7 Population Biology of Different *Clusia* Species in the State of Rio de Janeiro

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7.1 Introduction

"Simple-Sequence-Repeats" (SSR) or "Microsatellites" are repeats of short sequence motifs with a length of 1 to 6 bp, which can be replicated up to 100 times (Tautz 1993) and occur in the non-coding regions of eucaryotic and chloroplast genomes in a very high diversity (Gupta et al. 1994). These sequence repeats are the main cause for the length polymorphisms of microsatellites in populations (Schlötterer and Tautz 1992) and are very useful for molecular taxonomy and population genetics (Zhivotovsky and Feldman 1995). Due to the large number of microsatellites in eukaryotes, and a high diversity within species or populations, they are the most important markers for genomic mapping and relation studies (Kashi et al. 1997; Queller et al. 1993). Trimer primers are widespread, give the most suitable results (Hughes and Queller 1993), and were used in many different studies before (Becher et al. 2000; Beyermann et al. 1992; Echt and May-Marquardt 1997; Echt et al. 1996; Jarret et al. 1997). Successful amplification of polymorphic banding pattern was possible with trinucleotide primers such as AAC_7, AAG_8, and GTG_5 (Hale et al. 2001; Poulsen et al. 1993; Squirrell and Wolff 2001). Using AAC_7 we were able to identify ecotypes of Brazil pine [*Araucaria angustifolia* (Bert.) O. Ktze.; Hampp et al. 2000].

The genus *Clusia* comprises about 350 species (Willis 1973; Pipoly et al. 1998), which occur mainly in the tropical part of South America (Lüttge 1991). These plants are the only known dicotyledonous tree species that are able to perform crassulacean acid metabolism (CAM) and which possess the ability to switch between the C_3 and the CAM mode of photosynthesis. This enables them to conserve water, depending on environmental conditions (see also Chaps. 6 and 9). Certain *Clusia* species occur at quite different habitats with regard to subsoil and climatic conditions. It could thus be possible that this has resulted in site-specific diversity. In order to investigate this hypothesis,

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we performed an SSR analysis of genetic variability at the population level of three *Clusia* species, *C. parviflora* Engl., *C. fluminensis* Pl. et Tr., and *C. hilariana* Schlecht., growing at different sites along the coast of the state of Rio de Janeiro, Brazil. This was especially promising as the selected sites showed characteristic differences and thus a possible loss of genetic exchange due to habitat fragmentation. The sites mostly represent restingas with open vegetation on coastal sand dunes. *Clusia* is the main shrub of this vegetation structure and grows in the centres of the vegetation islands. Due to the severe conditions along the coast (e.g., salt stress), the vegetation is often destroyed, and the vegetation islands are dispersed, making genetic exchange difficult.

7.2 Population Studies on the Basis of Single Sequence Repeats

For our studies we collected leaf samples from nine locations along the coastline east to northeast of Rio de Janeiro (Fig. 7.1; Table 7.1). We took two leaves each from all *Clusia* plants we could find at a given site. DNA extraction,

