

## Comparison of Genetic Diversity of the Weed *Rubus alceifolius* in its Introduced and Native Areas

L. AMSELLEM<sup>1</sup>, T. LE BOURGEOIS<sup>1</sup>, J. L. NOYER<sup>1</sup>, and M. HOSSAERT-McKEY<sup>2</sup>

<sup>1</sup>CIRAD, BP 5035, F34032 Montpellier Cedex 1, France

<sup>2</sup>CEFE/CNRS, BP 5051, F34033 Montpellier, France

### Abstract

*Rubus alceifolius* Poir. (Rosaceae) was introduced into La Reunion island in the mid 19<sup>th</sup> century. Probable origins and precise time of its introduction are not well known. Some sources suppose that this bramble was introduced from Southeast Asia, others suggest that it may have first been introduced into Madagascar. This bramble has become a serious weed in native vegetation. The development of a biological control program is now being planned.

Prior to develop this biological control, we must first characterise the genetic diversity of the plant in every area of introduction and in native range. To estimate genetic diversity, 254 specimens of *Rubus* spp. were sampled within the area of introduction (La Reunion, Mayotte, Mauritius, Madagascar, Australia), and within its native range (Thailand, Laos, Vietnam, Sumatra and Java).

The technique we used is Amplified Fragment Length Polymorphism.

A matrix of distance between individuals was calculated according to the Simple Matching distance, and a tree was constructed according to the Neighbour-Joining Method.

In the tree, two well-separated groups are observed for *Rubus alceifolius*. In the first one, all provenances from within the native range are grouped. This group is characterised by a relatively high genetic diversity among individuals of the same locality. The second group includes all provenances from within the area of introduction. In this group, intra-locality variation is reduced, except for Madagascar, where polymorphism is comparable to that in populations from the native range.

The considerable separation between populations in the area of introduction and these from the native range, the intermediate position of individuals from Madagascar, and the lack of variability within each locality in the area of introduction, yield on the origins of introduced *Rubus alceifolius*. The reasons for insights a lack of polymorphism within each locality in the area of introduction are now being investigated.

**Keywords:** *Rubus alceifolius*, weed, genetic diversity, biological control, AFLP, La Reunion Island

### Introduction

*Rubus alceifolius*, Poir. (Rosaceae, subgenus *Malachobatus*, Focke) is a simple leaved Southeast Asian bramble. It was introduced into La Reunion island at the mid 19<sup>th</sup> century, but probable origins are not well known. It was also introduced in Madagascar, Mayotte, Mauritius and Australia (Queensland). This bramble was already signalled as a serious weed in La Reunion, in the early 20<sup>th</sup> century (*in* Lavergne, 1978), invading the

native vegetation and road edges on the island. Control is now necessary, to limit and if possible reverse the advance of this highly invasive species. The development of a biological control agent is now being planned with the support of the local authorities.

Prior to develop a specific biological control of this weed, we must first characterise the genetic diversity of the plant in the Reunion island, and compare it with its diversity in others places of introduction and in its native range. This could permit to focus a research of biological agents specialised on a particular strain of host-plant (Hasan, 1972), based on the comparison of the different genetic profiles obtained with a genetic approach, and thus to target the organisms the most highly specialised and efficient against this weed.

To estimate genetic diversity, we used the Amplified Fragment Length Polymorphism for specimens of *Rubus* spp sampled in the area of introduction and in its native range.

## Materials and Methods

### Plant material:

Two distinct areas were considered for the collection of the studied samples. The first is the native area where *Rubus alceifolius* exists in natural populations. The various sampled localities (Thailand, Vietnam, Laos, Java, Sumatra) represent all potential and possible origins of *R. alceifolius* in the Mascareignes and more precisely in La Reunion island, according to the various bibliographical sources (Cordemoy, 1892, Rivals, 1960; Friedmann, 1997, Jolivet, 1984, and Figier and Soulères, 1991). Another reference suggests that the plant may have first been introduced into Madagascar from Indonesia, and then introduced into La Reunion from Madagascan material (Owadally, 1960). The second area considered is the one of introduction (La Reunion, Mayotte, Madagascar, Mauritius, Australia). In order to test the capacity of discrimination of the tool we used, we have also joined to this study other species of *Rubus*, that are more or less closely related to the species studied (species with simple and compound leaves). 254 specimens of *Rubus* spp. (of which 224 *R. alceifolius*) have been sampled.

