

Spatial structure of genetic variation and primary succession in the pioneer tree species *Antirhea borbonica* on La Réunion

I. LITRICO,*† J. RONFORT,‡ R. VERLAQUE§ and J. D. THOMPSON*

*UMR 5175 Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, 1919 Route de Mende, 34293 Montpellier cedex 5, France, †UMR C53, Peuplement végétal et bioagresseurs en milieu tropical, Université de La Réunion, 7 Chemin de L'Irat, 97417 Saint Pierre, La Réunion, ‡UMR 1097, Diversité et génome des plantes cultivées, INRA- SGAP, Chemin de Mezouls 34130 Mauguio, France, §UMR 6116, Institut Méditerranéen d'Ecologie et de Paléocécologie CNRS — Université de Provence, Centre St-Charles, Laboratoire de Biosystématique et Ecologie méditerranéenne, 3 place Victor Hugo, 13331 Marseille cedex 3, France

Abstract

In habitats where colonization and extinction are recurrent, the distribution of gene frequencies among patches of suitable habitat may reflect the age structure of different populations. In this study, we quantify population genetic structure for a pioneer tree species, *Antirhea borbonica*, in a chrono-sequence of primary succession on the lava flows of the Piton de La Fournaise volcano (La Réunion). Using microsatellite loci and amplified fragment length polymorphism (AFLP) markers, we quantified genetic variation within and among populations for early- and late-succession populations in a landscape where extinction and recolonization are recurrent (the 'Grand Brûlé') and for late-succession populations in a more stable landscape. This study produced three main results. First, we detected no evidence that founder events increase genetic differentiation among colonizing populations; F_{ST} values among early- and among late-succession populations were similar. Second, we found no evidence for isolation by distance; genetic distance was not correlated with spatial distance within and among populations. Third, F_{IS} values are consistently high in all populations, despite the fact that *A. borbonica* populations are functionally close to dioecy and thus expected to have an outcrossing mating system. Multiple colonization events from different sources may limit differentiation among young populations and spatial isolation may enhance differentiation among late-succession populations. Ecological processes acting during colonization may create the conditions for spatial aggregation within pioneer populations, and thus contribute to the high F_{IS} values.

Keywords: dioecy, island, metapopulation, primary succession, Walhund effect

Received 20 September 2004; revision received 6 January 2005; accepted 20 January 2005

Introduction

The ecological processes of migration and colonization are crucial to species survival and can have a profound impact on the spatial organization of genetic structure within and among natural populations (Husband & Barrett 1996). There is thus a long tradition of work on the genetic consequences of different colonization dynamics (Slatkin 1977, 1993; Wade & McCauley 1988; Whitlock & McCauley 1990; Austerlitz *et al.* 1997; Le Corre & Kremer 1998). Indeed, data on the spatial genetic structure of populations

provide key information for reconstructing the history of migration and colonization and for our knowledge of the persistence and evolutionary potential of a species in a new environment (e.g. Hewitt 1996; Petit *et al.* 1997).

Common to all organisms, episodes of colonization can strongly affect levels of genetic differentiation among plant populations. Two particular situations illustrate this theme. First, where seeds colonize isolated sites (a new continental region or an island) as a result of long-distance dispersal, the colonizing population is immediately isolated from source populations. Genetic divergence can thus occur as a result of local founder events and/or the action of natural selection (Barrett 1996). Second, if colonization and extinction occur repeatedly and the habitat is ephemeral

Correspondence: Isabelle Litrico, Fax: 00 33 4 67 41 21 38; E-mail: isabelle.litrico@cefe.cnrs.fr

or short-lived, that is in species of open, disturbed or early-succession habitats, the distribution of gene frequencies among patches of suitable habitat may reflect the age structure of different populations (McCauley 1991). Such situations provide an illustration of metapopulation dynamics where subpopulations are interconnected during episodes of colonization. Unfortunately, the age structure of many plant metapopulations is difficult to ascertain (Husband & Barrett 1996; McCauley *et al.* 2001).

Colonization events frequently involve the establishment of small populations; hence, only a small sample of the genetic variability in the source population may be represented in the colonist population. This founder event may cause newly colonized sites to show higher differentiation than that observed among older populations which experience more gene flow (Whitlock & McCauley 1990; McCauley *et al.* 1995; Giles & Goudet 1997). The amounts of variability within colonist populations and levels of differentiation among them, relative to that among source populations, will however, depend on the mode of group formation. In the propagule pool model (colonist population formed from a single source), extinction and recolonization increase differentiation among colonist populations, whereas in the migrant pool model (several source populations potentially contribute to the colonization event), this may not be the case (Slatkin 1977; Wade & McCauley 1988; McCauley 1991). The spatial structure of genetic variation will thus depend not only on the genetic relatedness of founder individuals and the dispersal mode of the species in question but also on the relative rates of dispersal and arrival at a site, on the number and source of colonists, and on the number of habitat patches (McCauley 1991).

