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River dynamics shape clonal diversity and genetic structure of an Amazonian understorey herb

Matthias Schleuning[1]*, Thomas Becker[1,2], Giovana P. Vadillo[3,4], Thomas Hahn[4,5], Diethart Matthies[1] and Walter Durka[4]

[1]*Plant Ecology, Department of Biology, University of Marburg, Karl-von-Frisch Str. 8, 35043 Marburg, Germany;* [2]*Department of Ecology and Ecosystem Research, Albrecht von Haller Institute for Plant Sciences, University of Goettingen, Untere Karspuele 2, 37073 Goettingen, Germany;* [3]*Faculty of Biological Sciences, National University of San Marcos (UNMSM), Venezuela Av. 34, Lima 1, Peru;* [4]*Department of Community Ecology (BZF), UFZ-Helmholtz Centre for Environmental Research, Theodor-Lieser Str. 4, 06120 Halle, Saale, Germany; and* [5]*ITES Ecosystem Management, ETH Zurich, Universitaetsstr. 16, 8092 Zurich, Switzerland*

Summary

1. Clonal herbs are an important feature of the understorey of Amazonian forests. However, little is known about the environmental drivers determining the population genetics of these herbs and about the spatial scale of gene flow.

2. With amplified fragment length polymorphism markers, we analysed the clonal diversity and genetic structure of a clonal Amazonian herb (*Heliconia metallica*) in south-eastern Peru at two spatial scales. First, we sampled leaves in 24 patches differing in flooding frequency and canopy openness in 2 km^2 of a floodplain forest, and second in 21 riverine populations from the Andean foothills to the Amazon lowlands along a 550-km stretch of river.

3. At the small spatial scale in the floodplain forest, the clonal diversity of *H. metallica* was higher at flooded than at non-flooded sites, but clonal diversity did not increase with canopy openness.

4. At the large spatial scale, clonal diversity was very low in riverine populations at up- and downriver sites, suggesting that seedling recruitment was higher at mid-altitudes where the flooding intensity is intermediate. Genetic diversity of riverine populations monotonously increased downriver, indicating unidirectional gene flow mediated by hydrochory.

5. Genetic differentiation among riverine populations was very low (F_{ST} = 0.06) and followed an isolation-by-distance pattern, indicating a stepping-stone type of gene flow by seeds. Despite the much smaller spatial scale, genetic differentiation among patches in the floodplain forest was higher (F_{ST} = 0.16), due to spatially restricted gene flow in the forest understorey.

6. *Synthesis*. The genetic structure of *H. metallica* is the result of seedling recruitment being largely limited to flooded sites and of hydrochoric seed dispersal between populations growing on riverbanks. We conclude that river dynamics are the major determinant of the genetic structure of Amazonian plants and that largely undisturbed river systems, such as the Amazon, provide a crucial vector for gene flow, even at large spatial scales.

Key-words: AFLP markers, ecological genetics and ecogenomics, flooding regime, Heliconiaceae, hydrochory, Peru, population genetics, seed dispersal, tropical rain forest, unidirectional gene flow

Introduction

Environmental disturbance is a crucial factor shaping the demography and genetic structure of plant populations (Rusterholz, Kissling & Baur 2009; Vandepitte *et al*. 2010). In Amazonian forests, natural disturbances such as treefalls and flooding strongly affect the demography of both trees (King 2003; Stevenson 2007) and understorey plants (Svenning 2002; Schleuning, Huamán & Matthies 2008). During flooding events, water can disperse seeds of Amazonian plants over long distances (Moegenburg 2002), and thus potentially shapes the genetic structure of Amazonian plants. However, we still do

*Correspondence author. Biodiversity and Climate Research Centre (BiK-F), Senckenberganlage 25, 60325 Frankfurt (Main), Germany. E-mail: matthias.schleuning@senckenberg.de

not understand how these important environmental factors determine the genetic structure of plant populations in tropical floodplain forests.

Flooding regimes are important for many Amazonian plants: about 12% of Amazonian forests are currently affected by river dynamics and about 25% have been influenced in the recent past (Salo *et al.* 1986). In the interior of the floodplain forests, river dynamics have created a mosaic of microhabitats at different elevations above the river (King 2003; Schleuning, Huamán & Matthies 2008). Differences in flooding frequencies among microhabitats strongly affect patterns of seedling recruitment in the forest understorey (King 2003; Schleuning, Huamán & Matthies 2009). A second important process that influences demographic processes in the forest understorey is the formation of canopy gaps after treefalls (Svenning 2002; Stevenson 2007). Increased solar radiation on the forest floor results in a higher probability of flowering (Svenning 2002; Schleuning, Huamán & Matthies 2008) and of seedling recruitment (Montgomery & Chazdon 2002; Schleuning, Huamán & Matthies 2009). The effects of flooding and canopy gaps on plant demography suggest that both types of natural disturbance strongly affect the population genetics of Amazonian plants. In temperate forests, environmental gradients of light or water availability have been shown to cause differences in the genetic diversity of populations (e.g. Kudoh *et al.* 1999; Jacquemyn *et al.* 2005), but to our knowledge, no such studies have been carried out in tropical forests.

The effects of environmental disturbance on the genetic diversity of plant populations are particularly strong in clonal plants (Rusterholz, Kissling & Baur 2009; Vandepitte *et al.* 2010) as low probabilities of sexual reproduction and seedling recruitment reduce the ratio of sexually to clonally produced offspring and thus the clonal diversity of a population (Watkinson & Powell 1993). Although low rates of repeated seedling recruitment are sufficient to maintain the clonal diversity in a population (Watkinson & Powell 1993), many clonal plant species lack seedling recruitment after colonization, leading to a decrease in clonal diversity over time (Honnay *et al.* 2006). Because the genets of clonal plants are usually very long-lived (Ehrlén & Lehtilä 2002), even under environmental stress (Eriksson 1996), populations consisting of a single or a few genets can survive for a long time (Honnay & Bossuyt 2005). Although many understorey plants in tropical forests propagate clonally (Sagers 1993; Schleuning, Huamán & Matthies 2008), there are very few population genetic studies of these plants (but see Melendez-Ackerman *et al.* 2005; Cuartas-Hernández & Núñez-Farfán 2006), and no study has investigated the effects of environmental disturbance.

