a few very long ones (Figure 5).

$$\frac{1}{2N}$$

$$E[T_{MRCA}] = 2N$$

P(x)

2N   4N   6N

X
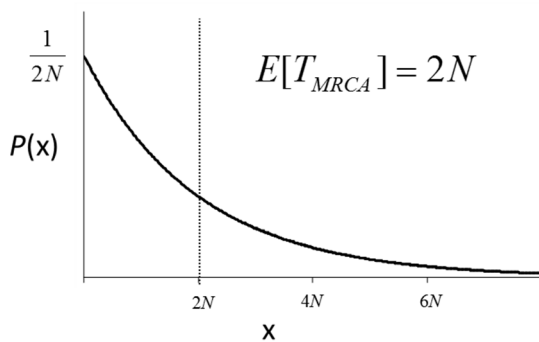
Figure 5 Probability density function of coalescence times for a sample of 2 sequences in a population of size N diploid individuals.

As a consequence, if the coalescent process is simulated multiple times, multiple genealogical tree shapes are typically obtained (Figure 6, illustration for *n*=10 sequences). Let's examine the coalescence for *n* sequences (Figure 4C), as opposed to two; this process is known as the *n*-coalescent. The following assumptions are made: each of the *n(n-1)/2* pairs coalesces independently, a single coalescence event can occur in a single generation and time can be represented as continuous. The probability of coalescence of *any* of the pairs in the previous generation is then

34

$$P_c = \frac{n(n-1)}{2} \frac{1}{2N} .$$

The expected time to coalescence of *any* of the pairs is then the inverse of this, namely $4N/(n(n\text{-}1))$, by the same logic as for the case of two sequences,

$$E(T_n) = \frac{4N}{n(n-1)} .$$

This shows that **the rate of coalescence in a population is dependent on population size and on the number of lineages** (alleles of the sample, followed backward in time) present at a given time (Figure 2C). The current state of the sample is thus the only thing that matters to model the following coalescence event. This **independence of successive coalescence events** is an important property of the coalescent, which allows the efficient modelling of genealogies.

Example: For a population of effective size 100 diploid individuals, in which we sample n=4 sequences, the expected time to coalescence of any of the pairs is 400/(4*3)=33.33 generations. If however, we are interested in the TMRCA of n=2 sequences in the sample, then the expected time to coalescence is 200 generations, as seen above. Thus the expected time to coalescence for the last two lineages is longer than any previous one. Genealogies in constant size populations are therefore commonly marked by long branches to the last coalescence event.
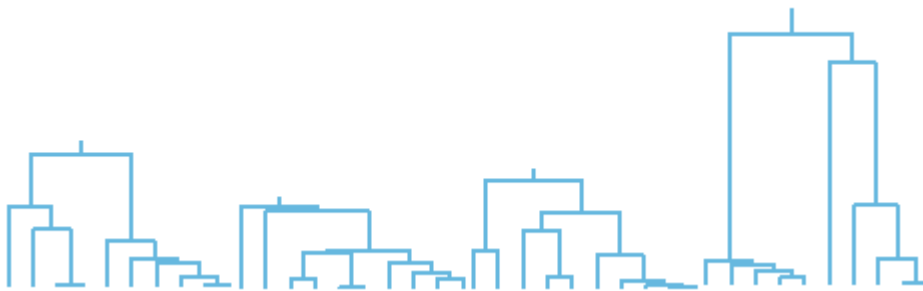
Figure 6 Random genealogical trees. The trees were generated using the same model, the standard coalescent for sample of size ten. Therefore, the variation among the trees reflects chance alone. From Rosenberg and Nordborg 2002.

3.3.2. Adding mutations onto the genealogy

Once a genealogy has been simulated, we wish to add mutations onto this genealogy. The conventional mutation model associated with the coalescent process is the infinite-sites model. The probability of a mutation occurring in a single generation is u; this is the mutation rate for the entire sequence considered. The number of mutations occurring on the sequence during t generations is Poisson distributed, with expectation equal to t x u. For our most simple genealogy of two sequences (the last coalescence event, top part of Figure 4C), the expected number of mutations – or number of "segregating sites", or number of pairwise differences between the two sequences – is then

$$E[\pi] = 2 \times u \times E[T_{MRCA}] = 4Nu = \theta .$$

The constant 2 is needed to represent the lineage length along which mutations occur; they occur independently along each of the two branches, and this **independence of mutations** is also an important property of the coalescent.

The quantity $4Nu$, the "population mutation parameter", is an important parameter describing the diversity in a population. It is usually written as a single parameter, θ.

Box 1 Gene trees vs. species trees

In population genetics, coalescent trees are used as a statistical tool to explore population history. Statistical properties of the coalescent reflect that the genetic data observed in a population are the outcome of a highly stochastic evolutionary process. It results that different genes in a population have typically very different genealogical trees, with varying topology and depth. Even within a gene, recombination can lead to different genealogical trees for different gene sections.

In phylogenetics, the basic model relates species to each other through a bifurcating genealogical tree, and species trees are commonly estimated on the basis of one or a few loci. Given the stochasticity of the coalescent process, how can the phylogenetic approach be valid?

This dilemma arises because gene trees are not the same as species trees. When time intervals between species branching events are much larger than time intervals beween lineage-branching events within a species, then gene trees are approximately similar to species trees, and the phylogenetic approach can be considered valid (Maddison 1997; Nichols 2001; Rosenberg & Nordborg 2002). This is the case for system A (**Error! Reference source not found.**), a "deep" phylogenetic tree: the gene tree ((red, green), blue) is concordant with the species tree ((a, b), c). In system B, with a shallower tree depth, the gene tree (red, (green, blue)) is incongruent (discordant) with the species tree.

Gene tree – species tree discordance has been analytically studied (Hudson 1992; Rosenberg 2002; Rosenberg & Degnan 2006), revealing that a substantial amount of time is required after the initial divergence of species before there will be a high probability of observing reciprocal monophyly at a sample of multiple loci. While reciprocal monophyly is not reached, the situation is one of so-called "incomplete linage sorting". In this situation, a signal of species divergence can nevertheless be extracted using multi-species coalescent approaches and sampling of multiple individuals/species and multiple loci, allowing species delimitation (Knowles & Carstens 2007; Mirarab *et al.* 2014; Rannala & Yang 2017)(**Error! Reference source not found.**C).

Which species tree depths occur in real-life situations? Tree depth can be expressed in "coalescent time units", i.e., as branch length in number of generations divided by $2N$. For example, for the species tree (((H, C), G), O) for human, chimpanzee, gorilla and orangutan, the time from the gorilla divergence to the split between humans and chimpanzees was estimated to 1.2 million years. Using an effective population size of $2N$ = 49 200 autosomal gene copies (24 600 individuals) and a generation time of 20 years (Rannala & Yang 2003), this branch length corresponds 60 000/49 200=1.2 coalescent time units. In the bird genus Passerina, conversely, species have been estimated to have effective population size N near 1 000 000 individuals and intervals between speciation events as small as ca. 100 000 generations (Carling & Brumfield 2008), suggesting branches as short as 0.05 coalescent time units (Degnan & Rosenberg 2009).

Besides incomplete lineage sorting, other reasons for gene tree – species tree discordance are horizontal gene transfer also known as introgression (Knowles *et al.* 2018), hybridization which affects full genomes, such as in homoploid hybrid speciation (Nieto Feliner *et al.* 2017), gene duplication and loss, and recombination (Degnan & Rosenberg 2009).
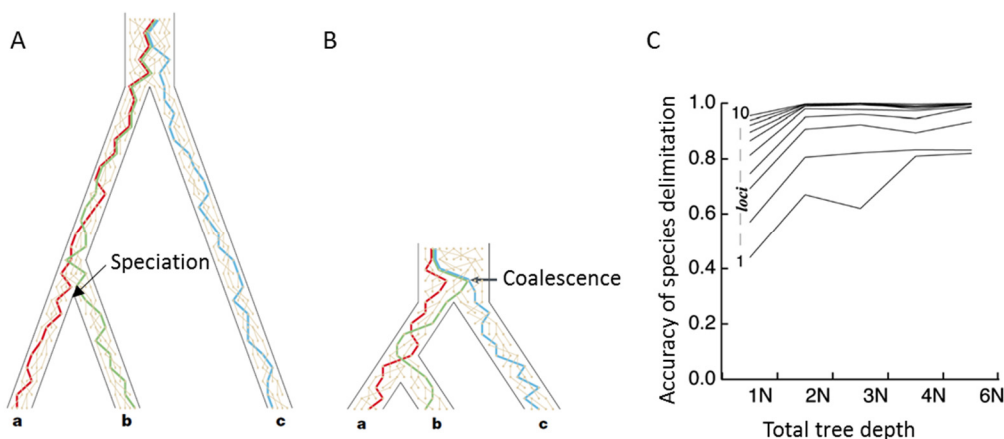

Figure 7 Gene trees vs. species trees and accuracy of species delimitation. A "Deep" species tree with brancing depth >>2N (one coalescent time unit) where phylogenetics approaches are generally valid; B, shallower species tree; C Accuracy of species delimitation with coalescent methods as a function of species tree depth (A and B from Deanan and

For the n-coalescent, the expected number of segregating sites is the sum over each coalescent interval,

$$E[S] = \theta \sum_{i=1}^{n-1} \frac{1}{i}$$ (Watterson 1975).

Example: In a population of 10^6 diploids, with a mutation rate of 10^{-9} per site per generation, the expected number of pairwise differences between $n = 2$ random sequences in a gene of 1500bp would be $E[\pi]$ = 4 x 10^6 x 10^{-9} x 1500 = 6. If we sample $n = 4$ sequences, the expected number of segregating sites in our sequence alignment would be $E[S]$ = $4Nu$ (1+½+⅓) = 6 x 1.833 = 11.

The variance in the number of segregating sites (mutations) is very large since it results from the combined stochasticity of the coalescent process, and the stochasticity of the mutation process.

3.3.3. Using the coalescent for population genetics

In statistical inference, we are interested in inferring information about a population of interest. To do so,

- we take a random sample from the population (e.g., a set of individuals that we sequence at one or several loci)
- we compute a statistic from the sample (from our set of sequences)
- we obtain information about the distribution of the statistic, using simulations or resampling
- using the distribution, we infer about the population based on the sample statistic

The inference can take the form of a point estimate of a population parameter of interest, an interval estimate such as a confidence interval, a credible interval (a set of values in Bayesian inference, containing a certain percentage of posterior belief), the rejection of a hypothesis, or the clustering of data points into groups (Konishi & Kitagawa 2007).

The most basic question in population genetics is whether a gene sequence evolves randomly, or "neutrally", in a population or whether, alternatively, it bears evidence of non-random evolutionary processes such as demographic expansion or contraction, directional or balancing selection, genetic hitchhiking or of being introgressed from a different population. Such different evolutionary histories affect the polymorphism pattern of gene sequences (Box 2).

We use the coalescent to simulate genealogies with mutations: typically millions of datasets are simulated under one or several evolutionary scenarios (models). Summary statistics computed from the observed data are then compared with the distribution of summary statistics computed from the simulated data to determine the likelihood of the observed data under the evolutionary model.

To tackle the question of neutral evolution using the sequences in a sample, we compute a set of statistics that capture different properties of the polymorphism pattern, e.g., the number and frequency of polymorphisms. Two straightforward statistics represent unbiased moment estimators of the population mutation parameter θ: Nei and Li's nucleotide diversity, π (Nei & Li 1979), and Watterson's estimator, θ_W (Watterson 1975).

Box 2. Population demographic scenarios can affect the shape of the coalescent tree and the distribution of mutations across DNA sequences (e.g., Navascués et al. 2006). In a constant size population, the last coalescence time is typically long, so that many mutations are located along the profound branches: this results in high-frequency polymorphisms shared by many sequences. In the case of population expansion (interpreted forward in time), genealogies typically have a comb-like appearance. Thus many mutations occur along terminal branches, which results in many rare polymorphisms, i.e., mutations that are singletons or shared by few sequences.
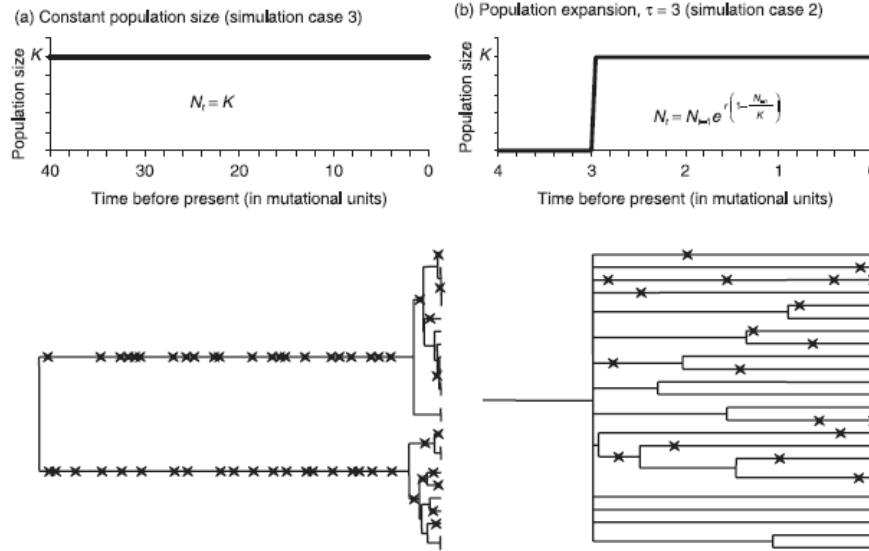
Figure 8 Coalescent process under two contrasting scenarios: constant population size and sudden population expansion. For each case, the demographic history and, in the same timescale, measured in mutational units (1 mutational unit = 1/2μ generations), the simulated genealogy of a random sample of genes is represented, with stars representing mutational events (from Navascués et al. 2006).

We saw that the number of expected pairwise differences in coalescent theory is equal to θ, therefore, the sample statistic, i.e., the average observed number of pairwise differences in a sample, is an unbiased estimator of θ. This estimator is the nucleotide diversity,

$$\pi = \sum_{ij} x_i x_j \pi_{ij} \, ,$$

where x_i and x_j are the frequency of sequences i and j, respectively, and π_{ij} is the number of pairwise differences between sequences i and j (Nei & Li 1979). Similarly, we saw that the expected number of segregating sites is a multiple of θ. Thus, Watterson's estimator (Watterson 1975)

$$\hat{\theta}_w = S / \sum_{i=1}^{n-1} \frac{1}{i} = S / a_n \, ,$$

where S is the number of segregating sites in a sample, is also an unbiased estimator of θ.

To evaluate neutral evolution, Tajima developed a statistic that represents the (normalized) difference between these two moment estimators, known as Tajima's D (Tajima 1989), $D = \dfrac{\pi - S / a_n}{\sqrt{\mathrm{Var}(\pi - S / a_n)}}$.

In a randomly evolving sequence in a contant-size population, D is expected to be zero as the two estimators of θ are expected to be equal (Figure 9). Comparing D computed from a sample to the distribution of D based on coalescent simulations will inform on how likely it is that the gene evolves neutrally in the population, given the evolutionary model used.
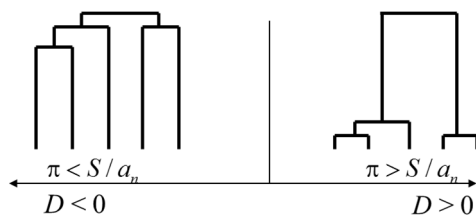
Figure 9 Variation of Tajima's D statistic as a function of the shape of genealogies. Negative D values indicate and excess of rare mutations, positive values indicate an excess of intermediate frequency mutations (from McVean 2005).

Besides Tajima's D, there are several other summary statistics and tests that can be used based on a sample to evaluate signatures of selection, taking advantage of data properties such as the site frequency spectrum of mutations (a summary of the allele frequencies of the various mutations in the sample) and the derived or ancestral state of mutations. A detailed review of the topic can be found in Nielsen (2005), written at a time where genomic data were basically only accessible for humans and a few other model organisms.

We can use coalescent simulations not only for parameter inference, or hypothesis testing, but also in the framework of choosing the most appropriate model to reflect the evolutionary history of a population. Model choice in population genetics is frequently done using Approximate Bayesian Computation (Beaumont *et al.* 2002; Cornuet *et al.* 2008; Csilléry *et al.* 2010). The procedure involves typically:

1. Simulation of data: millions of data sets are simulated under several different evolutionary scenarios, e.g., constant-size population, expanding population and contracting population;
2. Model fitting: some summary statistics are defined; we select the 1% (or another fraction) of simulated data (across models) with summary statistics closest to those computed from the observed data; from this subset of simulations, we determine the posterior probabilities (support) of the competing models and posterior distribution of parameter estimates.
3. Model checking: to make sure that the selected model and estimated parameters represent indeed a suitable model for the data, we simulate "pseudo-observed" data using the model and parameter estimates and evaluate, ideally with a summary statistic not used in model fitting, the match between observed and pseudo-observed data (Csilléry *et al.* 2010).

Alternative methods to infer population demographic history from coalescent simulations employ likelihood-based approaches, including full-likelihood methods (Hey & Nielsen 2007; Hey 2010) or summary-statistics-based likelihood approaches (Becquet & Przeworski 2007). These methods are computationally intensive, with computation time increasing with the number of loci. Therefore more recently developed methods seeking to take advantage of large genomic datasets increasingly rely on the site frequency spectrum of mutations, a type of summary statistic that does not depend on the amount of available genetic data (Adams & Hudson 2004; Excoffier *et al.* 2013). For instance, the fastsimcoal2 simulator allows to infer demographic parameters from the site frequency spectrum (SFS) of large genomic datasets under complex multi-population demographic scenarios using a composite-likelihood approach (Excoffier *et al.* 2013). Another method that uses the joint site-frequency spectra of multiple populations with a composite-likelihood approach does not rely on coalescent simulations to derive expectations but uses instead a diffusion based approach to compute the expected site frequency spectra (Gutenkunst *et al.* 2009). Other recently developed methods for population demographic inference include coalescent hidden Markov Models, that can take advantage of linkage disequilibrium information (Spence *et al.* 2018), and hierarchical Bayesian models that can infer phylogenetic relationships under isolation-with migration demographic scenarios (Hey *et al.* 2018).

4. Research contributions

4.1. Biogeography and phylogeography

Biogeography is the study of the distribution of species and ecosystems in geographic space and through geological time (Dansereau 1957; MacArthur & Wilson 2001). Phylogeography investigates the spatial distribution of biodiversity at a finer grain; it focusses on the distribution of genetic lineages within species or in closely related species in recent geological time, generally in the Pleistocene (Avise *et al.* 1987; Nielsen & Beaumont 2009; Hickerson *et al.* 2010).

4.1.1. Phylogeography of European tree species and species complexes

Since approximately the 1990s, researchers have used molecular markers to examine the Holocene (ca. the last 12,000 years) post-glacial colonization of the temperate tree flora towards higher latitudes (Petit et al. 2003; Soltis et al. 2006). The main aims of these studies were to go beyond insights from palaeopalynology and palaeobotany (Huntley & Birks 1983; Magri *et al.* 2006) and explore which glacial relict tree populations, i.e., refuge populations, contributed to the recolonization of the forests currently found in temperate and boreal latitudes. I contributed to investigating the impact of recent glacial – interglacial dynamics on population genetic processes with plastid DNA and nuclear microsatellite markers in several tree species in Europe.

Temperate tree species – In collaboration with Dr. Giovanni Vendramin (CNR, Italy), I described the geographically widespread sharing of plastid DNA haplotypes between common ash, *Fraxinus excelsior* L. (Oleaceae), and the closely related narrow-leaved ash, *F. angustifolia* Vahl, showing that both species experienced extensive historical gene flow during glacial isolation in refuge populations or during postglacial recolonization leading to near-complete haplotype sharing in sympatric regions (Figure 10; Heuertz, Carnevale, et al. 2006). In the sympatric distribution, higher genetic diversity in *F. excelsior* than in *F. angustifolia* reflected the more cold-tolerant temperament of the first species. We also discovered that the three southern European peninsulas as well as Turkey harboured glacial refuge populations for *F. angustifolia*, whereas *F. ornus* survived glaciations in the Appennine and Balkan peninsulas and Turkey without exchanging genes with the other two species (Figure 10).
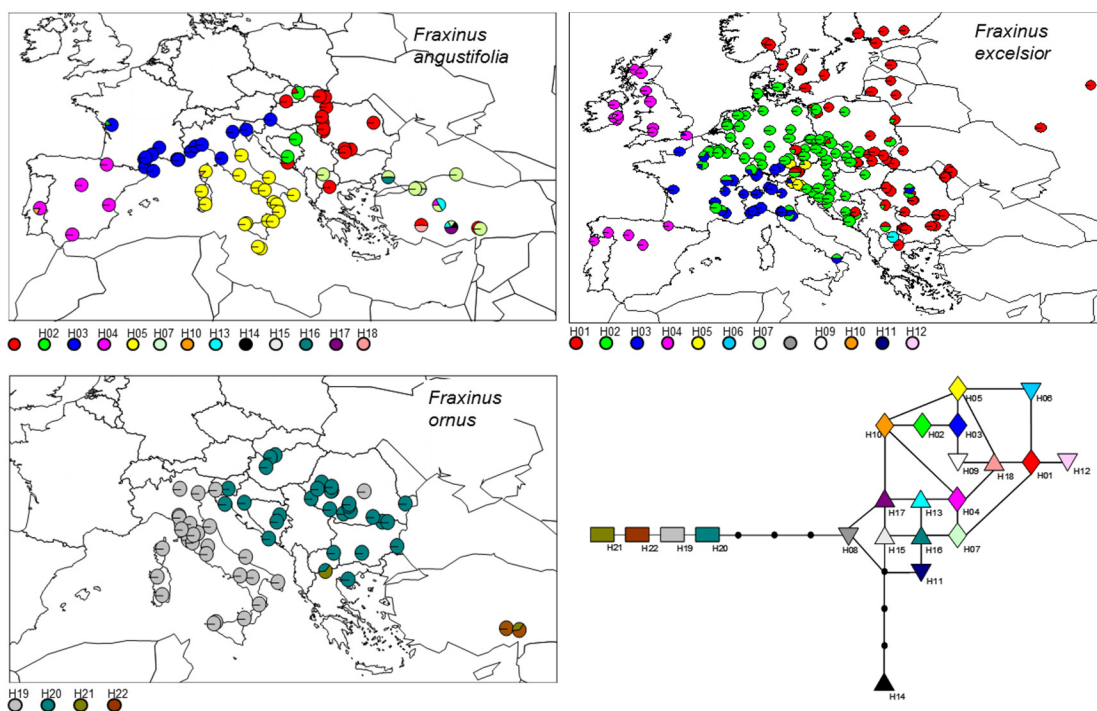
Figure 10 Plastid DNA haplotype distribution in three Fraxinus *species in Europ. From Heuertz, Carnevale et al. (2006).*

My subsequent work on *Fraxinus* focussed on the colonization of the northern range margin of the species in Norway in a collaboration with Mari Mette Tollefsrud (NIBIO, Norway). Using maternally transmitted plastid DNA markers to trace historical seed dispersal, we showed that the Norwegian coast was colonized by the main eastern European plastid DNA lineage (Tollefsrud *et al.* 2016). Nuclear genetic diversity declined towards the north, suggesting successive colonization bottlenecks (Figure 11; Tollefsrud et al. 2016).
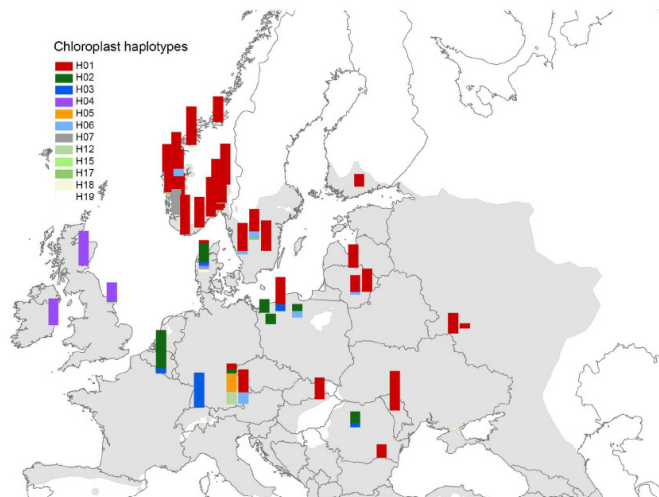
*Figure 11 Distribution of chloroplast haplotypes in 42 common ash (*Fraxinus excelsior*) population samples. From Tollefsrud et al. (2016).*

With the spread since the 1990s of ash dieback, an ascomycete pest causing large-scale mortality in European ash across Europe (Pautasso *et al.* 2013), this phylogeographic work of the *Fraxinus* host is of interest to many researchers. I am currently involved in a project, ASH-ADAPT, coordinated by Lene Rostgaard Nielsen at the University of Copenhagen to conduct an association genomics study and screen natural ash populations for tolerance to ash dieback and to another emergent pest, the emerald ash borer.

Mountain tree species – Mountain tree species do not experience the same latitudinal migration patterns as temperate species in response to climatic changes. Instead, they exhibit preferential elevational migration. In species that occur in multiple mountain ranges, genetic connectivity between range parts can be reduced due to the disjoint distribution. This isolation can lead to genetic divergence, possibly resulting in speciation. In a collaboration with Giovanni Vendramin and Santiago González-Martínez, I investigated the phylogeographic history of the European mountain pine species complex, *Pinus mugo* Turra s.l. (Heuertz *et al.* 2010). Populations within the Pyrenees (*Pinus uncinata* Ram.) and the Alps Pyrenees (*P. uncinata* and *P. mugo* s.s.) were well-connected through pollen-mediated gene flow since the last glaciation, and Alpine populations of *P. uncinata* exhibited a common gene pool with *P. mugo* s.s., so that geography was a better predictor of genetic structure in the complex than botanical species membership (Figure 12) (Heuertz *et al.* 2010).

Riparian tree species – I further participated in a study on phylogeographic structure in riparian trees of the genus *Populus*, contributing to the supervision of the PhD thesis of David Macaya-Sanz at the Universidad Autónoma de Madrid. In the Iberian Peninsula, the cold tolerant black popular *Populus nigra* L. displayed higher diversity and higher connectivity among river basins than white poplar *P. alba* L. (Macaya-Sanz *et al.* 2012). Despite strong genetic structure and frequent asexual propagation in white poplar, some growth traits displayed adaptive divergence between drainage and river basins ($Q_{ST} > F_{ST}$), highlighting the remarkable capacity of riparian tree populations to adapt to regional environmental conditions (Macaya-Sanz *et al.* 2012).
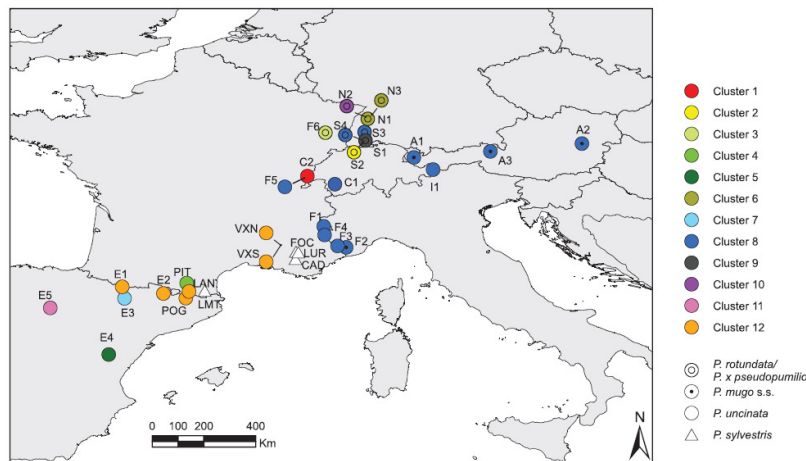
Figure 12 Spatial Bayesian cluster analysis (BAPS) showing genetically homogenous groups of populations of the Pinus mugo species complex in western Europe. From Heuertz et al. (2010).