Gradients of primary succession in which species colonize early successional stages and persist into late succession represent a potential illustration of age structure similar to that proposed for metapopulation dynamics, with the advantage that the history of extinction and colonization episodes is often fairly well known. On the island of La Réunion (Indian Ocean), the Piton de La Fournaise volcano regularly, at the rate of roughly one lava flow per year (Bachelery, personal communication), emits lava flows on the southeast slopes of the island, mostly into the caldera or Grand Brûlé. In this area, each lava flow is spatially distinct and lined by forest habitat. The recurrent nature of lava flows and the spatial pattern of population development and succession following extinction and colonization in the landscape of the Grand Brûlé have produced a matrix of different-aged vegetation communities. In this area, the natural lowland tropical rainforest occurs as fragmented pockets of habitat in a landscape of different-aged lava flows which represent a chrono-sequence of primary succession and whose ages (for those that have occurred in the last 300 years) are well known (Cadet 1977; Strasberg 1994). There is no seed bank on new lava; the

sources of colonization of individual lava flows are the fragments of forest that have been spared by lava flows (Thébaud & Strasberg 1997).

The purpose of this study is to quantify the structure of genetic variation among and within populations of a pioneer tree species *Antirhea borbonica* J.F. Gmel (Rubiaceae) which occurs in early- and late-succession populations within the Grand Brûlé and in other more stable communities outside of the region of intense volcanic activity on La Réunion. Using a combination of microsatellite loci and amplified fragment length polymorphism (AFLP) markers, we examine the following questions. First, is genetic differentiation among colonist (early succession) populations greater than that among older, late-succession populations? Second, does genetic distance increase with spatial distance within or among populations? Third, does the colonization process impact on genetic structure within young populations? The response to these questions provides novel information on the spatial pattern of genetic variation in a natural metapopulation whose age structure is associated with an ecological gradient of primary succession.

Materials and methods

Study species and population sampling

Antirhea borbonica ($2n = 44$, Verlaque, unpublished) is a woody tree species endemic to La Réunion and Mauritius. It is an abundant pioneer species on young lava flows on La Réunion, and persists into late succession where it maintains populations in climax rainforest (Strasberg 1996). *A. borbonica* produces small, tubular, white flowers which are pollinated by a range of insects (Lepidoptera, Diptera and Hymenoptera). Natural populations are functionally dioecious (Litrico *et al.*, unpublished), hence, the mating system is likely to be highly outcrossing (this species does not reproduce by vegetative means). Its reddish fleshy fruits are dispersed by birds, mostly native *Zosterops* spp. and introduced *Pycnonotus jocosus*. On La Réunion, *A. borbonica* is a frequent colonizer of young lava flows, that is early-succession habitats (some *c.* 25 years old), and is one of the most common native species in the Grand Brûlé (Strasberg 1994) where nearly all lava flows descend from the caldera towards the sea (Fig. 1). This species maintains populations in late succession where trees may be > 150 years old.

The study region (Fig. 1) is located on the islands of La Réunion and Mauritius in the Indian Ocean. Populations were sampled across the entire distribution of the study species. Thirteen populations were sampled on La Réunion (Fig. 1), in two main types of situation which differ dramatically in terms of rates of population turnover. First, nine of these populations occur inside the Grand Brûlé on the southeast flanks of the Piton de la Fournaise volcano at