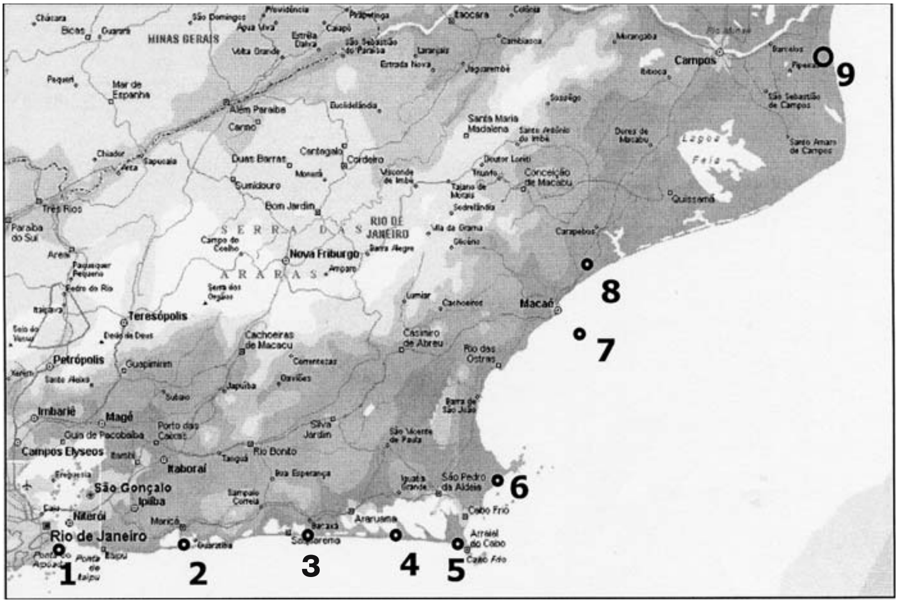


Fig. 7.1. Map of the state of Rio de Janeiro. Collection sites are marked with *circles*: 1, sugar loaf, city of Rio de Janeiro; 2, Maricá; 3, Jacarepia Nationalpark near Saquarema; 4, Figueira; 5, Arraial do Cabo; 6, Buzios; 7, Archipelago de Santana; 8, Jurubatiba Nationalpark near Macaé; 9, Iquipari near Campos de Goitacazes

Table 7.1. *Clusia* species and some properties of their habitats

Site		Vegetation (rainfall)	*Clusia* species
1 (S)	Sugar loaf	Rocky outcrops (1500 mm/a)	*Clusia parviflora*
1 (C)	Corcovado	Rocky outcrops (1500 mm/a)	*Clusia fluminensis*
2 (M)	Maricá	Wet restinga (1230 mm/a)	*Clusia fluminensis*
3 (S)	Jacarepia/Saquarema	Wet restinga (1000 mm/a)	*Clusia fluminensis*
4 (F)	Figueira	Dry restinga (800 mm/a)	*Clusia fluminensis*
5 (Ar)	Arraial do Cabo	Dry restinga (800 mm/a)	*Clusia fluminensis* *Clusia hilariana*
6 (B)	Buzios	Dry Forest (800 mm/a)	*Clusia fluminensis*
7 (A)	Archipelago de Santana	Rocky outcrops (~800 mm/a)	*Clusia fluminensis*
8 (J)	Jurubatiba NP/Macaé	Change of dry and wet Restinga (1164 mm/a)	*Clusia hilariana* *Clusia parviflora*
9 (I)	Iquipari/ Campos de Goitacazes	Transition from restinga into dry forest (800–1200 mm/a)	*Clusia hilariana* *Clusia spiritu-sanctensis*

analysis by PCR, and determination of banding patterns on polyacrylamide gels was as in Hampp et al. (2000).

7.2.1 *Clusia parviflora*

C. parviflora Engl. occurs at the coast only at two different sites, at the rocky vegetation of the sugar loaf and at the Restinga de Jurubatiba National Park, near Macaé (Fig. 7.1). This species showed the most obvious differences by use of the primer GTG_5 (Fig. 7.2).

The samples from the restinga site display clearly two different fragments with a size of approximately 1500 bp, while those of the sugar loaf have only one or even no fragment of this size. These differences are more conspicuous in the samples of the year 2001 (S1, J1) than in those of 2003 (S2, J2). Furthermore, a fragment at a size of approximately 800 bp which shows up in the samples of Macaé is extremely weak or absent in the sugar loaf samples. Likewise, a clear banding pattern appeared when using the primer AAG_8 (Fig. 7.3).