These contributions to phylogeographic studies in European tree species show that ecological requirements of tree species determine to a high extent the distribution of genetic diversity within species, and that the current geographic distribution of genetic diversity harbours the signature of past hybridization in the studied species complexes.

Funding

CYTOFOR – Measuring molecular differentiation of European deciduous forests for conservation and management. European Union, Agriculture and Fisheries (FAIR) specific RTD programme, CT97-3795. 1998 – 2001. PI: Rémy Petit. Funding to G.G. Vendramin, CNR.

Ash dieback in Norway – Cause, Impact and Control. The Research Council of Norway. 2011 – 2014. 5,050,000 NOK, 19,850€ to INIA-CIFOR. PI: Halvor Solheim. Coordinator for INIA-CIFOR: Myriam Heuertz.

ASH-ADAPT – Evolutionary potential of natural Fraxinus excelsior populations challenged by novel pests and pathogens. 2019-2021. PI: Lene Rostgaard Nielsen, Univ. Copenhagen.

Visiting scientist grant of Fonds National de la Recherche, Belgium (9 months, in 2008), to Myriam Heuertz. Host: Santiago González-Martínez, INIA-CIFOR (Spain).

Mentoring

Contribution to the supervision of PhD students

David Macaya Sanz, Universidad de Valladolid, 2015. Main supervisors: Santiago C González-Martínez and Christian Lexer. Filogeografía, diversidad e hibridación en *Populus* spp.

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Heuertz M, Teufel J, González-Martínez SC, Soto A, Fady B, Alía R, Vendramin GG (2010) Geography determines genetic relationships between species of mountain pine (*Pinus mugo* complex) in western Europe. *Journal of Biogeography* 37, 541–556.

Macaya-Sanz D, **Heuertz M**, López-de-Heredia U, de-Lucas AI, Hidalgo E, Maestro C, Prada A, Alía R, González Martínez SC (2012) The Atlantic-Mediterranean watershed, river basins and glacial history shape the genetic structure of Iberian poplars. *Molecular Ecology* 21, 3593–3609.

Tollefsrud MM, Myking T, Sønstebø JH, Hietala A, **Heuertz M** (2016) Genetic structure in the northern range margins of common ash, *Fraxinus excelsior* L. *PLOS ONE* 11, e0167104.

4.1.2. Plant biogeography and phylogeography in tropical Africa

Tropical Africa has undergone gradual aridification since the Miocene (23 to 5.3 Ma ago), with aridification starting in the southwest of the continent much earlier (ca. 17 Ma) than in the present-day Sahara (ca. 7 Ma, Figure 13) (Senut *et al.* 2009). Plant lineages in the south of the continent thus had time to adapt to a changing climate. Tropical forests became established in Central Africa due to subsidence of the Congo basin and Eastern African uplift since ca. 8 Ma, the latter also leading to aridification in Eastern Africa (Sepulchre *et al.* 2006).
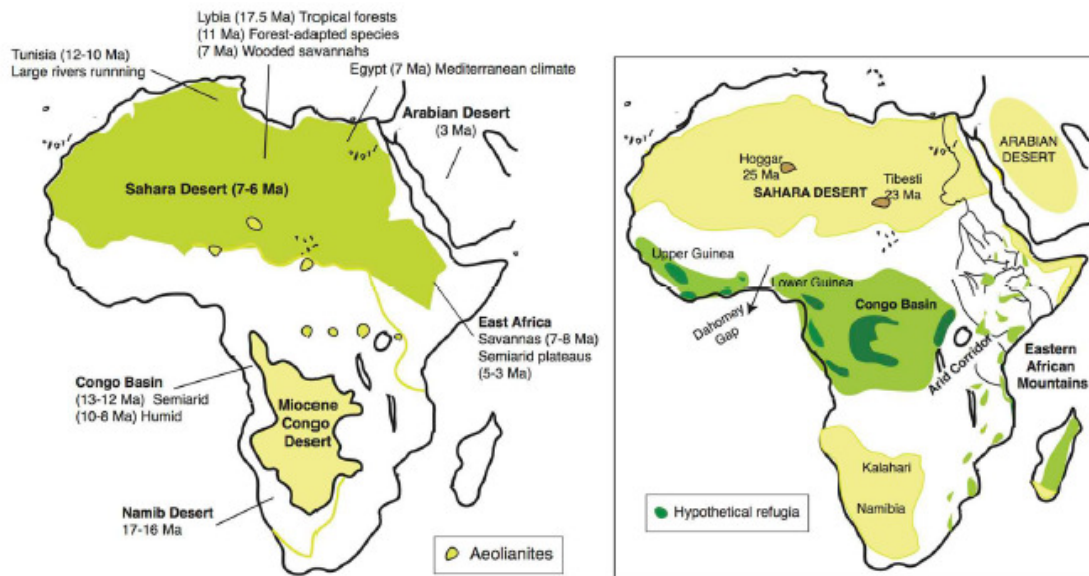
Figure 13 Main climatic events in Africa during the Miocene (left panel) and distribution of current vegetation types (right panel, with Guineo-Congolian rainforest in green). From Pokorny et al. (2015), adapted from Senut et al. (2009).

I became interested in evolutionary processes in African plants, especially trees, since my position as a Research Associate (2006-2008) of the Belgian National Fund for Scientific Reseach (FNRS) where I collaborated with the team of Olivier Hardy at the Université Libre de Bruxelles (ULB), and I pursued this work during my Ramón y Cajal Independent researcher position at INIA-CIFOR in Madrid, Spain (2010 – 2014), collaborating essentially with the team of Isabel Sanmartín at the Madrid Botanical Garden (RJB-CSIC).

Rand Flora – The Rand Flora is a well-known floristic pattern in which unrelated plant lineages show similar disjunct distributions in the continental margins of Africa and adjacent islands – Macaronesia-northwest Africa, Horn of Africa-Southern Arabia, Eastern Africa, and Southern Africa. These lineages are now separated by environmental barriers such as the arid regions of the Sahara and Kalahari deserts or the tropical lowlands of Central Africa (Figure 13, right panel). To provide a temporal framework for the Rand Flora pattern, I collaborated with Isabel Sanmartín's team to date the age of geographic disjunctions using published plastid DNA sequence data from 17 lineages in 12 angiosperm families (Figure 14). Most disjunctions were dated to the Miocene and Pliocene, coinciding with the onset of a major aridification trend, with sub-humid taxa (e.g., *Sideroxylon*, Sapotaceae) diverging earlier and those with more xeric affinities (e.g., *Campylanthus*, Plantaginaceae) diverging later (Pokorny *et al.* 2015). Some lineages (e.g., *Cicer*, *Campylanthus*) showed a long temporal gap between stem and crown ages, suggestive of extinction (Pokorny *et al.* 2015).

I also collaborated with Isabel's PhD student Mario Mairal on demographic simulations in the Rand Flora genus of bellflowers *Canarina* (Campanulaceae), more specifically in two emblematic species which are widely distributed in Afro-Macaronesian forests, a patchily distributed relict ecosystem: *Canarina eminii* Asch. & Schweinf., a palaeoendemic of the eastern Afromontane forests, and *Canarina canariensis* L. Vatke, a palaeoendemic of the Canarian laurel forest. All populations displayed

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signatures of a strong and recent decline of effective population size using coalescent simulations on nuclear microsatellite markers (Mairal *et al.* 2018). These results revealed that also locally widespread endemics may be alarmingly threatened, calling for urgent conservation action on the threatened ecosystems they inhabit.
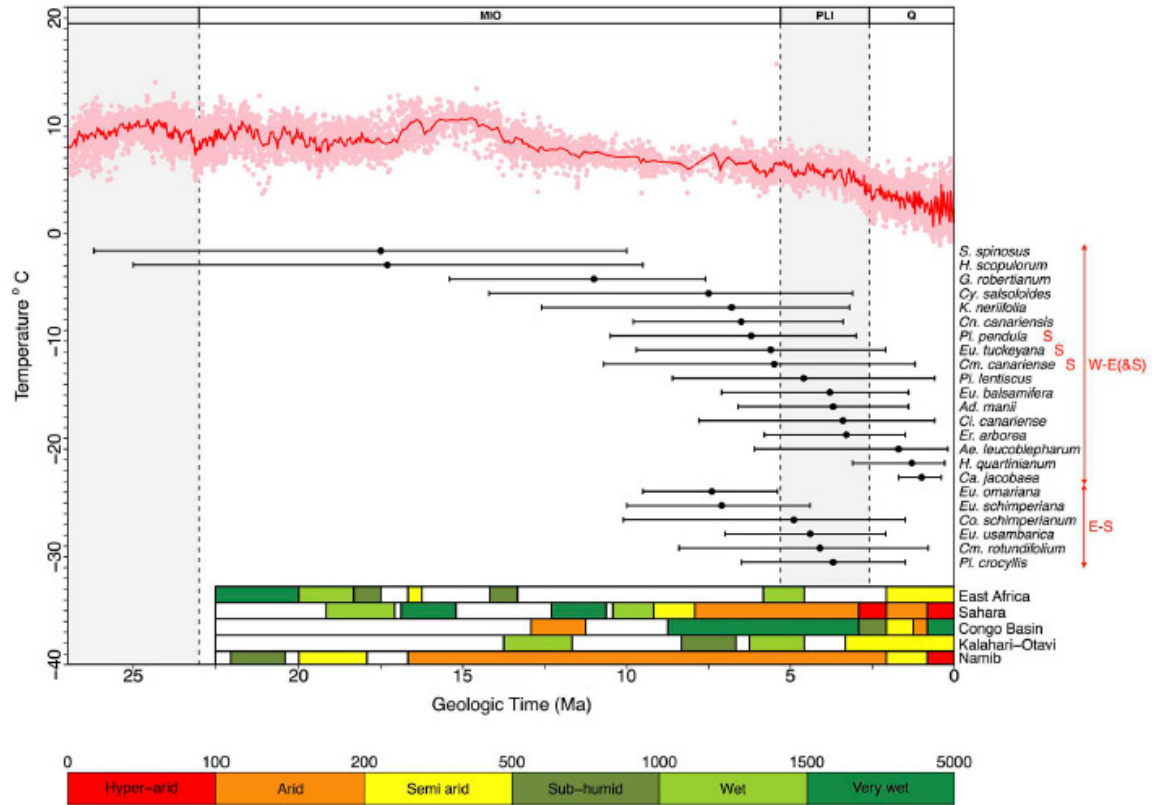

Figure 14 Estimated lineage divergence times for Rand flora lineages. W-E (&S): divergence times estimated between disjunct taxa distributed in Macaronesia-northwest-west Africa vs. Eastern Africa (a red S indicates presence in southern Africa); E-S: estimated divergence times between disjunct taxa distributed in southern Arabia-eastern Africa vs. southern Africa. From Pokorny et al. (2015).

Tropical rainforests – West and Central African tropical rainforests, known as Guineo-Congolian rainforests (Figure 13), constitute the second largest rainforest block in the world after the Amazon. These rainforests comprise ca. 10,000 plant species of which more than 40% are endemic (Droissart *et al.* 2018). Botanical surveys allowed to delineate three blocks of plant endemism in these forests: *Upper Guinea* in West Africa, from Sierra Leone to Ghana, *Lower Guinea* close to the Golf of Guinea, from southern Nigeria to the southwestern part of the Republic of Congo, and Congolia covering the Congo Basin (White 1983; Linder *et al.* 2012; Droissart *et al.* 2018). The vegetation history of these forests remained however contentious for a long time. Notably, there was poor knowledge on how plant endemism patterns were affected by putative barriers to plant migration, such as the Dahomey gap, a savannah region separating Upper and Lower Guinea, or the Cameroon Volcanic line, a mountainous region in western Cameroon. Palaeoecological data revealed that Plio-Pleistocene climatic oscillations led to forest contraction at the expense of savannah vegetation during cold and dry stages (Maley 1996; Vincens *et al.* 1999; Bonnefille 2007), and species endemism in forest herbs and shrubs with poor dispersal abilities such as Begoniaceae (Sosef 1994) or Rubiaceae (Robbrecht 1996) provided indications for the location of Plio-Pleistocene forest refuges (Maley 1996). Nevertheless, many questions remained on the location and structure of fragmented forests during these cold stages, and their impact on the constitution of today's rainforest vegetation in Africa.

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With the team of Olivier Hardy at the ULB, we sampled widespread African rainforest trees and conducted phylogeographic studies to characterize genetic structure and patterns of allele endemism, and thus reveal species-specific patters of past rainforest fragmentation and recolonization. While developing this research, I had the opportunity to mentor my first PhD student, Guillaume Koffi (co-supervision with Olivier Hardy), I contributed to mentoring other PhD students in the lab and I collaborated with postdocs. I continued this research line as a Ramón y Cajal researcher at INIA-CIFOR, mentoring PhD students Katharina Budde and Paloma Torroba-Balmori.

Single-species phylogeographic studies – The first wide-range phylogeographic studies in rainforest trees in west and Central Africa revealed taxon-specific signatures of genetic diversity and structure, with some common patterns at the large scale. Highly differentiated gene pools were found in Upper vs. Lower Guinea in the widespread dioecious tree species *Milicia excelsa* (Welw.) C.C. Berg (Moraceae), which has a broad ecological tolerance, as well as in the species *Santiria trimera* (Oliv.) Aubrév. (Burseraceae) typical of mature rainforests, suggesting effective isolation of Upper Guinean form Lower Guinean forests through several glacial cycles (Daïnou *et al.* 2010; Koffi *et al.* 2011). Within Lower Guinea, differentiation was less pronounced, and revealed North-South divergence in both *M. excelsa* and *S. trimera* as well as in the mature rainforest tree *Greenwayodendron suaveolens Greenwayodendron suaveolens* (Engl. & Diels) Verdc. *subsp. suaveolens* (Annonaceae) (Dauby *et al.* 2010). In *S. trimera* and *G. suaveolens*, endemic alleles were found in locations coinciding with previously proposed rainforest refuges in mountainous regions of Lower Guinea, especially in Cameroon (Maley 1996; Maley & Brenac 1998). These results indicated that these rainforest species persisted in the proposed refuges during Pleistocene cold stages (Dauby *et al.* 2010; Koffi *et al.* 2011). Conversely, in the widespread rainforest tree *Symphonia globulifera* L.f. (Clusiaceae), endemic alleles were not restricted to proposed rainforest refuges, indicating wide-spread long-term population persistence across a large region in Lower Guinea, probably owing to a broad ecological tolerance of the species, including the ability to persist in gallery forests (Budde *et al.* 2013).
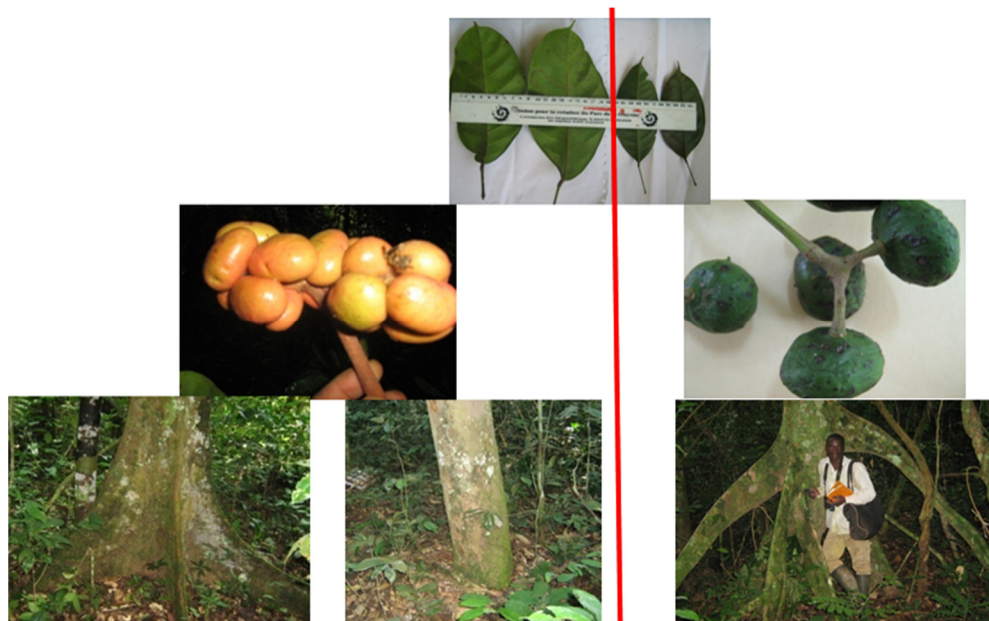

Figure 15 Phenotypic variation of distinct Santiria trimera *(Burseraceae) morphotypes. From Koffi (2010).*

These early phylogeographic studies were conducted on abundant and wide-spread, easy to recognize species in the Guineo-Congolian rainforests. Nevertheless, using a combination of molecular and morphological data, we discovered that several of these *a priori* species were commonly constituted of more than one (near) reproductively isolated species. Discriminant characters for these non-model trees are often found on flowers or fruits, which are difficult to sample because trees are tall and reproduction can be irregular. The two morphologically and genetically distinguishable varieties we

identified within *G. suaveolens* subsp. *suaveolens* in our study (Dauby *et al.* 2010) were later described as distinct species, *G. suaveolens* (Engl. & Diels) Verdc., and *G. gabonicum* (Le Thomas) Lissambou & Couvreur, comb. nov. (Lissambou *et al.* 2018). Similarly, in *Santiria trimera* (Burseraceae), we found that morphotypes with or without stilt roots corresponded to at least two species following the biological species concept in Central Africa (Figure 15; Koffi et al. 2010; Koffi 2010); this was later revised to be three species (Ikabanga *et al.* 2017). In the *Erythrophleum* (Fabaceae) species complex, two species were suspected, *E. suaveolens* (Guill. & Perr.) Brenan and *E. ivorense* A. Chev., but their distribution ranges were unknown. We delimited the species and characterized their respective ranges using morphological characters and genetic markers on a collection of field-collected samples and available herbarium vouchers, finding the best joint interpretation between field samples which only rarely have flowers or fruits available, and herbarium vouchers which suffer frequent misdetermination (Duminil *et al.* 2010).
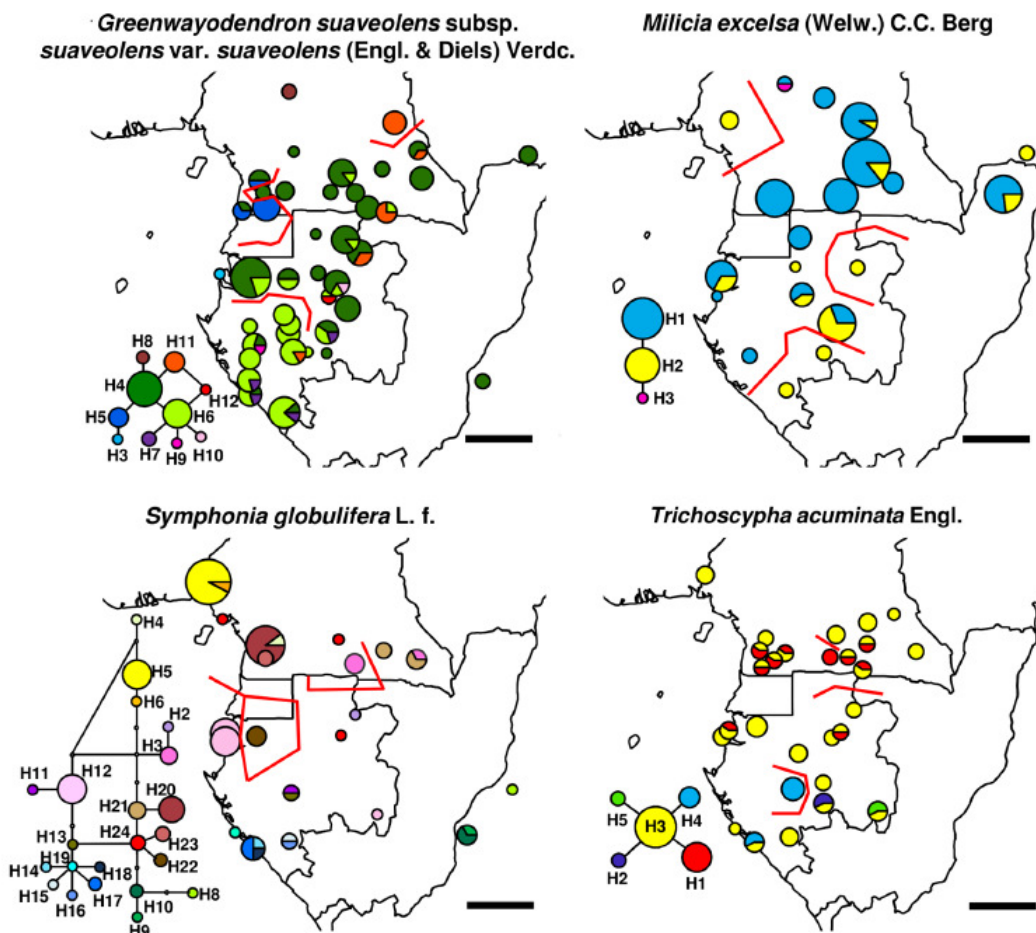
Figure 16 Distribution of plastid DNA haplotypes in four rainforest tree species in Lower Guinea, Africa. Sizes of circles are proportional to the sample sizes of each population, haplotypes represent variation at the trnC-ycf6 plastid DNA region and are colour-coded. Statistical parsimony networks of haplotypes are included for each species. Fram Heuertz et al. (2014).

Comparative phylogeography – To reconstruct the past vegetation history in the Lower Guinea region, I contributed to several studies using comparative and multi-species phylogeographic analysis approaches. We discovered that levels of plastid DNA diversity were widely variable in a set of 14 tree species which included early- to late-successional species of different forest strata. However, we were unable to identify any association between level of diversity and species life history traits nor any common phylogeographic pattern in most of these species (Heuertz et al. 2014). However, a common pattern of North-South genetic discontinuity was observed in Lower Guinea in four tree species for which larger sample sizes were available (n>100), pointing to limited gene flow across the climatic hinge (meteorological equator) (Figure 16; Heuertz et al. 2014). The same North-South genetic

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discontinuity was observed in a review paper on nuclear and plastid DNA-based phylogeographic patterns of 13 rainforest tree species in Lower Guinea (Figure 17; Hardy et al. 2013). We observed several congruent genetic discontinuities in the study region, which supported the theory of Plio-Pleistocene rainforest refugia, however the location of these discontinuities challenged the classically proposed refuge locations by Maley (Hardy *et al.* 2013).
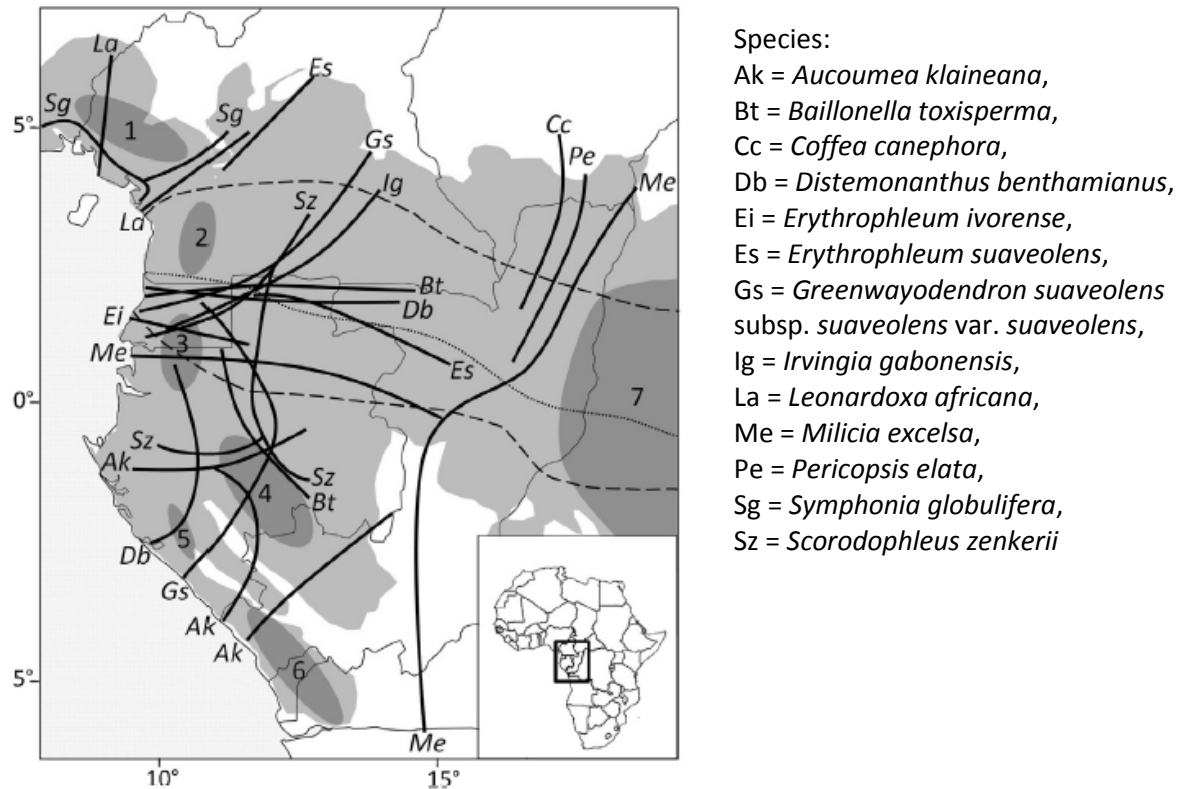

Species:
Ak = *Aucoumea klaineana*,
Bt = *Baillonella toxisperma*,
Cc = *Coffea canephora*,
Db = *Distemonanthus benthamianus*,
Ei = *Erythrophleum ivorense*,
Es = *Erythrophleum suaveolens*,
Gs = *Greenwayodendron suaveolens*
subsp. *suaveolens* var. *suaveolens*,
Ig = *Irvingia gabonensis*,
La = *Leonardoxa africana*,
Me = *Milicia excelsa*,
Pe = *Pericopsis elata*,
Sg = *Symphonia globulifera*,
Sz = *Scorodophleus zenkerii*

Figure 17 Approximate position of genetic discontinuities found within 13 rain forest tree species in Lower Guinea. Putative forest refugia during the Last Glacial maximum are depicted as dark grey shaded areas (modified after Maley, 1996): 1 = area surrounding the Cameroonian volcanic line, 2 = Ngovayang and surrounding massifs, 3 = Monts de Cristal, 4 = Massif du Chaillu, 5 = Monts Doudou, 6 = Mayombe, 7 = fluvial refuge of the Congo river. Genetic discontinuities were identified as the geographic limits between parapatric gene pools identified using nuclear microsatellites, except for Ig (RAPD data) and La (limits between parapatric clades of the chloroplast genome). Most species were not sampled on the eastern side of the map (except Cc, Me and Pe) so that the low density of discontinuities there might be an artifact. The two stippled lines delimit approximately the equatorial climate with two wet and two drier seasons, the latter being of equal intensity (in terms of rainfall) along the climatic hinge (dotted line); the boreal climate to the north is characterized by one dry and sunny season from December to February, while the austral climate to the South is characterized by one dry but cloudy (hence less harsh) season from June to August. From Hardy et al. (2013).