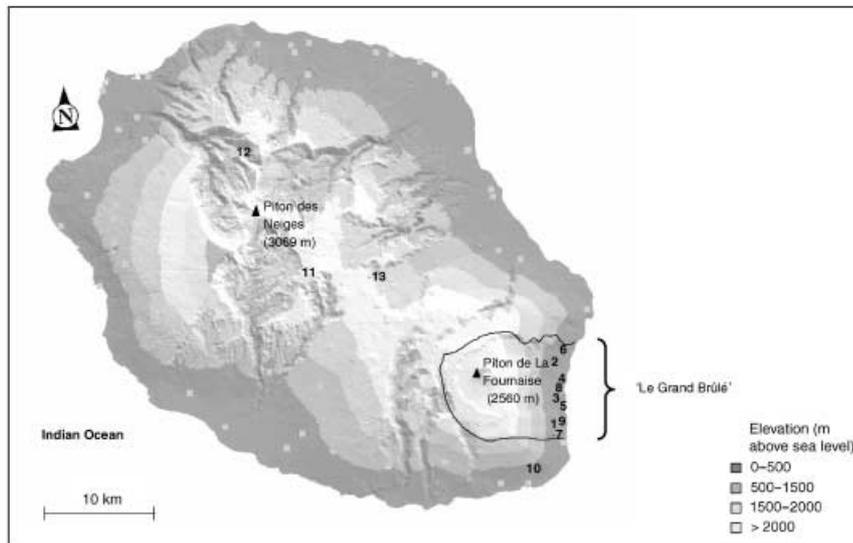


Fig. 1 The location of study sites of *Antirhea borbonica* on La Réunion. See text and Table 1 for details of each site and sample sizes.

< 300 m elevation, in a highly disturbed landscape where population extinction and recolonization are frequent. Of these nine populations, six (sites 1–6) are early-succession populations and three (sites 7–9) are late-succession populations (forest fragments spared by lava flows during the last 150 years). All the populations occur on independent lava flows of different ages except the three populations on the 1943 lava flow which nevertheless occur on different arms of this lava flow. All the early-succession populations are thus independent colonization events. The distances among these populations vary from 1 to 2 km to around 5 km. Second, four populations (sites 10–13) were sampled outside the Grand Brûlé in less fragmented climax forest: one (site 10) at < 300 m elevation in the only remaining piece of natural lowland tropical rain forest on La Réunion outside of the Grand Brûlé and three (sites 11–13) at 1000–1500 m elevation away from the active caldera. In addition, one population (site 14) was also sampled on the sister island of Mauritius (c. 300 km from La Réunion), in the Pétrin natural reserve. Sites 11–14 occur in a more stable landscape where volcanic activity ceased several thousand years ago.

A total of 366 individuals were sampled for genetic analysis in 2004 (Table 1). Trees of a roughly similar diameter at breast height were randomly sampled in a given population over an area of c. 2500 m². In eight populations (sites 1, 3, 4, 5, 7, 8, 10 and 13), the spatial location of all sampled trees was mapped. This was not done in all populations because of difficulties associated with accurately mapping individuals in dense vegetation. A 4-year study of the 13 populations on La Réunion has shown that they contain females and polliniferous plants with a sex ratio close to 1:1 except in one population (Litrico *et al.* unpublished). Sexual reproduction occurs every year in all populations, hence there is the potential for pollen and seed dispersal.

Microsatellite markers: procedure and analysis

Individuals were genotyped for seven of the 10 polymorphic microsatellite loci described in Litrico *et al.* (2004). Because of the probable occurrence of null alleles at loci *AntbT6F*, *AntbT10B*, and *AntbT7C*, these loci were excluded from the analysis. Plant DNA was extracted from leaves using the QIAmp Plant Kit (QIAGEN). Amplification conditions were for each locus in 20 µL including 0.1 µL of each 10 µM fluorescent-labelled primer and unlabelled primer, 2 µL of 10X manufactured reaction buffer, 2 µL of 25 mM MgCl₂, 0.3 µL of 20 mM dNTP mix, 0.08 µL of 5 units/µL *Taq* DNA polymerase (Eurogentec Red Gold Star) and 1 µL of genomic DNA. Polymerase chain reaction (PCR) was performed in the following conditions: 2 min of denaturation at 94 °C, 35 cycles of 30 s of initial denaturation at 94 °C, 30 s of annealing at suitable temperature (Litrico *et al.* 2004), 35 s of extension at 72 °C, and 5 min of elongation at 72 °C. One microlitre of diluted (1–20) PCR products was pooled in 15 µL of deionized formamide and 0.2 µL of Genescan-400XLROX size standard and analysed on an ABI PRISM3100 automated sequencer (Applied Biosystems). Subsequent analysis was carried out using GENESCAN analysis version 3.7 (Applied Biosystems). Fourteen individuals were replicated to check repeatability.