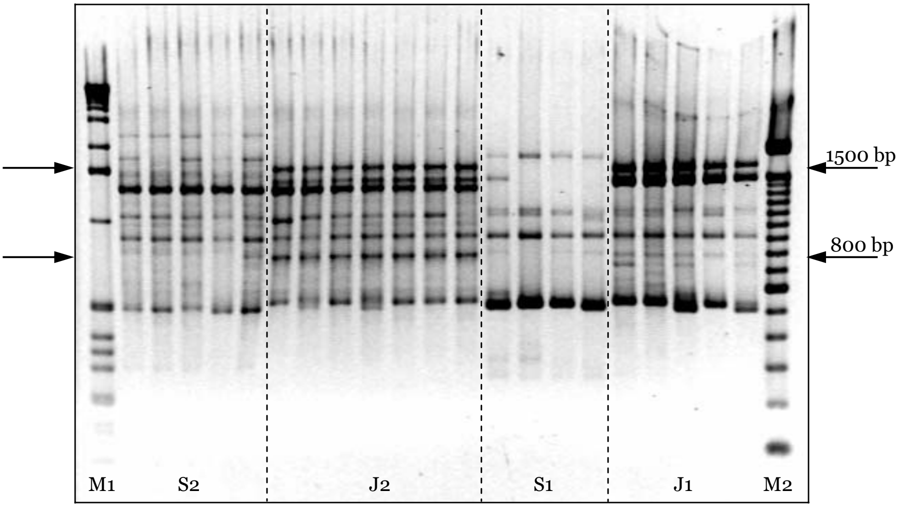


Fig. 7.2. Banding pattern of different samples of *C. parviflora* using the primer GTG_5. (Sample sites: S1, sugar loaf; J1, Restinga de Jurubatiba National Park, taken in 2001; S2, sugar loaf; J2, Restinga de Jurubatiba National Park, taken in 2003). M1 shows a 1-kb, M2 a 100-bp size marker ladder. Differences in the banding pattern are marked by *arrows*

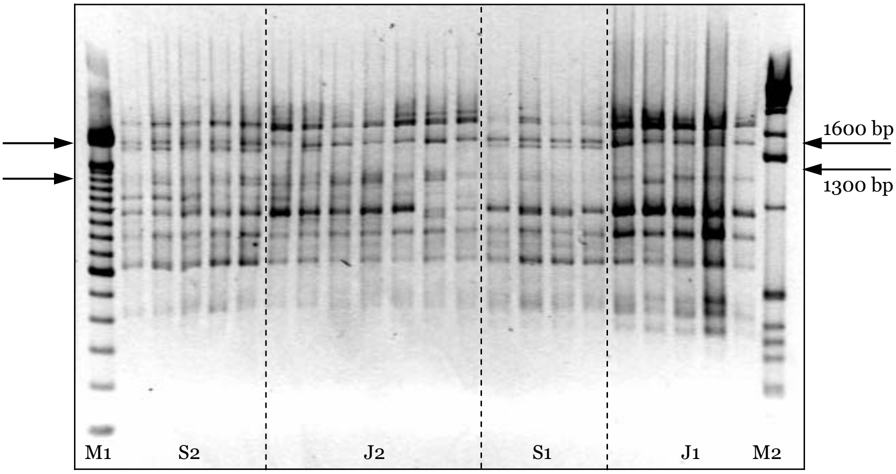


Fig. 7.3. Banding pattern of different samples of *C. parviflora* using the primer AAG_8 (for abbreviations see Fig. 7.2)

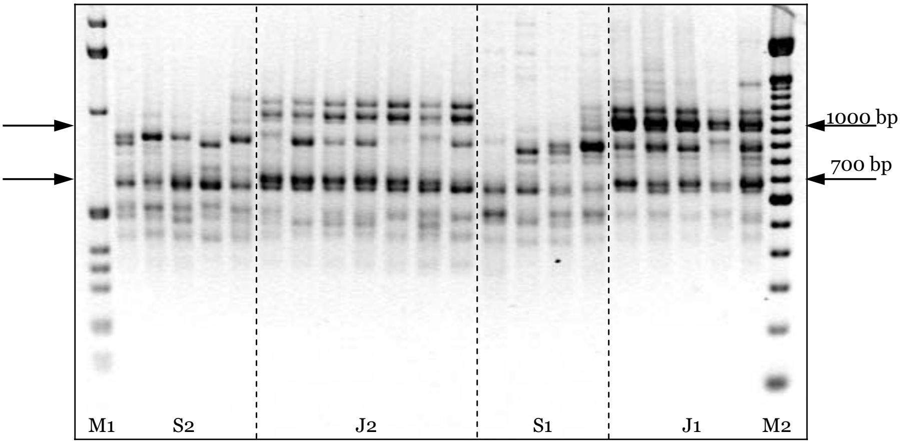


Fig. 7.4. Banding pattern of samples of *C. parviflora* using the primer AAC_7 (for abbreviations see Fig. 7.2)

At a size of ca. 1600 bp, the sugar loaf samples form a double band while the samples from the restinga display only a single band. By contrast, the samples of the sugar loaf exhibit only weak signals at a size of ca. 1300 bp while those from the restinga show one or two distinct signals. Finally, the third microsatellite primer, AAC_7, gave another polymorphic banding pattern (Fig. 7.4), which again clearly distinguished DNA samples from both sites. In a size range of between 1000 bp and 1100 bp, the samples from the restinga showed three distinct fragments while those from the sugar loaf site displayed only very weak or no bands. Similar differences were found at around 700 bp.