In another study, we examined high-resolution plastid DNA patterns in eight tree species in Lower Guinea and detected significant congruence in genetic distinctiveness patterns mostly among five of the eight species, with strong congruence in the north of the study region (Figure 18) (Dauby *et al.* 2014). High distinctiveness in coastal Cameroon vs. low distinctiveness further east is compatible with a scenario of past climate-related fragmentation affecting primarily the forests in eastern Cameroon and northeastern Gabon, whereas in western Cameroon forests would have persisted in one or several refugia. By contrast, low congruence among species was observed in the south of the study region (Gabon), which may reflect less drastic past forest fragmentation or a more complex history of vegetation changes. Finally, we also identified a potential impact of current environmental barriers, such as discontinuous rainforest distribution in southern Gabon, on spatial genetic structures (Dauby *et al.* 2014).
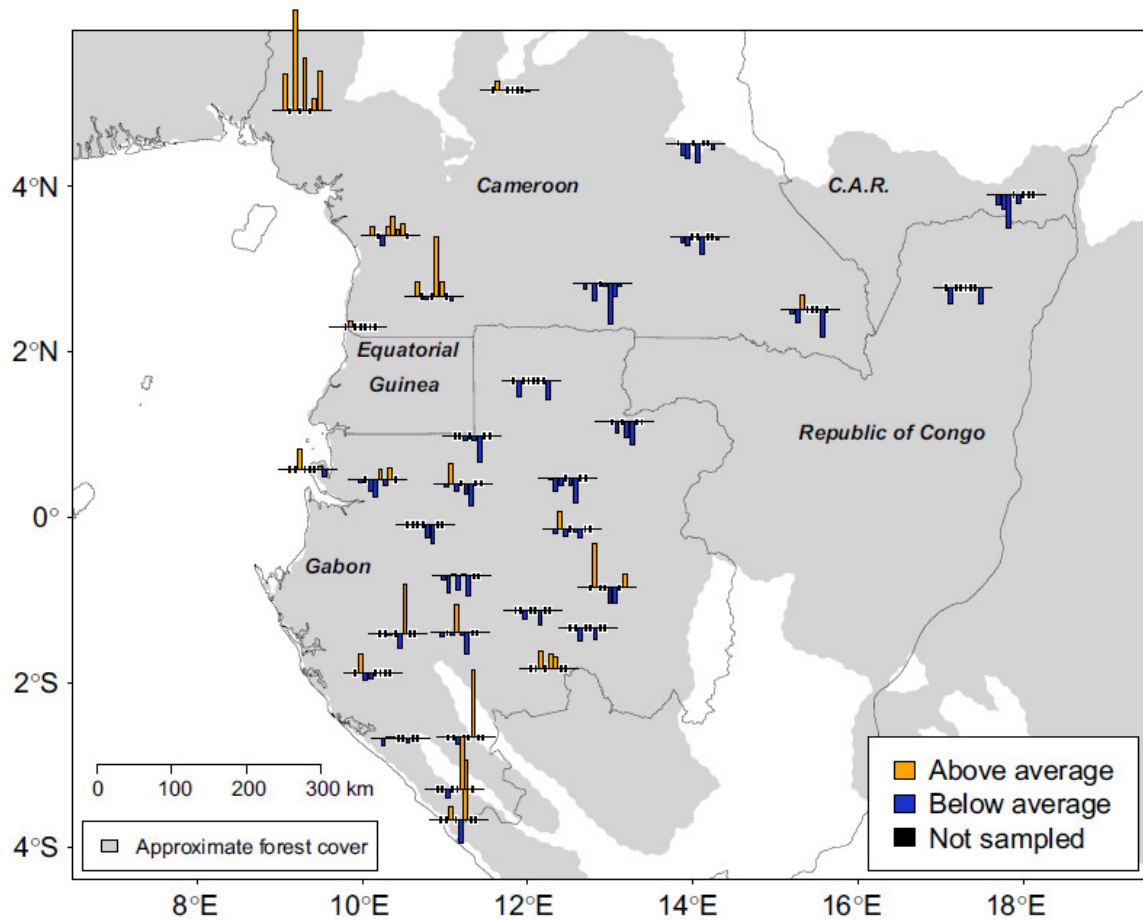
Figure 18 Genetic distinctiveness patterns in eight tropical tree species in Atlantic Central Africa. Distinctiveness above or below average is based on standardized pairwise genetic divergence (S'kij computed for each species) among sampling sites. Each barplot represents distinctiveness values, respectively, from left to right for the tree species Santiria trimera, Erythrophleum suaveolens, Greenwayodendron suaveolens, Afrostyrax kamerunensis, Afrostyrax lepidophyllus, Scorodophloeus zenkeri, Symphonia globulifera and Milicia excelsa. From Dauby et al. (2014).

Funding

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Mentoring

Master students

Miguel Rodríguez Blanco, Universidad de Valladolid, 2009. Filogeografía y estructura morfológica de dos árboles tropicales cercanos filogenéticamente: *Tricoscypha acuminata* y *T. oddonnii* (Anacardiaceae) en la región fitogeográfica de Baja Guinea, África Central.

Guillaume Kouamé Koffi, Université Libre de Bruxelles, 2007. Co-supervision with Olivier Hardy. Phylogeographie d'un arbre tropical en Afrique, *Santiria trimera*, Burseraceae.

PhD students

Guillaume Kouamé Koffi, co-supervision with Olivier J. Hardy, Université Libre de Bruxelles, 2006-2010. Genetic structure and ecology of a tropical tree with large morphological variation and a large distribution in Central Africa, *Santiria trimera*, Burseraceae.

Katharina B. Budde, co-supervision with Santiago C. González-Martínez, INIA-CIFOR Madrid and Universidad Complutense de Madrid, 2009-2014. Genetic structure of forest trees in biodiversity hotspots at different spatial scales.

Contribution to the supervision of PhD students

Gilles Dauby, Université Libre de Bruxelles, 2012. Main supervisors: Olivier J Hardy and Tariq Stévart. Structure spatiale de la diversité intra- et interspécifique en Afrique centrale.

Kasso Daïnou, Université de Liège, Belgium, 2012. Main supervisors: Jean-Louis Doucet and Grégory Mahy. Population genetic structure and conservation of *Milicia excelsa* (Moraceae), a tree species that is locally threatened from extinction in Central Africa.

Mario Mairal Pisa, Real Jardín Botánico-CSIC, Madrid and Universidad Autónoma de Madrid, 2015. Main supervisor: Isabel Sanmartín. Macroevolución y microevolución en sistemas insulares: el patrón Rand Flora en el género Canarina.

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Budde KB, González-Martínez SC, Hardy OJ, **Heuertz M** (2013) The ancient tropical rainforest tree *Symphonia globulifera* L. f. (Clusiaceae) was not restricted to postulated Pleistocene refugia in Atlantic Equatorial Africa. *Heredity* 11, 66–76.

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Dauby G., Duminil J, **Heuertz M**, Hardy OJ (2010) Chloroplast DNA polymorphism and phylogeography of a Central African tree species widespread in mature rainforests: *Greenwayodendron suaveolens* (Annonaceae). *Tropical Plant Biology* 3, 4–13.

Dauby G, Duminil J, **Heuertz M**, Koffi KG, Stévart T, Hardy OJ (2014) Congruent phylogeographic patterns of eight tree species in Atlantic Central Africa provide insights on the past dynamics of forest cover. *Molecular Ecology* 23, 2299–2312.

Duminil J, **Heuertz M**, Doucet JL, Bourland N, Cruaud C, Gavory F, Doumenge C, Navascués M, Hardy OJ (2010) CpDNA-based species identification and phylogeography: application to African tropical tree species. *Molecular Ecology* 19, 5469–5483.

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4.1.3. Phylogeography of Neotropical plants

Symphonia globulifera L.f. (Clusiaceae) is a widespread and hypervariable tropical tree species that can be considered a living fossil. The oldest fossil pollen attributable to the genus found in the Niger delta was dated to 45 Ma and the species colonized the Americas from Africa ca. 18 and 15 Ma ago (Dick *et al.* 2003). I collaborated with Christopher Dick of the University of Michigan to analyse SSR, ITS and plastid DNA data in a sample of Neotropical populations of this species (Central and South America): we documented within-species vicariance due to Andean uplift, genetic homogenization in Amazonia and strong among-population differentiation in mountainous topography in Mesoamerica (Dick & Heuertz 2008).

As a "Ramón y Cajal" researcher in Spain, I hosted Priciane Correa Ribeiro, PhD student at the University of Minas Gerais, Brazil, who obtained an internship to work on the phylogeographic history of two savannah tree species endemic of the Brazilian Cerrado, *Annona crassiflora* Mart. and *A. coriacea* Mart. (Annonaceae). Whereas *A. crassiflora* displayed a single haplotype and was inferred to have undergone a strong bottleneck, *A. coriacea* displayed a strong east-west divergence. Dating of a plastid DNA phylogeny indicated that diversification occurred in the western Cerrado during the Pliocene; followed by an early Pleistocene split, an probable eastward colonization, of the eastern haplotype group (Correa Ribeiro *et al.* 2016).

While in my current position at INRA, I hosted Bárbara Leal, PhD student at São Paulo State University, to work on phylogeography and population genomics of the bromeliad species *Pitcairnia lanuginosa* Ruiz & Pavón, a rupicolous herb with a disjunct distribution in the Brazilian Cerrado and the Andean Yungas. Cerrado and Yungas populations represented distinct phylogenetic lineages separated ca. 2.9 Ma ago. Very strong population genetic structure at SSRs with F_{ST}=0.73 among all populations, higher than the F_{CT}=0.17 between the Yungas and Cerrado lineages, suggested signatures of repeated dispersal and local persistence in small isolated populations (Figure 19; Leal et al. submitted).
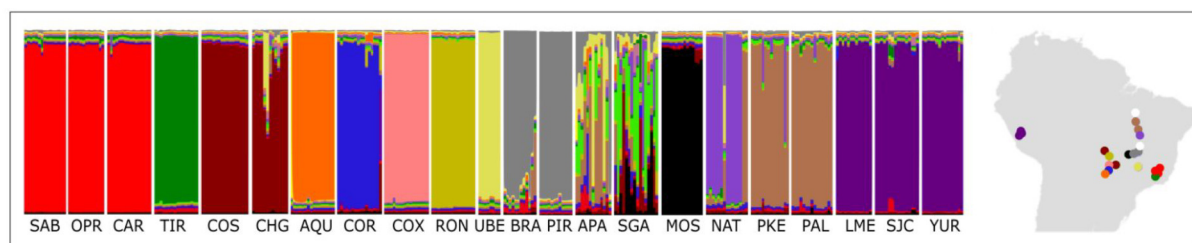

Figure 19 InStruct clustering analysis of SSR data on 318 individuals from 22 populations of Pitcairnia lanuginosa *for K=14. Colors represent distinct clusters, populations are colored in the map according to the most representative genetic cluster (except for highly admixed populations which are represented in white). From Leal et al. (submitted).*

Mentoring

Contribution to the supervision of PhD students

<u>Priciane Cristina Correa Ribeiro</u>, University of Minas Gerais, Brazil, 2014. Main supervisor: Maria Bernadete Lovato. CAPES Fellowship to INIA-CIFOR, April – Octobre 2013. História evolutiva de *Annona crassiflora* Mart. e *A. coriacea* Mart. (Annonaceae) e determinantes ambientais da divergencia genética e morfológica de populações no Cerrado.

<u>Bárbara Simoes Santos Leal</u>, São Paulo State University, 2018. Main supervisor: Clarisse Palma-Silva. Labex COTE mobility to INRA-Univ. Bordeaux, October-November 2016, and FAPESP Sandwich Fellowship February – July 2017. Population genomics and adaptive variation of the *Pitcairnia lanuginosa* complex (Bromeliaceae): development of ddRAD-Seq.

Publications

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Leal BSS, Araujo Graciano V, Neves Chaves CJ, Pillaca Huacre LA, **Heuertz M**, Clarisse Palma-Silva. Dispersal and local persistence shape the genetic structure of a widespread Neotropical plant species with a patchy distribution (submitted).

4.1.4. Diversification and speciation processes: roles of hybridization and tempo of evolution

Speciation is the evolutionary process by which populations evolve to become distinct species. Geographic isolation and natural selection both play important roles in speciation processes, acting in conjunction or separately (Coyne & Orr 2004). The classical model of speciation through cladogenesis proposes that new species form through a split of evolutionary lineages from an ancestral species, where each new species eventually forms a monophyletic clade: a subgroup of lineages that comprises its common ancestor at its base (Gould & Eldredge 1977; Howard & Berlocher 1998).

The phylogeographical studies I was involved in provided several examples of deviation from the classical model of speciation. An obvious reason why monophyletic groups are often not observed in tree species is that trees often bear large effective population size, leading to slow lineage sorting, as illustrated in the Introduction. Other reasons are geographically complex mechanisms of speciation that include population size changes, hybridization and distinct tempos of evolution.

Hybridization – Hybridization between divergent lineages or species is a common process in plant evolution (Soltis and Soltis 2009; Whitney et al. 2010; Abbott et al. 2013; Abbott, Barton, and Good 2016). Hybridization may slow down or reverse differentiation by allowing gene flow and recombination. It may accelerate speciation via adaptive introgression or cause near-instantaneous speciation by allopolyploidization (Abbott *et al.* 2013).

In a phylogeographic study on the tropical African rainforest understory herbs *Haumania danckelmanniana* (J. Br. & K. Schum.) Milne-Redh. and *H. liebrechtsiana* (De Wild. & Th. Dur.) J. Leonard (Marantaceae) in collaboration with Alexandra Ley (University of Halle, Germany) and Olivier Hardy, we delimited a total of five SSR-based gene pools across the two species (Ley *et al.* 2017). The highest differentiation in the dataset was not found between the two species, but between allopatric gene pools of *H. liebrechtsiana*. One *H. liebrechtsiana* gene pool was sympatric with *H. danckelmanniana* and a signature of interspecific introgression was identified. This study let us infer complex signatures of past rainforest fragmentation leading to allopatric speciation in these understory herbs, followed by population expansion leading to secondary contact and hybridization. The climate-driven forest fragmentation affected these sister species in a differential way, causing a

much deeper signature of genetic differentiation in *H. liebrechtsiana* than in *H. danckelmanniana* (Ley *et al.* 2017).

In a commentary paper in collaboration with Gonzalo Nieto Feliner (Madrid Botanical Garden, RJB-CSIC), we focussed on speciation by hybridization without polyploidization, a process known as homoploid hybrid speciation (HHS; Nieto Feliner et al. 2017). We critically examined the criteria used by Schumer and colleagues (Schumer *et al.* 2014) to define HHS, namely (1) a strong reproductive isolation (RI) mechanism between the putative parental and hybrid species; (2) genetic evidence of hybridization; and (3) RI mechanisms derived from hybridization itself. Schumer and colleagues argued that only four organisms meet their requirements to be considered homoploid hybrid species (one butterfly and three sunflower species). We argued in favour of less strict criteria to define HHS, to allow for a more broad scientific discussion on the contribution of hybridization to evolutionary novelty. We were particularly critical of Schumer et al.'s view on the origin of RI, and argued that if there is evidence that a hybridization event has given rise to an established, persistent, morphologically and ecologically distinct hybrid lineage, the recognition of the new lineage as originated through HHS should not depend on the need to demonstrate that hybridization was directly the cause of RI (Nieto Feliner *et al.* 2017). Our paper also set the stage for a new project on the genomics of hybridization in plant evolution recently awarded to Gonzalo Nieto Feliner, in which I am participating (RYVHON, see below).

I currently host a visiting PhD student, André Carneiro Muniz, from the University of Minas Gerais, Brazil, who is working on conservation genetics of a critically endangered legume tree, *Dimorphandra wilsonii* Rizzini (Fabaceae), of the Brazilian Cerrado – Atlantic forest ecotone. Our recent population genetic analyses identified a strongly negative inbreeding coefficient at most SSR loci in *D. wilsonii*, suggesting that this species is probably not an established species, but most probably represents the recurrent formation of F1 hybrids between the closely related congeners *D. mollis* Benth. and *D. exaltata* Schott with which it occurs in sympatry. This ongoing work illustrates that species delimitation based on morphology may not always capture persistent lineages, or established species.

Tempo of evolution – The rate of molecular evolution is linked to plant life history traits. Low rates of evolution are consistently associated with tree species that have long generation times, whereas herbs have short generation times and faster, but also more variable, evolutionary rates (Smith & Donoghue 2008). Similar associations of faster substitution rates in short-generation organisms have been observed in different animal groups (Nabholz *et al.* 2007; Thomas *et al.* 2010).

I contributed to a study led by Fernando Ojeda (University of Cádiz, Spain) where we examined evolutionary implications of a life history trait that lengthens generation time in the genus *Erica* (Ericaceae): the capacity of resprouting from the rootstock. The genus *Erica* harbours over 700 species following a radiation in the fynbos vegetation of the South African Cape Floristic Region (Linder 2003). Fire is an important driver in the adaptive processes leading to speciation in the fynbos. The genus *Erica* harbours "seeder" species that regenerate principally though seeds after fire, and "resprouter" species in which plants are not regularly killed by fire. The widespread *Erica coccinea* L. is a fynbos species that contains both seeder and resprouter populations, however it was unclear which life form was ancestral. We used phylogenetic approaches on nuclear ribosomal DNA and coalescent modelling on nuclear SSRs data, demonstrating the ancestral status of the resprouter form (Figure 20; Ojeda et al. 2016). Our study highlighted the role of fire in driving accelerated diversification in seeder lineages of highly speciose fynbos taxa in the Cape Floristic Region.
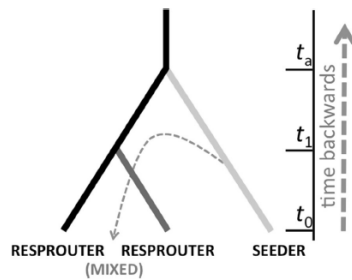
Figure 20 Summary illustration of the demographic events affecting seeder and resprouter populations of Erica coccinea, as inferred from coalescent simulations. Times are not scaled. From Ojeda et al. (2016).

As a Marie Curie Fellow at the University of Fribourg, hosted by Christian Lexer, I supervised Daniel Ducry, a Bachelor student, and Marjorie Berthouzoz, a Master student, on evolutionary processes in another CFR radiation, the sugarbushes (genus *Protea*). *Protea* are fire-adapted shrubs with an aerial seed bank; some of them also adapted to resprouting from the rootstock. Together, differentiation at plastid and and nuclear DNA markers indicated a stronger genetic structure and a more restricted dispersal for the resprouter species *Protea nitida* Mill. than for the seeder species *P. punctata* Meisn. Also, demographic simulations showed a population expansion for the seeder species *P. punctata* vs. a stable population size or decline for *P. nitida* (Lexer, Heuertz et al., unpublished results). These result suggest that increased genetic isolation may affect resprouter species in comparison with seeder species, and thus impact on their genetic diversity.

Funding

REVOL – Spatially explicit mechanisms of Recent EVOLution in an African biodiversity hotspot. European Commission, FP7-PEOPLE-2012-IEF, Marie Curie Action. Jul. 2013 – Aug. 2014. 149,546€. **PI: Myriam Heuertz**. Hosted at the University of Fribourg, Switzerland, host: Christian Lexer.

RHYVON - The role of natural hybridization in plant evolution: bridging the gap between theoretical models and empirical data. Plan Nacional I+D+I, Spain, 2018-2021. PI. Gonzalo Nieto Feliner.

Mentoring

Bachelor students supervision

Daniel Ducry, co-supervision with Christian Lexer, Université de Fribourg, Switzerland, 2014. Phylogeography of two *Protea* species from the Cape Floristic Region of South Africa.

Master students supervision

Marjorie Berthouzoz, co-supervision with Christian Lexer, Université de Fribourg, Switzerland, 2015. Microevolutionary drivers of species diversification and persistence in Cape Proteas.

Contribution to the supervision of PhD students

André Carneiro Muniz, Univ. Minas Gerais, Brazil, ongoing. Main supervisor: Maria Bernadete Lovato. CAPES fellowship to INRA UMR Biogeco, September 2018 – August 2019. Population genetics and species boundaries of *Dimorphandra* species (Leguminosae) from the Cerrado and Atlantic Forest: implications for conservation.

Publications

Ley A, **Heuertz M**, Hardy OJ (2017) The evolutionary history of central African rain forest plants: phylogeographical insights from sister species in the climber genus Haumania (Marantaceae). *Journal of Biogeography* 44(2), 308–321.

Nieto Feliner G, Álvarez Fernández I, Fuertes Aguilar J, **Heuertz M**, Marques I, Moharrek F, Piñeiro R, Riina R, Rosselló JA, Soltis P, Villa Machío I (2017) Is homoploid hybrid speciation that rare? An empiricist's view. *Heredity* 118, 513–516.

Ojeda F, Budde KB, **Heuertz M**, Segarra-Moragues JG, González Martínez SC (2016) Biogeography and evolution of seeder and resprouter forms of *Erica coccinea* (Ericaceae) in the fire-prone Cape fynbos. Plant Ecology 217, 751-761.

4.2. Population genetics and genomics

4.2.1. Spatial genetic structure and microevolutionary processes

Fine-scale spatial genetic structure (FSGS) refers to the non-random spatial distribution of genotypes within populations. FSGS is shaped by the complex interplay of microevolutionary processes including dispersal, local genetic drift and selection (Wright 1943; Vekemans & Hardy 2004). Plant breeding system, life form and population density all have effects on the strength of the FSGS pattern (Epperson 2003; Vekemans & Hardy 2004).

In their landmark paper on FSGS, Xavier Vekemans and Olivier Hardy compared a series of statistics to test for FSGS and developed a metric based on the decay of inter-individual kinship with spatial distance, the *Sp* statistic, which allows to quantify the strength of the FSGS pattern between populations, and even between species (Vekemans & Hardy 2004). Under conditions of drift-dispersal equilibrium, the theory of isolation by distance predicts a linear decay of genetic similarity between individuals with increasing spatial distance, or with the logarithm of spatial distance in two-dimensional populations (Wright 1943; Vekemans & Hardy 2004). In non-equilibrium populations, kinship is not expected to decay linearly with distance because temporal dynamics of establishment and demographic history can then affect FSGS patterns, resulting for example in locally co-ocurring gene pools or allele frequency gradients, or differences in FSGS between cohorts (Jones & Hubbell 2006; Hampe *et al.* 2010). Studying FSGS patterns and inferring their causes can be especially useful to identify pertinent eco-evolutionary processes in plant populations, which can help to design conservation strategies (Frankham *et al.* 2004).

I have conducted or contributed to a series of studies addressing local-scale microevolutionary processes, based on the characterization of FSGS.

Spatial and temporal processes determine FSGS in Mediterranean plants – Mediterranean environments are characterized by a high degree of patchiness and consequently many plant taxa, and species, have patchy and fragmented distribution ranges. This is the case for example for the snapdragons, *Antirrhinum* sp*.* (Plantaginaceae), for which a geographical process of speciation with repeated phases of isolation and secondary contact with hybridization has been inferred in the Western Mediterranean (Vargas *et al.* 2009). As a postdoc in the Madrid botanical garden under the supervision of Pablo Vargas, I worked with the critically endangered *Antirrhinum charidemi* Lange, a narrow endemic of the Cabo de Gata massif in Southern Spain. We found relatively high levels of genetic diversity across eight populations in the 8 x 4 km range, and weak to moderate population structure in this region of diverse topography (Forrest *et al.* 2017). Surprisingly, within a single population at a much narrower scale (ca. 150 m), we identified pronounced spatial genetic structure, which was best described by geography and altitude, i.e., by the population topography, comprising a central valley subpopulation as well as subpopulations dwelling on surrounding rocky outcrops, but not by corolla colour variation or the associated vegetation of individual plants (Figure 21; Heuertz et al., in preparation). Through simulations, we inferred that the central valley of the population was mostly a source of gene flow, whereas individuals installed on the surrounding rocky outcrops were mostly recipients of gene flow. Comparison of FSGS at plastid (Sp_p = 0.332) vs. nuclear markers (Sp_n = 0.020) resulted in average pollen to seed dispersal distances of > 4, suggesting that this very fine-scale genetic structure pattern was essentially shaped by narrow-ranging seed dispersal – the tiny snapdragon seed lack any dispersal adaptation – whereas wider-ranging pollen dispersal can be explained by the solitary bee pollinators being transported between population nuclei due to gusts of wind.
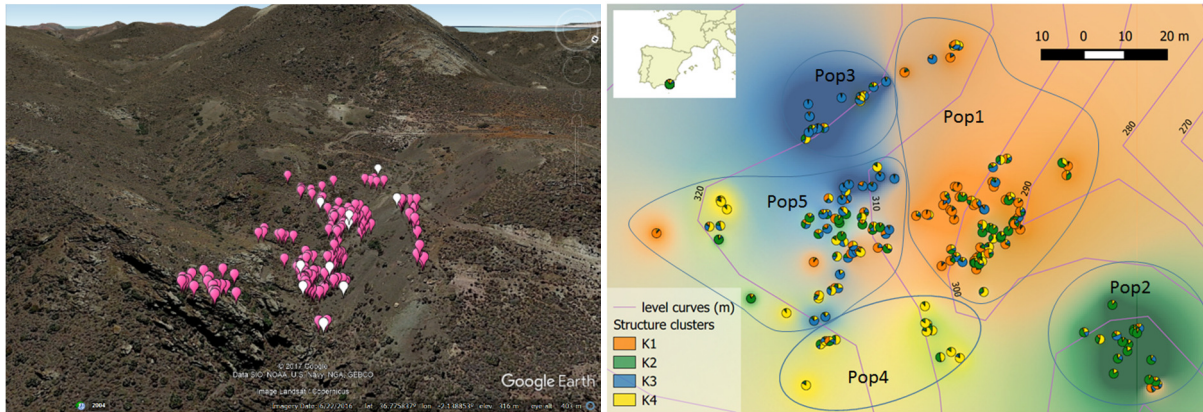
Figure 21Distribution of pink and white-flowered Antirrhinum charidemi *Lange individuals in the Barranco del Dragoncillo Blanco population in the Cabo de Gata massif, southeastern Spain (left) and distribution of STRUCTURE gene pools at 13 SSR loci (right). From Heuertz et al. in preparation.*

In an effort to characterize spatio-temporal variation in mating patterns in another Mediterranean plant, I contributed to a study in the wind-pollinated dioecious shrub *Pistacia lentiscus* L. (Anacardiaceae) led by Rafael Albaladejo (University of Seville). We examined progeny arrays from 30 and 26 mothers in consecutive years (2006 and 2007) and found three-fold variation in correlated paternity between years, translating to effective numbers of fathers per mother of 12 to 34 (Albaladejo *et al.* 2009). The effective pollen cloud of the population was structured into patches of high- or low genetic diversity, which were not constant across years despite constant weather conditions during flowering, thus suggesting other determinants such as inter-annual variation in phenology, or selection. Simulations indicated that in the studied population, low density and clumping of fathers aggravated pollen limitation, which is of special relevance in fragmented Mediterranean forests suffering ever greater anthropic pressure (Albaladejo *et al.* 2009).