Dynamic river systems may not only influence the genetic diversity within populations, but also the genetic differentiation among populations (Kondo, Nakagoshi & Isagi 2009; Hu *et al.* 2010), and are excellent model systems to study the genetic structure of plant metapopulations (Tero *et al.* 2003; Jacquemyn *et al.* 2006b). However, few studies have analysed the genetic structure of Amazonian plants, and all these studies have dealt with timber trees (Russell *et al.* 1999; Lemes *et al.* 2003; de la Torre *et al.* 2008). In temperate river systems, gene flow can link plant populations over long distances due to dispersal of seeds by water (Kudoh & Whigham 2001; Fér & Hroudová 2008; Kondo, Nakagoshi & Isagi 2009). Riparian plants can exhibit a stepping-stone type of gene flow between neighbouring populations (see Tero *et al.* 2003) that can lead to patterns of isolation by distance at drift–dispersal equilibrium (Hutchison & Templeton 1999) and to low genetic divergence along the river (Hu *et al.* 2010). Because seed dispersal by water is unidirectional, the genetic diversity in populations of riparian plants is expected to increase downriver (Barrett, Eckert & Husband 1993). In contrast to gene flow along rivers, gene flow in the temperate forest understorey is usually low and divergence among populations high, which is mostly due to spatially restricted seed dispersal (Auge *et al.* 2001; Jacquemyn *et al.* 2006a; Schmidt *et al.* 2009). Other than temperate forest herbs, most understorey plants from tropical forests are bird-dispersed, but tropical understorey birds have small home ranges and usually disperse seeds over short distances (Westcott & Graham 2000).

In this study, we apply dominant amplified fragment length polymorphism (AFLP) markers to study the effects of two important environmental factors (flooding and canopy gaps) and of seed dispersal by water and by birds on the genetic structure of a clonal Amazonian understorey herb (*Heliconia metallica* Planchon & Linden ex Hooker, Heliconiaceae). *H. metallica* grows patchily in the understorey and on riverbanks of Amazonian floodplain forests (Berry & Kress 1991; Schleuning, Huamán & Matthies 2008). Clonal propagation is much more important than seedling recruitment in established populations of *H. metallica* that can be very long-lived (Schleuning, Huamán & Matthies 2008). Ramets of *H. metallica* flower mainly in canopy gaps (Schleuning, Huamán & Matthies 2008) that are visited by their trap-lining hummingbird pollinators (Schleuning *et al.* 2010). The flowers are partially self-incompatible (Schleuning *et al.* 2010). Most of the fleshy fruits of *H. metallica* are eaten by frugivorous understorey birds that regurgitate and thus disperse the seeds. However, seed dispersal in the forest understorey is spatially restricted and about 35% of the ripe fruits fall to the ground without bird handling (Schleuning, Huamán & Matthies 2009). Thus, in populations growing close to streams and rivers, primary and secondary seed dispersal by water may also be important (see also Stiles 1979). The fleshy fruits, but not the seeds of *H. metallica*, are able to float. Seeds are able to germinate without bird gut passage (Schleuning, Huamán & Matthies 2009), and seedling recruitment from the short-lived seeds is much higher at flooded than at non-flooded sites (Schleuning, Huamán & Matthies 2009). With the detailed knowledge of the natural history of *H. metallica*, we aimed at identifying the environmental drivers of the population genetics of this herb at two spatial scales. At the small scale, we sampled leaves in 24 patches in a floodplain forest along a gradient of flooding frequency and canopy openness, and at the large scale in 21 riverine populations along a 550-km stretch of river. We tested four specific hypotheses: (1) At the small spatial scale, frequently flooded patches of *H. metallica* have a higher clonal diversity than non-flooded patches. (2) The clonal diversity in

a patch increases with canopy openness. (3) At the large spatial scale, the genetic diversity of riverine populations increases downriver because of unidirectional seed dispersal by water. (4) Gene flow by hydrochory is more effective than by ornithochory and results in lower genetic differentiation among riverine populations than among patches in the floodplain forest.

Materials and methods

STUDY SYSTEM

Among the over 200 *Heliconia* spp. (Heliconiaceae), which are widespread in the understorey of neotropical forests (Berry & Kress 1991), *H. metallica* occurs from Honduras to Bolivia (Berry & Kress 1991) and is common in both riverine and levee habitats in the floodplain forests of south-eastern Peru. Like most of its congeners, *H. metallica* spreads clonally by lateral rhizomes (Schleuning, Huamán & Matthies 2008). Rhizomes produce erect above-ground shoots (ramets) of up to 3.5 m height that can develop a single terminal inflorescence. Ramets eventually die after reproduction, but new shoots are formed from the rhizome (Schleuning, Huamán & Matthies 2008). The studied populations of *H. metallica* were located in the floodplain of the Madre de Dios River in south-eastern Peru (Fig. 1). Precipitation in the study area is 2200–3000 mm year^{-1}. Precipitation increases with altitude and decreases in the lowlands towards the East (Amazon Conservation Association, unpublished data).

SAMPLING PROCEDURE

To study the small-scale effects of environmental disturbance in a floodplain forest and the large-scale patterns along the river, we collected leaf samples of *H. metallica* in populations at the small and the large spatial scale. In January 2007, we collected *c.* 2 × 2 cm of leaf tissue from the most recently formed leaf of each ramet. Before leaf removal, we thoroughly cleaned the leaf surface with tissue paper to reduce contamination by epiphyllic algae and fungi. Samples were dried and stored in plastic bags filled with silica gel.

At the small spatial scale, we sampled 24 patches with *H. metallica* along a gradient of flooding frequency and canopy openness in *c.* 2 km^2 of a floodplain forest near Manu Wildlife Center (260 m a.s.l., 12°21′20″ S, 70°42′44″ W; site 8 in Fig. 1). Sampled patches were separated by at least 70 m from each other with only individual ramets in between (mean distance between sampled patches: 650 m). We chose patches both under closed canopy and in small canopy gaps, and sampled at different elevations above the river (maximum difference in elevation *c.* 6 m), ranging in distance to the river from 100 to 1100 m. Most patches of *H. metallica* in the floodplain interior are probably rather old (Schleuning, Huamán & Matthies 2008).