The banding pattern obtained with each of the three primers yielded differences between the two sites. Given that these two sites are located at a distance of ca. 150 km from each other, the lack of genetical exchange between these populations is not surprising. Furthermore, the different habitats could be responsible for these differences in the non-coding DNA regions.

7.2.2 *Clusia fluminensis*

C. fluminensis Pl. et Tr. is widely spread along the coast east of Rio de Janeiro, and this species is not found in the more northern parts of the state of Rio de Janeiro. *C. fluminensis* grows in all habitats of this region such as restingas (Maricá, Saquarema, Figueira), dry forests (Buzios), and rocky outcrops (Corcovado, Archipelago de Santana). It was only absent from the sugar loaf. In spite of this variety of habitats, there were relatively small differences in the banding patterns. Using the primer AAG_8, some variation at around 1700 bp was visible only in the Corcovado sample (C) (Fig. 7.5). At

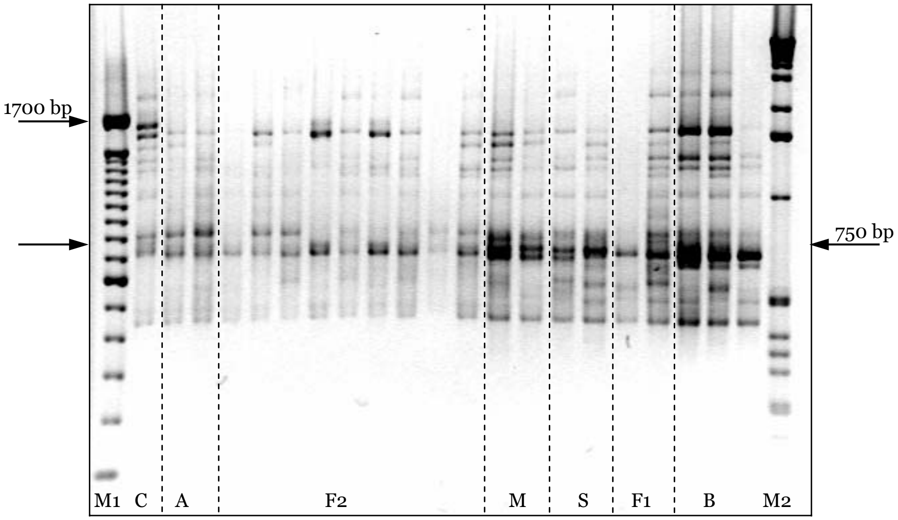


Fig. 7.5. Banding pattern of samples of *C. fluminensis* using the primer AAG_8 (M, Maricá; S, Saquarema; F1, Figueira; B, Buzios, taken in 2001; C, Corcovado; A, Archipelago de Santana; F2, Figueira, taken in 2003). M1 shows a 100-bp, M2 a 1-kb size marker ladder. Differences in the banding pattern are marked by *arrows*

about 750 bp other differences in the banding pattern occurred. The samples of Corcovado (C), Archipelago de Santana (A), and Figueira (F1, F2) yielded a single band while the samples of Maricá (M) and Saquarema (S) display an additional longer fragment, and the sample of Buzios (B) an additional smaller fragment.

The observed differences in fragment polymorphisms could be due to the climatic conditions of the different restingas. Maricá and Saquarema have more than 1000 mm rainfall per year and form a rather wet restinga, while the dry restinga in Figueira has only about 800 mm rainfall per year and thus the behaviour is more similar to the rocky sites of Corcovado and Archipelago de Santana with their increased water drain off. Buzios represents a special site because, after the separation of the tectonic plates some fragments of the African plate remained attached to the South American continent. This geological anomaly together with different subsoil, resulted in a dry forest vegetation instead of restinga and is the reason for numerous endemic plant species (Scarano et al. 2001). Owing to the different habitat, *C. fluminensis* grows in this area as a strangler on other trees, compared to a freestanding shrub in the open restinga vegetation. With this background the specific banding pattern of this provenience is not surprising.