Population fragmentation and adaptation to frequent fires increase FSGS in Mediterranean conifers
– While a visiting researcher at INIA-CIFOR in Spain in 2008, I had the opportunity to contribute to supervising PhD student Ana De-Lucas in a study on the determinants of FSGS in fragmented populations of an iconic Mediterranean conifer, the maritime or cluster pine, *Pinus pinaster* Aiton. Ana sampled four *P. pinaster* populations, located in continuous or fragmented range parts in Spain, and found a stronger FSGS in fragmented populations (De-Lucas *et al.* 2009b). This was expected, because in fragmented populations, non-random mating, increased drift due to small population size, genetic isolation, edge effects, low population densities and potential clumping of reproductive individuals can all lead to increased FSGS (Aguilar *et al.* 2008; Mona *et al.* 2014). We specifically inspected the effects of population size and genetic isolation in a simulation study tailored to the study species, by modelling pollen and seed dispersal kernals based on direct estimates obtained from seed trapping and paternity analysis in cluster pine (González-Martínez *et al.* 2006a; De-Lucas *et al.* 2008), vs. based on indirect estimates from observed FSGS in the populations of the study. Our simulations suggested that under fat-tailed dispersal (inferred from direct dispersal observation), small population size is a stronger determinant of FSGS than genetic isolation, whereas under normally distributed dispersal kernels, limited gene immigration is a stronger determinant of FSGS than small population size (Figure 22; Figure 22De-Lucas, González-Martínez, Vendramin, et al. 2009). We further found through simulations that assessing FSGS at short distance, < 100m as in our study, overestimated the true FSGS (Figure 22; Figure 22De-Lucas, González-Martínez, Vendramin, et al. 2009). Overall, a weak FSGS in cluster pine, with *Sp* values comprised between 0 and 0.026, reflected the effects on FSGS of breeding system – outcrossing species have lower *Sp* than mixed-mating or selfing species –, and life form – trees have lower *Sp* than herbs or shrubs (Vekemans & Hardy 2004).
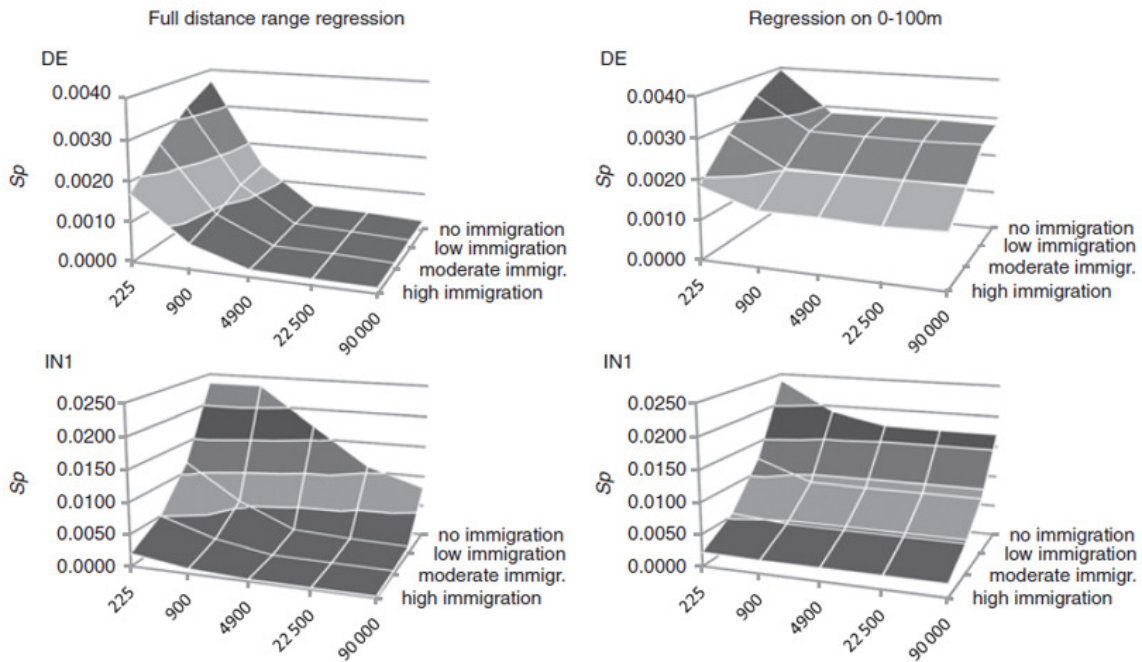
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Figure 22 Fine-scale spatial genetic structure simulated in populations under different population size and isolation scenarios. Scenario DE used power exponential distributions for seed and pollen dispersal, based on estimates obtained from seed trapping and parentage analysis, scenario IN1 used normal distributions based on gene dispersal estimates inferred from FSGS and population density. Under power exponential (fat-tailed) dispersal (DE), population size is a stronger determinant than isolation, whereas under normally distributed dispersal (IN1), isolation is a stronger determinant. When assessing FSGS at short distance (<100m, right side of figure), FSGS is overestimated, and more strongly so in the DE scenario. From De-Lucas et al. (2009).

In a follow-up study, we also investigated the origin of the cluster pine populations of the Castillian Plateau (northwestern Spain) using three nuclear SSR markers, considering several hypotheses including colonization from Mediterranean or Atlantic refugia, or a local origin. The Castillian Plateau gene pool was found to be distinct from Atlantic and Mediterranean gene pools, although a closer proximity with the Mediterranean gene pool was revealed (De-Lucas *et al.* 2009a), and later confirmed with more powerful SSR and SNP markers (Jaramillo-Correa *et al.* 2015).

As a "Ramón y Cajal" Researcher at INIA-CIFOR, I mentored my second PhD student, Katharina Budde, in a co-supervision with Santiago González-Martínez. One of the topics addressed in Katharina's thesis was the genetic correlates of adaptation to fire in Mediterranean conifers. Wildfires are major selective drivers shaping adaptive plant traits (Keeley *et al.* 2012; Pausas 2015). Aleppo pine, *Pinus halepensis* Mill, generally exhibits a seeder strategy, in which seeds are preserved in closed cones, known as serotinous cones. These cones persist in the canopy for years, and their opening is triggered by high fire-associated temperatures (Tapias *et al.* 2004). The level of serotiny and other fire response traits vary among populations, depending on variation in the fire regime (Hernández-Serrano *et al.* 2013). We investigated the effect of fire regime on the structure of genetic diversity within and among populations of Aleppo pine in the eastern Iberian Peninsula, where coastal populations have evolved under warm and dry conditions with frequent fires (HiFi, for high fire) whereas inland population in mountainous topography have evolved in subhumid climate with lower pressure from fire (LoFi, for low fire). Although FSGS was overall weak, we found that HiFi stands displayed a stronger FSGS than LoFi stands at SNP markers, which probably reflected the simultaneous post-fire recruitment of co-dispersed related seeds. SNPs with exceptionally strong FSGS, a proxy for microenvironmental selection, were only reliably identified under the HiFi regime (Figure 23; Budde et al. 2017). Our study suggested that an increasing fire frequency as predicted due to global change can promote increased FSGS with stronger family structures and alter natural selection in *P. halepensis* and in plants with similar life history traits.
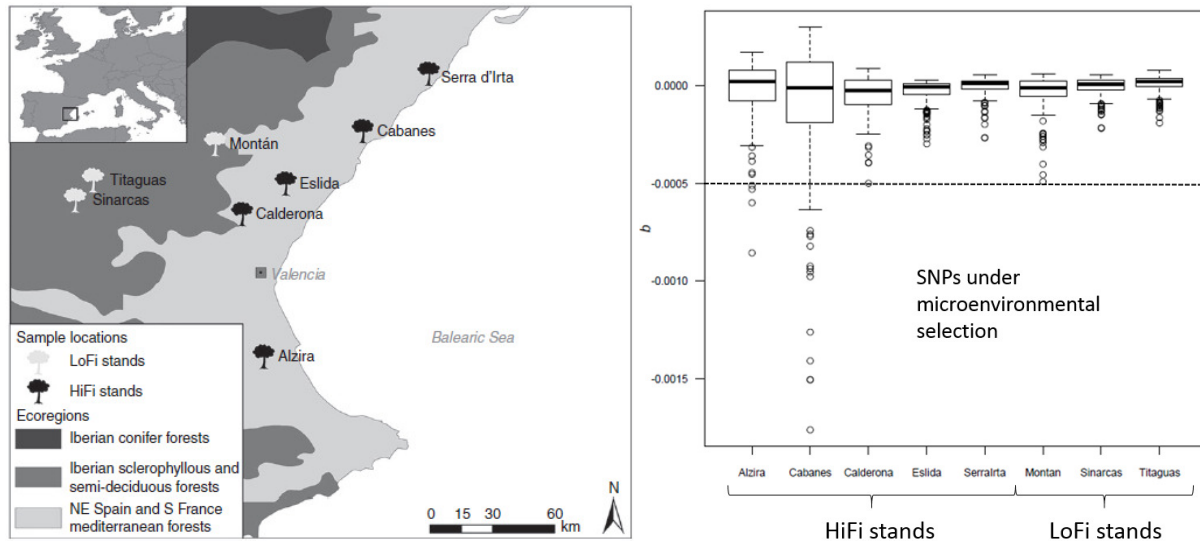
Figure 23 Location of studied Pinus halepensis stands in different fire regimes (left) and slope of the kinship – ln(distance) regression of SNP markers, reflecting FSGS per population (right). Outlier SNPs with exceptionally strong FSGS, a proxy for microenvironmental adaptation, were only found in HiFi stands. From Budde et al. (2017).

Forest management weakens FSGS in temperate trees – Collaborating with Giovanni Vendramin and Andrea Piotti of the CNR, Italy, I contributed to a study on the effects of management on the FSGS in common beech (*Fagus sylvatica* L.). We found that management regimes such as plantations, shelterwood systems or favouring spontaneous colonization generally decreased the level of substructure within stands compared with absence of management, or lower-intensity management (Piotti *et al.* 2013). These results suggested that indices characterizing different properties of population substructure can represent valuable indicators to monitor early effects of management, and more generally, of disturbance in common beech. My contribution in this paper concerned the development of simulations to assess the power of the four SSR markers used in a variety of FSGS contexts, and the hypothetical gain in the precision of indicator estimates had a larger marker number been used.

FSGS in inselberg bromeliads is shaped by breeding system, long-term isolation and adaptive divergence – While I was a SYNTHESYS fellow in Kew Gardens, I initiated collaboration with Christian Lexer and contributed to supervising the PhD thesis of Thelma Barbará on population genetic processes in inselberg bromeliads of the Brazilian Altantic forest. We investigated FSGS in nine populations of four species of the bromeliad genus *Alcantarea* (Barbará *et al.* 2008): strong differences in the strength of FSGS between species reflected known differences in the breeding system of these plants, with the strongest FSGS (*Sp* = 0.95) observed in *Alcantarea glaziouana* (Lemaire) Leme, a species that displays substantial inbreeding due to selfing. We identified biparental inbreeding, selfing and restricted seed dispersal as the main determinants of FSGS, with restricted pollen dispersal by bats contributing also to FSGS in some localities. The ability of *Alcantarea* spp. to colonize isolated inselbergs probably stems from flexible mating systems and an ability to tolerate inbreeding. Our estimates of short-ranging gene dispersal of σ_g = 7 to 27 m were consistent with a loss of dispersal function within inselbergs populations. Population subdivision associated with sympatric colour morphs in *A. imperialis* (Carrière) Harms was accompanied by between-morph differences in pollen and seed dispersal distances. Our results thus indicated a high potential for divergence with gene flow in these inselberg bromeliads.

Biogeographic history, breeding sytem, dispersal adaptations and microhabitat drive FSGS in tropical trees – Studies in tropical trees illustrate the diversity of the evolutionary processes that can affect their populations. In a collaboration with PhD student Kasso Daïnou of the University of Liège, I contributed to characterizing FSGS in the early successional *Milicia excelsa* (Welw.) C.C. Berg

(Moraceae), a threatened dioecious African tree species occurring typically at low density (ca. 10 adults per km^2; Bizoux et al. 2009). Open-habitat populations in Cameroon had extremely weak, non-significant FSGS, *Sp* ≤ 0.002, probably because of efficient wind-mediated pollen dispersal and seed dispersal by frugivorous bats. In closed forest, significant FSGS was detected, probably because of less efficient wind-dispersal of pollen in a closed canopy. Gene dispersal distances ranged from σ_g = 1 to 7.1 km, one order of magnitude higher than most estimates found in the literature for tropical tree species.

In a study with my PhD students Paloma Torroba-Balmori and Katharina Budde at INIA-CIFOR, Spain, we examined FSGS in seven African and Neotropical populations of another tropical tree, *Symphonia globulifera* L. f. (Clusiaceae), typical of mature rainforest. This species is adapted to local guilds of seed and pollen dispersers across its wide distribution range, with pollination typically assured by hummingbirds, perching birds and lepidoptera in the Neotropics, vs. sunbirds in Africa, and seed dispersal by bats and tapirs in the Neotropics vs. small mammals in Africa (Torroba-Balmori *et al.* 2017). African *S. globulifera* populations displayed a stronger FSGS than Neotropical populations at both plastid DNA and SSR markers (mean *Sp* = 0.025 vs. *Sp* = 0.008 at SSRs; Figure 24; Torroba-Balmori et al. 2017). All three African populations occurred in pronounced altitudinal gradients (in Cameroon and São Tomé), possibly restricting animal-mediated seed dispersal. Cyto-nuclear disequilibria in Cameroonian populations also suggested a legacy of biogeographic history to explain these genetic patterns. Conversely, Neotropical populations exhibited a weaker FSGS, which may reflect more efficient wide-ranging seed dispersal by Neotropical bats and other dispersers. The population from French Guiana displayed an association of plastid haplotypes with two morphotypes characterized by differential habitat preferences. These results highlight the importance of the microenvironment for eco-evolutionary processes within persistent tropical tree populations.

I further contributed to a study on local-scale evolutionary processes in *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul (Fabaceae), a widespread emblematic tree species typical of Seasonally Dry Tropical Forests (SDTFs) in South America, in the framework of the PhD thesis of Alejandra Goncalvez, in collaboration with her supervisor María García at Misiones University, Argentina, and Santiago González-Martínez. In a remnant population of the species in Northern Argentina, we observed a more pronoucend FSGS in saplings than adults, which was mainly explained by the joint dispersal of related seed in this partially selfing species (Goncalves *et al.* 2019). Despite high genetic diversity, high inbreeding suggests caution in using this remnant population as a seed source.

Conclusion – These studies on fine-scale spatial genetic structure in Mediterranean, temperate and tropical plants show that evolutionary processes related to breeding system, adaptation to dispersal vectors and adaptation to the environment operate at very local scales, and can contribute to divergence of subpopulations and produce genetic signatures of microenvironmental selection. Dispersal abilities and the availability of suitable habitat largely determine the spatial extension of populations and their levels of genetic diversity and genetic substructure.
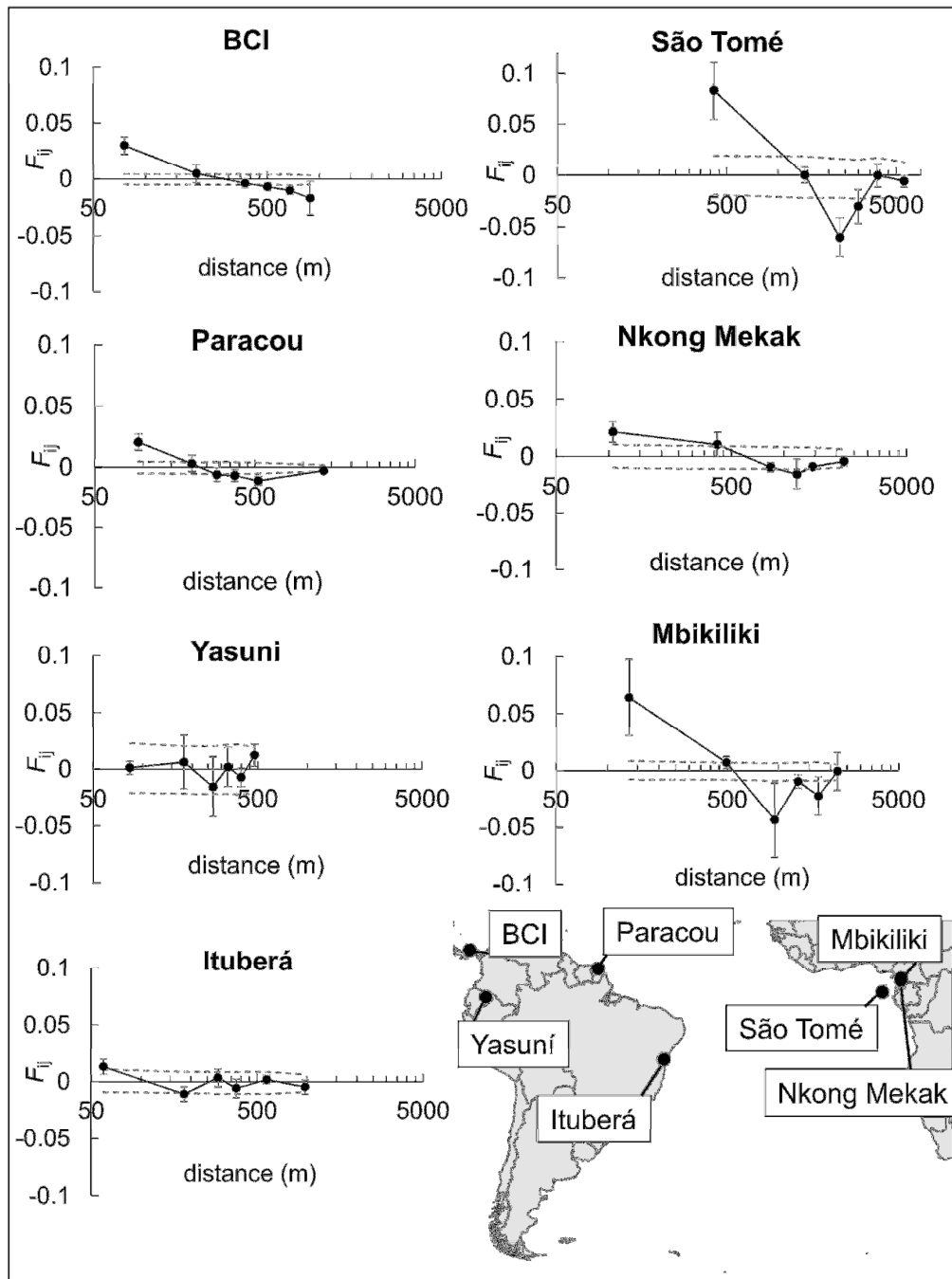
Figure 24 Location of Symphonia globulifera populations examined and kinship-distance relationships within populations. The mean jackknife estimate of the kinship coefficient F_{ij} is plotted per distance class, whiskers representing standard error; dashed lines represent the permutation-based 95% CI for absence of FSGS. From Torroba-Balmori et al. (2017).

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EU SYNTHESYS Visiting scientist grant to Royal Botanica Gardens Kew, Jan-Feb 2006, to **M. Heuertz** (2 months).

Biodiversity hotspots: evolution and conservation of forest trees. Funding: Ministerio de Ciencia e Innovación, Ramón y Cajal project. Jan 2010 – Dec 2014. 10,000€. **PI: Myriam Heuertz**. Hosted at INIA-CIFOR.

LINKTREE – Linking genetic variability with ecological responses to environmental changes: forest trees as model systems. Funding: BiodivERsA-ERANET, Jun 2009 – Jun 2012. 1,359,625€. PI: Santiago González-Martínez. Participants: INIA-CIFOR, INRA-Bordeaux, INRA-Avignon, Uppsala University, Universität Marburg.

TIPTREE – Scenarios for forest biodiversity dynamics under global change in Europe: identifying micro-evolutionary scale tipping points. ERAnet BiodivERsA-2. 2012-2015. 35,000€ to INIA-CIFOR. PI: Sylvie Oddou-Muratorio. Participants: INRA-URFM Avignon, Akdeniz University Antalya, INIA-CIFOR, European Forest Institute, ONF Fontainebleau, CNRS-UM Montpellier, INRA-BioGeCo Bordeaux, INRA-Ecofog Kourou, University of Marburg, Uppsala University, CNR-IGV Florence, Fed. of Swedish Forest Owners.

VAMPIRO – Efectos demográficos y selectivos del fuego en plantas Mediterráneas: variación molecular y pirofitismo. Ministerio de Ciencia e Innovación, Jan. 2009 – Dec. 2011. 181,379€. PI: Santiago González-Martínez. Participants: INIA-CIFOR, Universidad de Cádiz, CIDE – CSIC, Univ. Libre de Bruxelles.

AFFLORA – The assembly of African floras from lineages to populations: effects of historical climate changes and species ecology. Plan Nacional de I+D+I, Spain. Jan. 2013 – Dec. 2015. 193,050€. **PI: Myriam Heuertz**. Participants: Real Jardín Botánico-CSIC Madrid, INIA-CIFOR, ULB Brussels, INRA, Université de Yaoundé 1, CNARP Madagascar.

Mentoring

Master students

Katharina B. Budde, co-supervision with Santiago C. González-Martínez, Universidad Complutense de Madrid, 2010. Spatial genetic structure in tropical and temperate forest trees.

PhD students

Katharina B. Budde, co-supervision with Santiago C. González-Martínez, INIA-CIFOR Madrid and Universidad Complutense de Madrid, 2009-2014. Genetic structure of forest trees in biodiversity hotspots at different spatial scales.

Paloma Torroba Balmori, co-supervision with Ricardo Alía, INIA-CIFOR Madrid and Universidad de Valladolid, 2013-ongoing. Adaptive evolution in the tropical tree genus *Symphonia* (Clusiaceae).

Contribution to the supervision of PhD students

Kasso Daïnou, Université de Liège, Belgium, 2012. Main supervisors: Jean-Louis Doucet and Grégory Mahy. Population genetic structure and conservation of *Milicia excelsa* (Moraceae), a tree species that is locally threatened from extinction in Central Africa.

Ana I De-Lucas, Universidad de Valladolid, 2009. Main supervisors: Santiago C González-Martínez and Elena Hidalgo. Genetic structure in fragmented and continuous populations of maritime pine.

Thelma Barbará, Open University London, 2008. Main supervisor: Mike F Fay. Genetic structure of four Bromeliaceae species affected to inselbergs in Brazil.

Alejandra L. Goncalves, Facultad de Ciencias Naturales y Museo - Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina, 2014-2019. Main supervisors: María Victoria García and Rolando Rivera Pomar. Estructura genética espacial en paisajes fragmentados: Un estudio en poblaciones naturales de curupay (Leguminosae: *Anadenanthera colubrina* var. *cebil*).

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Albaladejo RG, González-Martínez SC, **Heuertz M**, Vendramin GG, Aparicio A (2009) Spatiotemporal mating pattern variation in a wind-pollinated Mediterranean shrub. *Molecular Ecology* 18, 5195–5206.

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Budde KB, González-Martínez SC, Navascués M, Burgarella C, Hernández-Serrano A, Lorenzo Z, Zabal-Aguirre M, Vendramin GG, Verdú M, Pausas JG, **Heuertz M** (2017) Increased fire frequency promotes stronger spatial

genetic structure and natural selection at regional and local scales in *Pinus halepensis* Mill. *Annals of Botany* 119(6), 1061–1072.

De-Lucas AI, González-Martínez SC, Vendramin GG, Hidalgo E, **Heuertz M** (2009) Spatial genetic structure in continuous and fragmented populations of *Pinus pinaster* Aiton. *Molecular Ecology* 18, 4564–4576.

De-Lucas AI, González-Martínez SC, Hidalgo E, Bravo F, **Heuertz M** (2009) Admixture, one-source colonization or long-term persistence of maritime pine in the Castilian Plateau? Insights from nuclear microsatellite markers. *Investigacíon agraria: sistemas y recursos forestales* 18, 3-12.

Forrest A, Escudero M, **Heuertz M**, Wilson Y, Cano E, Vargas P (2017) Testing the hypothesis of low genetic diversity and population structure in narrow endemic species: the endangered *Antirrhinum charidemi* (Plantaginaceae). *Botanical Journal of the Linnean Society* 183(2), 260–270.

Goncalves AL, García M, **Heuertz M**, González-Martínez SC (2019) Demographic history and spatial genetic structure in a remnant population of the subtropical tree *Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul (Fabaceae). *Annals of Forest Science* 76, 18.

Piotti A, Leonardi S, **Heuertz M**, Buiteveld J, Geburek T, Gerber S, Kramer K, Vettori C, Vendramin GG (2013) Within-population genetic structure in beech (*Fagus sylvatica* L.) stands characterized by different disturbance histories: does forest management simplify population substructure? *PLOS ONE* 8, e73391.

Torroba-Balmori MP, Budde KB, Heer K, González-Martínez SC, Olsson S, Scotti-Saintagne C, Casalis M, Sonké B, Dick CW, **Heuertz M** (2017) Altitudinal gradients, biogeographic history and microhabitat adaptation affect fine-scale spatial genetic structure in African and Neotropical populations of an ancient tropical tree species. *PLOS ONE* 12(8), e0182515.

4.2.2. Ecological genomics of local adaptation

Ecological genomics seeks to identify the genetic mechanisms that underly the responses of organisms to their natural environments (Ungerer *et al.* 2008; Morgan *et al.* 2018). The field addresses ecological and evolutionary questions in an interdisciplinary way, bridging the gap between ecological approaches, which have traditionally ignored the added value of genetics, and genetics, which has long focussed on characterizing gene function in model organisms without considering the effect of the natural environment. I have conducted, or contributed to a series of studies focussing on different aspects of interactions between genotype, environment, and phenotype, thus fitting broadly the concept of ecological genetics and genomics.

Genomic basis of phenology adaptation in *Picea abies* – As a postdoc at Uppsala University under the supervision of Martin Lascoux, I was interested in the genetic underpinnings of phenology variation across a latitudinal gradient in the conifer Norway spruce, *Picea abies* (L.) Karst. Norway spruce displays a latitudinal cline for the timing of seasonal growth cessation (bud set) which is triggered by photoperiod (Ekberg *et al.* 1979). This trait is under selection, as trees that fail to set bud in time incur a higher risk of damage due to early autumn frosts. Inheritance studies suggested that the character is controlled by many genes of predominantly additive effects (Ekberg *et al.* 1979).

We used a candidate gene approach to investigate the genetic control of bud set in Norway spruce. For this, we identified and resequenced 11 putative functional candidate genes from the photoperiod and vernalization pathways, as well as 11 anonymous control genes from expressed sequence tags (ESTs, Heuertz, de Paoli, et al. 2006). We chose candidate genes from the photoperiod and vernalization pathways because these were known to trigger photoperiod-mediated response in model organisms (Simpson and Dean 2002). For each gene, we sequenced an average of 47 haplotypes, representing seven populations.