We calculated the mean number of alleles per locus (n_a), Nei's unbiased expected heterozygosity (H_E) (Nei 1978), observed heterozygosity (H_O) & Wright's F_{IS} statistic according to Weir & Cockerham (1984) using GENETIX version 4.04 (Belkhir *et al.* 2001). The test for departure from Hardy-Weinberg equilibrium was conducted using 1000 permutations in each population using GENETIX version 4.04 (Belkhir *et al.* 2001). Spearman rank correlation coefficients were calculated to test for a relationship between F_{IS} and sample

Table 1 Mean (\pm SE) number of alleles per locus (N_a), Nei's diversity (H_E), observed heterozygosity (H_O), F_{IS} , the number of polymorphic AFLP bands (N_B) and the Shannon diversity index (I_S) from mature individuals in populations of *Antirhea borbonica*. Sites 1–6 are early succession populations in the Grand Brûlé, sites 7–14 are late-succession populations in the Grand-Brûlé (7–9), outside of Le Grand Brûlé (10–13) and on Mauritius (14)

Site number	Date of lava flow	Successional status	Microsatellite loci					AFLP markers			
			Sample size	N_a	H_E	H_O	F_{IS}	Sample size	N_B	H_E	I_S
1	1976	early	29	3.85	0.480 (0.2386)	0.363	0.26***	19	88.39	0.227 (0.1668)	0.360 (0.2238)
2	1961	early	29	3.82	0.445 (0.1678)	0.379	0.16***	19	94.84	0.276 (0.1621)	0.426 (0.2100)
3	1943	early	23	4.00	0.460 (0.3694)	0.290	0.38***	19	85.81	0.256 (0.1645)	0.396 (0.2252)
4	1943	early	26	3.57	0.425 (0.1842)	0.338	0.22***	12	87.74	0.246 (0.1601)	0.386 (0.2166)
5	1943	early	28	2.20	0.339 (0.2310)	0.202	0.42***	14	72.90	0.167 (0.1512)	0.275 (0.2197)
6	1931	early	21	2.83	0.478 (0.1838)	0.306	0.38***	11	74.84	0.214 (0.1726)	0.335 (0.2437)
7	100–300 years	late	25	2.85	0.452 (0.1971)	0.331	0.28***	15	87.10	0.213 (0.1448)	0.346 (0.2011)
8	100–300 years	late	30	3.14	0.417 (0.1960)	0.280	0.34***	16	88.39	0.231 (0.1516)	0.369 (0.2066)
9	100–300 years	late	30	3.42	0.410 (0.2293)	0.336	0.19***	20	91.61	0.244 (0.1590)	0.385 (0.2125)
10	c. 500 years	late	22	3.14	0.455 (0.2382)	0.261	0.44***	9	74.84	0.219 (0.1744)	0.342 (0.2448)
11	> 500 years	late	22	2.71	0.408 (0.2625)	0.311	0.25***	13	87.74	0.215 (0.1555)	0.346 (0.2108)
12	> 500 years	late	24	3.14	0.417 (0.1941)	0.217	0.49***	16	90.32	0.251 (0.1485)	0.397 (0.2004)
13	> 500 years	late	26	2.57	0.370 (0.1846)	0.256	0.32***	23	85.16	0.240 (0.1648)	0.376 (0.2260)
14	> 500 years	late	31	3.28	0.379 (0.2119)	0.302	0.21***	20	90.32	0.272 (0.1666)	0.417 (0.2231)

*** $P < 0.001$.

size. For eight populations (sites 1, 3, 4, 5, 7, 8, 10 and 13), spatial autocorrelation analyses were conducted using regressions of kinship coefficients (Loiselle *et al.* 1995) and geographical distances which were tested using a permutations test using the software from Hardy & Vekemans (2002).

F -statistics were calculated according to the Weir & Cockerham procedure (1984) and tested using 1000 permutations of individuals among populations or groups (GENETIX version 4.04, Belkhir *et al.* 2001). In order to test for isolation by distance, correlations between genetic distances (measured as F_{ST}) and the spatial distance between pairs of populations were calculated and tested, following the Mantel permutation procedure (GENETIX version 4.04, Belkhir *et al.* 2001). Also, in accordance with Rousset (1997), we conducted a multilocus regression $F_{ST}/(1 - F_{ST})$ estimate over the logarithmic transformation of the geographical distance for all pairs of populations. These regressions were computed using SPAGED1 (Hardy & Vekemans 2002). Assignment tests were performed on 366 individuals genotyped according to Bayesian method of Rannala & Mountain (1997) and with the Distance method developed by Nei *et al.* (1983) using the GENECLASS package (Piry *et al.* 2004).