The table 1 shows the sampling distribution used for our analysis of genetic diversity.

**Table 1.**  
**Sampling distribution of *Rubus alceifolius* and *Rubus* spp.**

Area	Reunion	Mavotte	Mauritus	Madagascar	Australia	Thailand	Vietnam	Laos	Java	Sumatra	Total
<i>Eff. Rubus alceifolius</i>	75	8	7	19	7	59	30	1	4	14	224
Eff. others <i>Rubus</i>	3	-	-	-	-	12	10	2	-	3	30
Total	78	8	7	19	7	71	40	3	4	17	254

A particular effort of sampling was made in La Reunion, in order to increase the resolution for this locality, and thus to minimise the risks of not observing a very localised polymorphism. Sampling took place in main biotopes of the island (Highlands and Lowlands) and on greatest possible populations where *Rubus alceifolius* was present. An effort was also accomplished in sampling intra and inter-populations individuals in Thailand. Final sampling included in this analysis was obtained after 2 independent readings of AFLP profiles. We retained only the individuals with no problem of migration on the gels, and having sure profiles in term of signal intensity.

According to the world distribution of *Rubus alceifolius* (Kalkmann, 1993; Friedmann, 1997), we can assume that our sample cover the main world genotypical variability of this bramble. However it misses some samples from India where *R. alceifolius* would be present in the Northeast part of the country.

### **Molecular method:**

The extraction of total DNA was proceeded according to the protocol of Bousquet *et al.* (1990), from about 100 mg of foliar tissues dried with Silicagel.

The technique used is the AFLP ("Amplified Fragments Length Polymorphism") (Zabeau and Vos, 1993). It is usually used to highlight molecular polymorphism applied to studies of genetic variability (Sharma *et al.*, 1996; Roa *et al.*, 1997, Escaravage *et al.*, 1998; Russel *et al.*, 1999).

AFLP technique consists in cutting out the genome of the individuals by a couple of enzymes, to mark radioactively the fragments thus obtained with g33P, and to make them migrate on a gel where circulates an electric field. The result is displayed on a photographic film put in contact a few days with this gel and impressed by radioactive radiation. Four couples of starters were tried. All of them were revealing polymorphism and thus were used for this study.

For each analysis, we used the 3 same Reunionese individuals as references. The purpose of this reference was not only to check the robustness and the reproducibility of the method, but also to gauge each gel on levels of known bands, in order to avoid any shifting or confusion from one gel to an other for a given couple of starter.

Among 554 band levels that could be observed, 491 of them was retained for the all study. The data were coded in a binary way (1 = presence; 0 = absence) on each band level and for each considered individual. From this matrix individuals x band levels, we calculated the index of similarity of Sokhal and Michener (or "Simple Matching") between all the individuals using the software "NTsys-PC" (Rohlf, version 1.80). This index then allowed us to draw a tree according to the algorithm of "Neighbor-Joining", using the software "PHYLIP" (Felsenstein, 1993).