We discovered low levels of nucleotide diversity and a fast decay of linkage disequilibrium, within a few hundred bases. Genetic structure reflected the three sampled rang parts, Baltico-Nordic, Alpine and Carpathian domains (Heuertz, de Paoli, et al. 2006). Mean Tajima's *D* and Fay and Wu's H across loci were both negative, indicating, respectively, an excess of rare variants and an exess of high-frequency-derived variants compared to the expected frequency spectrum in a standard neutral model. Using both neutrality statistics together, no robust genetic signatures for selection could be identified at any of the genes. We used coalescent simulations to examine the fit of the multilocus

data with expectations under several demographic models. Tests based on *D* and *H* led to the rejection of the standard neutral model and exponential growth model in the whole population as well as in the two main domains (Baltico-Nordic and Alpine). On the other hand, in all three cases the data were compatible with a severe bottleneck occurring some hundreds of thousands of years ago (Figure 25; Heuertz, de Paoli, et al. 2006).
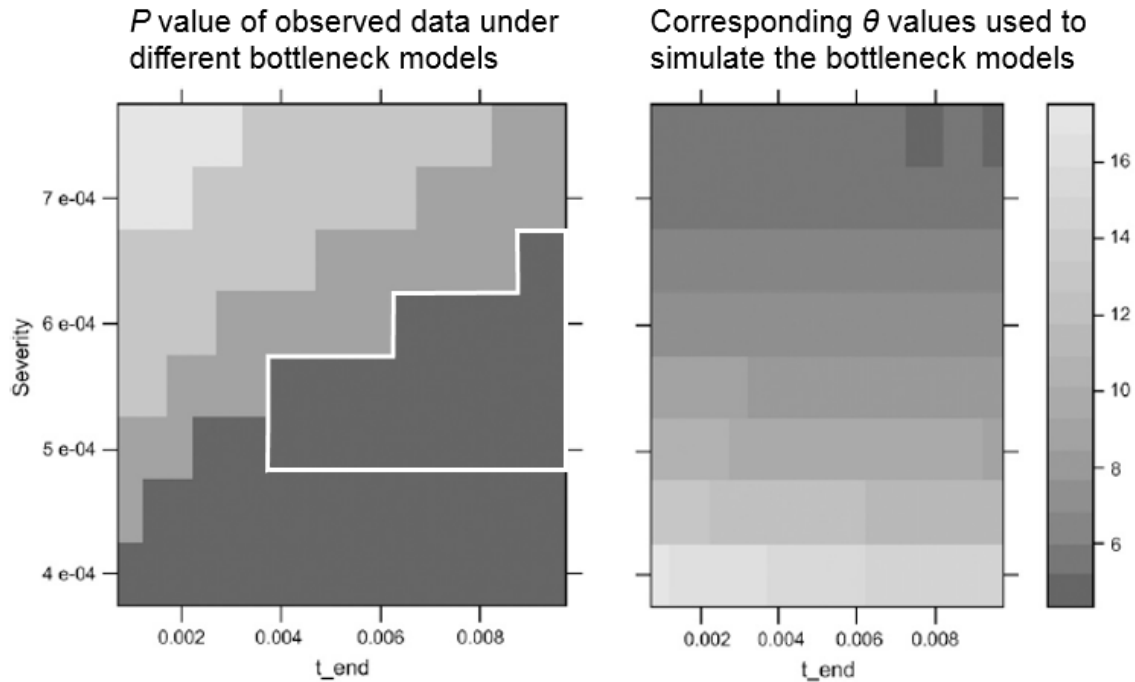

Figure 25 Evaluation of bottleneck models in Norway spruce using different combinations of bottleneck timing and severity. Left: P *values of Tajima's* D *of observed sequence data under the models: dark shading indicates bottleneck scenarios compatible with the observed data (P> 0.05); area highlighted in white indicates scenarios with realistic ancient population size. Right: theta values reflecting ancient population size used to model the scenarios. Modified from Heuertz, de Paoli et al. (2006).*

This study was one of the first to investigate the genetic basis of local adaptation in forest trees using candidate genes, and has been influential in the field. Our findings on demographic departures from equilibrium expectations and population structure in Norway spruce suggest that these aspects need to be accounted for when examining genetic signatures of selection and for association mapping studies, as failure to control for demography generates false positives in those types of studies.

In a second study on photoperiod adaptation of Norway spruce, we sequenced 19 candidate genes of three types; photoceptors, circadian clock genes and downstream targets, as well as control genes not a priori under selective pressure. Based on the control genes, we used coalescent simulations and an Approximate Bayesian Computation approach (ABC) to simulate realistic demographic models. We identified one circadian clock gene, *PaPRR3* (*Picea abies* pseudo response regulator 3) as systematically deviating from neutral expectations (Källman *et al.* 2014). This gene, which contains several non-synonymous mutations is indeed a good candidate for mediating photoperiod response in Norway spruce.

Early fitness consequences of exotic gene flow in Iberian pines – Gene flow from plantations of non-local tree provenances into natural stands of the same species is probably a widespread phenomenon, but its effects remain largely unexamined. Collaborating with Juanjo Robledo-Arnuncio and his PhD student Gregor Unger at INIA-CIFOR, we investigated early fitness consequences of intraspecific exotic gene flow, i.e., from surrounding non-local plantations, into natural relict stands of two Iberian pines, *Pinus sylvestris* L. and *P. pinaster* Ait. We assessed differences in survival among native, non-local, and F1 intraspecific offspring by tracking changes through time in the estimated proportion of each

parental crosstype, from seeds before dispersal to naturally established seedlings months after emergence. To estimate the proportion of each cross type, we used a maximum likelihood model previously developed by Juanjo Robledo-Arnuncio (Unger *et al.* 2014) which allows to tease apart male, female, native and exotic components of gene flow based on plastid and nuclear SSR genotypes.

We identified significant proportions of exotic male gametes before seed dispersal in the two pine relicts: 2.3% of *P. pinaster* seed and 15.2% of *P. sylvestris* seed were sired by exotic trees. Subsequently to seedling establishment, the frequency of exotic male gametes became nonsignificant in *P. pinaster*, and dropped by half in *P. sylvestris*. Exotic zygotic gene flow was significantly different from zero among early recruits for *P. sylvestris*, decreasing throughout seedling establishment. Seedling mortality resulted in small sample sizes for established seedlings, and temporal trends in exotic gene flow estimates were not significant, so we could not reject the null hypothesis of invariant early viability across parental cross types in the wild.

Fire and climate as drivers of local adaptation in *Pinus pinaster* – I contributed to two studies on adaptive genetic variation in maritime pine, *P. pinaster*, respectively addressing the genetic underpinnings of serotiny (the trait of displaying a persistent canopy seed bank of closed cones which typically relase seed after fire, Tapias et al. 2004), and the molecular basis of climate adaptation.

In the first study, part of the PhD thesis of Katharina Budde, we used an *in situ* association genetics approach to identify genotype-phenotype associations in an unstructured natural population of maritime pine in the eastern Iberian Peninsula. Serotiny was scored in the focal region and obtained for a wider geographic region from a previous study (Tapias *et al.* 2004); and a 384-plex SNP Oligo Pool Assay was genotyped in n=509 individuals including n=199 trees from the focal region. Seventeen common SNPs (MAF > 0.10) potentially associated with fire phenotypes in the eastern Iberian Peninsula (12 SNPs associated with serotiny detected in single-locus analysis and five additional SNPs detected in a stepwise mixed model) were used to construct a phenotypic predictive model based on RR-BLUP (ridge regression – best linear unbiased prediction). This model explained 29.15% of the phenotypic variation in serotiny in the region (Figure 26; Budde et al. 2014). When tested outside the focal range, a similar prediction power was found for nearby geographical regions from the same maternal lineage, but not for other genetic lineages of maritime pine.
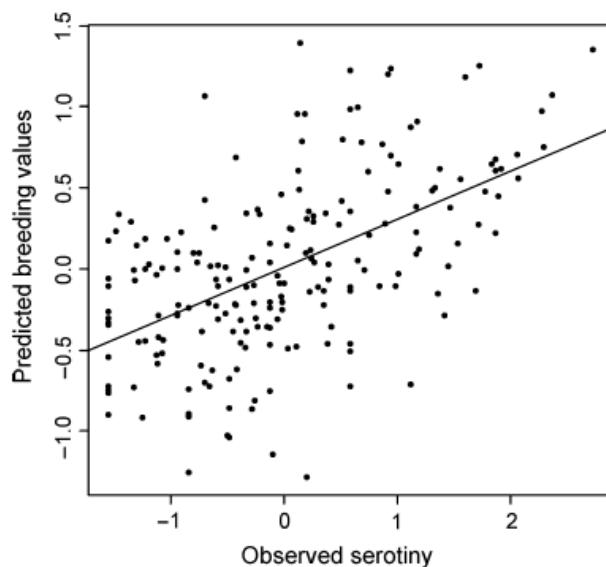

Figure 26 Correlation of predicted breeding values based on ridge regression in a mixed-effects modeling framework (RR-BLUP) and observed levels of serotiny (standardized) in the eastern Iberian Pinus pinaster *range. A linear trend is also shown. From Budde et al. (2014).*

Association genetics for ecologically relevant traits evaluated *in situ* is an attractive approach for forest trees, provided that traits are under strong genetic control and that populations are unstructured, with large phenotypic variability. *In situ* association studies like ours hold promise to identify the genetic basis of important traits in ecological keystone non-model species in their natural environments, where polymorphisms acquired their adaptive value.

In another study, we were interested in identifying SNPs associated with climate variables across the distribution range of maritime pine to help predicting adaptive genetic response under climate change in this long-lived tree species. Using logistic regression, latent factor mixed models, and Bayesian analyses of SNP–climate correlations, we retained a set of 18 SNPs (out of 266 candidate SNPs) potentially associated with climate, five of them involving amino acid-changing variants (Jaramillo-Correa *et al.* 2015). These relationships identified temperature as an important adaptive driver in maritime pine and demonstrated like in the previous study that selective forces are operating differentially in geographically discrete gene pools (Iberian Atlantic vs. Iberian Mediterranean gene pools, Figure 27). Representative populations from the maritime pine range were planted in a common garden at a site in northeastern Spain that corresponded to arid conditions for the species (average temperature 11.6°C, annual rainfall 502 mm, summer rainfall 101 mm, for the period 1975–2008) and their survival assessed after five years. The expected locally advantageous allele at the site of the common garden was determined for each of the 18 climate-associated SNPs. The frequency of these locally-advantageous alleles in the source populations was strongly correlated with breeding values (EBLUPs, empirical best linear unbiased predictors) for survival in the common garden (Figure 28, Jaramillo-Correa et al. 2015).
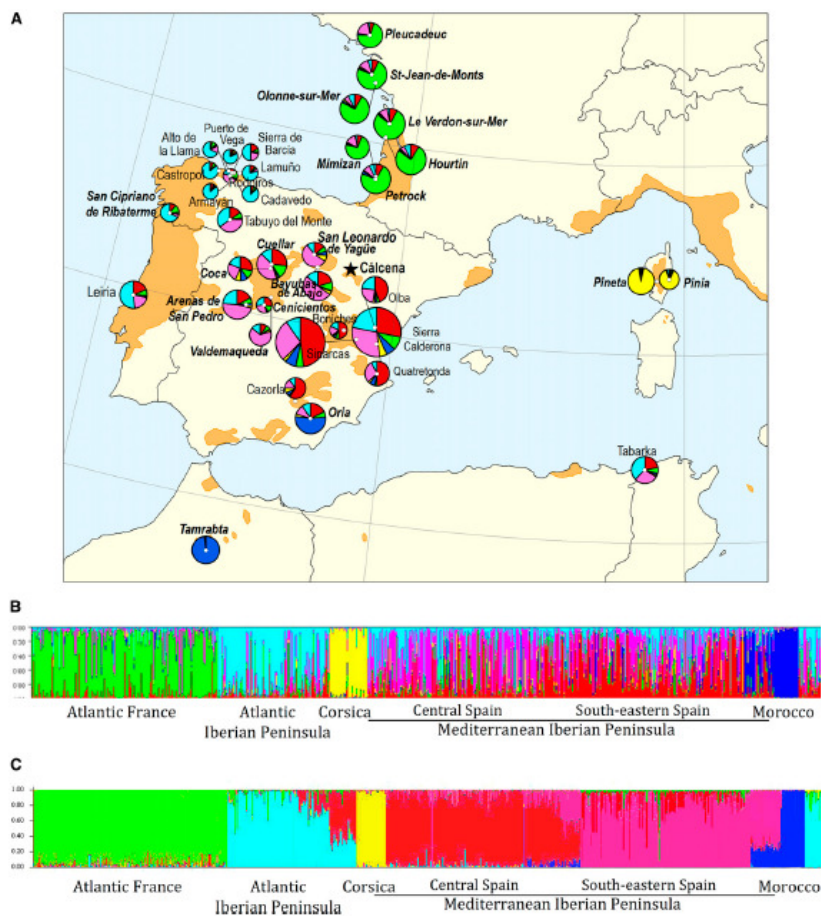

Figure 27 (A) Geographic distribution of the six gene pools obtained from (B) nine nuclear SSRs in 36 natural populations of maritime pine. (C) Genetic partition is also shown for 1745 control (i.e., putatively neutral) SNPs. The shading in A denotes the species natural range, and the star indicates the location of the common garden used to evaluate fitness (Cálcena, Spain). Populations included in the common garden are in boldface italics. Fraom Jaramillo-Correa et al. (2015).

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These results suggest that candidate-gene SNPs can be used to forecast the likely destiny of natural forest ecosystems under climate change scenarios. Geographically defined molecular proxies for climate adaptation can thus critically enhance the predictive power of range-shift models and help establishing mitigation measures for long-lived keystone forest trees in the face of climate change.
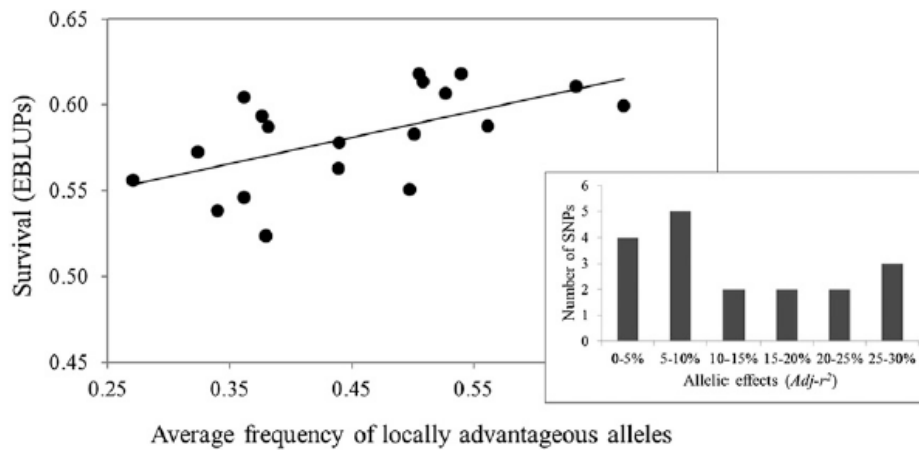

Figure 28 Scatter plot of average frequency of locally advantageous alleles in the common garden (Cálcena, Spain) at each source population and EBLUPs (empirical best linear unbiased predictors) for survival, a proxy for fitness. The inset shows the distribution of allelic effects for the 18 SNPs associated with climate. From Jaramillo-Correa et al. (2015).

Isolation by distance vs. isolation by environment as drivers of genetic divergence – To identify which factors might drive population divergence, potentially leading to speciation in the highly speciose South Africa Cape Floristic Region (CFR), I used restriction site associated DNA sequencing (RAD-Seq) in three species of Restionaceae, a characteristic adaptive radiation of the CFR (Linder 2003). This research was conducted during my Marie Curie Fellowship at the University of Fribourg, hosted by Christian Lexer.

The null hypothesis for population genetic divergence in the CFR is that of isolation by distance (IBD; Wright 1943), where differentiation can be explained by drift-dispersal processes alone. In alternative scenarios, coined 'isolation by environment' (IBE) and 'isolation by adaptation' (IBA), gene flow among populations living in different environments is limited primarily by selection against maladapted migrants (Nosil *et al.* 2009). IBE is related to IBA; the former is generally defined via environmental and the latter via adaptive phenotypic differences (Shafer & Wolf 2013). Both IBD and IBE hypotheses are plausible in the CFR: IBD would be expected considering that CFR climatic conditions have been temporally stable since the end of the Miocene, with a summer drought in the Western Cape and a more mesic climate in the East (Dupont 2011). IBE is possible since habitats are highly patchy in the CFR, incorporating climatic gradients, topographic variation, soil variation, aspect, micro-niches and the fact that many CFR plant taxa are characterized by weak dispersal abilities (Linder 2003).

We first examined the distribution of genomic diversity in ten range-wide populations of the dioecious herb *Restio capensis* (L.) H.P. Linder & C.R. Hardy, which displayed a weak differentiation among populations (F_{ST} = 0.056). A Bayesian generalized linear mixed modelling (GLMM) approach was used to explain different partitions of the genomic data using spatial, climatic and phytogeographic predictors. Modelling results indicated that population divergence across the CFR is mostly driven by IBE rather than IBD for both neutral and non-neutral markers (Figure 29, Lexer et al. 2014), consistent with genome hitchhiking or coupling effects during early stages of divergence. GLMM of plastid DNA and single divergent outlier loci from a Bayesian genome scan analysis confirmed the predominant role of climate and pointed to additional drivers of divergence, such as drift and ecological agents of selection captured by phytogeographic zones.
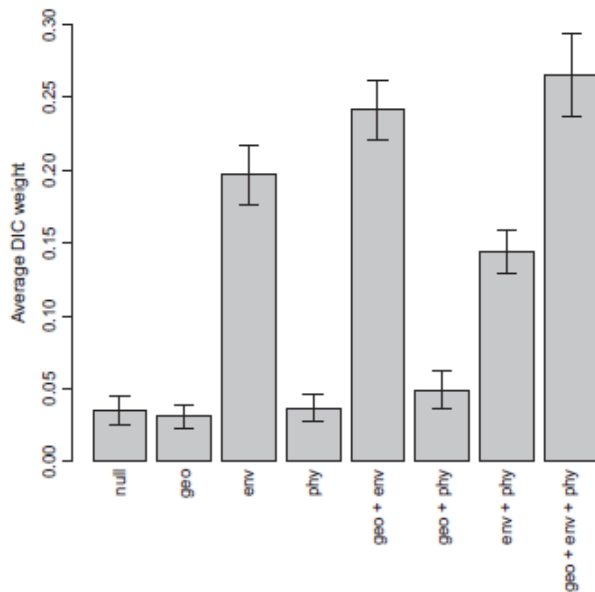
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Figure 29 Average deviance information criterion (DIC) weights (bars) and standard errors (whiskers) of mixed models predicting genetic divergence in 156 single outlier RAD-Seq polymorphisms with geographic distance (GEO), an environmental distance (ENV) based on climate data and phytogeographic zone membership (PHY). On average, models that include 'ENV' as predictor variable receive significantly stronger support (=greater DIC weights) than models that do not include this predictor. From Lexer et al. (2014).

The two other Restionaceae species examined, *Restio triticeus* Rottb. and *Hypodiscus aristatus* (Thunb.) C.Krauss., displayed a much stronger population genetic structure, $F_{ST} = 0.569$ and $F_{ST} = 0.456$, and a near-absence of admixed individuals (Figure 30) suggesting that these morphospecies contained divergent lineages. In *R. triticeus,* complete cyto-nuclear association supported the strong lineage divergence. In *H. aristatus*, higher sequencing depth was observed for one genetic cluster, Cl1 in green in Figure 30, than in another (Cl2 in yellow in Figure 30, Heuertz et al. unpublished results). This observation is compatible with the suggestion that both *H. aristatus* lineages probably correspond to distinct ploidy levels, triploids for genetic cluster Cl1, and tetraploids for Cl2 (Linder *et al.* 2017). An association of these cytotypes with an east-west climate gradient was observed, suggesting environment as a correlate of lineage isolation through ploidy differences.

We identified IBE as a driver of genetic as well as leaf trait divergence also in another system, the tree species *Annona crassiflora* Mart. (Annonaceae) endemic of the Brazilian Cerrado (Ribeiro *et al.* 2016), as part of Priciane Correa Ribeiro's PhD thesis, in which I mainly collaborated for the phylogeographic analyses. In this species populations were structured into three geographic groups across the Cerrado based on genetic data, vs. two groups based on leaf traits, the latter mostly reflecting an East-West divergence. IBE in both genetic and morphological markers was mainly due to temperature seasonality and precipitation of the warmest quarter. Populations that experienced lower precipitation summers and hotter winters had heavier leaves and lower specific leaf area. A region in the southwestern Cerrado had the highest genetic diversity and has probably acted as a climatic refuge for *A. crassiflora*. The genetic structure of *A. crassiflora* reflects the biogeographic history of the species more strongly than leaf traits, which are more related to current climate (Ribeiro *et al.* 2016).
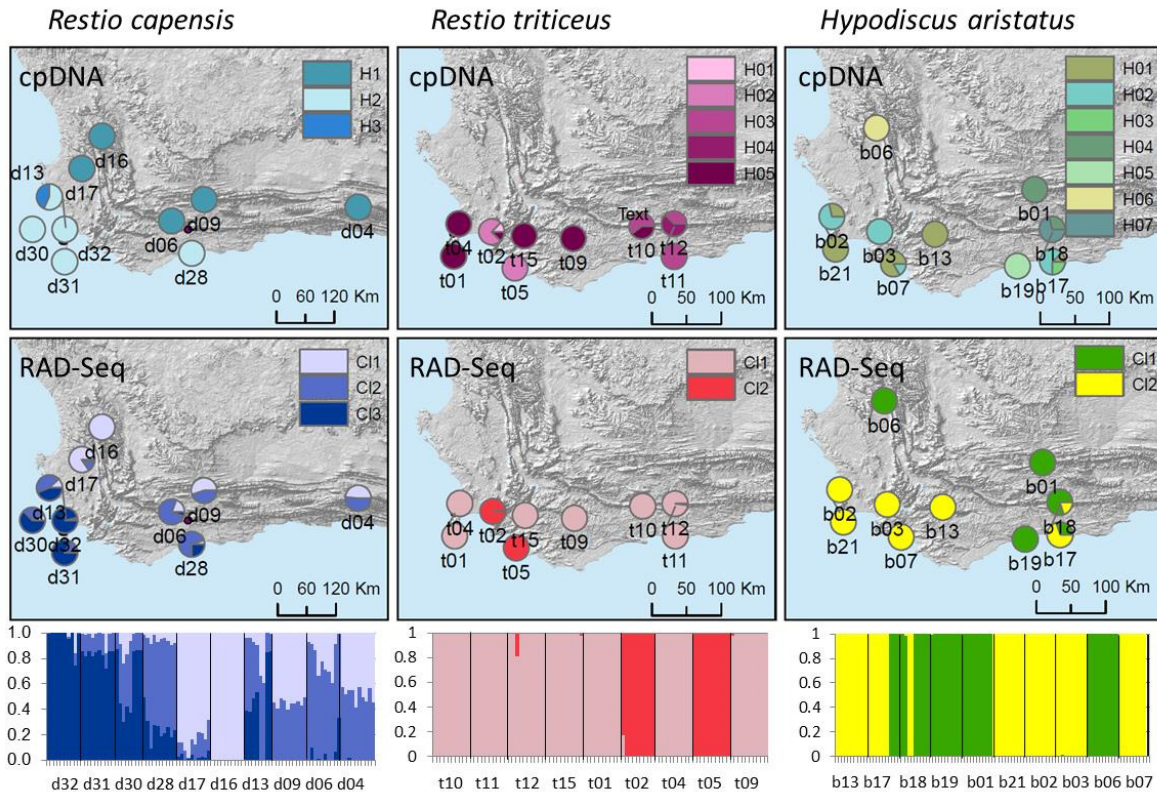
Figure 30 Spatial distribution of plastid DNA and RAD-Seq-based nuclear genetic diversity in populations of three restiad species in the Cape Floristic Region of South Africa. Upper row, distribution of plastid DNA haplotypes; mddle row, distribution of genetic clusters inferred from the best-supported number of clusters in a Bayesian genetic structure analysis (STRUCTURE); lower row, admixture proportions of individual specimens of each species from the same analysis. Heuertz et al. unpublished.

In the framework of Paloma Torroba-Balmori's PhD thesis, we were interested in the biogeographic history and the genetic underpinnings of local adaptation in the widespread tropical tree *Symphonia globulifera* L.f. across its Afro-Neotropical distribution. In collaboration with Thomas Parchman at the University of Nevada, we generated ddRAD-Seq data in nine populations, three from the Neotropics and six from Africa. We inferred population splits and mixtures from the genome-wide allele frequency data and discovered more ancient divergence events in Africa vs. the Neotropics in a full coalescent analysis (Torroba-Balmori et al. unpublished). The French Guiana *terra firme* ecotype, *Symphonia sp1*, potentially a different species (Baraloto *et al.* 2007b), was inferred to have a Neotropical origin (Figure 31). Differentiation outlier loci associated to climate and soil data were identified, however, effects of geography and environment may be confounded due to the strong population genetic structure in this ancient tree species. We are therefore currently exploring local adaptation at the within-population scale (like we did for pines, Budde et al. 2017), using these ddRAD-Seq data for which individuals have been georeferenced, as well as gene capture data produced in the ANR project FLAG, through a collaboration with Ivan Scotti (INRA, Avignon).
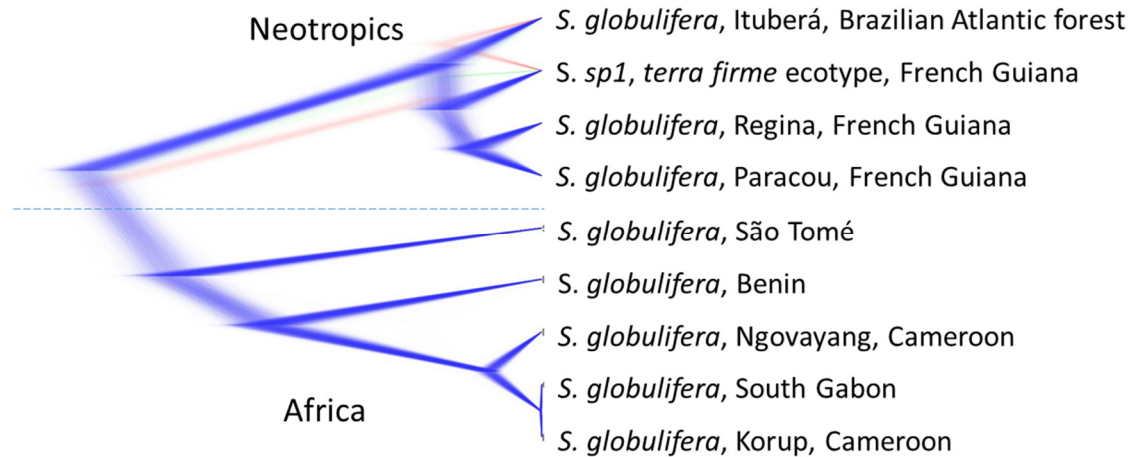
Figure 31 Full coalescent analysis (SNAPP) of evolutionary relationships among Symphonia globulifera *populations based on ddRAD-Seq SNPs.Torroba-Balmori et al., unpublished.*

Adaptive introgression – Population persistence through adverse climatic conditions such as those of the Pleistocene glaciations can be improved when plant species can reproduce vegetatively. I contributed to a study resulting from David Macaya-Sanz' PhD on the evolutionary consequences of clonal reproduction in white poplar, *Populus alba* L., which reproduces both clonally and sexually. We found that a few ancient (over a few thousand years old) and widespread genets dominate the Douro basin (North-Eastern Spain), both in terms of clone size and number of sexual offspring produced (Macaya-Sanz *et al.* 2016). Interestingly, large clones possessed two genomic regions introgressed from the more cold-tolerant *P. tremula* L., which may have favoured their spread under stressful environmental conditions. Despite the high levels of clonality and dominance of sexual reproduction by large clones, as well as a weak ancient (>0.1 Myr) decline in effective population size, the Douro population conserves considerable genetic diversity and low inbreeding. These results suggest that even extended levels of clonality do not represent a threat to population persistence in white poplar (Macaya-Sanz *et al.* 2016).