In the centre of each patch, we established a regular grid of 6 × 9 m with cords and sampled leaves from 12 ramets at equal distances of 3 m. In each grid, we counted the number of vegetative and reproductive ramets of *H. metallica* as a measure of patch density (range: 0.80–4.96 ramets m^{-2}, see Table S1a in Supporting Information), and calculated the proportion of reproductive ramets (range: 0–28%). In each patch, we took four hemispherical photographs above the *H. metallica* ramets (*c.* 1.8 m above ground) with a camera with fish-eye-converter (Nikon Coolpix 4500 with FC-E8 converter, Nikon, Tokyo, Japan) mounted on a levelled tripod. With the software Gap Light Analyzer (Frazer, Canham & Lertzman 1999), we determined the percentage of open sky in each photograph (i.e. canopy openness) and calculated mean canopy openness (range: 2.8–6.8%, Table S1a). During flooding events in the rainy seasons of 2005, 2006 and 2007, we visited the patches to record whether they were flooded or not. We distinguished patches that were frequently flooded (in all years, $n = 8$), occasionally flooded (in one or two years, $n = 8$), or never flooded ($n = 8$, Table S1a). From the 24 patches sampled, we chose a large, densely populated patch for an in-depth analysis. We marked a plot of 6 × 36 m with cords, and individually mapped and sampled each ramet of *H. metallica* growing in the plot ($n = 264$ ramets). To compare the genetic similarity of ramets within clones with that among siblings, we further sampled 79 seeds from 21 fruiting ramets growing in six of the 24 patches.

At the large spatial scale, we sampled 21 riverine sites with *H. metallica* from 500 m a.s.l. (12°52′08″ S, 71°22′29″ W) to 165 m a.s.l. (12°31′46″ S, 68°54′04″ W) along a 550-km stretch of

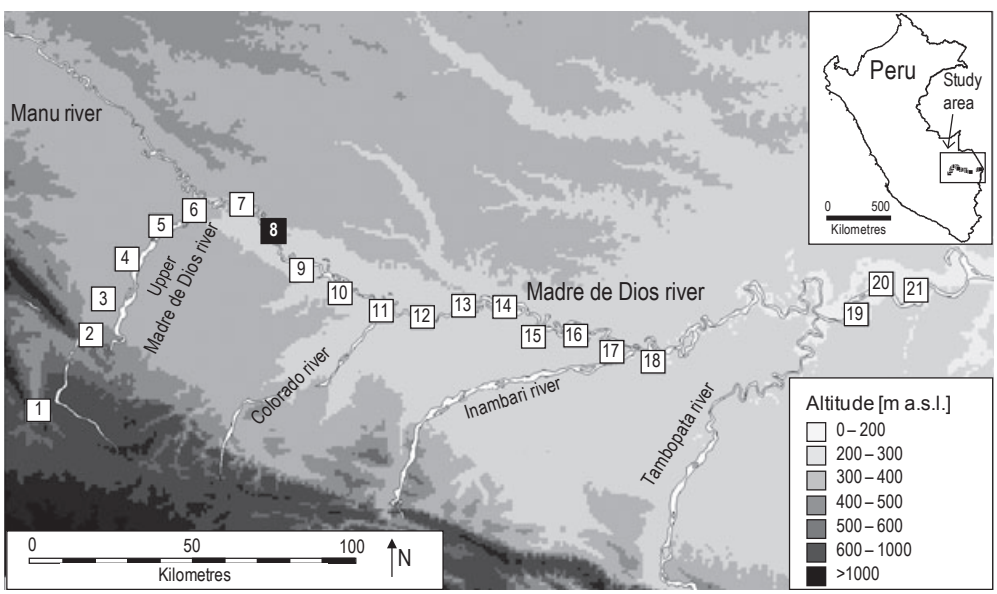

Fig. 1. Map of the study region with the location of the 21 study sites along the Madre de Dios River in southeastern Peru. Populations in the interior of the floodplain forest were studied at site 8, where 24 patches of *H. metallica* were sampled in an area of 2 km^2.

river (Fig. 1). The Upper Madre de Dios River is the altitudinal range limit of *H. metallica* in south-eastern Peru. To the East, *H. metallica* is less abundant because of a reduction in precipitation towards the savannah areas in adjacent Bolivia and Brazil. We determined the geographic coordinates and altitude a.s.l. of each site, and tracked the boat journey on the river with a GPS receiver. Track distances between sample sites were used to determine the distance between sites along the river (mean distance between neighbouring sites: 27.7 km, range: 11.6–135.0 km, Fig. 1). Distances along the river were very closely correlated with direct geographic distances ($r = 0.997$, Mantel-$P < 0.001$). In contrast to the floodplain patches, riverine populations grew next to the river, were linear in shape, and usually had an extension of more than 50 m; many of these populations might have established rather recently on the riverbanks. In each population, leaf samples from 12 ramets were collected at equal distances (*c.* 5 m) along a transect through the population. We counted the number of vegetative and reproductive ramets of *H. metallica* in a rectangle of 3 m width along each sample transect. We used the total number of ramets to calculate population density (range: 0.23–4.73 ramets m^{-2}, Table S1b) and also calculated the proportion of reproductive ramets for each site (range: 0–41%).

AFLP GENOTYPING

Plant genomic DNA was extracted from 10 to 15 mg of dried leaf tissue or seed, respectively, using the DNeasy 96 Plant Kit (Qiagen, Crawley, UK). Prior to DNA extraction from seeds, embryos were excised to remove the maternal tissue of the seed coat. The DNA was eluted in 50 μL of buffer AE obtaining concentrations of 16 ng $\mu L^{-1} \pm 2.5$ for leaves and 3.8 ng $\mu L^{-1} \pm 2.9$ for embryos. AFLP (Vos *et al.* 1995) analysis was performed with a modified version of the AFLP plant mapping kit protocol of Applied Biosystems (Carlsbad, CA, USA) (ABI, for details see Appendix S1).

We tested 26 primer combinations from which six were selected because of clear and scorable banding patterns: EcoRI-ACT-FAM/MseI-CTA, ACG-JOE/CAG, ACC-TAMRA/CAG, ACT-FAM/CTC, AAG-JOE/M-CTC and AAC-TAMRA/CTG. Fragment analysis was performed on an ABI Prism 310 genetic analyser with internal size standard GeneScan-500-Rox. The AFLP loci were scored with the Genographer 1.6.0 software (Benham *et al.* 1999), using fragments between 50 and 500 bp, and exported as a binary matrix. Out of a total of 625 leaf and 79 seed samples, we scored 180 fragments, of which 103 (57.2%) were polymorphic and used for the analyses. We performed replicate analyses of 16 samples to determine the reproducibility of AFLP genotyping and obtained an overall error rate of 3.1%.

