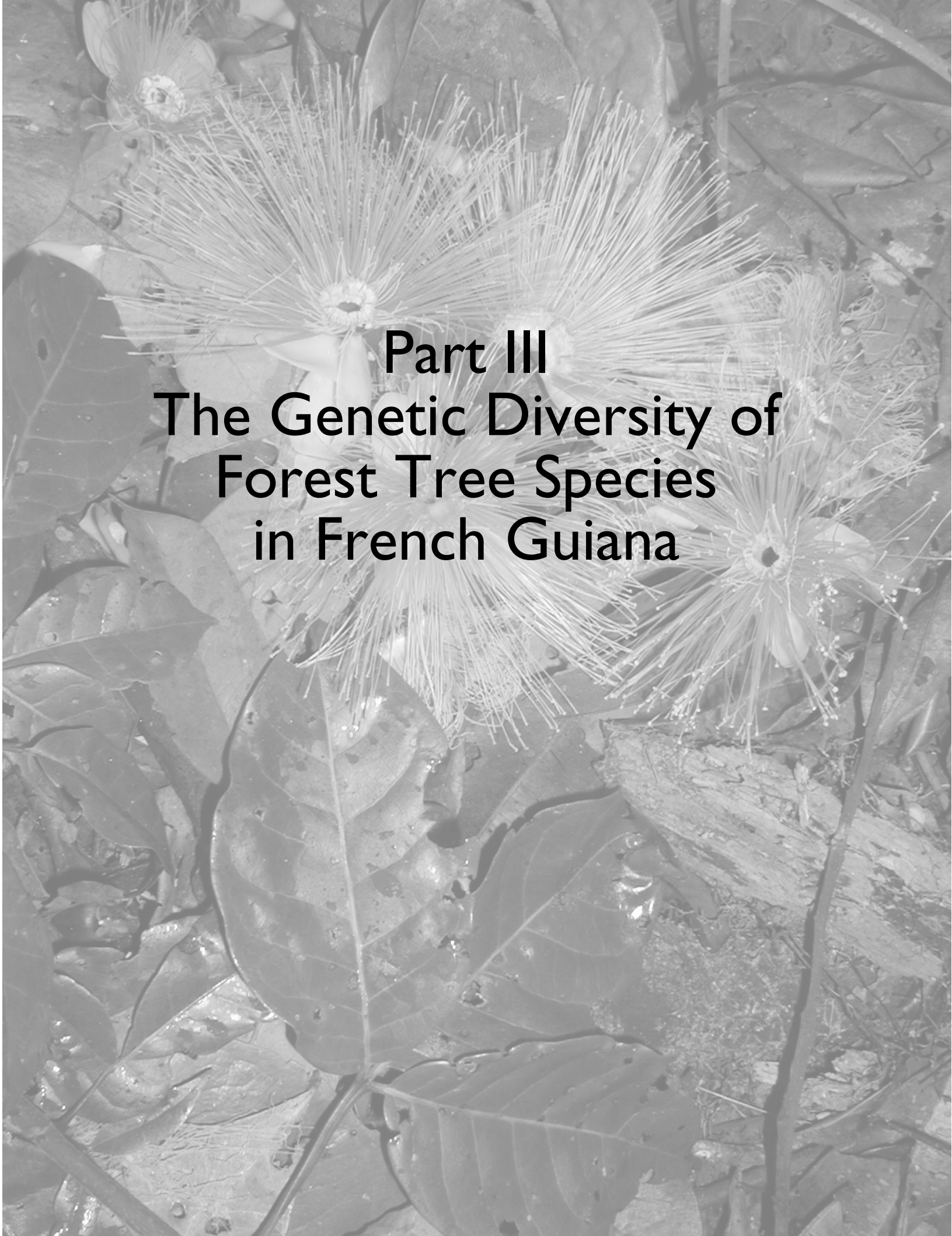

Part III
The Genetic Diversity of Forest Tree Species in French Guiana

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The genetic diversity of forest tree species in French Guiana

Antoine Kremer

In the early 1990s, INRA initiated a research project to describe and study the evolution of genetic diversity in the Guianan tropical forest. CIRAD joined the project shortly after it began. The basic objective of this project is to establish a general knowledge of the level and distribution of genetic diversity in tropical forests and its future dynamics. It is then planned to use this basic information to predict the future evolution of diversity as a response to various anthropic or non anthropic factors. The knowledge of the diversity within a species in terms of its different components (populations, trees..) is a prerequisite to the development of conservation strategies. In temperate zone forests, trees show reduced variation in life history traits, whereas there is an important variation in dispersion mechanisms and ecophysiological traits in the tropics. The INRA-CIRAD project has identified a set of 15 species exhibiting contrasting life history traits (pollen and seed dispersion, flowering phenology, succession, spatial distribution, etc.). For each of these species, the level and the spatial distribution of diversity within the Paracou stand are being described. Furthermore, genetic processes such as the mating system, the phenology of flowering, and gene flow are being monitored. Finally, we also intend to obtain insight into the evolutionary history of the species. The ultimate goal is to identify significant determinants of genetic diversity in tropical ecosystems that could be used in the future for a model of the evolution of diversity to serve as a basis for practical or more fundamental applications. The project is subdivided into three major steps.

Description and inventory of genetic diversity

The level and spatial distribution of genetic diversity are estimated for nuclear and cytoplasmic genetic markers for the 15 studied species within the Paracou stand. Methods are being developed that allow comparisons across species.

Dynamics and evolution of diversity

Basic genetic processes such as mating system, pollen and seed flow are being monitored in the Paracou stand by parentage analysis. These data are completed by further indirect measurements inferred from the spatial distribution of nuclear and cytoplasmic markers. Observations of floral and pollination biology and phenology

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are made in addition to genetic analysis. Investigations are also underway that will allow to retrace the recent migration routes and colonisation dynamics of the different species.

Modelling of genetic diversity

The information gained during the two first steps will be introduced into a model predicting the evolution of genetic diversity under different scenarios. The model will also take into account the particular features of tropical trees: multispecies (competition, status in succession) overlapping generations, metapopulation (extinction-colonisation).

We report here on the data and results for 10 of the 15 sampled species concerning the first two steps of the project.

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Chapter 1

Reproductive phenology and mating system of six tree species in Paracou stands

Henri Caron, Cyril Dutech, Eric Bandou

Abstract – The reproductive phenology of six tree species, *Dicorynia guianensis*, *Vouacapoua americana*, *Eperua grandiflora*, *Carapa procera, Virola michelii* and *Chrysophyllum sanguinolentum,* has been monitored in the Paracou stands over several years. A large variation of flowering rates exists among the species and from year to year. The tallest trees bloom and fruit more frequently than the smallest.

The allozyme analysis of parent trees and progenies showed that the studied species are largely outcrossed. However, the individual outcrossing rate is variable among both trees and years in mixed mating species.

Although the reproductive success of a logged population can be affected by the loss of effective reproductive trees, changes in flowering pattern, inbreeding and a decrease in seedling viability, impacts of silvicultural treatments were found only in *C. procera*.

This information can be of great interest for the forest manager who must maintain a minimal number of reproductive trees to ensure the regeneration of logged stands.

Keywords: Outcrossing rate, Flowering rate, Logging, Tropical forest tree, French Guiana

1. Introduction

Among the different biological traits of species, the mating system is of major importance in the determination of within-population genetic variations, especially in long lived woody species. As shown in the survey of genetic diversity of forest trees by Hamrick et al. (1992), selfing species exhibit significantly lower genetic diversity than do outcrossing species. In tropical rain forests, many tree species occur in low density and are unevenly distributed (Hubbel and Foster, 1983, Condit et al., 2000). These demographic traits can contribute to increasing self-fertilization. However, most tropical tree species possess mechanisms which encourage outcrossing (dioecy, monoecy, self-incompatibility) (Bawa and Krugman, 1986; Bawa, 1992). Of the 37 tropical tree species in which the mating system has been studied, about 50% were strictly allogamous and only 12% had an outcrossing rate lower than 0.80 (Murawski, 1995; Nason and Hamrick, 1997 for a review).