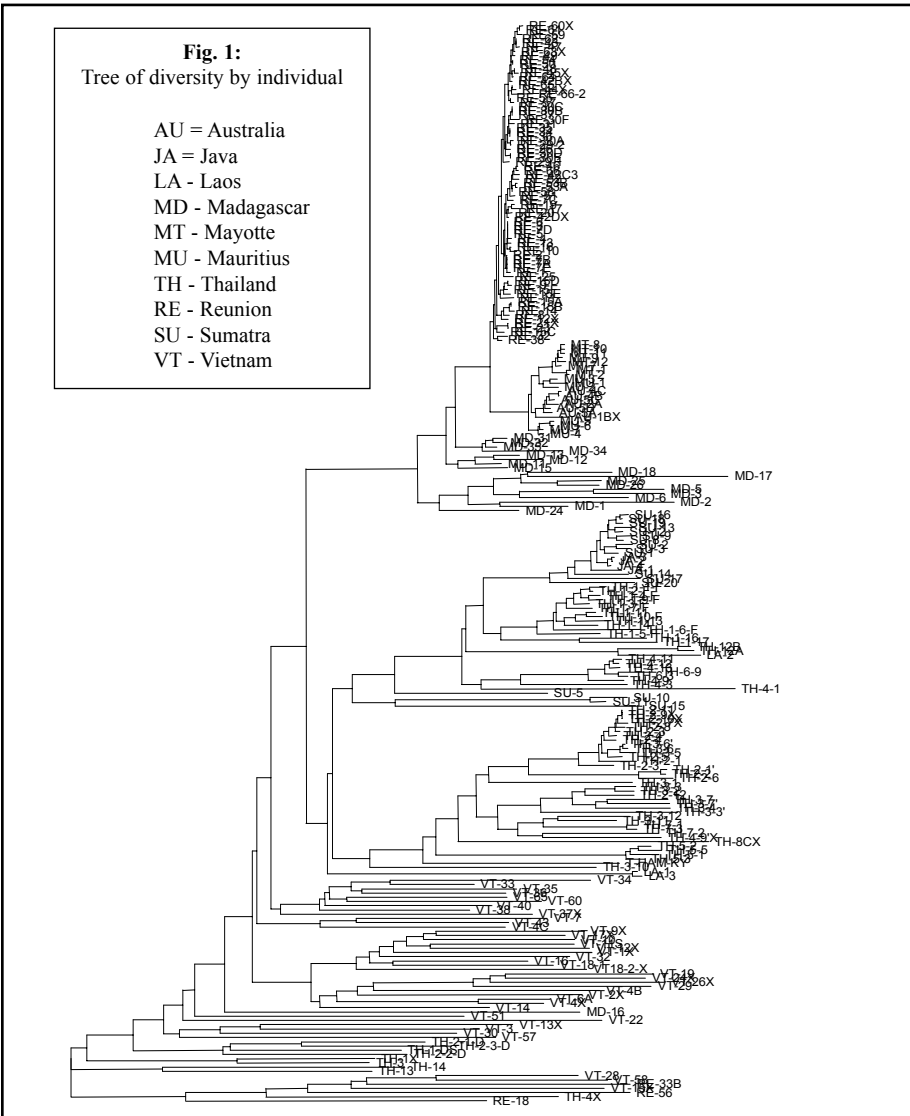
The other *Rubus* spp. used in the construction of the tree by individuals were omitted thereafter. Indeed, their consideration in this study was restricted to confront the reliability of the method. Once the reliability confirmed, only *Rubus alceifolius* was kept for the other analyses, in order to avoid a background noise resulting in lost of precision and resolution of results.

Finally, a Factorial Correspondence Analysis (FCA) was performed on the matrix individuals x band levels (SAS, 1996), in order to detect the major variables (or Dimensions) describing the specific associations among all the *R. alceifolius* sampled. Thus, it was possible to represent in the form of scatter plots all the individuals, since each dimension is represented in the form of axis.

**Results**

**Tree of diversity by individual:**

The tree is represented in figure 1. The *Rubus* spp. with compound leaves collected in La Reunion, in Thailand and in Vietnam are gathered together (*R. rosifolius*, *R. leucanthus*, *R. apetalus*, *R. ellipticus*, and *R. fraxinifolius*, representing 7 individuals). We considered this group as the outgroup, and then used it to root the tree. Then, are connected the *Rubus* spp. with simple leaves, (*R. rufus*, *R. blepharoneurus*, *R. parkeri*, *R. molucanus*, *R. clemens*, *R. polyadenus*). Finally are connected *Rubus alceifolius* from the various considered localities.



Two major groups of *R. alceifolius* differ from each other in this tree. The first group contains all the localities of the native area, and the second major group represents the localities of the area of introduction, which differentiate radically *Rubus alceifolius* of this group with the *Rubus* from Asia. Moreover, each locality of this area is well differentiated from the others.

However, we can notice that one Madagascan individual is placed in the native area, inside the Vietnamese locality.