AFLP markers: procedure and analysis

The AFLP protocol we used is that described by Vos *et al.* (1995). Plant DNA was extracted from leaves using the DNeasy Plant Mini Kit (QIAGEN). After digestion of 40 ng d'DNA with *EcoRI* (Biolabs) and *MseI* (Biolabs), adaptors

were ligated on both ends of genomic fragments and the preamplification was performed in 15 μ L including 5 μ L of diluted (1/10) DNA ligated, 1.5 μ L of 10 \times manufactured reaction buffer, 1.5 μ L of 25 mM $MgCl_2$, 0.7 μ L of 10 mM dNTP mix, 0.1 μ L of 5 units/ μ L *Taq* DNA polymerase (Eurogentec Red Gold Star), 0.7 μ L of each 5 μ M unlabelled primer. The selective PCR amplification was performed in the same conditions with the selective primer pair: fluorescent-labelled *EcoRI*-GACTGCGTACCAAAATCCG – and unlabelled *MseI*: ATGAGTCCTGAGTAAC (selective bases are in bold letters). Pre-amplification was for 2 min at 72 $^{\circ}C$, 2 min of denaturation at 94 $^{\circ}C$, 25 cycles of 30 s of initial denaturation at 94 $^{\circ}C$, 30 s of annealing at 56 $^{\circ}C$, 2 min of extension at 72 $^{\circ}C$, and 10 min of elongation at 72 $^{\circ}C$. Selective amplification was for 5 min of denaturation at 95 $^{\circ}C$, 30 cycles of 30 s of initial denaturation at 94 $^{\circ}C$, 1 min of annealing at 56 $^{\circ}C$, 1 min of extension at 72 $^{\circ}C$, and 10 min of elongation at 72 $^{\circ}C$. The selective amplification products were analysed on an ABI 3100 automated sequencer (Applied Biosystems). AFLP patterns were visualized with GENESCAN Analysis 3.7 (Applied biosystems), and the loci were typed as present or absent (i.e. above or below a threshold intensity). One individual per population was replicated.

Within-population estimates of genetic diversity were calculated using the POPGENE software version 1.32 (Yeh & Boyle 1997) incorporating F_{IS} values calculated for microsatellite loci. We calculated the number of polymorphic bands (N_B), Nei's expected heterozygosity (Nei 1973) (H_E) and Shannon's information index (I_S) (Lewontin 1972).

Among-population estimates of differentiation (F_{ST}) were calculated following Lynch & Milligan (1994) by integrating the overall F_{IS} value estimated from microsatellite loci. We performed 1000 random permutations of individuals among populations to assess genetic differentiation using AFLP-SURV software (Vekemans, X. 2002. AFLP-SURV version 1.0). In order to test for isolation by distance, correlations between genetic (measured as F_{ST}) and spatial distances between pairs of populations were calculated and tested following the Mantel permutation procedure (GENETIX version 4.04, Belkhir *et al.* 2001). In addition, we conducted a multilocus regression $F_{ST}/(1 - F_{ST})$ (Rousset 1997) estimate over log-transformation distance for all pairs of populations. These regressions were computed using SAS (2001) for AFLP markers.

Independence of markers

Given the outcrossing mating system of the study species, we did not expect to detect high levels of linkage disequilibrium. We nevertheless tested the possibility of linkage disequilibrium based on microsatellite markers within each population. Tests were made for each pair of loci by performing 1000 permutations using GENETIX version 4.04 (Belkhir *et al.* 2001). Linkage disequilibrium within populations was detected at the 5% level for 12 of the 294 possible tests for the microsatellite loci studied here. This value was less than that expected by chance at the 5% level (14.7).

In addition, for AFLP markers, to remove any bias introduced by the fact that two bands may appear for a given locus, we calculated the rates of among band association with the DDM (disequilibrium for dominant markers) software version 0.1 (Berthier, personal communication). DDM calculates, for each pair of markers (A , B), the mean distance D between the allelic values of two markers, where n is the number of individuals, $v(A,i)$ the allelic value (0 or 1) of individual i for the marker A :

$$D_{\text{mean}}(A,B) = \frac{1}{n} \sum_{i=1}^n |v(A,i) - v(B,i)|$$

To avoid redundancy, when the pair of markers had $D_{\text{mean}} < 0.05$ or > 0.95 , one was removed from the data set. From the 184 polymorphic AFLP fragments that could be used, 29 were removed ($D_{\text{mean}} < 0.05$ or > 0.95) from the data set prior to analysis. Analyses were thus conducted on a total of 155 polymorphic AFLP markers.

Results

Genetic diversity within populations: microsatellite markers

The mean number of alleles per locus for microsatellite markers was 7.14 and the mean number of alleles per locus

and per population varied from 2.2 to 4 (Table 1). Nei's expected heterozygosity values ranged from 0.339 to 0.481, that is values which are fairly low for microsatellite markers. Departures from Hardy-Weinberg equilibrium were observed in all populations. All F_{IS} values were high and significantly greater than zero (Table 1). There was no difference between F_{IS} values in early-succession (0.16–0.42) and late-succession (0.19–0.49) populations. Sample sizes per population were significantly correlated with F_{IS} values ($R_S = -0.56$, $P < 0.05$). However, this correlation is barely significant ($P = 0.044$) and only 29% of the variation is explained by the regression. In fact, this correlation appears to be the result of one single population whose high $F_{IS} = 0.49$ value is associated with a small sample size ($n = 24$). If this data point is removed, the correlation is nonsignificant, hence the consistently high F_{IS} values are unlikely to be resulting from small sample sizes.