The outcrossing rate of mixed mating tropical trees is highly variable spatially and temporally at both the population and the individual tree level (Hamrick and Murawski, 1990; Rocha and Lobo, 1998). Indeed, it has been shown to vary according to the density of

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flowering trees (*Cavanillesia platanifolia,* Murawski et al., 1991, 1992; *Shorea siamensi*s, Ghazoul et al., 1998), the pollinator foraging behaviour (*Tabebuia heterophylla,* Torregrossa et al., 1996; *Neobalanocarpus heimii,* Konuma et al., 2000), the impacts of human activities such as logging (*Shorea megistophylla,* Murawski et al., 1994; *Carapa procera,* Doligez and Joly, 1997) or landscape fragmentation (*Pinus merkusii,* Changtragoon and Finkerley, 1995; *Symphonia globulifera,* Aldrich et al., 1998; *Pterocarpus macrocarpus,* Liengsiri et al., 1998). Furthermore, individual fertilities are generally unevenly distributed within tree populations: between male trees for contribution to pollination (*Gleditsia triacanthos*, Schnabel and Hamrick, 1995; *Pithecellobium elegans,* Chase et al., 1996; *Cecropia obtusifolia,* Kaufman et al., 1998), as well as between female trees in reproductive success, as illustrated by many experimental results (*Gleditsia triacanthos,* Schnabel et al., 1998; *Symphonia globulifera,* Aldrich et al., 1998). In addition, the number and the spatial distribution of flowering trees, the intensity of flowering, the degree of synchrony in the flowering periods are factors which can modify the effective pollen flow within populations. Finally, the fruiting success depends on the viability and the compatibility of the received pollen, on the nutrient and water uptake of the trees and on the intensity of predation during the maturation period. All these parameters are highly variable at both individual and population levels (Sabatier, 1983; Sampson et al., 1990; Newstrom et al., 1991; Ter Steege and Persaud, 1991; House, 1992 and 1993; Loubry, 1993 and 1994; Ghazoul et al., 1998).

In French Guiana, data about the mating systems of forest trees are scarce (Doligez and Joly, 1997, Caron et al., 1998). This lack of information is a serious gap in understanding the biological causes which shape the dynamics of genetic diversity. In addition, sufficient knowledge of the reproductive behaviour of species is essential to develop any plan of sustainable management. Since 1993, we have been studying the mating system of six tree species in the Paracou natural rainforest stands. These species belong to a panel chosen to represent a large range of life history traits which are known to influence the genetic diversity and the mating system of trees (Hamrick et al., 1992). In parallel to this study of the mating system, phenological observations were carried out to measure annual flowering variation, intensity of blooming among individuals, and synchrony of flowering periods within the populations. We tried to understand how these factors may influence the reproduction and especially the outcrossing rates in the different species. In some cases, we evaluated the impact of logging on phenology and its consequences on the mating system.

2. Material and methods

2.1. Species studied

The six species are canopy or subcanopy trees (Table 1). The spatial distribution of *Dicorynia guianensis* (Caesalpiniaceae), *Vouacapoua americana* (Caesalpiniaceae) and *Eperua grandiflora* (Caesalpiniaceae) is clumped. The other three species, *Carapa procera* (Meliaceae), *Virola michelii* (Myristicaceae) and *Chrysophyllum sanguinolentum* (Sapotaceae), tend to be randomly or regularly distributed, although *C. procera* can be aggregated in lowlands (Doligez and Joly, 1997; Forget et al., 1999; and see the results in Part IV, Chapter 1, Table 1). The local density of trees with a trunk diameter at breast height (DBH) larger than 10 cm varied between 2.4 individuals per ha (*V. michelii*) to 8.4 individuals per ha (*V. americana*) on the Paracou plots. According to the floral syndrome, the species are likely to be pollinated by different kinds of insects. *C. sanguinolentum* could be additionally pollinated by bats. *V. michelii* is dioecious and the other five species have a bisexual breeding system.

2.2. Sampling

To determine the phenological status of trees, random samples of 50 to 500 individuals of each species were examined twice or three times during flowering periods for several consecutive years over the entire plots of Paracou (see Part I for a general presentation of the plots).

To study the synchronism of flowering, we monitored the development of flowers on all the potential reproductive trees in *D. guianensis* (DBH > 18 cm) and in *C. procera* (DBH > 14 cm). These observations were made in plot 16, once or twice a week during the reproductive period in 1995 and 1996. The presence or absence of flowers was assessed on each sampled tree using binoculars (8×40).

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Table 1
Life history traits of studied species in Paracou stands: distribution area, local density in Paracou (trees/ha), height class (C: canopy tree; SC: sub canopy tree), local spatial distribution, sexual system, flowering phenology, pollination agents, seed dispersal mode

Species	*Distribution area*	*Density (DBH >10 cm)*	*Height class*	*Spatial distribution*	*Sexual system*	*Flowering phenology*	*Pollination agents*	*Seed dispersal mode*
Vouacapoua americana	Guiana shield	8.4	C	Large patch (> 10 ha)	Hermaphroditic	Irregular synchronous	Insects (?)	Rodents < 30 m
Eperua grandiflora	Guiana shield	6.2	C	Patch	Hermaphroditic	Irregular synchronous	Insects (?)	Gravity < 20 m
Dicorynia guianensis	Guiana shield	6.2	C	Small patch (0.5 ha)	Hermaphroditic	Regular synchronous	Insects	Wind < 50 m
Carapa procera	Africa, Amazonia Guiana shield	6.4	SC	Random	Monoecious	Regular spread	Insects	Rodents < 30 m
Chrysophyllum sanguinolentum	Guiana shield Amazonia	3.2	SC	Random	Hermaphroditic	Regular spread	Insects, bats	Monkeys > 50 m
Virola michelii	Guiana shield Amazonia	2.4	C	Random	Dioecious	Regular spread	Insects (?)	Bats, monkeys kinkajou > 50 m

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2.3. Evaluation of the mating system

The estimation of the outcrossing rate was based on the comparison of multilocus arrays among adult trees and their offspring. Eighteen to 47 adult "trap" trees were sampled throughout the Paracou plots for each species. On each "trap" tree, 10 to 63 seeds collected on the ground under the maternal tree were sown in the greenhouse or laboratory. Three to nine isozymes coding for 3 to 10 gene loci were scored. Total proteins were extracted from cotyledons on offsprings, and from fresh leaves for adult trees. Protocols for extraction were adapted from Liengsiri et al. (1990). Standard starch gel electrophoresis and staining methods were used to separate and identify allelic products (Zanetto et al., 1996).

Outcrossing and selfing rates were estimated at the population and individual tree level using the multilocus mixed mating model (Ritland and Jain, 1981; Ritland's MLT software 1990). This model is based on several assumptions: independent segregation of alleles at each marker locus, no allele selection or mutation between fertilization and progeny assay, homogeneity of pollen pool composition over maternal trees, random mating for outcrosses. Despite many biases to these assumptions in natural populations, simulations have shown that the model is quite robust.

This maximum-likelihood method allowed calculation of the multilocus estimates of outcrossing rate t_m, the mean of the monolocus outcrossing rate t_s, and estimates of pollen and ovule allele frequencies, p and q respectively.

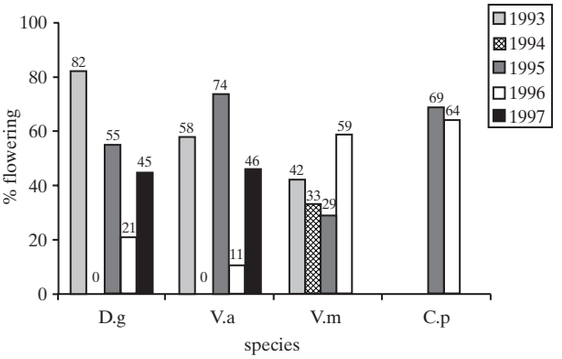

Fig. 1. Yearly variation of flowering rate (%) of *D. guianensis, V. americana, V. michelii* and *C. procera* for a five year period.

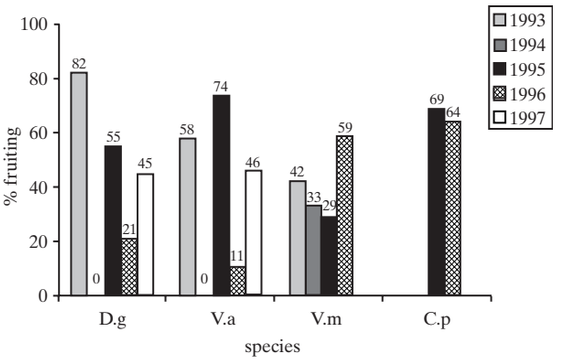

Fig. 2. Yearly variation of fruiting rate (%) of *D. guianensis, V. americana, E. grandiflora, V. michelii* and *C. sanguinolentum* for a five year period.