Conclusion – The recent deployment of genomic approaches to studying the evolution of forest trees and other non-model plant species in their natural environments has greatly enhanced our understanding of the interplay between the genome and environment at a level of detail and depth not possible only a few years ago. I contributed to two reviews that, respectively, reviewed the state of the art of forest genomics on the occasion of the 50th anniversary of the journal Annals of Forest Science (Plomion *et al.* 2016), and summarized contributions in ecological genomics of forest trees presented at the international IUFRO (International Union of Forest Research Organizations) conference 'Genomics and Forest Tree Genetics' held in Arcachon in May 2016 (Holliday *et al.* 2017). Through the application of genomic approaches, important insights have been gained on the structure, functioning, and evolution of forest tree genomes (Plomion *et al.* 2016) and the genomic underpinnings of local adaptation (Holliday *et al.* 2017). Genomic approaches are now applied to respond to the main current and future challenges for natural and plantation forests: conservation of forest genetic resources under land use change and climate change and associated threats, while ensuring the provisioning of a vast diversity of forest-based products and ecosystem services (Plomion *et al.* 2016; Holliday *et al.* 2017).

Genetic basis of budset in Norway spruce. Carl Tryggers Foundation, Sweden. 2005 – 2006. 378.000 SEK. PI: Niclas Gyllenstrand. Participants: Uppsala University, Swedish University of Agricultural Sciences.

Cooperation INRA-FORMAS (Institut Nacional de la Recherche Agronomique, France - Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning). Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning. 2004 – 2005. 100,000 SEK. PI: Martin Lascoux.

Range shift and adaptive response in Scandinavian Picea abies populations. Philip Sörensen Foundation, Sweden. 2004 – 2006. 150.000 SEK. PI: Martin Lascoux. Participants: Uppsala University.

VAMPIRO – Efectos demográficos y selectivos del fuego en plantas Mediterráneas: variación molecular y pirofitismo. Ministerio de Ciencia e Innovación, Jan. 2009 – Dec. 2011. 181,379€. PI: Santiago González-Martínez. Participants: INIA-CIFOR, Universidad de Cádiz, CIDE – CSIC, Univ. Libre de Bruxelles.

LINKTREE – Linking genetic variability with ecological responses to environmental changes: forest trees as model systems. Funding: BiodivERsA-ERANET, Jun. 2009 – Jun. 2012. 1,359,625€. PI: Santiago González-Martínez.

FLAG – Génétique écologique des arbres forestiers : interactions entre flux de gènes et variabilité environnementale dans la détermination de l'adaptation locale et du potentiel d'adaptation. ANR, France. 2012-2015. 306,065€. PI: Ivan Scotti. **Coordinator for INIA-CIFOR: Myriam Heuertz**.

TIPTREE – Scenarios for forest biodiversity dynamics under global change in Europe: identifying micro-evolutionary scale tipping points. ERAnet BiodivERsA-2. 2012-2015. 35,000€ to INIA-CIFOR. PI: Sylvie Oddou-Muratorio.

FLUGAL – Interaction between gene flow and local adaptation in forest tree species: new analytical and experimental approximations. Plan Nacional de I+D+i, Spain. Jan. 2010 – Dec. 2012. 207,757€. PI: Juan José Robledo Arnuncio.

AFFLORA – The assembly of African floras from lineages to populations: effects of historical climate changes and species ecology. Plan Nacional de I+D+I, Spain. Jan. 2013 – Dec. 2015. 193,050€. **PI: Myriam Heuertz**.

REVOL – Spatially explicit mechanisms of Recent EVOLution in an African biodiversity hotspot. European Commission, FP7-PEOPLE-2012-IEF, Marie Curie Action. Jul. 2013 – Aug. 2014. 149,546€. **PI: Myriam Heuertz**. Hosted at the University of Fribourg, Switzerland, host: Christian Lexer.

Mentoring

PhD students

Katharina B. Budde, co-supervision with Santiago C. González-Martínez, INIA-CIFOR Madrid and Universidad Complutense de Madrid, 2009-2014. Genetic structure of forest trees in biodiversity hotspots at different spatial scales.

Paloma Torroba Balmori, co-supervision with Ricardo Alía, INIA-CIFOR Madrid and Universidad de Valladolid, 2013-ongoing. Adaptive evolution in the tropical tree genus *Symphonia* (Clusiaceae).

Contribution to the supervision of PhD students

David Macaya Sanz, Universidad de Valladolid, 2015. Main supervisors: Santiago C González-Martínez and Christian Lexer. Filogeografía, diversidad y hibridación en Populus spp.

Priciane Cristina Ribeiro Correa, University of Minas Gerais, Brazil, 2014. Main supervisor: María Bernadete Lovato. História evolutiva de *Annona crassiflora* Mart. e *A. coriacea* Mart (Annonaceae) e determinantes ambientais da divergencia genética e morfológica de populações no Cerrado.

Publications

Budde KB, **Heuertz M**, Hernández-Serrano A, Pausas JG, Vendramin GG, Verdú M, González-Martínez SC (2014) *In situ* genetic association for fire serotiny, a fire-related trait, in Mediterranean maritime pine (*Pinus pinaster* Aiton). *New Phytologist* 201, 230–241.

Heuertz M, De Paoli E, Källman T, Larsson H, Jurman I, Morgante M, Lascoux M, Gyllenstrand N (2006) Multilocus patterns of nucleotide diversity, linkage disequilibrium and demographic history of Norway spruce (*Picea abies* (L.) Karst). *Genetics* 174, 2095–2105.

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Jaramillo-Correa JP, Grivet D, Lepoittevin C, Sebastiani F, **Heuertz M**, Garnier-Géré P, Alía R, Plomion C, Vendramin GG, González-Martínez SC (2015) Molecular proxies of climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics* 199(3), 793–807.

Källman T, De Mita S, Larsson H, Gyllenstrand N, **Heuertz M**, Parducci L, Suyama Y, Lagercrantz U, Lascoux M. (2014) Patterns of nucleotide diversity at photoperiod related genes in Norway spruce [*Picea abies* L. (Karst)], *PLOS ONE* 9, e95306.

Lexer C, Wüest R, Mangili S, **Heuertz M**, Stölting KN, Pearman P, Forest F, Salamin N, Zimmermann N, Bossolini E. 2014. Genomics of the speciation continuum in an African plant biodiversity hotspot I: drivers of population divergence in *Restio capensis* (Restionaceae). *Molecular Ecology* 23, 4373–4386.

Macaya-Sanz D, **Heuertz M**, Lindtke D, Lexer C, González-Martínez SC (2016) Causes and consequences of large clonal assemblies in a poplar hybrid zone. *Molecular Ecology* 25, 5330–5344.

Plomion C, Bastien C, Bogeat-Triboulot M-B, Bouffier L, Déjardin A, Duplessis S, Fady B, **Heuertz M**, Le Gac A-L, Le Provost G, Legué V, Lelu-Walter A-M, Leplé J-C, Maury S, Morel A, Oddou-Muratorio S, Pilate G, Sanchez L, Scotti I, Scotti-Saintagne C, Segura V, Trontin J-F, Vacher C (2016) Forest tree genomics: 10 achievements from the past 10 years and future prospects. *Annals of Forest Science* 73, 77–103.

Ribeiro P, Souza ML, Muller LAC, Ellis VA, **Heuertz M**, Lemos-Filho JP, Lovato MB (2016) Climatic drivers of leaf traits and genetic divergence in the tree *Annona crassiflora*: a broad spatial survey in the Brazilian savanna. *Global Change Biology* 22(11), 3789–3803.

Unger GM, **Heuertz M**, Vendramin GG, Robledo-Arnuncio JJ (2016) Assessing early fitness consequences of exotic gene flow in the wild: a field study with Iberian pine relics. *Evolutionary Applications* 9(2), 367–80.

4.3. Development of genetic resources and contributions in applied conservation genetics

4.3.1. Genetic and genomic marker developement

Development of SSR markers – With my PhD student Guillaume Koffi at ULB, we developed nuclear SSR markers in the African rainforest tree *Santiria trimera* (Oliv.) Aubrév. (Burseraceae), a morphotaxon that contains three putative biological species (Koffi *et al.* 2010). We tested SSR development through sequencing of SSR-enriched clonal libraries, without success. Using a pyrosequencing-based SSR discovery assay, we succeeded in developing seven SSRs in *S. trimera* that can be successfully genotyped across the three morphotypes, with endemic alleles in each morphotype (Koffi *et al.* 2012). In collaboration with Jérôme Duminil, we simultaneously attempted identifying SSRs in two African timber tree species of the genus *Erythrophleum* (Fabaceae). In this pair of species, the classical approach of sequencing SSR-enriched clonal libraries worked, allowing us to characterize nine SSRs that are polymorphic in both species of this complex (Duminil *et al.* 2011).

Genomic markers giving access to functional variation: Case of *Symphonia* sp. – The lack of a tropical model tree species with available genomic resources has long limited our population genetics work on tropical trees, hindering the investigation into functional genetic variation and the study of local adaptation. We thus decided to choose a suitable model system in which to undertake genomic resource development. The genus *Symphonia* (Clusiaceae) was selected for its particularly interesting evolutionary history, with one widespread species, *S. globulifera* L. f. in Africa and the Neotropics, and some 20 species endemic to Madagascar (Perrier de la Bâthie 1951; Abdul-Salim 2002), raising questions on the scale of local adaptation, both in within species and between closely related species. At the same time, challenging species delimitation questions arise in Madagascar, where a recent radiation most probably occurred, producing a complex of closely related species. After examining the phylogeographic history of *S. globulifera* L.f. in the Neotropics (Dick & Heuertz 2008) and Africa (Budde *et al.* 2013), and the determinants of fine-scale spatial genetic structure in multiple populations (Torroba-Balmori *et al.* 2017), we used high-throughput sequencing approaches to develop genomic resources that allow access to functional DNA variation. Mostly in collaboration with Sanna Olsson, the postdoc I supervised in the framework of the *AFFLORA* project of the Spanish National Research Plan, and the bioinformatics unit of the University of Málaga, we constructed a low-coverage

fragmented draft genome for a *S. globulifera* individual from Cameroon and used it as a resource for marker mining. Against the genome draft, we mapped a published *S. globulifera* transcriptome constructed based on plant material from the Guiana Shield, which allowed us to discover loci and pre-screen them for polymorphism across a wide geographic range. We identified 923 high-quality SNPs and developed 18 genic SSRs that were polymorphic in African and Neotropical populations (Olsson *et al.* 2017). Our approach of using plant materials from different range parts for marker discovery and polymorphism screening is particularly interesting for widespread tropical or endangered taxa with limited DNA availability.

To delimit *Symphonia* species from Madagascar with Sanna Olsson and my PhD student Paloma Torroba-Balmori, we developed an additional set of SNP markers based on RNA-Seq, and genotyped them at the Bordeaux Genome Transcriptome Facility in the framework of a TREES4FUTURE grant to Paloma. We discovered that some Malagasy species of *Symphonia* are tetraploid, which added difficulty to the species delimitation question. This work is not published yet.

Funding

Mentoring

PhD students

Guillaume Kouamé Koffi, co-supervision with Olivier J. Hardy, Université Libre de Bruxelles, 2006-2010. Title: Genetic structure and ecology of a tropical tree with large morphological variation and a large distribution in Central Africa, *Santiria trimera*, Burseraceae.

Paloma Torroba Balmori, co-supervision with Ricardo Alía, INIA-CIFOR Madrid and Universidad de Valladolid, 2013-ongoing. Adaptive evolution in the tropical tree genus *Symphonia* (Clusiaceae).

Publications

Duminil J, Koffi GK, Debout G, Sebastiani F, Vendramin GG, **Heuertz M**, González-Martínez SC, Hardy OJ (2011) Isolation of SSR markers for two African tropical tree species *Erythrophleum ivorense* and *E. suaveolens* (Fabaceae, Caesalpinioideae). *American Journal of Botany* 98, E106–E108.

Koffi KG, **Heuertz M**, Jans R, Hardy O, Vendramin GG, Duminil J (2012) Characterization of new microsatellite loci isolated from *Santiria trimera* (Burseraceae). *American Journal of Botany* 99, E334–E336.

Olsson S, Seoane-Zonjic P, Bautista R, Claros MG, González-Martínez SC, Scotti I, Scotti-Saintagne C, Hardy OJ, **Heuertz M** (2017) Development of genomic tools in a widespread tropical tree, *Symphonia globulifera* L.f.: a new low-coverage draft genome, SNP and SSR markers. *Molecular Ecology Resources* 17(4), 614–630.

4.3.2. Applied conservation genetics

Wildlife forensics – In collaboration with Alain Frantz, I contributed to a study for the Luxembourg Administration for Nature and Forests on the genetic characterization of the red deer population in Luxembourg and adjacent regions and to test the hypothesis that a focal group of deer had been illegally released by recreational hunters. We genotyped SSR markers on DNA from non-invasively sampled hair of the focal group of animals and from tissue samples from hunting campaigns available from the authorities. We characterized the spatial genetic structure of the deer population, detecting barriers to gene flow created by a combination of urban areas, motorways and a river valley used for viticulture. The four focal animals could be excluded with a high probability from the three genetic subpopulations discovered in the region and it was therefore likely that they had been released illegally (Frantz *et al.* 2006).

Genetic markers applied to resource management in grapevine – In the return phase to the Gabriel Lippmann Research Centre in Luxembourg (2005) after my postdoc mobility in Uppsala, I worked on a research project on grapevine cultivar identification and pedigree inference, as well as capacity building in genetic marker technologies, in collaboration with colleagues from several countries from Eastern Europe, foremost with Svetlana Goryslavets at the Magarach Institute of Vine and Wine in Yalta, Ukraine. Collections of grapevine genetic resources in eastern European countries were in a neglected state after the economic transition in the 1990s. We found high genetic diversity in a Ukrainian collection of grapevine varieties using SSRs and resolved their relatedness to French and eastern European cultivars (Heuertz *et al.* 2008). Wine cultivars were significantly differentiated from table grape cultivars and a Bayesian method detected two genetic clusters, one containing mainly table grape and Muscat accessions and the other mainly wine grape accessions from throughout Europe, including Ukraine (Heuertz *et al.* 2008). Our paper highlighted the contribution of Muscat and European winegrapes in the breeding design of Ukrainian varieties and suggested that selection and historical and contemporary movement of germplasm are major factors shaping the structure of the grapevine gene pool. In a second paper, we determined the pedigree of four popular commercially cultivated grapevine varieties in Ukraine: Antey Magarachskii, Rubinovyi Magaracha, Granatovyi Magaracha and Rubin Golodrigi (Goryslavets *et al.* 2010).

In a follow-up study, collaborating with PhD student José Carlos Santana of the University of Valladolid, we used similar methods to solve the origins and patterns of relatedness in grapevine cultivars grown on the Castillian plateau in Spain. We assessed 421 (*Vitis vinifera* ssp. *sativa*) and four alleged wild grapevine samples (putative *Vitis vinifera* ssp. *sylvestris*) using six SSRs. The cultivated data set yielded 117 unique genotypes, including 13 homonyms (same name for genetically different accessions), and 27 previously unreported genotypes, almost one-fourth of the nonredundant genotypes (Santana *et al.* 2010). Nonredundant genotypes were examined at another 16 nuclear and three chloroplast SSRs. Three differentiated genetic clusters were detected among them, separating (1) Muscat-type accessions and interspecific Vitis hybrids, (2) accessions from France and the western Castilian Plateau, and (3) accessions from the central Castilian Plateau together with local table grapes. Three of the four wild-collected samples were likely to represent genuine *Vitis vinifera* ssp. *sylvestris* (Santana *et al.* 2010).

Conservation of forest genetic resources – I participated in the editing of technical guidelines for forest managers as a country representative for Luxembourg, from 1999 to 2005, in EUFORGEN (European Forest Genetics Resources Program), a European network on forest genetic resources conservation (Pliûra & Heuertz 2003; Pliura & Heuertz 2012).

I also participated in a prospection mission on the conservation status of cluster pine genetic resources in Morocco in the framework of a project of the Spanish Cooperation Agency. This prospection led to the publication of a paper in French targeted at a forestry-related readership (Wahid et al. 2009).

Funding

Plant genetic resources in southeastern Europe III: grapevine genetic resources: Luxembourg Ministry of Foreign Affairs and International Plant Genetic Resources Institute (IPGRI). 2004-2006. PI: Jean François Hausman.

Diversidad y conservación de recursos genéticos de coníferas en la Península Ibérica y Marruecos. Agencia Española de Cooperación Internacional – PCI. Jan. 2006 – Dec. 2006. 8.400€. PI: Santiago C. González Martínez. Participants: INIA-CIFOR; Université Cadi Ayyad, Morocco.

Mentoring

Contribution to the supervision of PhD students

José Carlos Santana Pérez, Universidad de Valladolid, 2011. Main supervisor: Elena Hidalgo. Caracterización genética de variedades de vid en Castilla y León con fines de conservación.

Other mentoring activities

Training of scientists from East European countries (Moldova, Ukraine) in molecular analysis techniques.

Publications

Frantz AC, Tigel Pourtois J, **Heuertz M**, Schley L, Flamand MC, Krier A, Bertouille S, Chaumont F, Burke T (2006) Genetic structure and assignment tests demonstrate illegal translocation of red deer (Cervus elaphus) into a continuous population. *Molecular Ecology* 15, 3191–3203.

Goryslavets S, Risovanna V, Bacilieri R, Hausman JF, **Heuertz M** (2010) A parentage study of closely related Ukrainian wine grape cultivars using microsatellite markers. *Cytology and Genetics* 44, 29–37.

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Pliûra A, **Heuertz M** (2003) EUFORGEN Technical Guidelines for genetic conservation and use for common ash (*Fraxinus excelsior*). International Plant Genetic Resources Institute, Rome, Italy. 6 pages. ISBN 92-9043-567-4.

Pliûra A, **Heuertz M** (2012) EUFORGEN Guía técnica para la conservación genética y utilización del fresno común (*Fraxinus excelsior* L.). Foresta Madrid. España. 6 p., ISSN 1575-2356.

Santana SC, **Heuertz M**, Arranz C, Rubio JA, Martínez-Zapater JM, Hidalgo E (2010) Genetic structure, origins and relationships of grapevine cultivars from the Castilian Plateau (Northern-Central Spain). *American Journal of Enology and Viticulture* 61, 214–224.

Wahid N, González-Martínez SC, Alía R, Boulli A, **Heuertz M**, 2009. Exploration et conservation des ressources génétiques du pin maritime au Maroc. *Forêt Méditerranéenne* 30, 240–256.

5. Eco-evolutionary processes in tree species complexes: ongoing work and perspectives

5.1. Evolutionary relationships and genomic signatures of local adaptation in tropical tree species complexes

The main research line I currently develop concerns the evolutionary relationships between species in tropical tree species complexes and their implications for species coexistence and adaptive evolution. A variety of ecological mechanisms have been proposed to explain species coexistence in highly diverse communities such as tropical rainforests (Hubbell 2001; Molino & Sabatier 2001; Volkov *et al.* 2005; Comita *et al.* 2010). These studies have proposed deterministic or stochastic processes relating to competition between species, or to disturbance, to explain species coexistence, but they have mostly ignored the role of genotypic or phenotypic variation within species.

To study eco-evolutionary processes in tropical tree species complexes, the first steps to resolve are 1) genetic species delimitation and its match with morphological species identification, and 2) clarify the evolutionary history of closely related species, including distinguishing the role of shared ancestral variation vs. modern hybridization. Adaptive evolutionary processes can only be investigated reliably once these questions on the evolutionary history of the study species or populations are addressed. The inference of adaptive processes can then rely on genomic data along with complementary information, e.g., on phenotypic trait variation or environmental variation. The main geographic region where I conduct my current research is the Neotropics, in particular the Guiana Shield, but I also work on studies in Africa, through continuation of research I developed before joining INRA, or through collaborations.

5.1.1. Species delimitation and evolutionary relationships

Case of *Symphonia* spp. in Madagascar – The genus *Symphonia* (Clusiaceae) harbours ca. 20 species endemic of Madagascar, and one wide-spread species, *S. globulifera* L.f., distributed accross tropical Africa and the Neotropics (Perrier de la Bâthie 1951; Abdul-Salim 2002). The valid botanical species descriptions in the *Symphonia* species complex in Madagascar (Perrier de la Bâthie 1951) are based on only a subset of the currently available botanical collections (e.g., collections deposited at the Missouri Botanical Garden Herbarium, MO, at the Herbarium of the National Natural History Museum in Paris, P, our collections deposited in the Herbarium of the Real Jardín Botánico-CSIC, Madrid, MA). Morphological characters, both vegetative and reproductive, are widely variable even within species and display considerable overlap between species (own observations). The endemic species of Madagascar have most likely evolved in a recent radiation on Madagascar, whereas there is currently only weak evidence for speciation within the widespread *S. globulifera* (Abdul-Salim 2002; Baraloto *et al.* 2007a; Torroba-Balmori *et al.* 2017).

To investigate species delimitation as a preliminary step before investigating adaptive evolution, we sampled 414 *Symphonia* sp. individuals from nine sites in Madagascar (mostly in the framework of the Spain-funded AFFLORA project, in 2013 and 2014) across a representative but non-exhaustive range where species of the genus can be found, in the rainforests of eastern Madagascar. We reached a preliminary species determination based on morphology for n=308 samples using the identification key and species descriptions of Perrier de la Bâthie (1951). This determination was considered too uncertain as a basis for evolutionary inference. We thus resorted to genetic species delimitation on "blind" samples, e.g., without considering the morphological information. Sequences of plastid DNA or the nuclear ribosomal internal transcribed spacer (ITS) led to poor resolution of putative evolutionary units. Thus, we genotyped 20 genic SSR loci that we previously developed (Olsson *et al.* 2017) and conducted genetic species delimitation based on the genetic clustering approach

implemented in STRUCTURE (Pritchard *et al.* 2000). A hierarchical analysis identified three major clusters which were then further subdivided, allowing us to identify a total of twelve putative species in our sample, with a first cluster of three closely related tetraploid species, a second cluster that harboured eight putative species, all of them diploid, and a third diploid cluster that was mostly confined to a single location, the island of Nosy Mangabe in North-East Madagascar, where within-population structure was identified, but interpreted as representing a single species (Figure 32, Budde et al., in prep). We are cautious at this stage and call these units putative species, considering that genetic clusters, especially allopatric ones, may in fact belong to the same species (see also Sukumaran and Knowles 2017). Genetic species delimitation was congruent with a priori morphological identification in five taxa only; the three closely related tetraploid species were well-distinct from all other species and could also be distinguished among each other (Figure 32).
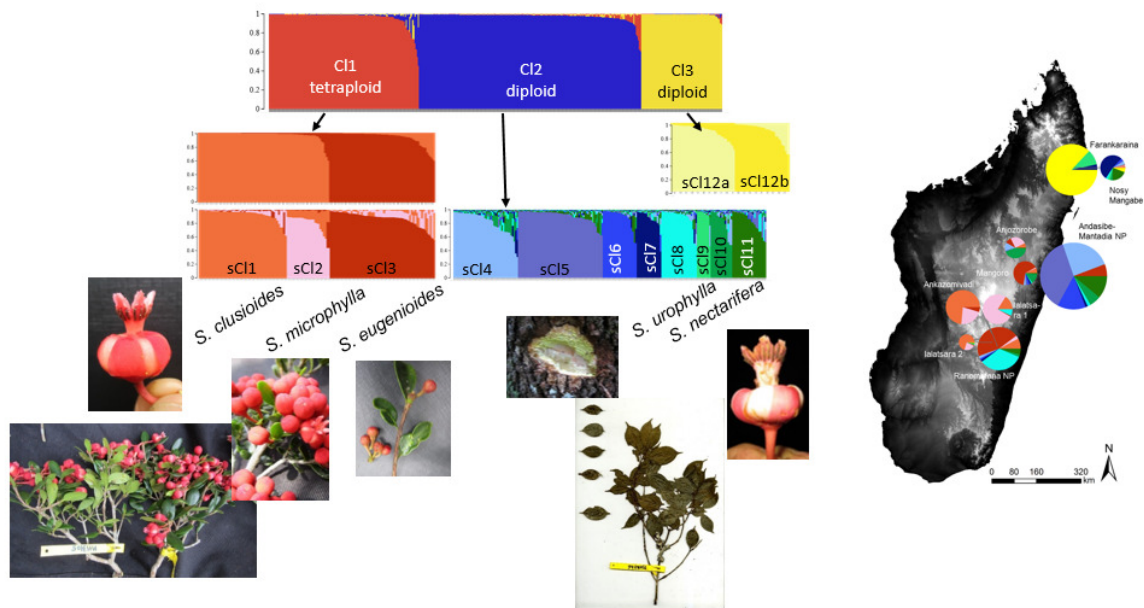

Figure 32 Genetic species delimitation in the genus Symphonia (Clusiaceae) in Madagascar based on 20 genic SSR markers and the STRUCTURE software. From Budde et al., in prep.
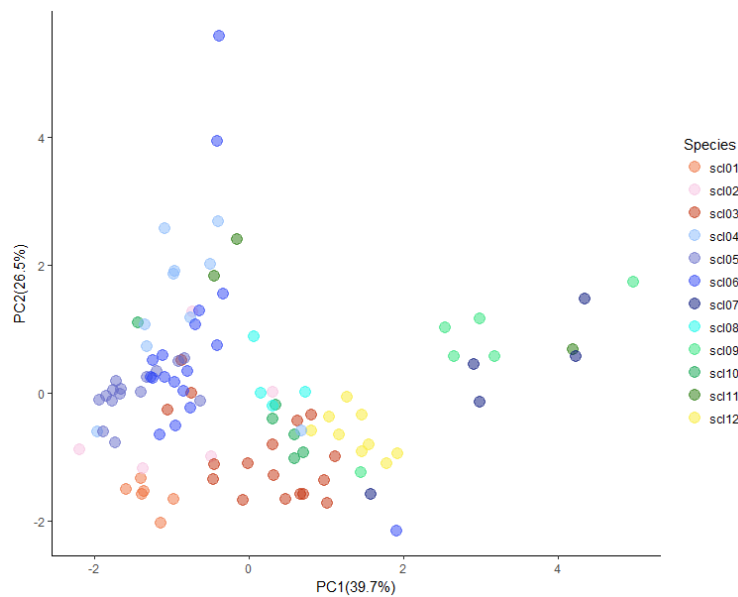

Figure 33 Principal component analysis on leaf morphological traits in the twelve putative Symphonia species identified with genic SSRs in Madagascar. From Budde et al., in prep.