*Rubus alceifolius* of Queensland is placed in the middle of those of Mauritius and Mayotte.

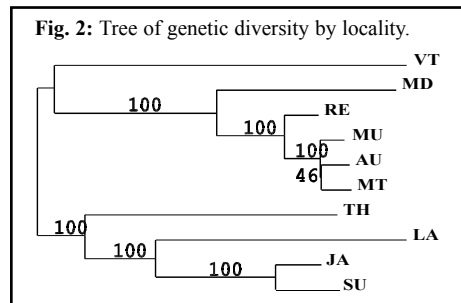
### Intra-locality variability:

The variability among individuals within the area of introduction is very low, except for Madagascar. These very low variability, make us able to conclude that in each locality of the introduced area the individuals represent a clone. For Madagascar, we can notice a great diversity in this locality. Concerning the native area, there is a great heterogeneity among the individuals within a locality. The highest variability within locality is observed in Vietnam. In Thailand, genetic diversity seems a little lower. The strong resemblance among the Javanese individuals between themselves is certainly due to the fact that only 4 individuals were used in this locality, and that nothing is known about their spatial distribution. In Sumatra, there is low apparent variability among the individuals.

### Tree by locality:

The tree obtained, when individuals are grouped by locality, is represented in figure 2.

The area of introduction and the native areas are very well separated, except however of Vietnam, which is situated as a brother-group of the area of introduction, but with a large distance among them.



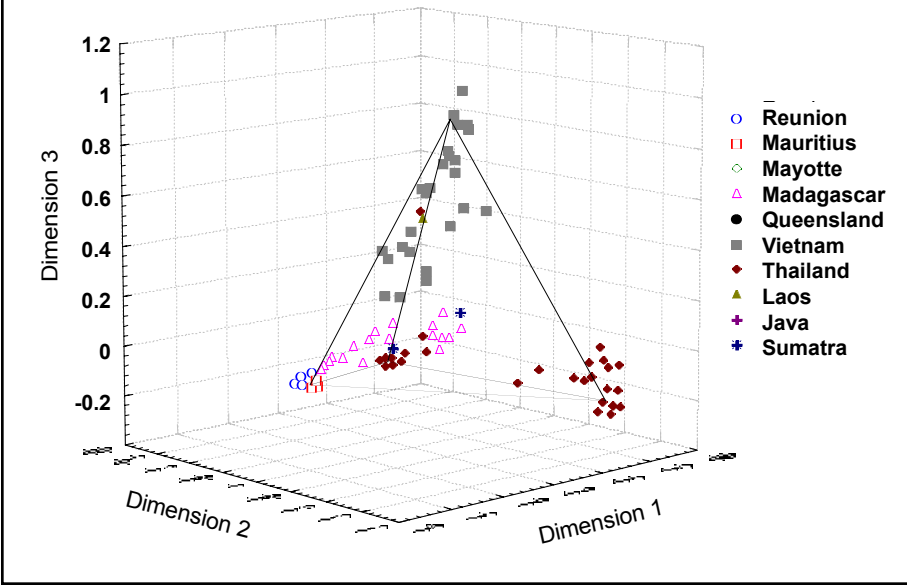
### Factorial Correspondence Analysis:

The first 3 dimensions of the FCA. explain alone 41,44% of the total variance of the collected observations, the third one participating to less than 9% of the variance.

The representation in a 3 dimensional visualisation for the whole of the individuals is given in figure 3. The spatial visualisation of this scatter plot is facilitated by including all the individuals in a volume of a pyramid type. The triangular base of this pyramid, is represented by the area of introduction (except Madagascar), Thai populations 2 and 3, and the native area (except Vietnam). The “left profile” of the pyramid shows the highest Vietnamese individuals at the top, and the Madagascan individuals at the bottom according to one of the height of the base.

Representation using FCA give the same results as the tree obtained before: the area of introduction is compact and unequivocally separated from the native area. The Madagascan individuals make the transition between these two areas with the Vietnamese one. The native area itself is divided into two groups with one consisting of Thai populations 2 and 3, and an the other one consisting of the Thai populations 1, 4, 5, 6, Java and Sumatra.