3. Results

3.1. The studied species exhibit a high variability of flowering and fruiting behaviour for different years and individual trees

The mean population flowering and fruiting rates of 5 out of 6 species varied widely over the years. In contrast, the flowering rate in *C. procera* was of similar magnitude in 1995 and 1996 (Fig. 1, 2).

The percentage of flowering trees and the diameter of the smallest flowering tree increased in the largest diameter classes, particularly in low flowering years such as 1996 and 2000 (Table 2). In addition, a clear relationship between regularity of flowering and diameter can be observed in the data sets (i.e *C. procera*, Fig. 3): the biggest trees flowered most often.

For all species, the minimal diameter of flowering trees was smaller than the minimal diameter of fruiting trees. The difference between flowering and fruiting diameter depends on species, from less than 1 cm for *V. americana* to 7 cm for *V. michelii*. These observations allowed a preliminary estimate of the density of potential reproductive trees in Paracou (Table 3).

In 1995, significant differences between flowering rate and fruiting rate were observed in every species except *V. americana* (Table 4).

No relationship was found between the intensity of flowering and tree diameter in *D. guianensis* in 1996

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Table 2
Flowering rate (%) according to diameter classes in *V. americana* and *D. guianensis*

Year	*Flowering rate (%)*	*Minimal DBH (cm)*	*DBH < 40*	*DBH > 40*	
		V. americana			
1995	74	19.4	70	78.8	
1996	5.3	40.6	0	10	
		D. guianensis			
				40 < DBH < 60	*DBH > 60*
1995	46	26.7	25	62	67
1996	16	33.7	7	25	25
1997	50	25	34	65	100
2000	28	31	15	52	73

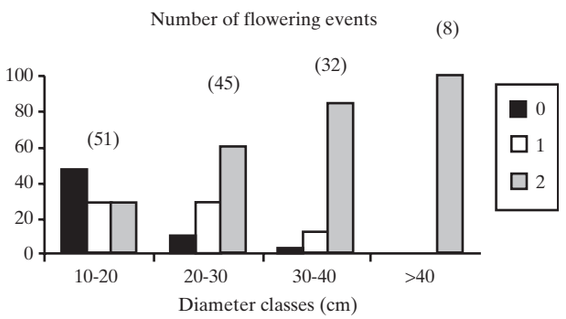

Fig. 3. Number of individual flowering events according to diameter classes in *C. procera* in 1995 and 1996 ((*n*): sample size in the diameter class).

and 2000 (unpublished data), but in *C. procera,* the most intense production of flowers was observed among the largest trees in 1995 and 1996.

The flowering and fruiting rates did not show any spatial pattern across the Paracou plots. There were no significant differences between silvicultural treatments despite the variability of tree density (Table 5). We only observed that the smallest flowering trees grew under the most open conditions.

Table 3
Minimal diameters of flowering and fruiting trees which were observed in Paracou

	Minimum flowering DBH (cm)	*Minimum fruiting DBH (cm)*	*Mean density of potential fruiting tree (tree/ha)*
V. michelii	15.5	23.8	0.9
C. sanguinolentum	*	25	1.6
E. grandiflora	28	30.7	2.1
D. guianensis	18.3	22	3.9
C. procera	12.5	16.8[a]	5.1
V. americana	19.4	20	6.2

[a]after Doligez (1996).

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Table 4
Flowering and fruiting rate (%) in Paracou in 1995

1995	*D. guianensis*	*V. americana*	*V. michelii*	*E. grandiflora*	*C. procera*
Flowering	46 (314)	74 (424)	28.6 (56)	83 (188)	69 (139)
Fruiting	33 (297)	71 (107)	8 (96)	23.2 (99)	*

(*n*): sample size.

Table 5
Flowering rate (%) according to increased logging intensity (T1 < T2 < T3) in 1995

	Control plots	*All logged plots*	*T1*	*T2*	*T3*
			D. guianensis		
Density (DBH > 18 cm) (tree/ha)	5.5	3.9	3.9	2.7	5
Flowering 1995	34.9 (63)	44 (159)	35.3 (51)	54.5 (33)	45.3 (75)
			V. americana		
Density (DBH > 19 cm)	6	6.1	8.5	7.1	0.7
Flowering 1995	75.8 (78)	70.8 (274)	63.2 (125)	77.6 (147)	*
			E. grandiflora		
Density (DBH > 28 cm)	5.4	2.4	2.4	2.4	2.4
Flowering 1995	85 (118)	73.2 (71)	65 (23)	89.6 (29)	57.9 (19)

(*n*): sampling size.

3.2. The within-population synchrony of flowering is very different in *D. guianensis* and *C. procera*

The flowering behaviour of *C. procera* and *D. guianensis* trees was observed in detail, in plot 16 (25 ha).

The floral phenology of *C. procera* was studied during 1995 and 1996. Two-thirds of those trees having a DBH > 14 cm bloomed. The flowering spread over a long period from September to February, with two "peaks": the first occurred in November, when the majority of trees bloomed. The second occurred in January and was less pronounced. During this period, some trees bloomed for the first time during the season and a few large trees bloomed a second time. The spikes lived during two to three weeks, but on a given tree the different spikes bloomed at different times. Our observations showed that trees at the beginning (less than 5% of the observed trees in plot 16 flowered in September 1996) and at the end (February) of the reproduction period were few: these trees probably received pollen from only a few conspecifics which bloomed at the same time.

The proportion of flowering trees increased with diameter classes in *C. procera*: only 35 to 45% of individuals with a DBH < 20 cm bloomed in 1996 and 1995, respectively. In contrast, almost 90% of

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trees with a DBH < 30 cm bloomed, and all the individuals with a DBH < 40 cm bloomed each year.

In 1996, the flowering rate of the *D. guianensis* population was low (26 out of 72 trees with a diameter greater than 30 cm). Only one flowering time was observed from the beginning of January until the beginning of February; all trees but one bloomed simultaneously; one tree produced flowers two weeks later. The intensity of flowering (number of flowers per tree) was variable between individuals and there was no clear relationship between tree diameter and the flowering rate.

3.3. All studied tree species are largely outcrossed

E. grandiflora, *V. michelii* and *C. sanguinolentum* were strictly allogamous. Only *V. americana* had an outcrossing rate estimation below 0.8 ($t_m = 0.63$ in 1993). For *D. guianensis* and *C. procera*, the mean population selfing rate estimates were significantly different from 0, and remarkably stable in 1993 and 1995 despite different flowering rates (Table 6).

In *C. procera*, the difference between multilocus (t_m) and single locus (t_s) estimations of the population outcrossing rate was significantly different from 0 ($p < 0.01$) probably due to positive assortative matings (Doligez and Joly, 1997). Our field examination of flowering periods in 1995 and 1996 supported the hypothesis of a temporal structure due to phenological groups in this species.

For the outcrossing species *C. sanguinolentum* (7 trees) and *E. grandiflora* (4 trees), all individual outcrossing estimates were not significantly different from 1. In contrast, for the mixed mating studied species, individual outcrossing rates less than 1 were found in one tree out of the five *C. procera* and in 15 trees out of the 21 *D. guianensis* analyzed. However, no relationship was found between the outcrossing rate and the density of neighbouring flowering trees (see Dutech, 1995; Caron et al., 1998).

Table 6
Multilocus (t_m) and monolocus (t_s) population outcrossing rates of six tree species in Paracou

Species	*Year*	N_f	N_s	*No loci*	*Population flowering rate*	t_m (S.E)	t_s (S.E)
D. guianensis	1993	37	680	4	82	0.83 (0.04)	0.79 (0.04)
D. guianensis	1995	32	729	4	55	0.83 (0.05)	0.84 (0.06)
D. guianensis[1]	2000	18	300	4	28.5	0.95 (0.04)	0.99 (0.06)
C. procera[2]	1993	47	483	10	*	0.78 (0.05)	0.69 (0.05)
C. procera[3]	1995	30	520	5	*	0.80 (0.03)	0.73 (0.03)
V. michelii	1994	21	343	4	32	0.93 (0.05)	0.93 (0.05)
C. sanguinolentum	1995	31	682	3	*	1.03 (0.05)	1.02 (0.05)
V. americana	1993	18	184	3	58	0.63 (0.09)	0.63 (0.11)
E. grandiflora	1995	18	272	3	83	1.11 (0.16)	1.14 (0.17)

N_f, N_s: number of open pollinated progenies and total number of analysed seedlings.
[1]Latouche-Hallé unpublished data.
[2]Doligez 1996.
[3]Dutech 1995.