We found no evidence for a negative relationship between spatial distance and kinship coefficients within populations as, for a given spatial distance, the kinship coefficient between pairs of individuals had highly variable values in all tested populations (site 1, $P > 0.1$; site 3, $P > 0.1$; site 4, $P > 0.5$; site 5, $P > 0.5$; site 7, $P > 0.1$; site 8, $P > 0.5$; site 10, $P > 0.5$; site 13, $P > 0.5$).

Genetic diversity within populations: AFLP markers

Nei's expected heterozygosity values ranged from 0.168 to 0.276 for AFLP markers. Shannon diversity indices ranged from 0.275 to 0.426.

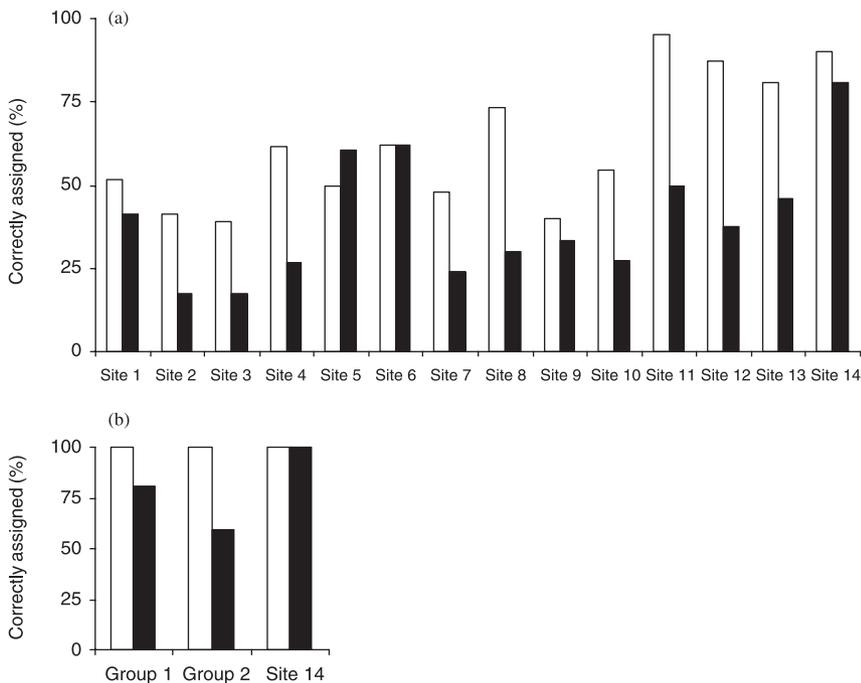
Genetic differentiation among populations: microsatellite markers

Microsatellite loci showed significant genetic differentiation among all populations ($F_{ST} = 0.10$; $P < 0.001$) and all populations on La Réunion ($F_{ST} = 0.09$; $P < 0.001$). Genetic differentiation among the nine populations located inside the Grand Brûlé was significant ($F_{ST} = 0.04$, $P < 0.0001$), but less than that among the four late-succession populations located outside of the Grand Brûlé ($F_{ST} = 0.19$, $P < 0.001$). Within the Grand Brûlé, genetic differentiation among early-succession (sites 1–6) populations ($F_{ST} = 0.04$, $P < 0.001$) was similar to that among late-succession (sites 7–9) populations ($F_{ST} = 0.04$, $P < 0.001$) (Table 2). Hierarchical analyses showed only slight, albeit significant ($F_{ST} = 0.0052$, $P < 0.05$), differentiation among early- and late-succession populations in the Grand Brûlé.