IDENTIFICATION OF CLONES

In order to identify clones, i.e. genetically identical ramets, we used the method of Douhovnikoff & Dodd (2003) to distinguish clones from closely related siblings and to calculate a threshold of similarity above which ramets were considered to belong to the same genet. We determined the Jaccard similarity between the 79 siblings from the 21 seed families (120 within-family comparisons), and between 92 ramets growing in the 6 × 36 m plot (4186 within-genet comparisons) because 92 of the 96 genotyped ramets in this plot belonged to the same genet. Siblings and clones were distinguished by a threshold Jaccard similarity T defined by the following equation: $T = (SD_{clones} \times mean_{sibs} + SD_{sibs} \times mean_{clones})/(SD_{sibs} + SD_{clones})$, with $mean_{sibs} = 0.728$, $SD_{sibs} = 0.058$, $mean_{clones} = 0.966$, $SD_{clones} = 0.019$, where mean and SD are the mean and standard deviation of the pairwise Jaccard similarities, respectively. The formula resulted in a threshold of $T = 0.901$, corresponding to seven mismatches. We used the function clone of AFLPDAT (Ehrich 2006) to define clonal phenotypes and detected 190 genets in floodplain patches and riverine populations. Each genet occurred only in a single population, and more than half of the genets (57%) occurred only once.

CLONAL AND GENETIC DIVERSITY

To estimate intrapopulation clonal diversity, we determined clonal diversity R as $R = (G - 1)/(n - 1)$, where G is the number of genets and n is the number of sampled ramets (Dorken & Eckert 2001), as well as the complement of the Simpson index D, and the evenness E based on the Simpson index (Arnaud-Haond *et al.* 2007). The different measures of clonal diversity (G, R, D) were closely correlated ($r > 0.89$), and spatial variation in clonal diversity was only analysed in terms of clonal diversity R.

Analyses of genetic diversity were performed at the genet level and restricted to populations that contained at least four genets, because estimators of genetic variation strongly depend on sample size. We calculated Nei's gene diversity, H_e, and the percentage of polymorphic loci, P_m, with AFLP-SURV 1.0 (Vekemans *et al.* 2002), estimating allele frequencies with the square-root method assuming an inbreeding coefficient of $F = 0.0$. H_e and P_m were closely correlated ($r = 0.73$) and genetic diversity among riverine populations was compared in terms of percentage of polymorphic loci. In the floodplain interior, genetic diversity was not analysed, because 15 of 24 patches consisted of less than four genets.

In the floodplain interior, we tested the effect of flooding regime on the clonal diversity in the 24 patches with ANOVA and partitioned the overall flooding effect into two orthogonal contrasts of flooded vs. non-flooded, and frequently vs. occasionally flooded patches. Moreover, we used simple regression analyses to investigate the effects of canopy openness, patch density, and proportion of reproductive ramets on clonal diversity. At the large spatial scale, we used the river distances between neighbouring sample sites to determine the position of each population along the river. The position of the population at the altitudinal range margin of *H. metallica* was set to zero. We carried out simple regression analyses to test the effects of river position, population density, and proportion of reproductive ramets on clonal diversity. Because of the lower abundance of *H. metallica* on the riverbanks of up- and downriver sites, we expected a hump-shaped effect of river position on clonal diversity and incorporated an additional quadratic term of river position into the regression model. Furthermore, we analysed the effects of river position, population density, and proportion of reproductive ramets on genetic diversity. To adjust for the different number of genets in the populations, we additionally included the number of genets in multiple regression models.

We examined the normality of the residuals of all linear models and angular-transformed clonal diversity R to improve model residuals. Angular transformation of the proportion of reproductive ramets and of the proportion of polymorphic loci was not necessary. Analyses were carried out with R 2.10.1 (R Development Core Team 2009).

GENETIC STRUCTURE WITHIN AND AMONG POPULATIONS

In order to detect whether floodplain patches and riverine populations showed a small-scale genetic structure, we analysed the spatial

genetic autocorrelation of ramets and genets with the program SPAGeDI (Hardy & Vekemans 2002), pooling data from the two spatial scales. We used the relationship coefficient for dominant markers, defined by Hardy (2003), as a measure of genetic relatedness. To determine spatial coordinates, we used sampling positions along transects or sampling grids for the ramets and centroid coordinates for the genets.

To estimate genetic differentiation among populations, we calculated Nei's genetic distance (Nei 1972) and *F*-statistics (Wright 1951), incorporating only populations that contained at least four genets. Both measures were calculated with AFLP-SURV 1.0 following the approach of Lynch & Milligan (1994), estimating allele frequencies with the square-root method at $F = 0.0$. Genetic population structure was further investigated by analyses of molecular variance (AMOVA) with Arlequin 3.0 (Excoffier, Laval & Schneider 2005). In order to test for isolation by distance among populations, we analysed whether Nei's genetic distances between pairs of floodplain patches or riverine populations were related to geographic distances or to river distances, respectively, using Mantel-tests (10 000 iterations, vegan package 1.17-2 in R 2.10.1, Oksanen *et al*. 2010). In addition, we applied a Bayesian approach to cluster individuals into 'panmictic' phylogroups using BAPS 5.2 (Corander, Sirén & Arjas 2008). We ran both a non-spatial and a spatial population mixture analysis with the maximal number of groups, *K*, ranging from 1 to 20 with three replicates that identified the most likely partitioning according to marginal log-likelihood.

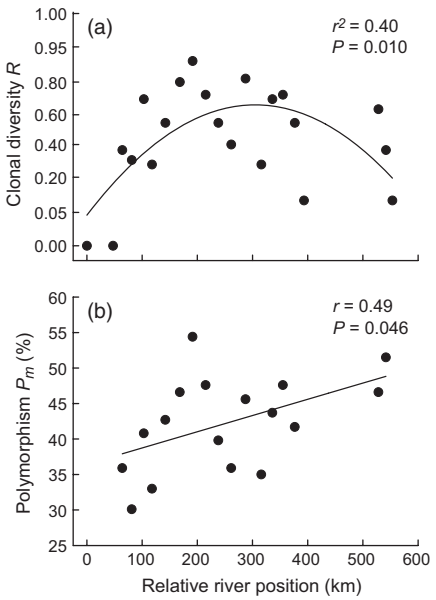

Fig. 3. Effect of the position of a population on the Madre de Dios River on (a) clonal diversity in 21, and (b) percentage polymorphism in 17 populations of *H. metallica*. River distances between populations were used to estimate the river position of each population; population 1 was set to zero. Note angular scale for clonal diversity *R*.