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Logging intensity did not seem to affect the outcrossing rate of *D. guianensis*; in contrast, in *C. procera*, the outcrossing rate clearly decreased in logged plots compared to unlogged plots (Table 7).

4. Discussion

4.1. An irregular flowering and fruiting schedule is a common trait of tropical tree species

Data recorded from the investigated species show large variations of flowering and fruiting rates among years. For example, the percentage of flowering trees of *D. guianensis* and *V. americana* was high in 1993 and 1995 while it was nul or low in 1994 and 1996. This could be the result of environmental effects: the high flowering rates observed can be linked to the severe drought that occurred during October and November of the previous year. Reciprocally, the lack of dry season was followed by the absence of flowering in *D. guianensis* and *V. americana* in 1994. However, in 1995, the reduced rainfall observed from December to February had a negative impact on fruiting of species, with a long period of fruit maturation in *D. guianensis*, *V. michelii*, *E. grandiflora,* but not in *V. americana* in which flowering and fruiting occurred in a very short period in March and April.

4.2. Flowering and fruiting are subjected to size effects

In all observed species, the percentage of flowering and fruiting trees increases as a function of diameter class, especially in low flowering years. As a result, the contribution to reproduction is uneven among trees, as was already shown in *Gleditsia triacanthos* (Schnabel and Hamrick, 1995; Schnabel et al., 1998). The density and the spatial distribution of the tallest trees would be of capital importance for the reproductive success of the population: too low a density of big trees can decrease the number of flowering trees and increase mating among relatives. The increase of these crossings may induce the expression of recessive lethal alleles, resulting in a higher mortality and decreasing the reproductive success in the overall population.

Furthermore, the consequences of the size effect on reproductive success may be more pronounced for species which exhibit asynchronous flowering, such as *C. procera* (Doligez and Joly, 1997) or for species such as *V. michelii*, *Cecropia obtisufolia* (Alvarez-Buylla and Garay, 1994) or *Brosimum alicastrum* (Hamrick and Murawski, 1990), which are dioecious.

4.3. Tropical species exhibit low variations of their mating system

Among the six studied species, three were strictly outcrossing, whereas the remaining species exhibited mixed mating. The life history traits of outcrossing species contributing to allogamy, are dioecy (*V. michelii*), synchrony of flowering times (*V. americana*, *D. guianensis, E. grandiflora*) and self-incompatibility mechanisms (*D. guianensis, C. sanguinolentum* and *E. grandiflora).* Outbreeding contributes to maintaining genetic diversity in spite of low density of potential reproductive trees and/or the aggregated spatial distribution of trees in these species.

In *C. procera* and *D. guianensis,* which exhibited a mixed mating system, we did not find a significant relationship between the density of neighbouring flowering trees and the outcrossing rate, either at the

Table 7
Outcrossing rate in logged and control plots in Paracou stand (1993)

	D. guianensis		*C. procera**	
	N_f	t_m (e.t)	N_f	t_m (e.t)
Logged plots	15	0.84 (0,07)	14	0.63 (0.08)
Unlogged plots	23	0.85 (0,04)	33	0.85 (0.05)

N_f: number of progenies.
*: after Doligez and Joly (1997).

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population level or at the individual level. The mean population outcrossing rate seems to be stable in spite of different flowering rates, in contrast with theoretical models which predict that the outcrossing rate is equal to 0 or to 1 for panmictic populations (Charlesworth and Charlesworth, 1987). This could be explained on one hand by the structure of the populations: the *C. procera* population is divided into phenological groups, and in *D. guianensis*, trees are patchily distributed and, on the other hand, by the genetic load which induces early elimination of inbred progenies (Caron, 2000).

4.4. Logging can induce a lower effective population size and disturbance in pollination mechanisms

The effective population size can decrease due to the death of reproductive trees during or after exploitation, in addition to the extracted trees. Some species are more sensitive to the indirect effects of logging, such as *Sextonia rubra* in the Paracou stand (Durrieu de Madron, 1994). Too low a number of big trees can prevent any reproductive event for a long time, because the tallest trees are the main contributors to regeneration. Furthermore, the spatial pattern of the remaining reproductive trees must also be taken into account to ensure the maintenance of an effective population size. Big trees should be maintained close enough to allow pollen exchanges. However, White et al. (2002) recently found that in some tropical angiosperm tree species, such as *Swietenia humilis*, increased pollen flow can counteract the spatial isolation of reproductive trees.

The disturbance of the forest ecosystem can alter the phenological rhythms, the flowering rates, or the pollination biology of the species. The impact of habitat destruction should be variable according to the species ecological traits, as shown by the comparison of *C. procera* and *D. guianensis*. In *C. procera,* silvicultural treatments affected the mating system, as shown by Doligez and Joly (1997). They hypothesized that opening of the canopy was unfavorable to pollinator movements and reduced inter-tree pollination efficiency and the outcrossing rate. These consequences were not observed in *D. guianensis,* which seems to be adapted to low density.

In French Guiana today, the minimal logging tree diameter is 55-60 cm. Considering our results, this management rule seems to be correct, all the more so because the logging intensity applied at Paracou was higher than standard procedures. However, these results must be considered with caution as the the phenomena observed at Paracou may not be fully representative of what happens in the entire forest-managed area.

5. Conclusion

This work has taught us two things. On one hand, we need more discriminant tools in order to study more finely the mechanisms of genetic diversity dynamics such as the mating system, pollen flow or seed dispersal, and to know the effective contributors to the regeneration cohorts. As a result, we are currently developing hypervariable genetic markers for several species. On the other hand, the impact of logging on the genetic diversity of tree species will have to be investigated elsewhere, in forests that had been exploited on a larger spatial scale and over a longer time than on the Paracou stands.

Acknowledgements

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Chapter 2

Multilocus assessment of levels of genetic diversity in tropical trees in Paracou stands

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Abstract – The level of the within-population genetic diversity was assessed using dominant genetic markers for 10 tropical tree species in the Paracou plots in French Guiana. Simulations were conducted on experimental data sets in order to estimate the phenotypic and the genetic diversity, their associated sampling variance and optimal sample sizes. There was a wide range of variation of the level of genetic diversity between the species and pairwise differences were significant, using either RAPDs or AFLPs markers. Among the life history traits considered, the size of the distribution area was clearly an indication of the level of diversity: species with large distributions exhibited higher levels of diversity than did endemic species. Among the biological characteristics of species, the distribution area was the only obvious factor which discriminated groups of species at the local scale. Additional biological and demographic data have to be assessed in order to identify the determinants of genetic diversity and its dynamics.

Keywords: Dominant markers, Sample size, Sampling variance, RAPDs, AFLPs, Rainforest, French Guiana

1. Introduction

The level and the distribution of genetic diversity in natural plant populations have been recognized as prerequisite information for conservation strategies and sustainable management of forests (Namkoong et al., 1996). Surveys of genetic diversity have been conducted in numerous temperate species, and similar efforts are currently ongoing for tropical trees (Boyle, 1996).

Hamrick et al. (1992) demonstrated the significant relationship which exists between genetic diversity and the ecological characteristics of woody plant species: woody species with large geographic ranges, that are out-crossing and that disperse their seeds by wind, harbor more genetic diversity within populations than do species with other combinations of traits. Similar conclusions were obtained for tropical species by Loveless (1992). However, tropical tree species exhibit a wider range of diversity in life history traits, habitats, demographic structures and spatial distributions that may result in different patterns of genetic diversity.

During the last decade, there has been an increasing interest in the study of population genetics of tropical trees. Surveys of genetic diversity were conducted in different parts of the tropics (Forgen news, 1997). Investigations were also extended to the study of genetic processes which shape diversity such as as mating system (Lee et al., 2000) and gene flow (Chase et al., 1996; Konuma et al., 2000). Finally, there have

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also been attempts to evaluate human interference such as logging (Gillies et al., 1999; Lowe et al., 2002; Murawski et al., 1994) or fragmentation (Aldrich et al., 1998; Cardoso et al., 1998; Collevatti et al., 2001; White et al., 2002) on the level and structure of genetic diversity. Unfortunately, the methodologies (sampling design, tools and data analysis) were often too discordant to allow easy comparisons of within-population diversity.

Nevertheless, some general trends stand out.