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Leaf morphological traits exhibited a large variation in the Symphonia species complex: individuals from the same genetic species often clustered together in a principal component analysis although there was considerable overlap between clusters (Figure 33, Budde et al., in prep).

Some putative hybrids were identified in this analysis based on their admixed genotypes. Further research at the within-population scale should allow us to identify which species/gene pools preferentially hybridize today. Information on the geographical distribution of gene pools, patterns of pairwise differentiation between gene pools and estimates of their genetic diversity will allow us to construct alternative scenarios of past population history, which we can test using coalescent simulations (Cornuet *et al.* 2008; Excoffier *et al.* 2013). Such approaches can inform on past population splits and the rates of past and recent gene flow between species or gene pools (Leroy *et al.* 2017).

We are currently also using alternative approaches to improve our knowledge on the evolutionary history of this complex, notably the construction of a backbone phylogeny on the basis of RNA-Seq data conducted in 20 *Symphonia* spp*.* samples (work led by Sanna Olsson at INIA-CIFOR Madrid, who was a postdoc in the AFFLORA project). This initiative is not straightforward because of the close relatedness of the species, the poor morphological species identification, potential past and ongoing hybridization, and the difficulty of identifying true orthologs in a system with ploidy differences. Based on the RNA-Seq data, we developed and genotyped 144 SNP loci (section 4.3.1) for phylogenetic inference, but the analysis resulted in poor clade delimitation and poor support of clades, illustrating that currently the genic SSRs are the most powerful markers we have for genetic species delimitation in *Symphonia*. These results lend support to the hypothesis of recent speciation in *Symphonia* in Madagascar.

Case of *Eschweilera* species in French Guiana – The Lecythidaceae family is represented by ecologically important tree species in lowland forests in many parts of Amazonia, including some species with very wide distribution ranges encompassing the Amazon Basin and the Guiana Shield (ter Steege *et al.* 2006, 2013; Mori *et al.* 2017). We are currently using molecular markers for species delimitation in the *Bertholletia* clade, the clade of the Brazil nut tree, a Neotropical clade characterized by its zygomorphic flowers that harbours ca. 125 species in four genera: *Lecythis*, *Eschweilera*, *Corythophora* and *Bertholletia* (Huang *et al.* 2015; Mori *et al.* 2017). Within the *Bertholletia* clade, ten subclades have been resolved (Huang *et al.* 2015; Mori *et al.* 2017), identifying the genera *Lecythis* and *Eschweilera* as polyphyletic, with five and three subclades, respectively, whereas *Corythophora* is monophyletic and *Bertholletia* monotypic. The *Parvifolia* clade harbours 63 species of the genus *Eschweilera*; ca. half of the species included in the *Bertholletia* clade. The *Parvifolia* clade, highly diverse and phylogenetically poorly resolved, is currently the main focus of our studies.

We used SSRs to genotype 152 individuals representing 11 species of the genus *Eschweilera* all belonging to the *Parvifolia* clade, sampled in nine forest inventory plots in French Guiana, as part of the MSc internship of Sana Miloudi (2018). The amplification across species was successful, illustrating their close genetic relatedness. We detected five gene pools and observed a good correspondence between morphological and genetic delimitation for *Eschweilera sagotiana* Miers and the undescribed morphospecies *E. sp3* (which resembles *E. grandiflora* (Aubl.) Sandwith), and to a lesser extent for *E. decolorans* Sandwith and *E. micrantha* (O.Berg) Miers (Figure 34, Heuertz et al. submitted).
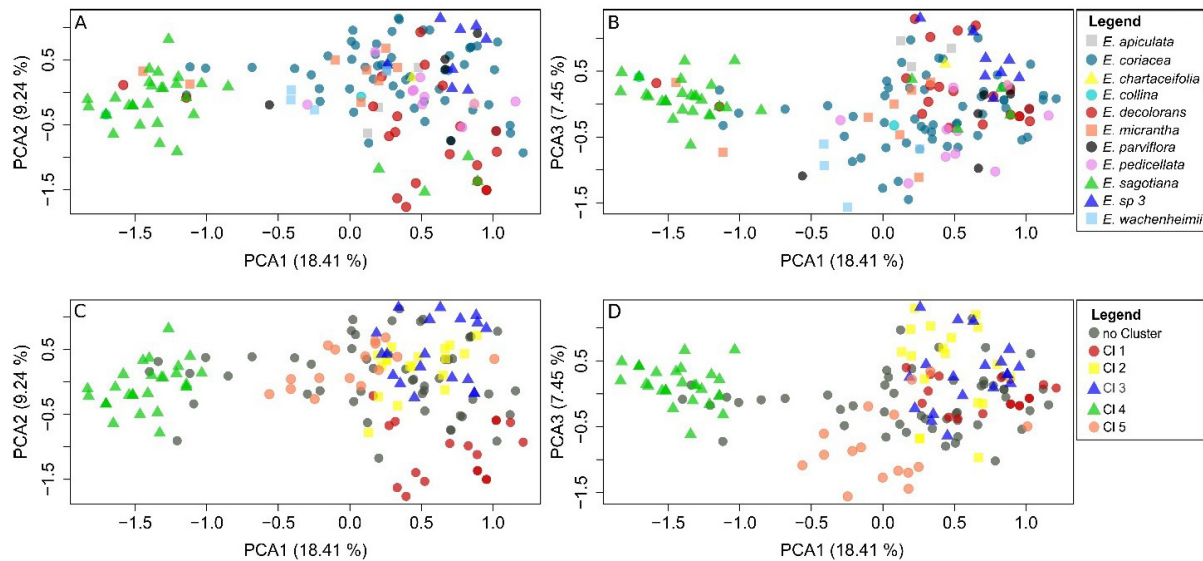
Figure 34 Principal component analysis (PCA) of 152 Eschweilera individuals genotyped at four SSR loci. Colours and symbols indicate the species determination of samples in plots A and B and the assignment to STRUCTRE clusters (threshold > 0.875 ancestry proportion) in plots C and D.

Using resequencing data of 134 nuclear amplicons (defined from a pilot ddRAD-Seq experiment in the framework of the MSc thesis of Quentin Jehanne in 2016) for a wider array of samples from the *Bertholletia* clade, we uncovered the expected phylogenetic relationships previously described in the clade (Huang, Mori, and Kelly 2015), as illustrated in an unrooted phylogenetic network (Figure 35). We however obtained poor support for species within the *Parvifolia* clade using phylogenetic approaches. Population genetic approaches were more successful for gene pool delimitation but identified substantial mismatches between putative genetic and morphologically identified species (Heuertz *et al.* 2018). The higher suitability of population genetics clustering approaches for species delimitation was expected, considering that several of the tropical tree species from the *Parvifolia* clade are considered Amazonian "hyperdominants", i.e., hyperabundant species with census sizes of 0.4 to 4 x 10^9 individuals estimated across Amazonia (ter Steege *et al.* 2013). Species with such large census sizes are also expected to have large effective population sizes *Ne*, and thus to conserve a high level of ancestral polymorphism, potentially shared between species.

The analysis of this resequencing dataset still needs careful validation because of the likely inclusion of paralogous genes in these initial analyses. Based on SSR data, we earlier thought that *Eschweilera* species from the *Parvifolia* clade might be tetraploid; however, we now believe that they are diploid (based on genome size estimates from flow cytometry) but that they bear a strong signature of a past genome duplication event affecting the order Ericales to which the Lecythidaceae belong (Shi *et al.* 2010). We are currently investigating which percentage of the genome bears a signature of duplication by examining patterns of heterozygosity indicative of paralogous genes in our pilot ddRAD-Seq data as well as on sequence capture data in the Parvifolia clade shared by Dr. Dick's lab using the Lecythidaceae-wide bait set (Vargas et al. 2019) in the framework of the MSc thesis of Thomas Folituu (2019).
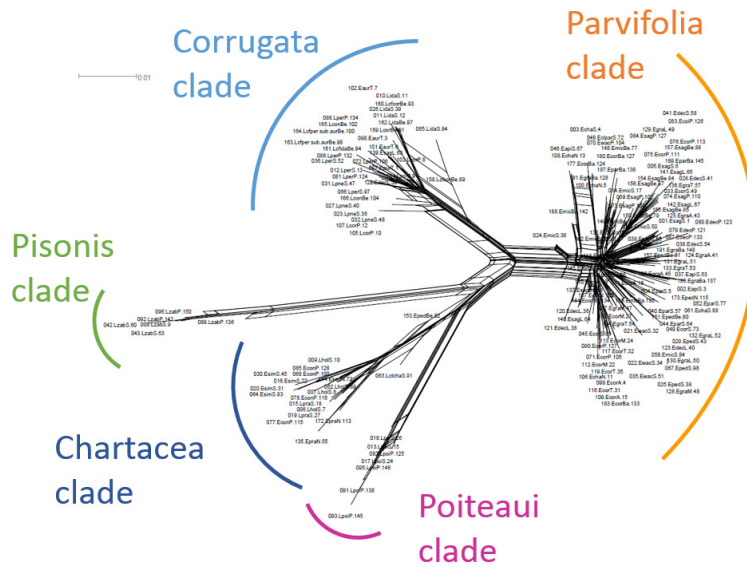
Figure 35 Unrooted phylogenetic network of 96 Lecythidaceae samples belonging to the Bertholletia clade based on amplicon resequencing and analysis in the Splitstree spoftware. From Heuertz et al. (2018).

With the objective of accessing functional genetic variation for future studies on evolutionary processes in the Lecythidaceae and also to design nuclear genetic markers for phylogenetic approaches in the family, we conducted an RNA-Seq experiment in *Eschweilera* and *Lecythis* species (*E. coriacea* (n=2), *E. sagotiana* (n=1), *L. congestifolia* (n=1), *L. persistens* (n=1)) at the Genome Transcriptome Facility of Bordeaux (PGTB). In collaboration with the team of Dr. Christopher Dick (University of Michigan), we identified orthologous genes in these and seven additional transcriptomes from other genera and designed a bait set for sequence capture in the family. The bait set was tested by capture *in silico* on genome skimming data derived from a previous publication of Dr. Dick's team (Thomson *et al.* 2018), which allowed to obtain a preliminary nuclear backbone phylogeny for the family, although on a small set of species (Vargas et al. 2019). Some conflict was observed between the plastome and nuclear phylogenies of the family (Figure 37, Vargas et al. 2019).
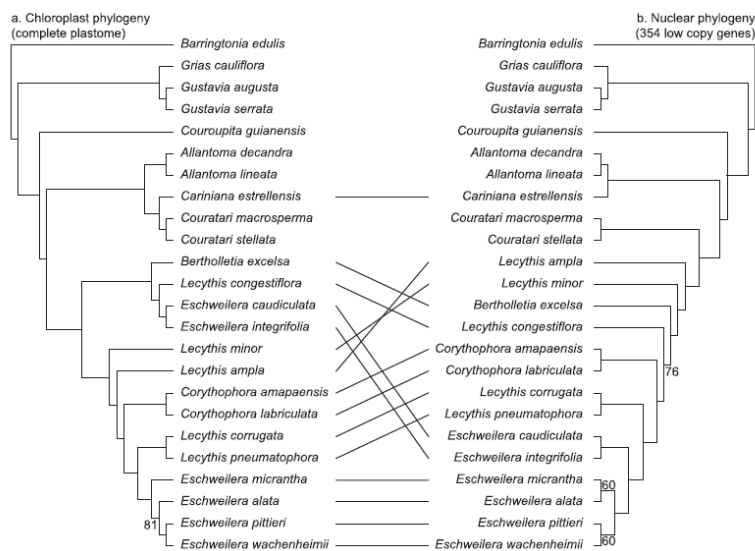

Figure 36 Comparison between the maximum likelihood phylogenies derived from (a) the complete plastome alignment of Thomson et al. (2018) and (b) 354 nuclear markers. All nodes have a bootstrap support of 100 unless noted otherwise. Lines between taxa indicate a conflicting position between the two topologies. From Vargas et al. (2019).

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5.1.2. Exploration of genomic signatures of local adaptation

Case of *Symphonia spp.* in Madagascar – The nine sampling sites for *Symphonia* species cover a steep climatic gradient, from coastal wet climate in the northeast to drier inland climate in the southeast, and the distribution of SSR gene pools shows a marked geographic structure (Figure 32). Furthermore, in the intensively sampled site of Andasibe National Park, we observed that sympatric species (defined based on SSR gene pools) occupy overlapping but locally divergent habitat niches, potentially reflecting differential adaptation to water and nutrient availability. These preliminary signatures of species turnover both at the local scale and at the scale of the sampling range suggest that *Symphonia* species are most probably differentially adapted to their local environments in Madagascar. *Symphonia* in Madagascar is thus potentially an attractive system to test for adaptive evolution at the level of the species complex. The data we currently have to examine adaptive evolution in this system are limited: 144 genic SNPs and 20 genic SSRs, as well as 20 transcriptomes. Once a consolidated phylogeny can be established, adaptive evolution can be explored along the phylogeny, taking advantage of the availability of loci with structural annotation. We can for instance apply i) the neutral substitution test, as implemented in CRANN (Creevey & McInerney 2003); this test identifies genes with more or fewer non-synonymous or silent substitutions than expected under a model of neutral evolution. An excess of substitutions leading to amino acid replacements is interpreted as a signature of positive selection whereas an excess of silent substitutions is interpreted as negative selection; ii) the ratio of non-synonymous to synonymous substitution rates, ω, which, if heterogenous among lineages, indicates adaptive evolution, as implemented in PAML (Yang 2007). To re-construct the evolutionary history of life-history and phenotypic traits, we plan to use the program BayesTraits (Pagel & Meade 2006) which allows to test hypotheses about models of evolution, about ancestral states and about correlations among pairs of traits.

Case of *Symphonia globulifera in Africa* – We recently compiled a ddRAD-Seq data set in the framework of the Erasmus Mundus MSc thesis of Janan Gawra (2018), co-supervised by Katharina Budde, Olivier Hardy and myself, on *S. globulifera* across the African tropics, from Upper Guinea to the Congo Basin. Using this dataset, we aim to identify candidate genes for adaptive evolution i) based on population genetic structure and genomic features, ii) associated with climatic/environmental variables, in the framework of a postdoctoral project proposal submitted to the Idex program of the University of Bordeaux (January 2019) with Franck Monthe (PhD, Université Libre de Bruxelles 2019). After characterizing population genetic structure, we will identify candidate loci for positive selection as those which are outliers for particularly strong differentiation among populations (Beaumont & Nichols 1996; Foll & Gaggiotti 2008). We expect that this approach can help to validate suspected ecotypes in the species (Budde *et al.* 2013), and potentially identify loci involved in adaptive evolution that contrasts between populations. We will also screen for loci whose allele frequencies vary according to environmental variables (de Villemereuil & Gaggiotti 2015; Gautier 2015). The ddRAD sequence catalog (the full sequences on which the SNP-matrix is based) will be annotated using blast-based methods such as FullLengtherNext (Benzekri *et al.* 2013) and Provean (Choi *et al.* 2012). These annotations will be needed to explore functions of loci or pathways putatively under selection, but also to bioinformatically infer the frequency of deleterious alleles, a signature of genetic load (suboptimal fitness) in populations (Henn *et al.* 2015). This genomic approach to explore genetic load is justified by an increasing body of evidence in humans that selection on complex phenotypic traits involves a high number of genes (is highly polygenic) and that these traits are actually commonly under negative, not positive, selection (Zeng *et al.* 2018).

Case of *Eschweilera* species in French Guiana – We have gathered a resequencing dataset of 134 nuclear regions in 102 individuals belonging to eleven *Eschweilera* species of the *Parvifolia* clade sampled in nine forest inventory plots in French Guiana, by increasing the dataset presented in Figure 35. As soon as the paralog problem (Section 5.1.1) is solved, we will construct a phylogenetic tree on this data, and then proceed to detecting adaptive evolution along the phylogeny, as suggested for the case of *Symphonia* in Madagascar above.

Funding

COLLEVOL – Testing Collective Evolution in Neotropical Trees. Labex CEBA (ANR [Agence Nationale pour la Recherche], France), annual project. 2016-2017. 20,000€. **PI: Myriam Heuertz**.

NEOTROPHYL – Inferring the drivers of Neotropical diversification using an integrative macroevolutionary approach. Labex CEBA (ANR, France), strategic project. 2016-2019. 250,000 € (20,000 € to INRA UMR Biogeco). PI: Frédéric Delsuc.

AFFLORA – The assembly of African floras from lineages to populations: effects of historical climate changes and species ecology. Plan Nacional de I+D+I, Spain. Jan. 2013 – Dec. 2015. 193,050€. **PI: Myriam Heuertz**.

ADAPMADA – Adaptive radiation in Symphonia, Clusiaceae, in Madagascar. EU TREES4FUTURE grant to Paloma Torroba-Balmori, 2, to the Bordeaux Genome Transcriptome Facility (PGTB), 2015.

Mentoring

Supervision of MSc sudents

Quentin Jehanne, co-supervision with Stéphanie Mariette, Université de Bordeaux, 2016. Exploration de méthodes pour la détection de SNPs sur données de RAD-Seq et confirmation expérimentale de polymorphismes.

Sana Miloudi, co-supervision with Katharina Budde, Université de Bordeaux, 2018. Phylogeography of the hyperdominant *Eschweilera coriacea*.

Janan Gawra, co-supervision with Katharina Budde, Olivier Hardy, ERASMUS MUNDUS TROPIMUNDO, Université Libre de Bruxelles, 2018. An update to the pylogeography of *Symphonia globulifera* in Africa.

Thomas Folituu, co-supervision with Olivier Lepais, Université de Bordeaux 2019. Investigating diploidization in the tropical tree species complex Eschweilera (Parvifolia clade, Lecythidaceae).

Supervision of PhD students

Paloma Torroba Balmori, co-supervision with Ricardo Alía, INIA-CIFOR Madrid and Universidad de Valladolid, 2013-ongoing. Adaptive evolution in the tropical tree genus *Symphonia* (Clusiaceae).

Katharina B. Budde, co-supervision with Santiago C. González-Martínez, INIA-CIFOR Madrid and Universidad Complutense de Madrid, 2009-2014. Genetic structure of forest trees in biodiversity hotspots at different spatial scales.

Supervision of Postdocs

Katharina B. Budde, postdoc of the Idex excellence program of the Université de Bordeaux, Sept. 2016 – Aug. 2018. Drought stress in tropical ecosystems: adaptive allele sharing in tree species complexes.

Sanna Olsson, postdoc in the AFFLORA project "The assembly of African floras from lineages to populations: effects of historical climate changes and species ecology." Jan 2014 – June 2015, INIA-CIFOR Madrid.

Publications

Vargas OM, **Heuertz M**, Smith SA, Dick CW (2019) Target sequence capture in the Brazil nut family (Lecythidaceae): Marker selection and in silico capture from genome skimming data. *Molecular Phylogenetics and Evolution*, 135: 98–104.

Heuertz M, Jehanne Q, Budde KB, Caron H, Tysklind N, Scotti-Saintagne C, Guichoux E, Molino JF, Sabatier D, El Mujtar V, Franc A, Garnier-Géré P, Mariette S. Talk. Evolutionary history of tropical tree species complexes: species delimitation and adaptive genetic variation in the Brazil nut clade (Lecythidaceae). In: Challenges in tropical ecology and conservation - Global perspectives. Proceedings of the European Conference of Tropical Ecology, GTOE18, Paris 26-29 March 2018.

5.2. Local-scale adaptation in tropical tree species complexes: an integrative approach

I currently co-supervise, with Bruno Hérault (CIRAD) as the main supervisor and Niklas Tysklind (INRA, UMR Ecofog) as an additional advisor, the PhD of Sylvain Schmitt, entitled "Ecological genomics of niche exploitation and individual performance in tropical forest trees". In his PhD thesis, Sylvain is using complementary approaches to reach an integrative comprehension of the interactions between the genetic constitution of tree species complexes, the abiotic and biotic environment in which these species thrive, and the phenotypic variation that the tree species exhibit (Figure 37).

He is conducting his work based on the forest inventory plot of Paracou (https://paracou.cirad.fr/) established in the 1980s and managed by CIRAD, where tree diameter at breast height (DBH) has been measured every year or every two years in six undisturbed plots of 6.25 ha size, and every five years in one 25 ha plot. Twelve 6.25 ha plots have undergone three distinct wood extraction treatments. All individual trees are georeferenced and detailed habitat characterization is available. Associations between tree species and specific habitats have been characterized in Paracou (Allié *et al.* 2015) and the effects of species' functional traits on their ontogenetic growth trajectories have been described (Hérault *et al.* 2011). The Paracou inventory site is thus excellently suited to conduct research that combines ecological and evolutionary approaches to investigate local adaptation from the within-species scale to the between species complexes scale.

As a first approach to characterize the realized niche of selected species complexes, Sylvain used a Bayesian framework to model the probability of presence of individuals using as predictors an integrative habitat variable, the topographic wetness index (TWI, wet habitats have high TWI), and an integrative index of competitive interaction, the neighbour crowding index (NCI) based on the DBH of neighbouring trees within a radius of 20m from the focal tree. The results suggest pervasive local-scale niche differentiation of tree species within complexes (Figure 38, Schmitt et al., in prep); note in particular the niche differentiation for *Symphonia* and *Eschweilera* (Parvifolia clade).
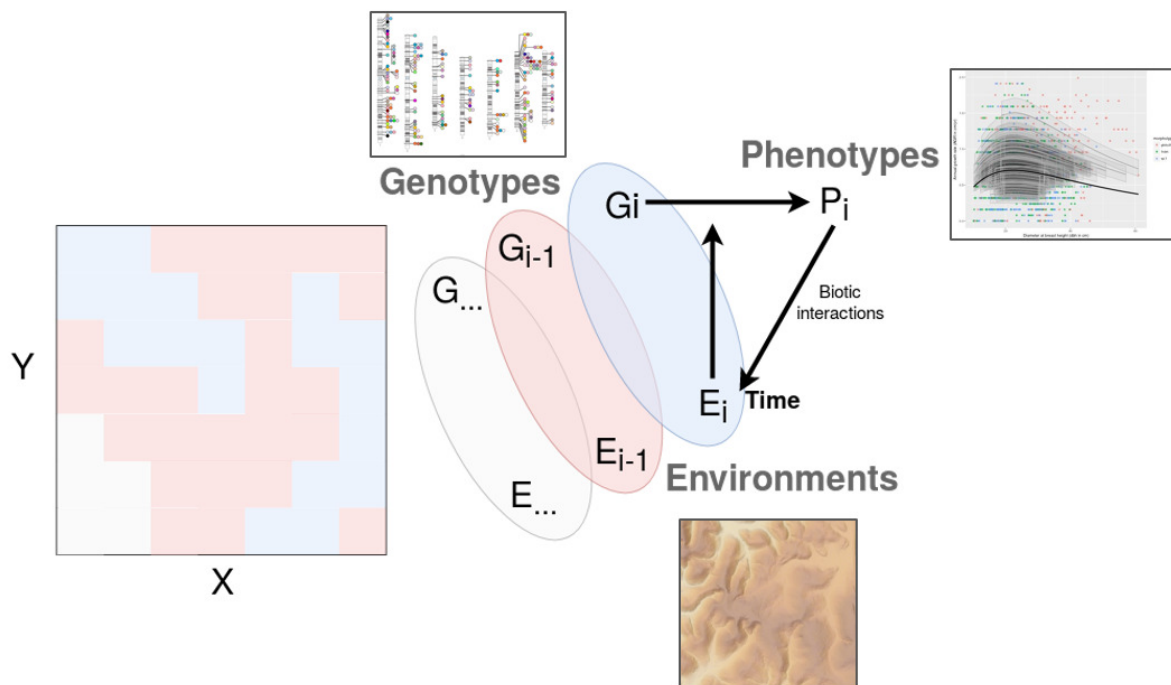

Figure 37 Conceptual scheme of interlinks between genotypes, phenotypes and environment in tropical tree species complexes investigated at the local scale in the PhD work of Sylvain Schmitt. Phenotypes include simple phenotypic traits, such as leaf traits, as well as integrative fitness-related phenotypes such as growth (the inset shows a growth model as a function of DBH with maximum growth rate inferred per individual).
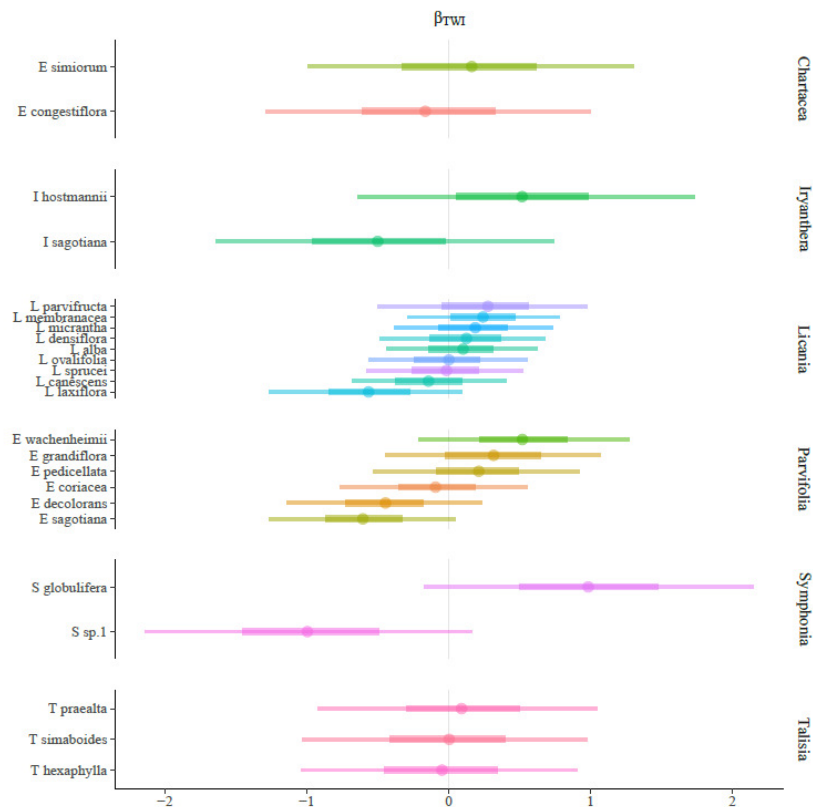
Figure 38 Effect of the topographic wetness index (TWI, high values are indicative of wet habitat) on species distribution within species complexes. TWI posterior is represented for each species complex and species with mean (circle), 50% credibility interval (thick segment) and 95% credibility interval (thin segment). The species complexes are given by their clade names (right side of figure): Chartaceae and Parvifolia clades harbour species of the genus Eschweilera (Lecythidaceae); Iryanthera (Myristicaceae), Licania (Chrysobalanaceae), Symphonia (Clusiaceae), Talisia (Sapindaceae). From Schmitt et al. in prep.