Location-dependent differences in the banding pattern of the non-coding DNA regions of this species could also be identified with the primer AAC_7 (Fig. 7.6). The samples taken from Maricá (M), Buzios (B), and Corcovado (C)

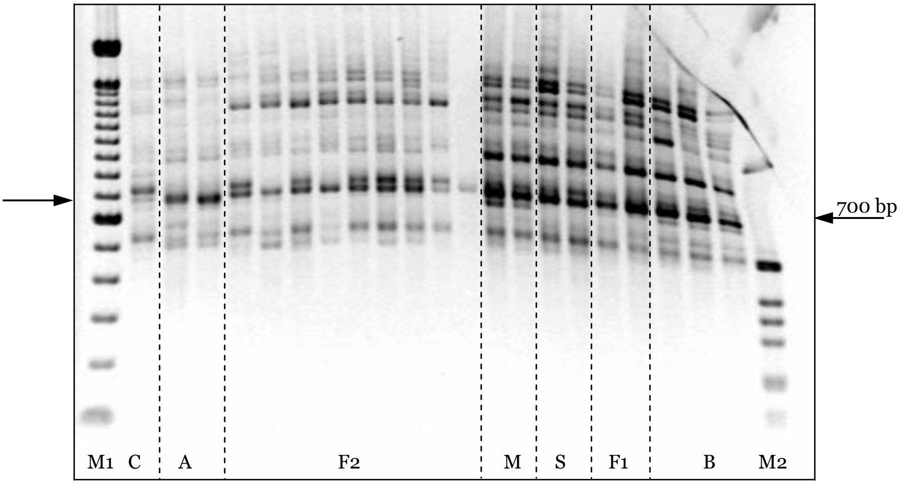


Fig. 7.6. Banding pattern of samples of *C. fluminensis* using the primer AAC$_7$ (for abbreviations see Fig. 7.5)

clearly show three different fragments at a size of around 700 bp, while those from Archipelago de Santana (A) exhibit only one fragment, and those from Saquarema two fragments at the respective size. The samples of Figueira (F1, F2) display only a weak third fragment. The distinctly different pattern of the sample of the Archipelago de Santana could be due to the island habitat. The distance to the coast renders genetic exchange for this location possibly more difficult. In addition, the sites of *C. fluminensis* at the coast are more restricted to the south. The banding pattern of the sample taken from Saquarema could be related to the site-specific subsoil properties, characterized by a higher moisture compared to the other restinga sites and, in consequence, a close-fitting marsh vegetation (Cirne and Scarano 2001). Furthermore, at this site *C. fluminensis* is not a dominating shrub but grows in the underwood, because the humid soil favours trees and bigger shrubs.

7.2.3 *Clusia hilariana*

C. hilariana Schlecht. occurs in the Restinga de Jurubatiba National Park near Macaé, at Iquipari near Campos de Goitacazes, and at Arraial do Cabo (Fig. 7.1). These sites are located in the eastern part of the state of Rio de Janeiro and include mostly regions where *C. fluminensis* does not occur. Both species demonstrate a spatial separation with an overlapping area at Arraial do Cabo. *C. hilariana* is the highest shrub in the restinga, forms the crown of the vegetation islands, and delivers the shade for germination and growth of other plants. The area at Macaé consists of dune walls with valleys in between.

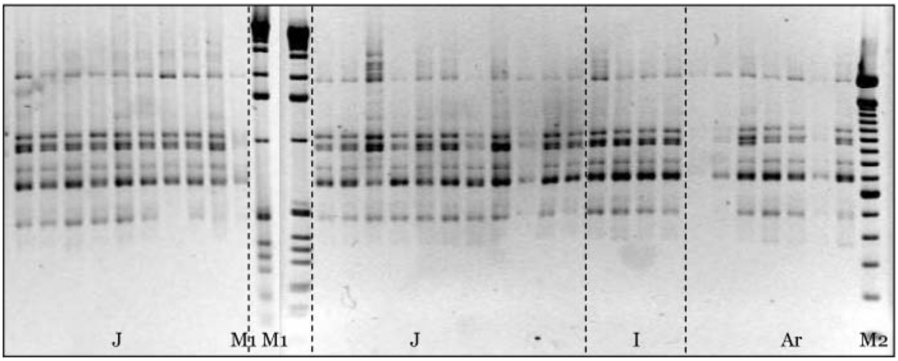


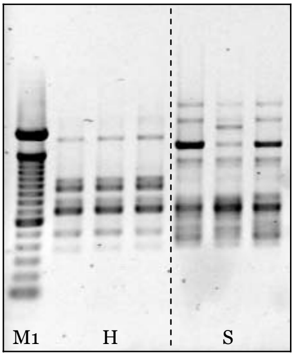
Fig. 7.7. Banding pattern of samples of *C. hilariana* using the primer AAG_8 (J, Restinga de Jurubatiba National Park; I, Iquipari near Campos de Goitacazes; Ar, Arraial do Cabo, taken in 2001). M1 shows a 1-kb, M2 a 100-bp size marker ladder