The tree species which exhibit the lowest diversity levels have narrow or fragmented distribution areas and/or are subjected to overcollecting (*Prunus africana*, Dawson and Powell, 1999; *Tabebuia heterophylla*, Roux, 1999; *Pinus merkusii*, Changtragoon and Finkerley, 1995) or to domestication (*Calliandra calothyrsus*, Chamberlain, 1998) as opposed to other species, such as *Pentaclethra macroloba* (Hall et al., 1994), which is widespread from Nicaragua to Amazon basin and still locally abundant.

The species with the highest level of diversity are widespread (*Dryobalanops aromatica*, Lee et al., 2000; *Caryocar brasilense*, Collevatti et al., 2001; *Caesalpinia echinata*, Cardoso et al., 1998 and *Eugenia uniflora*, Margis et al., 2002), and are preferentially out-crossed. However, there is no consistent trend between diversity and pollination or seed dispersal modes. For instance, *D. aromatica* is pollinated by honey bees, which are known to forage over long distances and the species exhibits a high diversity; however *C. brasiliense*, which is pollinated by small bats that forage within restricted areas, also exhibits a high level of diversity. Other examples of species with contrasted dispersion distances and similar levels of diversity are *E. uniflora* or *C. echinata*. In contrast to previous results (Loveless, 1992), recent data indicate that species with high population densities (*D. aromatica*) or patchy distribution (*C. brasiliense*, *C. echinata* and *E. uniflora*) may harbor high levels of diversity.

The initial hypothesis of the genetic project of INRA and CIRAD was that in complex tropical ecosystems, a large range of diversity levels among tree species was expected in regard to the high variability of life history traits. Thus, the aim of the survey of genetic diversity in Paracou was to identify key biological or demographic attributes responsible for inter-specific differences in genetic diversity. A set of 15 species, representing as much as possible the diversity of breeding systems, of dispersal mechanisms and of distribution patterns, was sampled in the Paracou stands to identify the relationship between life history traits and genetic diversity.

The levels of genetic diversity have been monitored, using dominant markers, on these 15 species. The tree species that are present in the Paracou stands exhibit a large variation in their distribution range, from widespread species such as *Symphonia globulifera* and *Carapa procera*, which are distributed in tropical Africa and in tropical Americana, to endemic species, *Dicorynia guianensis*, *Moronobea coccinea* and *Vouacapoua americana*, which are restricted to the Guyana shield. More than half of the studied species are obligate out-breeders (Caron, 2000). Some species are locally randomly distributed *(Virola michelii* and *Sextonia rubra)* or show clear aggregation in clusters (*Cecropia sciadophylla*, *V. americana* and *D. guianensis*) (see Part IV, Chapter 1). The seed dispersal distances can vary from a few meters (*Eperua grandiflora*, Forget, 1992) to several hundred meters (*Chrysophyllum sp*, Julliot, 1997).

We chose to monitor diversity with multilocus molecular techniques such as RAPDs (Williams et al., 1990) and AFLPs (Vos et al., 1995). RAPDs markers have been widely used to study patterns of genetic diversity in plant species (see Nybom and Bartish, 2000, for a review). AFLPs have been more recently used to analyze the genetic structure of populations of tropical trees (Cardoso et al., 2000; Lowe et al., 2002; Muluvi et al., 1999; Russel et al., 1999). Both techniques have the advantage of assessing the diversity at the whole genome level, and can be implemented on any species without additional sequence information, but they suffer from a major drawback: they behave generally as dominant markers, which hampers the distinction of dominant homozygotes from heterozygotes. Hence we developed a technique inspired by Lynch and Milligan (1994) that allows to estimate the genetic diversity based on allelic frequencies instead of restricting the analysis of the phenotypic diversity based solely on the frequencies of bands.

The aims of the present work are to:

(i) compare the phenotypic and genetic diversity for each species and to compare the results among species;

(ii) compare the level of diversity for each species, obtained with the two different marker techniques, RAPDs and AFLPs. There is no background information on the genomic regions that are sampled by the two marker techniques. Our objective is

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therefore to test whether the two methods rank the species for their diversity in a similar way;

(iii) investigate whether the levels of within-population diversity were related to life history and ecological characteristics of tree species.

2. Materials and methods

2.1. Tree species

A long-term genetic project on the description and evolution of genetic diversity has been undertaken on 15 so-called "model-species". The species were selected according to several life history traits (distribution, flowering phenology and dispersion), and demographic attributes (spatial distribution and densities). The species were sampled within the range of variation of these traits and attributes. We report here on the results that have been obtained so far on a set of 10 species (see Table 1 and Chapter 1, Part III).

2.2. Sampling

In the absence of any information on the spatial distribution of the genetic diversity, trees were sampled within each of the four subgroups of plots (T_0, T_1, T_2 and T_3, see Part I for the description of the Paracou stands) according to the density of the species. Depending on the species and its density, a subset of each population was collected. The sample size varied from 36 to 91 individuals. For all species but *S. rubra*, adult trees were sampled. As it was impossible to extract DNA from adult tissues in *S. rubra*, collections were made on seedlings growing next to adult trees.

2.3. Molecular analysis

DNA was extracted from cambial tissue, except for *C. procera* and *S. rubra* for which leaves were used. The procedure of cambium collection is described in Degen et al. (2001). Extraction methods were applied following a modified CTAB procedure (Doyle and Doyle, 1987).

Amplification, migration and staining protocols for RAPDs are described in Bodénès et al. (1997). Amplification reactions were carried out using 6–14 primers per species, from Operon technologies. A preliminary survey permitted to select primers which gave reproducible and informative marker patterns (for details, see Degen et al., 2001).

AFLP markers were obtained following the protocol of Vos et al. (1995). Total genomic DNA was digested with the restriction enzymes *EcoRI* and *MseI*. A selective pre-amplification was carried out using a *EcoRI* primer without additional nucleotide and a *MseI* primer carrying two selective nucleotides. Final selective PCR reactions used *EcoRI* primers with two nucleotides and *MseI* primers with four nucleotides (Table 2).

2.4. Data analysis

RAPD and AFLP fragments were scored as presence (1) or absence of a band (0), and a matrix of phenotypes was assembled. The level of diversity within a population can therefore be estimated at two different levels:

1. The phenotypic level. For a given RAPD or AFLP fragment (locus), the phenotypic diversity (H_p) is the probability that two trees (phenotypes) taken at random within the population are different.

2. The genetic level. For a given RAPD or AFLP fragment, the genetic diversity (H_g) is the probability that two alleles controlling the expression of a band and taken at random within the population are different.

Let P and Q be the frequency of the presence and the absence of the fragment in a population, then the phenotypic diversity is:

$$H_p = 1 - p^2 - Q^2$$

Similarly the genetic diversity can be written as:

$$H_g = 1 - p^2 - q^2$$

where p and q are the frequencies of the alleles responsible for the presence or the absence of the RAPD or AFLP fragment.

The genetic diversity H_g can be calculated from observed frequencies P and Q as shown in Mariette et al. (2002):

$$H_g = \frac{-1}{(1-F)}\left(F - \sqrt{F^2 + 4(1-F)Q}\right)\left[1 + \frac{1}{2(1-F)}\left(F - \sqrt{F^2 + 4(1-F)Q}\right)\right]$$

where F is the fixation index in the population. The difference between H_g and H_p can be inferred from the previous equations (Caron, 2000):

$$H_g - H_p = \frac{-1}{(1-F)}\left(F - \sqrt{F^2 + 4(1-F)Q}\right)\left[1 + \frac{1}{2(1-F)}\left(F - \sqrt{F^2 + 4(1-F)Q}\right)\right] - 2Q(1-Q)$$

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Table 1
Ecological characteristics of the species studied at Paracou

Species	*Families*	*Distribution area*	*Spatial distribution*	*Floral phenology*	*Pollination agents*	*Seed dispersion agents*
Eperua grandiflora	Caesalpiniaceae	Guiana shield	Small clump	Irregular synchrony	Insects	Gravity <30 m
Vouacapoua americana	Caesalpiniaceae	Guiana shield	Big clump	Irregular synchrony	Insects	Rodents <30 m
Dicorynia guianensis	Caesalpiniaceae	Guiana shield	Small clump	Regular synchrony	Insects	Wind <50 m
Chrisophyllum sanguinolentum	Sapotaceae	Guiana shield	Clump	Regular spread	Insects	Monkeys >50 m
Carapa procera	Meliaceae	Africa; Amazonia	Clump	Regular spread	Insects	Rodents <30 m
Virola michelii	Myristicaceae	Guiana shield	Dispersed	Regular spread	Insects	Birds, kinkajou >50 m
Sextonia rubra	Lauraceae	Guiana shield	Dispersed	Irregular synchrony	Insects	Gravity <50 m
Symphonia globulifera	Clusiaceae	Africa; Meso-América Amazonia	Clump	Regular spread	Hummingbirds perching birds	Gravity + rodents >30 m
Moronobea coccinea	Clusiaceae	Guiana shield	Dispersed	Regular	Hummingbirds	Gravity + rodents <50 m
Cecropia sciadophylla	Moraceae	Amazonia	Clump	Continuous	Wind	Bats >50 m