Differentiation values based on assignment tests produced similar results. Population assignment showed that 228 of 366 (62.3%) individuals were accurately assigned with the Bayesian method and 146 of 366 (39.9%) with the Distance method (Fig. 2a). With the Bayesian and Distance methods, wrongly assigned individuals had a fairly equal

Table 2 Pairwise F_{ST} values (nonsignificant estimates are in italic) estimated from mature individuals among populations of *Antirhea borbonica* on La Réunion and Mauritius based on polymorphic microsatellite loci (upper matrix) and AFLP (lower matrix) markers

Site number	Date of lava flow	Successional status	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1976	early	—	0.007	0.037	0.012	0.043	0.101	0.008	0.050	0.022	0.090	0.086	0.134	0.071	0.138
2	1961	early	0.018	—	0.020	0.019	0.062	0.096	0.005	0.049	-0.002	0.112	0.082	0.127	0.122	0.148
3	1943	early	0.105	0.066	—	0.035	0.055	0.095	0.007	0.052	0.007	0.067	0.052	0.119	0.041	0.074
4	1943	early	0.106	0.065	0.066	—	0.001	0.101	0.007	0.058	0.029	0.076	0.105	0.148	0.061	0.152
5	1943	early	0.292	0.218	0.148	0.139	—	0.167	0.043	0.050	0.046	0.118	0.076	0.116	0.033	0.166
6	1931	early	0.397	0.324	0.322	0.296	0.382	—	0.091	0.064	0.103	0.022	0.144	0.213	0.124	0.127
7	100–300 years	late	0.220	0.152	0.078	0.093	0.045	0.327	—	0.051	0.012	0.088	0.090	0.129	0.072	0.121
8	100–300 years	late	0.292	0.217	0.018	0.153	0.182	0.114	0.145	—	0.052	0.129	0.116	0.174	0.132	0.173
9	100–300 years	late	0.328	0.250	0.244	0.211	0.287	0.034	0.242	0.045	—	0.130	0.089	0.127	0.140	0.164
10	c. 500 years	late	0.084	0.068	0.081	0.060	0.234	0.361	0.175	0.235	0.283	—	0.217	0.228	0.102	0.108
11	> 500 years	late	0.106	0.068	0.048	0.028	0.140	0.326	0.092	0.175	0.240	0.048	—	0.181	0.180	0.245
12	> 500 years	late	0.144	0.095	0.054	0.036	0.061	0.309	0.042	0.134	0.220	0.097	0.029	—	0.248	0.276
13	> 500 years	late	0.385	0.306	0.315	0.289	0.383	0.052	0.328	0.132	0.047	0.344	0.324	0.313	—	0.286
14	> 500 years	late	0.103	0.070	0.080	0.032	0.223	0.318	0.151	0.203	0.241	0.047	0.075	0.960	0.306	—

**Fig. 2** Percentage of individuals correctly assigned (a) into each population or (b) into populations inside the Grand Brûlé (group 1), outside the Grand Brûlé on La Réunion (group 2) or the population on Mauritius (site 14), using the Bayesian (open bars) and Distance methods (closed bars).

probability of being assigned to a range of different populations. When assignment tests were performed using populations grouped according to their location either within the Grand Brûlé (group 1), outside of the Grand Brûlé on La Réunion (group 2) or on Mauritius (site 14) as sources for assignment, either 100% (Bayesian method) or 76.8% (Distance method) of individuals were correctly assigned (Fig. 2b).

Microsatellite markers showed no evidence for isolation by distance. The Mantel permutation test for a relation between F_{ST} and spatial distance produced a nonsignificant

result ($Z = 2358.32$; $P > 0.05$), as did the multilocus regression of F_{ST} over $1 - F_{ST}$ ($P > 0.05$).

Genetic differentiation among populations: AFLP markers

Amplified fragment length polymorphism markers produced similar results to those for microsatellites, with an $F_{ST} = 0.19$ ($P < 0.001$) among all populations (La Réunion and Mauritius), $F_{ST} = 0.19$ ($P < 0.001$) among all populations on La Réunion, $F_{ST} = 0.20$ ($P < 0.001$) among all populations in the Grand Brûlé, and $F_{ST} = 0.22$ ($P < 0.001$) among late-succession

populations outside the Grand Brûlé (Table 2). Inside the Grand Brûlé, genetic differentiation among early-succession (sites 1–6) populations ($F_{ST} = 0.20$, $P < 0.001$) was similar to that among late-succession (sites 7–9) populations ($F_{ST} = 0.15$, $P < 0.001$). The higher values obtained with AFLP markers relative to microsatellite markers may reflect their dominant character which may cause them to overestimate patterns of differentiation (Isabel *et al.* 1999; Kravtsovskii *et al.* 1999).

There was no evidence shown by AFLP markers for isolation by distance. The Mantel permutation test for a relation between F_{ST} and spatial distances among populations gave a nonsignificant result ($Z = 3485.89$; $P > 0.1$), as did the multilocus regression of F_{ST} over $1 - F_{ST}$ ($P > 0.1$).