Results

CLONAL AND GENETIC DIVERSITY

Clonal diversity *R* differed strongly among patches in the interior of the single floodplain forest studied (mean: 0.16, range: 0–0.46, Table S1a). Consistent with our first hypothesis, *R* was much higher in flooded than in non-flooded patches ($F_{1,22} = 20.9$, $P < 0.001$, Fig. 2), but frequently flooded and occasionally flooded patches did not differ ($F_{1,14} = 0.07$, $P = 0.791$). In contrast to the second hypothesis, *R* did not increase with canopy openness ($r = -0.12$, $P = 0.563$). Moreover, clonal diversity was not influenced by patch density ($r = -0.15$, $P = 0.496$) or the proportion of reproductive ramets ($r = -0.13$, $P = 0.532$).

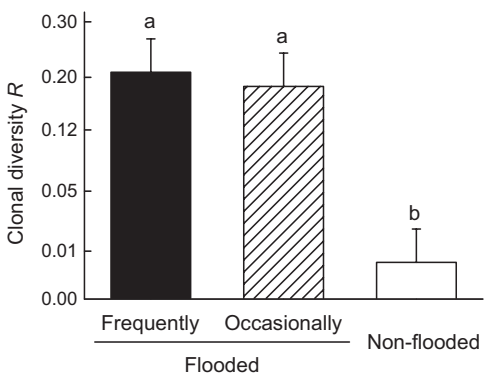

Fig. 2. Effects of different flooding regimes on the clonal diversity in 24 patches of *H. metallica* in a floodplain forest. Different letters indicate significant differences between flooding regimes in a *post hoc* test (Tukey, $P < 0.01$); note angular scale for clonal diversity *R*.

In riverine populations, mean clonal diversity *R* was 0.47 (range: 0–0.90), Simpson's index *D* 0.74 (range: 0–0.98), and Evenness *E* 0.56 (range: 0–1, Table S1b). The effect of river position on clonal diversity *R* was best described by a quadratic regression ($r^2 = 0.40$, $P = 0.010$, Fig. 3a), indicating that clonal diversity *R* was highest in populations at the middle river course and lower in up- and downriver populations. Clonal diversity *R* was not related to population density ($r = 0.31$, $P = 0.174$) or the proportion of flowering ramets in a population ($r = 0.02$, $P = 0.936$).

The average of Nei's gene diversity H_e for riverine populations was 0.22 (range: 0.20–0.26) and of percentage of polymorphism P_m 42.3% (range: 30.1–54.4%, Table S1b). In line with our third hypothesis, percentage polymorphism monotonously increased downriver ($r = 0.49$, $P = 0.046$, Fig. 3b). We found an increase in genetic diversity even when we adjusted for the number of genets in a population ($\beta = 0.40$, $t = 2.94$, $P = 0.011$), and when we removed two outlier populations that strongly affected the partial regression coefficient ($\beta = 0.18$, $t = 2.28$, $P = 0.042$). Percentage polymorphism P_m was not related to population density ($r = 0.19$, $P = 0.459$) or the proportion of flowering ramets of *H. metallica* ($r = -0.40$, $P = 0.115$); incorporating the number of genets in a multiple model did not change the results qualitatively.

GENETIC STRUCTURE WITHIN AND AMONG POPULATIONS

The analysis of spatial genetic autocorrelation over all samples revealed a significant spatial structure at both the ramet and genet level. Relationship coefficients of genets decreased within

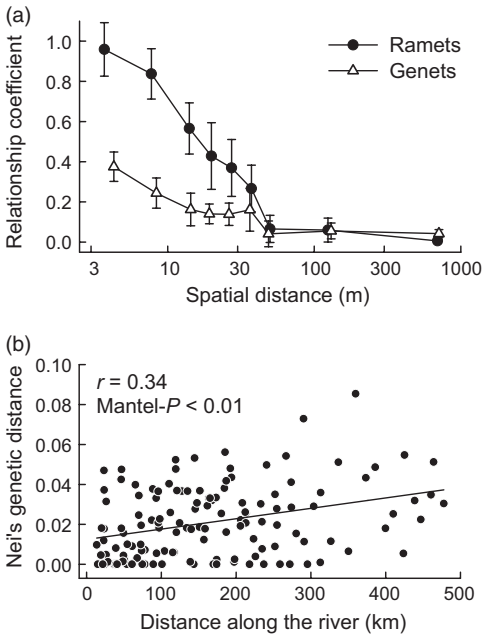

Fig. 4. Isolation by distance within and between populations of *H. metallica*. (a) Spatial autocorrelogram of relationship coefficients of ramets and genets over all populations. Mean relationship coefficients and their 95% confidence intervals are given; note log-scale for spatial distance. (b) Nei's genetic distances between pairs of 17 riverine populations in relation to distance along the river.

patches (at distances < 10 m), suggesting that gene flow is spatially very restricted. Spatial autocorrelation declined steeper for ramets than for genets (Fig. 4a). At distances < 10 m, relationship coefficients of ramets were very high, but dropped to levels similar to those of genets at distances > 50 m. Accordingly, a genet never occurred in more than one population, and ramets belonging to the same genet extended over a maximum distance of 55 m.

F-statistics indicated that 16% of the genetic variation resided among patches in the floodplain forest ($F_{ST} = 0.158$, SE $= 0.075$, $P < 0.001$), but only 6% among riverine populations at the large spatial scale ($F_{ST} = 0.062$, SE $= 0.231$, $P < 0.001$). Nei's genetic distances between pairs of populations were higher between patches in the floodplain forest ($0.051 \pm 95\%$ CI 0.011) than between riverine populations ($0.021 \pm 95\%$ CI 0.003). The higher differentiation within the floodplain than along the river is consistent with our fourth hypothesis suggesting that gene flow by ornithochory is less effective than by hydrochory. Genetic distances were not related to geographic distances in the floodplain interior ($r = 0.035$, Mantel-$P > 0.40$), but increased with distance along the river among riverine populations ($r = 0.338$, Mantel-$P < 0.01$, Fig. 4b).