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Table 2
Additional nucleotides used in the primer-enzyme combinations (EcoRI+2, MseI+4)

V. michelii	*V. americana*	*E. grandiflora*	*D. guianensis*	*C. sanguinolentum*
CG/ACAA	AC/ACAC	GC/ACAC	AC/ACAC	TG/CGGA
CG/TACC	AC/ ACAG	AC/TACC	AC/ACAG	CG/CTTA
CG/ACAG	AC/ACAT	CC/ACAG	AC/ACAT	TG/CTTG
GC/TACC	AC/ACAA	CC/TACC	AC/ACAA	CT/CGGA
GC/ACAA	CC/ACAG	GT/ACAG	CC/ACAG	CG/GCGT
GT/ACAA	CC/ACAA	GT/TACC	CC/ACAA	GT/CGGA
	CC/ACAC	CG/TGTC	CC/ACAC	AC/CGGA
	CC/ACAT		CC/ACAT	GC/CGGA

For the estimation of H_g and its sampling variance, we considered the formulae of the expected value and variance of a function of several variates (Kendall and Stuart, 1977). In doing so, we considered that H_g depended on only one variate Q, and consequently that the value of F was known for each species, and constant among the different loci. For 7 out of the 10 investigated species, F could be estimated from codominant markers (either isozymes or microsatellites). In our study, F values were available for all species except *M. coccinea*, *S. rubra* and *C. sciadophylla*. We assumed that the estimated value was the true value.

For L loci, the estimator over all loci is:

$$\widehat{H} = (1/L)\sum_{i=1}^{L} \widehat{H}_{gi}$$

As Lynch and Milligan (1994) showed that the bias introduced in the estimation of q due to a small sample size was substantial when the null allele was rare, we calculated the genetic diversity restricted to the subset of fragments that showed an observed frequency smaller than (1–(3/N)), where N is the population sample size. Any fragment that exhibited a higher frequency than (1–(3/N)) in a species was removed from the whole data set. H_{g2} is the genetic diversity computed on this subset of fragments.

The two molecular techniques (RAPD and AFLP) allow to randomly sample markers throughout the genome. A number of markers (DNA fragments) are detected in the genome and amplified by PCR if the recognition site of the primers used in each technique corresponds to the template DNA. Polymorphism is scored at each fragment and a mean estimate is calculated over all fragments. Since the markers are randomly selected within the genome, the mean estimate is expected to provide an unbiased estimate at the whole genome level, regardless of the fragments that were amplified in each species.

However, comparisons of levels of diversity between species require to estimate the sampling variance including the sampling within the genome. The sampling variances of the phenotypic and genetic diversity (H_g and H_{g2}) were estimated using resampling methods. Since comparisons among species are made, the sampling variances were calculated so that they comprise the two sampling processes: sampling of loci within the genome and sampling of trees within the population, respectively inter- and intra-locus variance (V_{inter} and V_{intra}, Nei and Roychoudhury, 1974). The two variances were estimated by resampling methods: V_{inter} was estimated by bootstrapping over loci and V_{intra} by bootstrapping over individuals.

As a result the total sampling variance V is:

$$V = V_{inter} + V_{intra}$$

Comparisons of H_p and H_g among species were made using the Z-test (Zhang and Allard, 1986). The estimates of the phenotypic, genetic diversities and their associated sampling variances were computed with a home-made software package (Haploid, Hapdom, Boot; Kremer and Labbé, unpublished).

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3. Results

3.1. Phenotypic vs. genetic diversity

We analyzed graphically the equation giving the difference (H_g–H_p), according to the value of frequency Q and the fixation index F (Fig. 1). We considered F ranging between –0.30 and +0.30, encompassing values usually observed in forest trees. Two major conclusions can be drawn from Fig. 1.

- The genotypic diversity is equal to the phenotypic diversity in a limited number of situations, when Q varies between 0.35 and 0.45. Therefore, estimates of H_g are recommended rather than H_p. The difference is inflated when the fixation index (F) decreases.

- The estimation of H_g is only slightly affected by F, except when Q is lower than 0.20. As a result, assuming that all loci have the same value, F does not introduce an important bias in the estimation of H_g.

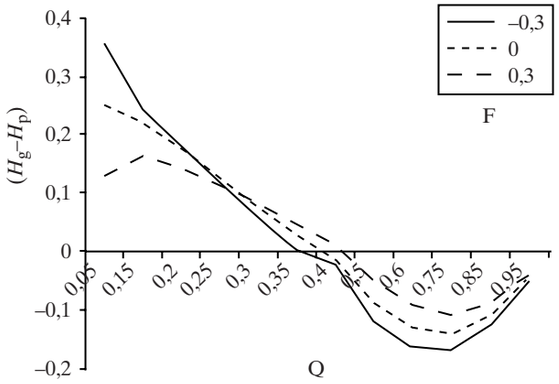

Fig. 1. Variation of the difference (H_g–H_p) as a function of frequency of the recessive homozygote (Q) value.

3.2. The components of the sampling variance

Using the experimental data sets, the two components of the variance were estimated (Fig. 2). There are

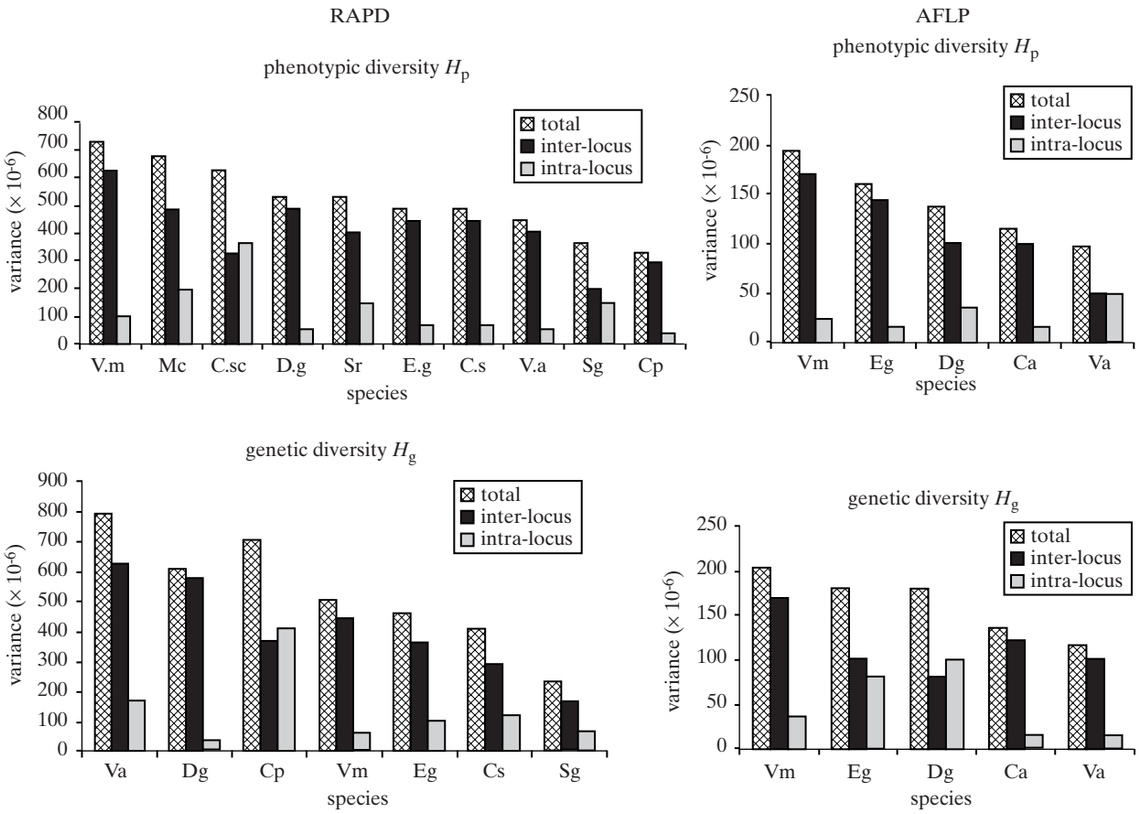

Fig. 2. Total, inter- and intra-locus variances of H_p and H_g, using RAPDs and AFLPs (*Virola michelii*, *Moronobea coccinea*, *Cecropia sciadophylla*, *Dicorynia guianensis*, *Sextonia rubra*, *Eperua grandiflora*, *Chrisophyllum sanguinolentum*, *Vouacapoua americana*, *Symphonia globulifera* and *Carapa procera*).