The *Symphonia* and *Eschweilera* (*Parvifolia* clade) complexes were chosen for the collection of genomic and leaf functional trait variation. Sylvain has conducted two sampling campaigns in 2017 and 2018, with the support of the Ecofog mixed research unit and interns Anne Baranger and Émilie Ducouret, to sample leaf material from > 400 individuals in each complex, to store in silica gel for DNA analysis on the one side, and to measure leaf functional traits such as leaf area (LA), mass per area (LMA), dry matter content (LDMC), thickness (LT) and chlorophyll content (CC), on the other side.

To assess the role of tree ontogeny and abiotic environment on leaf functional trait variation, Sylvain developed a hierarchical Bayesian model. Principal component analysis (PCA) highlighted differences in trait values between complexes and between species within complexes, with LA and LT showing stronger species differentiation than LMA, LDMC and CC (Figure 39). These results can be interpreted with regard to the leaf economics spectrum, a framework that conceptualises the fast to slow return on investments of nutrients and dry mass in leaves, and operates largely independently of growth form, plant functional type or biome (Wright *et al.* 2004). Diameter at breast height, representing indissociable effects of ontogeny and access to light, was positively correlated with leaf thickness and leaf mass per area and negatively with leaf area, indicating an increasingly conservative strategy for the leaf economics spectrum for increasingly large trees in luminous environments (see also Hérault *et al.* 2011). Our study (Schmitt et al., in prep) also showed that higher topographic position of trees resulted in a shift from acquisitive to conservative, i.e. fast to slow, strategies, both across and within species. These results suggest that the conservation of functional plant strategies previously shown at the community and species scale (Díaz *et al.* 2016; Bruelheide *et al.* 2018) can be extended to the within-species level. Functional trait variability in response to the environment at the within-species level could increase the species' tolerance to environmental filtering and thus contribute to species coexistence (Clark 2010).
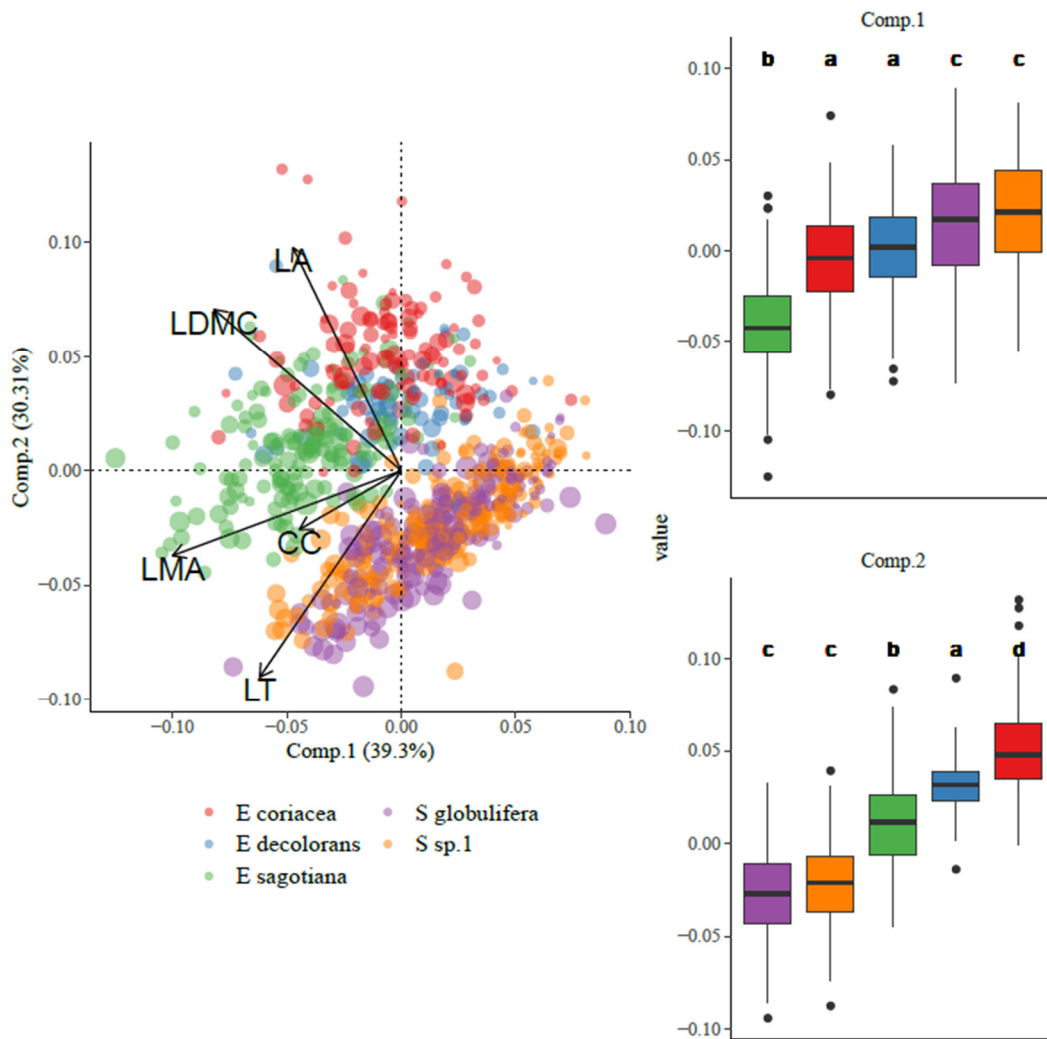
Figure 39 Principal Component Analysis (PCA) of leaf traits in Eschweilera and Symphonia species and segregation on the two first axes. Circle and box colours indicate the species, whereas size of circles indicates individual diameter at breast height. Species segregation has been investigated by Anova, **** indicates a p–value < 0.0001 and letters indicate post-hoc groups investigated by Tukey Honest Significant Differences. Abbreviations of traits: LA, leaf area; LMA, leaf mass per area; LDMC, leaf dry matter content; LT, leaf thickness; CC, chlorophyll content. From Schmitt et al. in prep.

In parallel, in the framework of Sylvain's PhD, we are conducting resequencing experiments in both the *Symphonia* and the *Eschweilera* (*Parvifolia* clade) complexes to explore the genotype-phenotype and genotype-environment associations. In *Symphonia*, where two putative species display differential habitat adaptation with *S. globulifera* occurring most frequently in seasonally flooded valley bottoms and *S. sp1* preferentially in terra firme forest (Figure 38), we are conducting a sequence capture experiment funded in part through the EFPA project LOCOCAP (Low Cost Capture). We designed a new bait set targeting a total of about 1.5 Mbp of sequence variation comprising anonymous sequences, functional sequences and functional candidate sequences differentially expressed between the two putative species (Tysklind *et al.*, in prep). In the *Eschweilera* (*Parvifolia* clade) complex, the Lecythidaceae-wide bait set (Vargas *et al.* 2019) is not well-suited to capture variation at shallow evolutionary depth, which is why we are currently designing a new bait set essentially using the *Eschweilera* (*Parvifolia* clade) transcriptomes generated (Vargas *et al.* 2019). The work will be funded by labex CEBA projects NEOTROPHYL and RARETREE, as well as the GUYAMAZON project LECYTOMICS. For the characterization of genotype-phenotype and genotype-environment associations, we will consider single-locus associations as well as polygenic adaptation signatures (Guan & Stephens 2011; Racimo *et al.* 2018), and we plan to investigate signatures of deleterious variation and negative selection (Henn *et al.* 2015; Zeng *et al.* 2018).

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Sylvain's work is expected to yield insights into the links between environment and phenotypes (available results), genotypes and environment, as well as genotypes and phenotypes. Once these links are better understood, Sylvain will explore how genotypes interact with the environment to explain functional trait variation, and ultimately, complex integrative fitness-related phenotypes such as growth. We hope to collect further evidence on the implications of these eco-evolutionary processes for species coexistence, specifically, to support the hypothesis that intraspecific (genetic and phenotypic) variation increases the species' tolerance to environmental filtering (Clark 2010), and will thus decisively contribute to species coexistence.

Funding

Mentoring

Supervision of PhD students

Sylvain Schmitt, co-supervision with Bruno Hérault, CIRAD. Univ. Bordeaux, 2017-ongoing. Ecological genomics of niche exploitation and individual performance in tropical forest trees.

5.3. Eco-evolutionary dynamics and implications of commonness vs. rarity in Amazonian tree congeners

The Lecythidaceae are an attractive system to study eco-evolutionary processes in Amazonian rainforests as shown in chapters 5.1. and 5.2. Many tree species of this family have wide distribution ranges in Amazonia (ter Steege *et al.* 2006, 2013; Mori *et al.* 2017). Extending their study beyond the local and regional contexts of French Guiana to other parts of the Amazon basin appears thus an attractive approach to uncover general mechanisms in their eco-evolutionary dynamics. We recently obtained funding to address questions on local adaptation, ecological genomics and evolutionary dynamics in Lecythidaceae communities in a three-region comparative scheme between French Guina, Central Amazonia and western Amazonia, through the projects LECYTOMICS (Guyamazon program for Franco-Brazilian cooperation, 2019-2020) and RARETREE (labex CEBA annual projet 2019-2020).

In the project **LECYTOMICS – *Species boundaries and ecological genomics in the face of global change in the Brazil nut family (Lecythidaceae)***, in a collaboration between UMR Biogeco, UMR Ecofog (French Guiana), Christopher Dick's team at the University of Michigan and Maristerra Lemes' team of INPA, Manaus (Brazil), we will use a replicated design between Paracou, French Guiana, and the Biological Dynamics of Forest Fragments Project (BDFFP), Manaus, Brazil. The objectives are the following:

1. To investigate genetic species boundaries, especially between closely related species, to evaluate the congruence of genetically and morphologically delimited species, and to characterize the evolutionary relationships between allopatric samples of the same species vs. those between

sympatric samples of different species. These analyses will allow us to shed light on the extent of ancestral shared polymorphism vs. recent hybridization. For example, *E. coriacea* (DC.) S.A.Mori, *E. pedicellata* (Rich.) S.A.Mori and *E. wachenheimii* (Benoist) Sandwith are relatively common in both French Guiana and Central Amazonia, allowing for such comparative analysis.

2. To characterize the local niches of these species based on microenvironmental variation, and to the extent possible, data on competition. Then, to explore the relationships between the identified functional strategies and genetic variation and thus, evolutionary potential.

3. To use molecular data to examine the historical population demography and colonization history of the human-harvested Brazil nut, *Bertholletia excelsa*, and establish how the genetic constitution of the species varies according to type and intensity of human impact.

We will use forest invenoty data and will generate sequence capture data in the *Eschweilera* (*Parvifolia* clade) species complex using the bait set under development in Sylvain Schmitt's thesis in a set of species from French Guiana and in a partially overlapping set of species from Manaus, Brazil. We will furthermore generate ddRAD-Seq data in the emblematic Brazil nut tree, *Bertholletia excelsa* Humb. & Bonpl. across multiple sampling sites ranging from eastern to western Amazonia. The results of the project, obtained in a robust multi-site design, are expected to improve our understanding of past and present gene pool dynamics in tropical rainforest tree species communities facing anthropogenic influence and global change. As such, the results can contribute to the design of strategies of rainforest conservation and sustainable management and use of tropical tree products.

In the project **RARETREE – *Eco-evolutionary implications of commonness vs. rarity in Amazonian tree congeners*** we will use a replicated design between French Guiana and Amazonian Ecuador to gain further insights into the mechanisms of species coexistence and the evolution of related common and rare tropical tree species (*Eschweilera*, *Parvifolia* clade) across Amazonian regions. To reach the objectives, we will develop and build on an emerging scientific collaboration between UMR Biogeco, UMR Ecofog, Christopher Dick's team at the University of Michigan and Pieter van 't Hof and Gonzalo Rivas-Torres' team at Universidad San Francisco de Quito. The following objectives will be addressed:

1. To build the ecological niches occupied by *Eschweilera* species at local scale based on individual competition indices derived from forest inventories, and microenvironmental variables.

2. Based on targeted sequencing, to estimate current and historical effective population sizes, *Ne*, to test the prediction that common species have large *Ne*, indicative of high adaptive potential, and to examine whether rare and common species are equally persistent through time. We developed this hypothesis based on a previously observed positive (albeit weak) relationship between genetic diversity (allelic richness at microsatellite markers) and species abundance of eight closely related *Eschweilera* species (*Parvifolia* clade, Lecythidaceae) in French Guiana (Heuertz *et al.* submitted).

3. To estimate scenarios of genetic introgression between common and rare species, to test the prediction of high introgression rate in common species, increasing their *Ne*, and of lower introgression rates and lower *Ne* in rare species, due to a trade-off between reproductive assurance vs. avoidance of genetic assimilation. We will also assess if rare species preferentially introgress functional genetic variants, increasing their adaptability.

Through both LECYTOMICS and RARETREE projects we expect to discover whether current ecological niche breath associates with long time evolutionary processes in tropical tree species complexes, i.e., if niche breath associates with historical effective population size. Extending the study of eco-evolutionary processes in tropical tree species complexes to several Amazonian regions offers opportunity for uncovering general mechanisms, providing insights that are useful in other tropical regions. In the framework of RARETREE, we will also organize a Lecythidaceae workshop at the 2nd Latin American Congress of Biogeography in August 2019 in Quito, Ecuador, to foster scientific exchange and future collaboration, in combination with a field sampling mission to UNESCO Yasuní Biosphere Reserve and Tiputini Biological Station.

Publications

Heuertz M, Caron H, Scotti-Saintagne C, Petronelli P, Engel J, Tysklind N, Miloudi S, Chave J, Molino JF, Sabatier D, Budde KB. The hyperdominant tropical tree Eschweilera coriacea (Parvifolia clade, Lecythidaceae) shows higher genetic heterogeneity than sympatric Eschweilera species in French Guiana. Plant Ecology and Evolution (submitted).

5.4. Eco-evolutionary resilience of forest species communities and provision of ecosystem services

My mid- to long-term priorities for research perspectives are 1. to pursue my research line on eco-evolutionary processes in tree species and tree species complexes presented in this chapter, integrating evolutionary approaches with ecological approaches as needed, and 2. to use the insights gained on these eco-evolutionary processes in integrative approaches to contribute to forest ecosystem conservation and the sustainable management of forest ecosystem resources for the continued provision of ecosystem services. Both aspects will require building on, and extending my network of collaborations.

5.4.1. Comparative patterns of diversification in major tree species clades of Amazonia

Given the important ecological roles of hyperabundant tropical tree species, or hyperdominants *sensu* ter Steege et al. (2013), and thus their pertinence for evolutionary biology and conservation, it will be a strategic choice to continue elucidating eco-evolutionary processes in tropical tree genera and families that harbour such hyperdominants. Investigation of local-scale adaptive evolution in hyperdominants (chapters 5.2 and 5.3) can be complemented by the use of biogeographic approaches that explicitly test diversification scenarios at deeper evolutionary time frames. While lowland Amazonia harbours tremendous species richness and has been recognized as the primary source of Neotropical biodiversity (Antonelli *et al.* 2018), we still lack a basic understanding of how Amazonian biodiversity was assembled over evolutionary timescales. Diversification of Amazonian lineages has been strongly linked to the Andean orogenetic process (Antonelli *et al.* 2009; Luebert & Weigend 2014), but diversification processes within Amazonian lowlands are less well characterized. With Dr. Jérôme Chave from EDB Toulouse, we have submitted a PhD project to labex CEBA to address diversification in the common Amazonian plant families Sapotaceae, Chrysobalanaceae, and Lecythidaceae, in a dated biogeographic context. The objectives are the following:

1. To explore the potential of predominantly lowland Amazonian plant lineages to diversify across the large expanse of Amazonia, and in peripheral lowland moist tropical forest areas linked to the Andean orogenetic context in Colombia (Magdalena Valley and Chocó region). The PhD candidate is expected to complement existing genome skimming data sets (e.g., Bardon *et al.* 2016) by adding more species and improving the geographical representation within species. The candidate will retrieve plastome sequences from the data and will explore extracting low-copy nuclear genomic information as well, since genome skimming data can prove a valuable data source for phylogenomic inference using the nuclear genome (Vargas *et al.* 2019). Dated phylogenetic trees will be produced and biogeographic scenarios tested.

2. To focus on selected species complexes and to assess the likelihood that they may contain cryptic species. Recently, it has been proposed that tropical tree species of Africa may contain as many as 50% more species, based on careful phylogeographic work on some 23 species (Olivier Hardy, pers. comm.). The vast expanse of territory covering Amazonia makes it even more likely that cryptic plant species exist there. This goal necessitates to focus on a more limited number of species, and to target species with large distribution ranges. One good candidate for conducting this study is *Licania heteromorpha* Benth. (Chrysobalanaceae).

5.4.2. A framework to assess eco-evolutionary resilience and adaptive capacity in forest ecosystems

In the interest of conservation and sustainable management of forest genetic resources, it is important to assess the bearing of eco-evolutionary processes in tree species or species complexes for the ecosystems in which these species occur. A bit more than a decade ago, the concepts of "*keystone species"*, i.e. a plant or animal that plays a unique and crucial role in the way an ecosystem functions, and of "*extended phenotype"* have been proposed to highlight the role of emblematic tree species for their forest ecosystems (Whitham *et al.* 2003, 2008). These concepts help to convey the importance of some forest trees for the functioning of ecosystems, but we are today in a greater need for a general framework on how eco-evolutionary processes in individual species can affect the conservation status of the ecosystems in which they thrive, under global climate change and under anthropogenic influence through management and land use change.

I am currently contributing to the development of a general framework for risk assessment in forest ecosystems under the influence of management and climate change hazards as part of the construction of the EU H2020 Research and Innovation Action (RIA) project *FORCES – Forest-based Solutions for Biodiversity, Climate Action and Ecosystem Services*, coordinated by Dr. François Lefèvre of INRA URFM, Avignon. The first-stage application has been submitted to the European Commission in February 2019. FORCES is a project that harnesses multi-disciplinary and transdisciplinary expertise and a wide range of actors to launch evidence-based local Innovation Actions, i.e., suites of territory-specific, cross-sectoral policies and initiatives, in forest ecosystems, and supports their long-term implementation and assessment. FORCES will launch diverse types of nature-based solutions (NBS), i.e., nature-based management actions aimed at fulfilling specific goals such as restoration, erosion control etc., across a set of selected Innovation Action Areas (IAA) supported by associated Research Sites and Observatories (RSO) in Europe and Latin America (CELAC countries), addressing cross-scale issues. FORCES is expected to contribute to strengthening European capacity to develop innovative solutions for and by its forests, to support forest-based attainment of sustainable development goals (SDGs) in other regions, and to promote NBS for landscape and ecosystem conservation, restoration and development. To reach this goal, FORCES has four objectives:

1. Exemplify the potential of NBS for achieving biodiversity, climate action and ecosystem services goals in a range of forest systems across Europe and Latin America.

2. Improve knowledge on the drivers of resilience and adaptive capacity of forest social-ecological systems in the context of climate change, as well as synergies and trade-offs across scales.

3. Provide innovative tools, methods and guidance to support NBS design, implementation and assessment.

4. Promote multi-actors' engagement in NBS for ecosystem conservation, restoration and management to support climate change adaptation and mitigation.

As a transdisciplinary Research and Innovation Action (RIA) project, FORCES consists of two action-oriented work packages (WP1, WP2), three research-oriented work packages (WP3, WP4, WP5), one work package for multi-targeted dissemination, communication and open science (WP6), and one work package for project coordination and management (WP7). I am serving as the leader of WP4 on *Eco-evolutionary resilience and adaptive capacity of forest ecosystems*. The central objective of WP4

is to develop a robust and generic risk assessment protocol for forest ecosystems to evaluate climate- and management related risks, building on the IUCN Red List of Ecosystems (RLE) protocol.

Ecosystem risk assessment should balance the need for specificity – to support consistent, quantitative evaluation of risk – with the need for generality – to support application of common theoretical concepts across a wide variety of ecosystems. A risk assessment protocol may be expected to identify ecosystems at risk of losing biodiversity, ecological functions and/or ecosystems services, three complexly inter-related and important objects for conservation. The IUCN Red List of Ecosystems (RLE) framework represents an ecosystem risk assessment protocol that has chosen the loss of biodiversity as a primary focus (Keith *et al.* 2013). Biodiversity, encompassing variation from within species to across landscapes, may be potentially less important for the short-term processes operating in ecosystems, but it is crucial for the longer-term maintenance of ecosystem functions and services under substantial predicted future environmental change, i.e., their 'resilience' (Oliver *et al.* 2015). In FORCES, we will develop a generic risk assessment protocol for forest ecosystems starting from the IUCN RLE framework, with preserving biodiversity as the highest hierarchical criterion.

An important conceptual difference exists between the IUCN RLE approach and the risk assessment framework we wish to develop: the RLE approach fundamentally aims to assess the consequences of loss of biodiversity, especially the risk of non-linear, sudden degradation, known as ecosystem collapse. WP4's protocol will be adapted to assessing the consequences of diverse management options, e.g., potential nature-based solutions (NBS) under future climates, especially those that aim to enhance biodiversity and/or ecosystem functions and services. We will thus develop a risk assessment framework aimed at quantifying the probability, direction and magnitude of change, positive or negative, to biodiversity and its associated functions and services.

To be able to quantify change in an ecosystem, it is necessary to first define the focal ecosystem, which can be done based on the IUCN RLE descriptors: i) the biotic complex or assemblage of species; ii) the associated abiotic environment or complex; iii) the interactions within and between those complexes; and iv) the physical space in which these operate. Second, it is necessary to identify indicators of ecosystem defining features based on biodiversity, composition or function that will be used to diagnose changes in biodiversity, and its consequences (e.g., on ecosystem services).

WP4's risk assessment protocol for forest ecosystems will go beyond the IUCN RLE protocol in two important ways: 1. It will examine **a representative set of indicators** of ecosystem defining features that integrate the diversity in characteristic biota through hierarchical levels of organization, including genetic and/or functional variability of characteristic species and/or communities, and investigate their effect on ecosystem processes and services (e.g., including carbon cycle) to gain insight on the effect of biodiversity on ecosystem functioning and eco-evolutionary resilience. 2. It will **integrate the specific risk factors** that lead to positive or negative symptoms on the ecosystem. We will specifically focus on the effects of management and climate-related hazards, as well as their interaction on the resilience of ecosystems. Conceptually, addressing specific types of hazards and their interaction adds an additional level of uncertainty to the risk assessment protocol.

5.4.3. Genomic biodiversity knowledge for resilient ecosystems

In a rapidly changing environment the resilience of ecosystems depends ultimately on species adaptability.

The COST action G-BIKE – *Genomic BIodiversity Knowledge for resilient Ecosystems* coordinated by Dr. Cristiano Vernesi of Fondazione Edmund Mach, San Michele all'Adige, Italy, that has recently been launched (March 2019) will enable standard and routine genetics or genomics tools for assessing, monitoring and managing the genetic resilience and related adaptive potential of wild and captive populations. Although genetic data can be obtained for most organisms, the standardization of protocols for detecting and monitoring species' genetic diversity, and their potential for adaptation, is still lacking. G-BIKE will assist scientists and practitioners across the EU and particularly in COST

Inclusiveness Target Countries to integrate genetic and evolutionary knowledge into conservation planning policies, and to promote cross-border management and long term monitoring programs of evolutionary potential in order to ensure persistence of populations and species, and ultimately the continued supply of nature-based ecosystem services. Considering the drastic impacts of climate change during the coming decades, G-BIKE is especially urgent. The following aims will be accomplished by involving a balanced representation of scientists and practitioners from a diversity of countries: 1) clearly articulating for managers how genetic diversity can support ecosystems; 2) developing and testing best practice protocols for monitoring genetic diversity in time and space; 3) providing an online forum on emerging tools; 4) connecting all stakeholders through networking and training opportunities; 4) building a network of conservation genetics labs; 5) building a foundation for long term impact. Results will be disseminated in easy-to-read summaries for practitioners and outreach to the public at Natura 2000 sites, botanic gardens and zoos as well as in scientific publications.

G-BIKE will address its aims through networking activities, specifically, training schools, workshops and short term scientific missions, in relation to five workig groups (WGs): WG1 – Implementing genetics into management; WG2 – Monitoring of genetic diversity; WG3 – Genomics and Ecosystem Services; WG4 – Biotechnological Assessment; WG5 – Knowledge sharing.

Within G-BIKE, I serve as the leader of WG3. Our objectives are 1. Providing the Practitioners community scientifically sound evidence of the importance of genomic variation for adaptation. 2. Identification of major ecosystems to be analysed with genomic tools on a continental scale. We are currently still in the process of defining the exact thematic presentation of WG3. The work will likely start with a compilation of scientific evidence of the importance of genomic variation for resilience and adaptation. Specifically, it will be useful to demonstrate links between the loss of genetic diversity and the associated loss in ecosystem functions and services. Studies and approaches that establish a direct link between biodiversity and ecosystem services will be especially attractive, for instance, metatranscriptomics studies can simultaneously quantify biodiversity and biodiversity-derived function, e.g., in soils or marine environments. It will also be helpful to categorize ecosystem services into the four categories defined in the 2005 Millennium Ecosystem Assessment (MA), Supporting services, Provisioning Services, Regulating services and Cultural services.

CONGENAfrica – Conservation genomics of harvested tropical rainforest timber trees in Africa is a postdoctoral project proposal, submitted with Franck Monthe (PhD, Université Libre de Bruxelles 2019) that proposes a concrete application of genomic approaches to inform the conservation and management of emblematic harvested rainforest trees in African tropical rainforests (TRFs). CONGENAfrica proposes to infer the phylogeographic history, population dynamics and role of tree species' life history traits (LHTs) in shaping the genetic structure of ten African tree species widely distributed across the Guineo-Congolian tropical rainforest (five Meliaceae species, one Clusiaceae and four Fabaceae species). Some of these species are well known for their timber, best exemplified by *Entandrophragma cylindricum* (trade name sapelli, Meliaceae) and *Afzelia bipindensis* (trade name doussié, Fabaceae), and are threatened from intense exploitation. In rainforest species of the genus *Entandrophragma*, standard genetic markers (microsatellites) revealed weak genetic differentiation within species (Monthe Kameni 2019), probably as a consequence of extensive gene flow, but the power of these molecular markers was low. Genomic data may thus be useful to identify undetected genetic structure and to improve the characterization of spatial genetic variation. In objective O1, we will generate genomic genotyping-by-sequencing (GbS) markers in five widely distributed species of the genus *Entandrophragma* across their distribution ranges to resolve large-scale genetic structure and to date divergence events. We expect improved resolution of clusters for GbS vs. microsatellites, as observed in other TRF trees (Piñeiro et al., unpublished; Gawra 2018). In objective O2, we will consider evolutionary processes within genetic clusters. Specifically, we will investigate the tempo of diversification and the history of past population size changes (bottlenecks, expansions) in Entandrophragma species, and additionally in four Fabaceae and one Clusiaceae species based on data

available through collaborations (Piñeiro et al., unpublished; Gawra 2018). This will allow us to assess the role of species' LHTs on their evolutionary history. In objective O3, the GbS data of O1 and O2 will be used to examine genomic signatures of adaptive evolution and to identify putative drivers for adaptive evolution in each genetic cluster, an approach which has not yet been applied for timber tree species in Africa. We will produce geographic maps of genetic indices reflecting evolutionary potential as well as genetic load for ten TRF tree species, which will represent invaluable genetic and genomic information for the design of conservation and management strategies.

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