**Fig. 3:** Factorial Correspondence Analysis on the *Rubus alceifolius* individuals (n=224), according to the Dimensions 1, 2, and 3.



### Discussion:

In the tree by individuals, the separation between the *Rubus* species with compound leaves and those with simple leaves coincide with their systematic position. The AFLP method was also able to differentiate the various studied localities, even the populations within a same locality (Thailand). Moreover, *R. moluccanus* (VT-3 and VT-30, placed in the group of *Rubus* with simple leaves) and *R. alceifolius*, regarded as its sister-species, are well separated. These results show that there is enough differences in the genetic profiles of these 2 species, and that the molecular tool we used here is able to detect such differences.

A so strong genetical difference between the Southeast Asian *Rubus alceifolius* and the several localities where it has been introduced, can be due to the fact that the taxon is not the same in the two considered areas. Indeed, we can assume that *R. alceifolius* in the area of introduction is in fact a subspecies or a botanical variety morphologically identical to the Asian *Rubus alceifolius*, and thus indistinguishable but genetically different. If true, this could explain why it is impossible to link together the individuals present in the two areas, but would locate them on the tree as being the closest taxa in this study. This point could explain why it is not possible to determinate an Asian source of the bramble in the area of introduction.

Each locality within the area of introduction can be considered to have a single “clone” on its whole territory, each time a little bit different. This data is essential and hopeful to initiate a biological control of *Rubus alceifolius* in La Reunion island. If an agent is found to be enough efficient and specialised to this weed, there good reasons to think that an efficient control of this bramble is possible in this island. However, if the agent is too specialised and specific, it is possible to facilitate the appearance of a new

genotype which the agent doesn't recognise. Then the vacant niches would be occupied by this new genotype of *R. alceifolius*, out of control of the agent.

The great proximity of genetic profiles of each locality within the area of introduction, contrasts seriously with the strong diversity observed in Madagascar. A possible assumption is that this bramble was first introduced in Madagascar, and genetically differentiated there. Our results show some genetic proximity among individuals from Madagascar and from Vietnam. Even if the molecular tool used doesn't allow us to conclude about the geographical origin of the Madagascan *Rubus*, it seems to indicate that the Vietnamese *Rubus* is the one having the most genetic proximity with the Madagascan one.

Then, from this zone of secondary diversity, we can pretend that *R. alceifolius* would have disseminated from Madagascar towards the neighbourhood islands (Mauritius, La Reunion, and Mayotte), and then from Mauritius to Queensland.

## Conclusions

This work has shown that Reunion population of *Rubus alceifolius* seems to be genetically homogeneous, which is in favour of a biological control.

This work had to clarify the conditions of the introduction of *Rubus alceifolius* in the Mascareignes, because of the vagueness about the geographical origins and time of its arrival in this area. Even if this goal was not reached in this study, it seems however that possible scenarios of the course of this bramble from its native range in Southeast Asia to the Mascareignes and particularly in La Reunion are clarified in the light of our results. Indeed, Madagascar and Vietnam seems to do the junction between the two areas considered here. This could be a clue for the research of potential strains of specific agents to initiate a biological control of this weed in La Reunion.

But before to concentrate on the agent to control this bramble, we have now to deepen our knowledge of the life history and the biology of *Rubus alceifolius*. In the first time, we have to test the different hypothesis done in this study, to the light of the results collected. The data analysed here raise new questions as for the nature of the Asian *Rubus alceifolius* and those introduced. We can also wonder why a species not presenting any sign of major predominance in its native area can become so aggressive towards the other species of the vegetable communities in certain localities where it was introduced. A comparative study on the biology of the reproduction, with a co-dominant molecular tool (microsatellites) will be done from germinated seeds from introduced and native populations of this bramble, to test the relative ratios of allofecondation, autofecondation, and apomixis in the two areas, and to detect an eventual switching towards a higher potential of asexual reproduction in the area of introduction. In the same way, a count of the chromosomal chart of *R. alceifolius* in Asia and in the Mascareignes will be undertaken to test the hypothesis of a change in the ploidy level between these two geographic areas, and then susceptible to explain the invasive characters of *Rubus alceifolius* in the area of introduction. Nybom (1986) investigated 11 species of *Rubus* from the subgenus *Malachobatus* (*R. alceifolius* was not represented). All of them are polyploid (from tetra to octoploid). Also, knowing that, in the genus *Rubus*, hybridisation between close species is very frequent and give viable and fecund offspring (Richards, 1986, Nybom, 1986, Nybom, 1988), it is possible to believe that introduced *Rubus alceifolius* can be an hybrid of Asian *Rubus alceifolius* with a closely related species, and then changed in its ploidy level and genetic pool. The next experiments will be about these characters.