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important differences of the total sampling variance among the species, as a result of the different sampling efforts that were attributed to each species. The highest intra-locus variance is for *C. sciadophylla*, which has also the lowest sample size. Interestingly, the inter-locus sampling variance is the major component of the total sampling variance in all species (except *C. sciadophylla)*, and exceeds in some cases up to 10 times the within locus sampling variance. The same pattern of the sampling variance was obtained when AFLP markers were used. As for RAPDs, the inter-locus variance is much higher than the intra-locus variance. However, the total variance is lower for AFLPs than for RAPDs.

We empirically tested the impact of the sample size (number of loci and number of trees) by resampling different numbers of trees and loci and computing the coefficient of variation of the phenotypic diversity (Fig. 3).

In the RAPD data set, the coefficient of variation of phenotypic diversity estimates decreased rapidly when the number of sampled trees decreased between 10 and 40 and became stabilized beyond 50. The curve constantly decreased when the number of loci decreased from 15 to 60 loci. In the same way, in the AFLP data set, the stabilization occurred beyond 30 individuals and 150 loci (Fig. 3a,b). Similar observations were made when the coefficient of genetic diversity estimates varied as a function of the number of trees and of the number of loci (results unshown).

3.3. Comparison of the levels of diversity among the 10 species

The estimates of phenotypic diversity using RAPDs exhibited a broad range of variation, ranging from $H_p = 0.17$ (*V. americana*) to $H_p = 0.39$ (*S. globulifera*) Fig. 4). Pairwise comparisons of phenotypic diversity

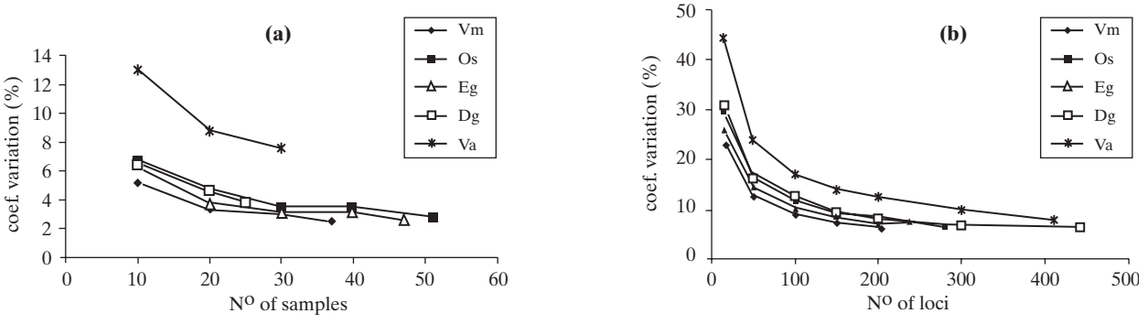

Fig. 3. Coefficient of variation as a function of number of samples (a) and of loci (b) in 5 species, with AFLP markers.

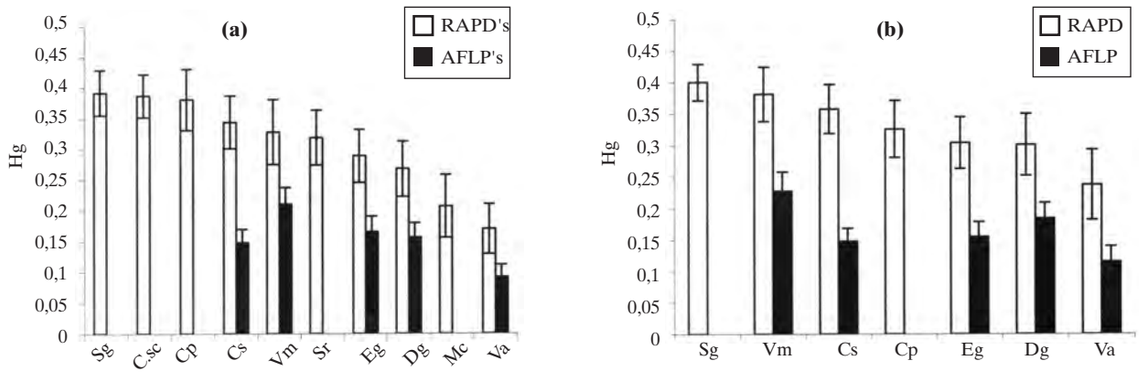

Fig. 4. (a) Comparison of the level of within-population phenotypic diversity of 10 species (*Symphonia globulifera*, *Carapa procera*, *Cecropia sciadophylla*, *Chrisophyllum sanguinolentum*, *Virola michelii*, *Sextonia rubra*, *Eperua grandiflora*, *Dicorynia guianensis*, *Moronobea coccinea*, *Vouacapoua americana*) in Paracou stand. (b) Comparison of the level of within-population genetic diversity of seven species (*Symphonia globulifera*, *Virola michelii*, *Chrisophyllum sanguinolentum*, *Carapa procera*, *Eperua grandiflora*, *Dicorynia guianensis*, *Vouacapoua americana*).

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were calculated using the *Z*-test. The 10 species could be clustered in five groups showing significant differences among them (Table 3).

For seven species, we could estimate the fixation Index (*F*), because codominant data were available (either microsatellites or isozymes). Consequently we computed for these species their genetic diversity. For all species but *C. procera*, the genetic diversity was equal or higher than the phenotypic diversity, as predicted by the theoretical calculations (Table 3).

In addition, for these species we also calculated the genetic diversity (H_{g2}) on the subset of fragments that showed an observed frequency smaller than (1–(3/*N*)), where *N* is the population sample size, as recommended by Lynch and Milligan (1994).

The coefficient of correlation was significant between H_p and H_g estimates ($r^2 = 0.75$, Student test: $P < 0.02$), and between H_g and H_{g2} ($r^2 = 0.86$, $P < 0.001$).

3.4. Comparison of AFLP and RAPD

In general the estimates of the diversity computed with AFLP markers were lower than for RAPD markers, and this observation also holds for their associate sampling variance: the variance for AFLPs was lower than for RAPDs. However, despite the differences of diversity in absolute values, the two marker systems rank the species in the same order except for *Chrisophyllum sanguinolentum* (Table 3). This is also shown by the correlation between the measures of diversity (either phenotypic or genetic) computed between the two marker systems (Fig. 5).

4. Discussion

4.1. Measuring diversity to compare species

Our investigations have shown that multilocus markers can be used for comparing diversity levels across species, provided that adequate guidelines are used. First of all, a sufficient number of loci (markers) are needed. Our empirical comparisons have shown that at least 150 loci are needed, whereas 30 individuals are sampled. These differences in sampling efforts between individuals and loci were predicted earlier by theoretical studies (Nei, 1987). They were confirmed here by the differences in intra- and inter-locus sampling variances. Whatever the species studied, the inter-locus variance was always higher than the intra-locus variance.

With a few exceptions, the two marker systems (RAPD and AFLP) ranked the two species in the same order. However, the diversity estimated with RAPD markers was always higher than for AFLP markers. The difference may be due to higher mutation rates in regions amplified by RAPDs primers; it can also result from a poorer reproducibility of RAPD markers. The sampling variance for RAPD markers is also higher than for AFLP markers; therefore the former marker system is less powerful for statistical comparisons between species.

4.2. Genetic diversity and life history traits

As found in earlier allozyme-based studies (Loveless, 1992), a closed relationship occurred in our data set between the geographic range and the level of within population diversity. Widespread species such as *S. globulifera* and *C. procera*, which have a transcontinental distribution spreading from Africa to America, have a higher diversity level than do endemic species such as *D. guianensis*, *M. coccinea* and *V. americana*, which are restricted to the Guianan shield. Species exhibiting intermediate levels of diversity (*V. michelii*, *C. sanguinolentum*, *C. sciadophylla*) have rather large regional distribution areas. In a recent meta analysis of diversity calculated with multilocus dominant markers, Nybom and Bartish (2001) found no association at all between geographic range and within population diversity.