The CAM performing plant, *C. hilariana*, grows at the dry and hot dunes, while the C_3-CAM-intermediate plant, *C. parviflora*, which uses C_3 photosynthesis in this area, occurs in the valleys, providing better water supply. Again, we wanted to find out, whether the diverse habitats could have created ecotypes, which could be identified by SSR. With regard to dune walls with variable distances to the ocean, samples were taken from the first, second and fourth dune (on the third dune, *Clusia* did not occur). Using the primer AAG_8, the banding pattern was identical for all samples (Fig. 7.7). The same was true for the other primers used in this investigation. We thus conclude that the sites were of such high similarity (all represent dry restinga on a sandy soil) that only one ecotype has evolved.

7.3 Relationship of *Clusia spiritu-sanctensis* to other *Clusia* Species

The species *C. spiritu-sanctensis* G. Mariz et Weinberg was discovered not long ago and thus the phylogenetic classification is not yet completely clear. This species has a strong morphological similarity to *C. hilariana*. A comparison of the banding pattern of *C. spiritu-sanctensis* with that of the other species (primer AAG_8) indicated specific relationships. Compared to *C. hilariana*, there were many differences in the banding pattern (Fig. 7.8), and only one fragment looked identical while each of the other fragments were found only in one species. In contrast, *C. spiritu-sanctensis* and *C. fluminensis* displayed largely homologous banding patterns (Fig. 7.9). The dominating fragments with a size of 450, 700 and approximately 2000 bp existed in each of the samples, as well as a weaker fragment with a size of 1000 bp and two fragments of 1300 bp. Similar data were obtained with the other primers. This could be

Fig. 7.8. Banding pattern of samples of *C. hilariana* and *C. spiritu-sanctensis* using the primer AAG_8 (H, *C. hilariana*; S, *C. spiritu-sanctensis* from Iquipari near Campos de Goitacazes). M1 shows a 100-bp size marker ladder



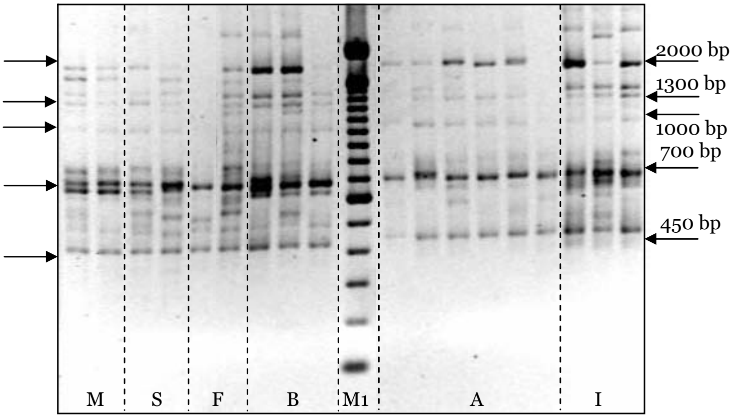


Fig. 7.9. Banding pattern of samples of *C. fluminensis* and *C. spiritu-sanctensis* using the primer AAG_8 (*C. fluminensis*: M, Maricá; S, Saquarema; F, Figueira; B, Buzios; A, Arraial do Cabo; I, *C. spiritu-sanctensis* from Iquipari near Campos de Goitacazes). M1 shows a 100-bp size marker ladder. Differences in the banding pattern are marked by *arrows*

taken as evidence that both species are closely related or even identical, which is in accordance with conclusions from a comparative sequence analysis of the ITS region as well as other molecular studies (Gustafsson and Bittrich 1999, 2002).

7.4 Conclusion

Population studies of different *Clusia* species along the coast of the state of Rio de Janeiro using short tandem repeat primers indicate a fragmentation of the *C. parviflora* populations, which may have been caused by the distance between the two sites as well as by the ecological differences between the two habitats. *C. fluminensis* exhibited differences between the diverse populations, but it is less clear whether this can be assigned to either the spatial separation of the habitats or the site specific conditions. *C. hilariana* in contrast, showed no SSR variations between the populations. This could be the result of still functional exchange of genetic information, or to an only recent disruption of this exchange.

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