---

**References**

- Bousquet, J., L. Simon, and M. Lalonde. 1990.** DNA amplification from vegetative and sexual tissues of trees using Polymerase Chain Reaction. *Can. J. Forest. Res.*, 20: 254-257.
- Cordemoy, E.J. 1896.** Flore de l'île de La Réunion. (Klincksieck, Paris).
- Escaravage, N., S. Questiau, A. Pornon, B. Doche, and P. Taberlet. 1998.** Clonal diversity in a *Rhododendron ferrugineum* L (Ericaceae) population inferred from AFLP markers. *Mol. Ecol.*, 7, 975-982.
- Felsenstein, J. 1993.** PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Figier, J. et Soulerès, O. 1991.** Le problèmes des plantes exotiques envahissantes. Bois et Forêts des Tropiques, n°229, 3ème trimestre 1991, CIRAD ed. : 31-34.
- Friedmann, F. 1997.** 81. *Rosaceae*. In : Bosser, J., Cadet, T., Marais, W, Guého, J. (1997). Flore des Mascareignes. La Réunion, Maurice, Rodrigues. ORSTOM ed. 1-11.
- Hasan, S. 1972.** Specificity and host specialisation of *Puccinia chondrillina*. *Annals of Applied Biology*, 72, 257-263.
- Jolivet, P. 1984.** *Phaedon fulvescens* Weise (Col. Chrysomelidae chrysomelinae), un auxiliaire possible dans le contrôle des *Rubus* aux tropiques. Bulletin de la société linnéenne de Lyon, 7 Septembre, 235-245.
- Kalkmann, C. 1993.** "*Rosaceae* in Flora Malesiana, Vol. 11, 227-351, (A Foundation Flora Malesiana éditeur).
- Lavergne, R. 1978.** Les pestes végétales dans l'île de La Réunion. *Info-nature*, 16, 9-59.
- Nybom, H. 1986.** Chromosome number and reproduction in *Rubus* subgen. *Malachobatus*. *Pl. Syst. Evol.*, 152, 211-218.
- Nybom, H. 1988.** Apomixis versus sexuality in blackberries (*Rubus* subgen. *Rubus*, Rosaceae). *Pl. Syst. Evol.*, 160, 207-218.
- Owadally, M. 1980.** Agriculture Sucrière, Ile Maurice, 59: 76-94.
- Richards, A.J. 1986.** Plant Breeding Systems. Unwin Hyman, London.
- Rivals, P. 1960.** Les espèces fruitières introduites à La Réunion (Notes historiques et biologiques). Travaux du laboratoire forestier de Toulouse. Vol. 1, Art. 3, 96 pages.
- Roa, A.C., M.M. Maya, M.C. Duque, J. Tohme, A.C. Allem, and M.C. Bonierbale. 1997.** AFLP analysis of relationships among cassava and other *Manihot* species. *Theor. Appl. Genet.*, 95, 741-750.
- Russell, J.R., J.C. Weber, A. Booth, W. Powell, C. Sotelo-Montes, and I.K. Dawson. 1999.** Genetic variation of *Calycophyllum spruceanum* in the Peruvian Amazon Basin, revealed by amplified fragment length polymorphism (AFLP) analysis. *Mol. Ecol.*, 8, 199-204
- Sharma, K., M.R. Knox, and T.H.N. Ellis. 1996.** AFLP analysis of the diversity and phylogeny of *Lens* and comparison with RAPD analysis. *Theor. Appl. Genet.*, 93, 751-758.
- Zabeau, M., and P. Vos. 1993.** Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application, publication no : EP 0534858.