In contrast to the results of Hamrick et al. (1992) and of Nybom and Bartish (2001), the mating system was not an obvious correlate of the within-population diversity: the lowest out-crossing rate estimates ($t_m = 0.6$) were found for *S. globulifera* and for *V. americana*, whereas species of the intermediate group, *C. sciadophylla*, *C. sanguinolentum*, *V. michelii*, *S. rubra* and *E. grandiflora* are obligated out-crossing species (Caron, 2000). However, this data must be interpreted cautiously because the genetic diversity is shaped by long-term mechanisms whereas out-crossing estimates are usually observed within single years and usually exhibit high between-year variations.

The impact of gene flow and dispersal mechanisms on diversity is less clear and requires more investigations. Information about pollination distance is scarce and imprecise for the studied species. *S. globulifera* and *M. coccinea* are pollinated by birds (Bittrich and Amaral, 1996; Gill et al., 1998). They can distribute pollen over long distances but are

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Table 3
Sample size (n), total number of bands (l), phenotypic (H_p) and genetic (H_g), (H_{g2}) diversity using RAPDs and AFLPs

Species	*I.A.1. RAPD*								*I.A.2. AFLP*							
	n	l	H_p	s	H_g	s	H_{g2}	s	n	l	H_p	s	H_g	s	H_{g2}	s
V. michelii	92	37	0.328 (0.027)	ab	0.381 (0.022)	ab	0.396 (0.022)	ab	37	207	0.211 (0.014)	a	0.228 (0.015)	a	0.325 (0.016)	a
C. sanguinolentum	75	48	0.358 (0.022)	ab	0.357 (0.020)	ab	0.375 (0.019)	ab	51	281	0.148 (0.011)	b	0.148 (0.011)	cd	0.210 (0.017)	bc
E. grandiflora	65	60	0.289 (0.022)	b	0.303 (0.021)	b	0.315 (0.021)	c	47	236	0.166 (0.013)	b	0.155 (0.012)	bc	0.202 (0.016)	bc
D. guianensis	91	61	0.268 (0.023)	bc	0.300 (0.025)	bc	0.351 (0.022)	bc	25	442	0.157 (0.012)	b	0.184 (0.013)	b	0.303 (0.016)	ab
V. americana	66	68	0.170 (0.021)	c	0.237 (0.028)	c	0.299 (0.032)	c	30	411	0.092 (0.010)	c	0.115 (0.013)	d	0.232 (0.023)	b
S. globulifera	94	66	0.390 (0.018)	a	0.404 (0.015)	a	0.404 (0.015)	a								
C. procera	65	46	0.387 (0.017)	ab	0.329 (0.023)	b	0.329 (0.023)	bc								
M. coccinea	89	57	0.206 (0.026)	c												
S. rubra	47	58	0.319 (0.023)	b												
C. sciadophylla	36	52	0.381 (0.026)	ab												

H_{g2} is the genetic diversity according to Lynch and Milligan recommendations. s (a,b,c,d: significantly different groups at 5% level, Z-test).

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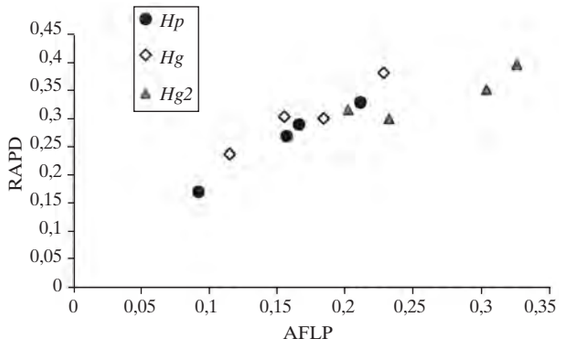

Fig. 5. Correlation between diversity estimates using two different dominant marker systems (RAPDs and AFLPs) (H_p: $r^2 = 0.978$, Student test, $P < 0.02$; H_g: $r^2 = 0.926$, $P < 0.05$; H_{g2}: $r^2 = 0.800$, n.s).

known to forage in restricted neighborhoods when flowering trees are dispersed (Franceschinelli and Bawa, 2000). The other species are supposed to be insect pollinated and the range of pollination distance could be very large. In the same way, seed dispersal is restricted for *V. americana*, *M. coccinea* and *C. procera* (by rodents), *D. guianensis* (by wind), *E. grandiflora*, *S. rubra* (by gravity) and potentially large for *S. globulifera*, *C. sciadophylla* (by bats), *V. michelii* (by kinkajou, birds and monkeys) and *C. sanguinolentum* (by monkeys) (Forget, 1988; Forget and Sabatier, 1997; Ter Steege, 1990; Julliot, 1997). Although some crude estimates of physical dispersion have been documented, the effective distances of seed dispersal (seeds from which reproductive trees of the next generation will originate) are not well known.

However, there are some indirect indications of the potential impact of gene flow on levels of diversity. When comparing the diversity estimates that we obtained with the spatial genetic structure of the species in Paracou (Degen et al., 2001), there is a clear trend for lower diversity for those species exhibiting stronger spatial structures (*D. guianensis*, *M. coccinea* and *V. americana*). These results suggest that reduced gene flow as detected by strong spatial structure may have some impact on the level of diversity.

We also investigated the potential relationship between abundance and spatial distribution of the species, with its genetic diversity. The different studied species exhibited quite an important variation for their density and distribution. *S. globulifera* (10.6 tree/ha with DBH ≥ 10 cm) and *V. americana* (8.4 tree/ha with DBH ≥ 10 cm) are abundant whereas *V. michelii* (2.4 tree/ha with DBH ≥ 10 cm) has a low density. *V. americana*, *D. guianensis* and *C. sciadophylla* are strongly aggregated, but *M. coccinea*, *S. rubra*, *V. michelii* are more evenly distributed. The more frequent species, *S. globulifera* and *V. americana*, are at the extreme ranks for the diversity level. These contrasted results confirmed the absence of a clear relationship between density and genetic diversity, which had been already pointed out by others. Shapcott (1999) found a significant negative correlation between population genetic diversity and within population density in understory palm species of the genus *Pinanga*, in Borneo, in contrast to the results of Hamrick and Murawski (1991) who found, in Panama, that species at low population densities (<0.5 individuals/ha) were less genetically variable than species which occurred at high density.

As the review of Hamrick et al. (1992) indicated, we did not find any clear relationship between the successional status of the species and their level of diversity. Even if our results indicate that late successional species (*V. americana* and *M. coccinea)* exhibited lower diversities than early successional species (*C. sciadophylla)*, these results may be misleading because the late successional species are also of endemic origin.

The evolutionary history of species must have played a significant role in shaping the present genetic structure of the species (Hamrick et al., 1992). Particularly, the quaternary and holocene events are assumed to have differently impacted on the genetic structure of species. The species with stricter ecological needs and weaker colonization abilities (*V. americana*, *E. grandiflora*), which could have experienced severe population bottlenecks, might have lost more genetic diversity than species with a history of larger and more continuously distributed populations (*S. globulifera*). Paracou forest is located in coastal plain, probably a recent colonization zone. There are some comparative analyses of diversity with inland sites. Differentiation (F_{st}) between inland and coastal populations varied from 0.08 in *V. americana* (Dutech, 2001) to 0.046 for *C. sciadophylla*, 0.035 for *C. procera* and 0.042 for *D. guianensis* (Caron and Chevallier, 1999). However, these observed differentiation values were much lower than the mean value for most tropical trees ($G_{st} = 0.109$, isozymes) (Loveless, 1992), probably because the populations

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sampled in our studies were too close. Historical trends may only be identified from differentiation trends observed on a large sample of populations, including widely separated populations.

5. Conclusion

The estimation of the level of the genetic diversity of species is the first step in a program of sustainable management of genetic resources. The multi-allelic markers, such as RAPDs and AFLPs, were rapid tools to monitor diversity in different species for comparative purposes.

As expected, the range of variation of the level of genetic diversity between tree species of Paracou was large. The only clear trend observed was the strong relationship between diversity and distribution range of the species. Endemic species to the Guiana shield exhibit lower diversity that species having a regional or a continental distribution. Other life history traits did not indicate any clear relationship with genetic diversity. Many life history traits probably interact with each other.

Given the present state of knowledge for these traits, additional data concerning the demography and the reproductive biology of species are necessary. Furthermore, the genetic inventory will need to be enlarged to other populations for the same species, and to be increased to a larger number of species in order to construct a model of prediction of genetic diversity in tropical species. We are currently conducting monitoring of genetic diversity using AFLP markers on more than 40 species over several geographic populations in order to meet these goals.

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