The Bayesian cluster analysis at the genet level revealed four gene pools (log marginal likelihood -6853, Fig. S1 in Supporting Information), that where rather closely related with pairwise Nei's distances ranging from 0.029 to 0.143. Gene pools 1 and 2 where present both along the entire river and in the floodplain forest with most populations being a mixture of both pools. Pools 3 and 4 were almost exclusively restricted to several (11) or a few (2) floodplain patches, respectively. In contrast to the non-spatial analysis, only a single gene pool was found in the spatially informed cluster analysis (log marginal likelihood -7001), underlining the low genetic divergence among populations.

Discussion

ENVIRONMENTAL EFFECTS ON CLONAL AND GENETIC DIVERSITY

Flooding, but not canopy openness strongly affected the clonal diversity of *H. metallica*. Clonal diversity was much higher at flooded than at non-flooded sites, because seedling recruitment of *H. metallica* is largely limited to flooded sites (Schleuning, Huamán & Matthies 2009). Our study supports the conclusion from a demographic study that non-flooded populations are maintained only by means of clonal propagation (Schleuning, Huamán & Matthies 2008). These populations could be remnant populations that have survived for several decades or even centuries after their sites were no longer flooded (Schleuning, Huamán & Matthies 2008) and may have lost their clonal diversity over time. Alternatively, non-flooded populations could have been established by a single colonization event during an extreme flood and experienced strong founder effects. Although not only flooding, but also the formation of canopy gaps, increases seedling recruitment (Schleuning, Huamán & Matthies 2009), gaps did not affect clonal diversity. This might be due to the ephemeral character of small canopy gaps that increase light availability in the understorey of tropical forests only for about 2 years (Smith, Hogan & Idol 1992; Schleuning, Huamán & Matthies 2008).

In temperate understorey herbs, environmental conditions strongly affect the clonal diversity of populations, but effects depend on the environmental driver and the plant species considered. High soil moisture increases seedling recruitment and clonal diversity of *Paris quadrifolia* (Jacquemyn *et al.* 2005), while human trampling decreases clonal diversity of *Anemone nemorosa* (Rusterholz, Kissling & Baur 2009). Canopy gaps affect clonal diversity both positively (Kudoh *et al.* 1999) and negatively (Vandepitte *et al.* 2010), depending on the responses of sexual and clonal offspring to the increase in light availability (Matlaga & Horvitz 2009; Vandepitte *et al.* 2010). The results from our study suggest that flooding influences the genetic structure of plant populations in Amazonian floodplain forests more strongly than ephemeral canopy gaps.

The position along the river influenced the clonal diversity of riverine populations of *H. metallica*. In contrast to populations along the middle course, upriver and downriver populations only consisted of a single or a few different genets. Accordingly, the overall abundance of *H. metallica* was lower on the riverbanks in the Andean foothills and in the Eastern lowlands. Differences in both clonal diversity and overall abundance could be due to differences in flooding regimes along the river because the flow velocity strongly decreases from the Andean foothills to the Eastern lowlands (Goulding

et al. 2003), providing suitable intensities of flooding for seedling recruitment mainly at mid-altitudes (see Schleuning, Huamán & Matthies 2009). Alternatively, changes in soil properties or longer drought periods in the Eastern lowlands could limit the distribution of *H. metallica* (see Schleuning, Huamán & Matthies 2008). A lower clonal diversity at the distributional margins is consistent with previous studies reporting that sexual reproduction and seedling recruitment decrease at the range margins of plant species (Eckert 2001; Jump & Woodward 2003; Beatty *et al*. 2008).

The genetic diversity of *H. metallica* increased downriver. To some extent, this could be the consequence of rare seedling recruitment in upriver populations, resulting in low frequencies of multilocus genotypes in these populations. However, in our analyses, we only included populations with at least four genets and statistically accounted for different numbers of genets per population. Moreover, differences in seedling recruitment cannot explain the high-genetic diversity in downriver populations where clonal diversity was comparatively low. The monotonous increase of genetic diversity in riverine populations of *H. metallica* is rather consistent with the hypothesis that unidirectional gene flow leads to an accumulation of genetic diversity in downstream populations (Barrett, Eckert & Husband 1993). In the Madre de Dios river system, gene flow by hydrochoric seed dispersal from lowland tributaries (Colorado, Inambari and Tambopata River, see Fig. 1) could further contribute to the accumulation of genetic diversity in downriver populations. Increasing genetic diversity downriver was found in several plant species of temperate river systems (Lundqvist & Andersson 2001; Liu, Wang & Huang 2006; Fér & Hroudová 2008; Chen *et al*. 2009; Pollux *et al*. 2009), but was not evident in many others (Lundqvist & Andersson 2001; Imbert & Lefèvre 2003; Tero *et al*. 2003; Hu *et al*. 2010). According to these studies, the relative contribution of dispersal systems other than hydrochory and the importance of gene flow by pollen determine the distribution of genetic diversity along river systems. In *H. metallica*, gene flow by ornithochorous pollen and seed dispersal seems to be far less important than water-mediated seed dispersal downriver. Therefore, we assume that hydrochory is an important driver of the population genetic structure of plant species growing along rivers with strong dynamics, such as the Amazon and its tributaries.

SPATIAL GENETIC STRUCTURE AT DIFFERENT SPATIAL SCALES

We found that the extension of genets of *H. metallica* was up to 50 m and that those genets were much larger than the genets of understorey herbs of temperate forests (Kudoh *et al*. 1999; Jacquemyn *et al*. 2005; Vandepitte *et al*. 2010). Genets of *H. metallica* could be many decades or even centuries old because rhizome growth, in particular at closed-canopy sites, is rather slow (maximum of 65 cm year^{-1}; Schleuning, Huamán & Matthies 2008). Nevertheless, relationship coefficients of ramets of *H. metallica* dropped to background levels at distances > 50 m, indicating that genets were patchily distributed and colonization of new sites was exclusively due to the recruitment of seedlings and not to the dispersal of clonal propagules.

In the one floodplain forest studied, the genetic differentiation among patches of *H. metallica* was pronounced (F_{ST} = 0.16). Patches clustered into four different gene pools, although all were situated within a small area of 2 km^2 of a floodplain forest. Strong genetic differentiation has also been found in studies of clonal herbs of temperate forests (Auge *et al*. 2001; Jacquemyn *et al*. 2006a). In tropical floodplain forests, gene flow by pollen or seeds appears to be low. Recruitment of seedlings of *H. metallica* in the floodplain mostly occurs adjacent to reproductive ramets indicating that ornithochorous seed dispersal of *H. metallica* is spatially restricted (Schleuning, Huamán & Matthies 2009). However, in *H. metallica*, pollen flow between patches is likely to occur and flowers of solitary ramets receive pollen of high quality from distant sources (Schleuning *et al*. 2010). This is not surprising because hermit hummingbirds, the predominant pollinators of *H. metallica*, forage along regularly followed routes (i.e. trap lines) of up to 1 km length (Stiles & Wolf 1979). Nevertheless, pollen flow between patches of *H. metallica* is apparently not sufficient to prevent the formation of a strong spatial genetic structure in the floodplain.

The lack of replication of the small-scale study of spatial genetic structure of *H. metallica* could affect the generality of our findings. Detailed sampling was only feasible in a single floodplain forest where trails provided easy access to the forest interior and where flooding frequencies were monitored over a 3-year period. However, the study site might be representative for many other sites along the middle course of Amazonian rivers where spatially extended floodplain forests have been formed along river meanders (Salo *et al*. 1986; Foster 1990). Although each floodplain may be unique in its flooding history, in water and soil chemistry and in the relative proportions of currently flooded and non-flooded areas (Salo *et al*. 1986), these forests share a heterogeneous micro-topography, due to variation in elevation above the river at a small spatial scale (Foster 1990; King 2003). Moreover, they harbour similar pollinator and frugivore bird communities in the forest understorey (Robinson & Terborgh 1990). Thus, we assume that the spatial genetic structure of *H. metallica* is similarly strong in other Amazonian floodplain forests.

Genetic differentiation among riverine populations of *H. metallica* (F_{ST} = 0.06) was much lower than among the patches in the one floodplain forest (F_{ST} = 0.16). Differences in population age can contribute to these differences because recently founded populations of clonal plants are less differentiated from each other than old populations (Jacquemyn *et al*. 2006b). In *H. metallica*, floodplain populations are presumably older than riverine populations that might have rather recently established on the riverbanks. More importantly, in plants that are dispersed by water, like *H. metallica*, gene flow is likely to be higher in habitats close or adjacent to the water flow (Kudoh & Whigham 2001; Hu *et al*. 2010). Consistent with this conclusion, in *H. metallica* almost all riverine populations clustered into two closely related gene pools, indicating

frequent genetic exchange among populations. Furthermore, pairwise genetic distances between riverine populations increased with distance along the river, suggesting a stepping-stone type of gene flow by hydrochoric seed dispersal. The accumulation of genetic diversity in downriver populations reflects the same processes. In the same Peruvian river system, a stepping-stone type of hydrochory-mediated gene flow has been suggested for the floodplain tree *Cedrela odorata* (de la Torre *et al.* 2008). We propose further studies to investigate whether this type of gene flow is typical for Amazonian plants and whether the genetic structure of Amazonian plants differs in general between floodplain and riverine habitats.

Conclusions

The population genetic structure of the Amazonian understorey herb *H. metallica* is the result of seedling recruitment being largely limited to flooded sites and of clonal propagation after colonization. Because of higher seedling recruitment, the clonal diversity of *H. metallica* was higher at flooded than at non-flooded sites, but the formation of ephemeral canopy gaps did not increase clonal diversity. In the one floodplain forest studied, founder effects and genetic drift shape the population genetic structure more than gene flow because seed dispersal is spatially restricted and seedling recruitment rare, resulting in strong genetic divergence at a small spatial scale. In contrast, gene flow is rather high between riverine populations, probably due to frequent events of hydrochoric seed dispersal between neighbouring populations. This results in increasing genetic diversity downriver and low genetic divergence among riverine populations. We conclude that river dynamics are the major determinant of the genetic structure of Amazonian plants and that largely undisturbed river systems, such as the Amazon, provide a crucial vector for gene flow, even at large spatial scales.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. A description of the AFLP genotyping procedure.

Fig. S1. Results from a non-spatial Bayesian cluster analysis with BAPS (Corander, Sirén & Arjas 2008) based on genets of *H. metallica*.

Table S1. A table with important environmental predictors and with the estimates of clonal and genetic diversity of *H. metallica* in 24 patches in a floodplain forest, and in 21 riverine populations.

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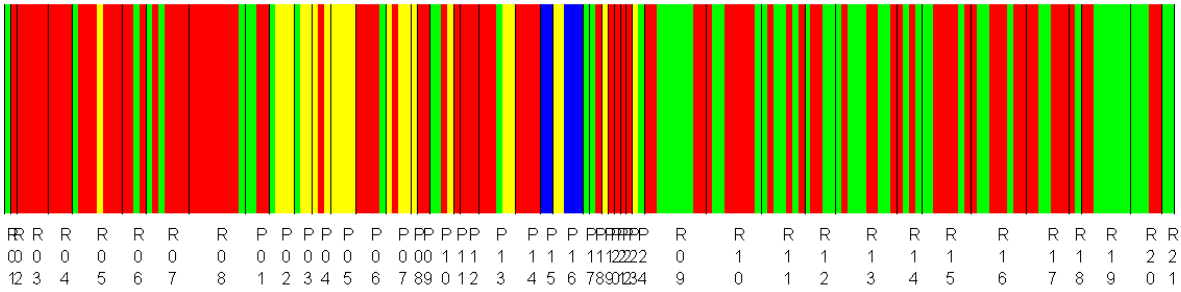
Figure S1*Non-spatial Bayesian cluster analysis with BAPS (Corander*et al. *2008) based on genets of* H. metallica*. Four different gene pools were identified (cluster 1: red, 2: green, 3: yellow, 4: blue). Genets are represented by vertical bars, populations are separated by black lines. Riverine populations are ordered by position along the river (R01−R21), with the floodplain patches (P01− P24) situated near population R08.*



Reference

Corander, J., Sirén, J. &Arjas, E. (2008) Spatial modelling of genetic population structure. *Computational Statistics*, **23**, 111–129.

Table S1*Clonal and genetic diversity of* H. metallica *in (a) 24 patches in a floodplain forest, and (b) in 21 riverine populations along 550kmof river*.n*: sample size;* G*: number of genets;* R*: clonal diversity;* D*: complement of Simpson index;* E*: clonal evenness;* P_m*: percentage of polymorphic loci;* H_e*: gene diversity;*P_m *and* H_e*are given for populations with >4 genets.*

(a) Floodplain patches

Patch	Flooding	Canopy openness [%]	Ramets per m²	*n*	*G*	*R*	*D*	*E*	P_m	H_e
P01	Frequent	4.01	3.89	10	4	0.33	0.71	0.59	35.0	0.231
P02	Frequent	3.48	4.78	12	4	0.27	0.46	0.00	30.1	0.199
P03	Frequent	3.57	1.69	12	3	0.18	0.32	0.00	-	-
P04	Frequent	6.07	2.28	12	3	0.18	0.62	0.74	-	-
P05	Frequent	3.45	1.26	12	4	0.27	0.56	0.29	24.3	0.175
P06	Frequent	3.78	2.31	12	5	0.36	0.73	0.51	28.2	0.180
P07	Frequent	2.93	3.00	12	4	0.27	0.71	0.71	29.1	0.206
P08	Frequent	3.42	1.26	12	1	0.00	0.00	-	-	-
P09	Occasional	4.07	1.94	11	2	0.10	0.55	0.99	-	-
P10	Occasional	4.27	1.28	12	4	0.27	0.56	0.29	41.7	0.249
P11	Occasional	3.77	1.28	12	1	0.00	0.00	-	-	-
P12	Occasional	3.73	2.19	12	3	0.18	0.67	0.85	-	-
P13	Occasional	3.46	1.35	12	6	0.46	0.68	0.00	36.9	0.205
P14	Occasional	2.83	0.80	12	4	0.27	0.65	0.54	26.2	0.199
P15	Occasional	4.04	2.15	12	2	0.09	0.17	0.00	-	-
P16	Occasional	3.58	1.30	12	5	0.36	0.79	0.71	36.9	0.223
P17	Non	2.94	1.81	12	1	0.00	0.00	-	-	-
P18	Non	3.01	1.56	12	2	0.09	0.17	0.00	-	-
P19	Non	3.31	1.80	12	1	0.00	0.00	-	-	-
P20	Non	3.99	3.50	12	1	0.00	0.00	-	-	-
P21	Non	6.75	3.30	10	1	0.00	0.00	-	-	-
P22	Non	3.08	2.43	12	1	0.00	0.00	-	-	-
P23	Non	4.10	4.96	12	1	0.00	0.00	-	-	-
P24	Non	3.46	1.30	12	2	0.09	0.30	0.36	-	-

(b) Riverine populations

Site	Altitude [ma.s.l.]	Ramets per m²	*n*	*G*	*R*	*D*	*E*	P_m	H_e
R01	496	2.17	12	1	0.00	0.00	-	-	-
R02	407	0.23	12	1	0.00	0.00	-	-	-
R03	362	0.83	12	5	0.36	0.73	0.51	35.9	0.221
R04	324	1.08	11	4	0.30	0.78	0.87	30.1	0.202
R05	301	2.29	11	8	0.70	0.93	0.51	40.8	0.199
R06	285	1.63	12	4	0.27	0.80	0.96	33.0	0.224
R07	268	3.75	12	7	0.55	0.91	0.84	42.7	0.217
R08	260	1.47	11	9	0.80	0.95	0.00	46.6	0.230
R09	252	1.34	11	10	0.90	0.98	0.00	54.4	0.260
R10	240	4.73	12	9	0.73	0.94	0.50	47.6	0.221
R11	231	4.27	12	7	0.55	0.89	0.75	39.8	0.224
R12	226	3.47	11	5	0.40	0.82	0.76	35.9	0.212
R13	219	3.66	12	10	0.82	0.95	0.00	45.6	0.217
R14	214	0.92	12	4	0.27	0.65	0.54	35.0	0.240
R15	209	4.66	11	8	0.70	0.95	0.76	43.7	0.213
R16	201	3.69	12	9	0.73	0.95	0.75	47.6	0.235
R17	196	2.44	12	7	0.55	0.86	0.56	41.7	0.222
R18	189	3.37	12	2	0.09	0.17	0.00	-	-
R19	182	1.84	12	8	0.64	0.94	0.86	46.6	0.214
R20	167	1.10	12	5	0.36	0.73	0.51	51.5	0.264
R21	166	3.77	12	2	0.09	0.55	1.00	-	-

Appendix S1 *A description of the AFLP genotyping procedure.*

For the restriction of the genomic DNA and ligation of the adapters, 6 µl DNA solution (*ca.* 60–120 ng DNA) were added to 5 µl of restriction-ligation (RL) reaction mix. The RL reaction mix contained 0.05 µl H_2O (HPLC-grade, Merck), 0.55 µg BSA (New England Biolabs, NEB), 1.1 µl 0.5 M NaCl, 5 U *EcoR*I (NEB), 1 U *Mse*I (NEB), 1.1 µl T4 DNA ligase buffer (NEB), 67 U T4 DNA ligase (NEB), 50 pmol*Mse*I-adapters and 5 pmol*Eco*RI adapters. Restriction-ligation was carried out at room temperature overnight.

For the following preselective amplification (PCRI), 4 µl of 1:5 diluted RL product was combined with 16 µl of PCR I reaction mix. When DNA from seeds was analysed, the RL product was not diluted. The reaction mix contained 30 ng of *Mse*I- and *EcoR*Ipreselective primers each, 4 pmoldNTPs (Roth, Germany), 2 µl (10x) PCR buffer + $(NH_4)_2SO_4$ (Fermentas, Germany), 30 pmol $MgCl_2$ (Fermentas), 0.8 U Taq polymerase (Fermentas) and 9.64 µl H_2O. The thermocycler protocol started with 72.0°C for 2 min followed by 20 cycles of 94.0°C (0.2 min), 56.0°C (0.3 min) and 72.0°C (2 min) and a final step at 60.0°C for 30 min. PCRs were performed with an EPPENDORF Mastercycler gradient.

Prior to the selective amplification (PCRII) the PCRI product was diluted 1:10 with H_2O. For the selective PCR, 1 µl of the dilution was added to 5 µl of AFLP Amplification Core Mix (Applied Biosystems, Foster City) and 3.0 and 0.6 pmol of *Mse*I and *EcoR*I selective primers respectively. The selective amplification started with 94.0°C for 2 min followed by 10 cycles of 94.0°C (0.2 min), 66.0°C (0.3 min, decreasing 1°C per cycle) and 72.0°C (2 min) and 20 cycles of 94.0°C (0.2 min), 56.0°C (0.3 min) and 72.0°C (2 min), and a final step at 60.0°C for 30 min.