Discussion

Our study of the spatial genetic structure of populations of the pioneer tree species *Antirhea borbonica* in early- and late succession populations on La Réunion has produced two key results. First, genetic differentiation among early-succession populations is similar to that among late-succession populations. Second, F_{IS} values are high in all populations, despite the fact that this species should be highly outcrossing. Our discussion is focused on how different ecological processes associated with colonization and establishment during primary succession and the mating system of this species contribute to these results.

No enhanced differentiation among pioneer populations

Within the Grand Brûlé, where population extinction and colonization are recurrent features of the population biology of all native species, estimates of genetic differentiation among early-succession populations are similar to those among late-succession populations of *A. borbonica*. This result suggests that founder effects during colonization do not cause enhanced divergence among colonist populations, as reported previously in herbaceous pioneer populations of *Silene* species in primary (Giles & Goudet 1997) and secondary (McCauley *et al.* 1995) successions and in tree species (Richardson & Higgins 1998; for review: Petit *et al.* 2004). The low levels of differentiation among populations in the Grand Brûlé, combined with the results of the assignment tests, indicate that the process of colonization of individual lava flows may involve different source populations located around the newly formed lava flows. Such multiple colonization events will be facilitated by the proximity of source fragments of forests to new lava flows. Indeed, ecological studies of recolonization on the lava flows of the Grand Brûlé have shown that a wave of gradual colonization from the different margins towards the centre of new lava occurs for most species, including *A. borbonica* (Thébaud & Strasberg 1997; Prugnolle 1999).

The slightly higher variation in levels of genetic differentiation among the late-succession populations outside of the Grand Brûlé may be the result of several causes, such as greater spatial isolation, independent colonization of different habitats on the slopes of both the older and now extinct Piton des Neiges volcano and the flanks of the Piton de la Fournaise (Fig. 1), biotype depletion during succession (Gray 1993), and disturbance caused by cyclonic activity. Our study does not allow us to distinguish among these alternatives.

A final result of interest here is that the population on Mauritius does not appear to be more genetically distinct from populations on La Réunion than are the latter among themselves. This result strongly suggests that the colonization of La Réunion (which was formed at c. 3 Ma) was from Mauritius (formed at c. 8 Ma) and that this colonization did not involve a strong founder effect on diversity. Once again our results attest to the lack of a strong founder event, even after long-distance colonization.

Why are F_{IS} values consistently high?

A key result of this study is that F_{IS} values are significant in all populations, despite functional dioecy (Litrico *et al.*, unpublished) and thus a predominantly outcrossing mating system. This would suggest that high F_{IS} values are not the result of inbreeding. It may be driven by the ecological processes associated with dispersal and colonization which may create localized spatial structure within populations. Indeed, previous work on the spatial distribution of *A. borbonica* has shown significant spatial aggregation within both early and late succession populations (Prugnolle 1999; Litrico 2001; J. Gilles *et al.*, unpublished).

The joint effects of dispersal limitation, facilitation, and nucleation can significantly affect the spatial pattern of colonization of open areas by fleshy-fruited species (Debussche & Lepart 1992; Debussche & Isenmann 1994; Herrera *et al.* 1994) and herbaceous and woody species which establish pioneer populations in situations of primary succession (Wood & Del Moral 1987; Drake & Mueller-Dombois 1993). As we now discuss, our study provides a potential illustration of how the processes associated with dispersal limitation, facilitation and nucleation may shape the genetic structure of colonist populations in primary succession.

First, the development of spatial population genetic structure may be enhanced by dispersal limitation. As mentioned previously, colonization of lava flows in the Grand Brûlé occurs gradually from the forest edges which border lava flows towards the centre, as witnessed by a gradual decrease in abundance of most species, including *A. borbonica*, towards the centre of recent lava flows (Thébaud & Strasberg 1997; Prugnolle 1999). In addition, fruit dispersal away from maternal trees is limited, most

fruit falling under or around the canopy (I. Litrico, personal observation). In primary succession, installation limitation may be observed over extremely short distances because of the stressful nature of the habitat (Del Moral 1993; Lichter 2000). On the lava flows of the Grand Brûlé, seed germination and seedling establishment of *A. borbonica* are likely to be strongly limited by the availability of suitable microsites. On La Réunion, new lava is chemically uniform but structurally variable (Bachelery 1981 as cited by Thébaud & Strasberg 1997), and seedling establishment during colonization is restricted to cracks and crevices which occur at low densities on otherwise barren substrates (Thébaud & Strasberg 1997; I. Litrico, personal observation).

The second is facilitation, a key element in the process of primary succession (Connell & Slatyer 1977; Callaway *et al.* 1990; Bertness & Callaway 1994), which probably enhances spatial aggregation of pioneer plants on the lava flows. On the barren surface of new lava, percolation is rapid and available resources are scarce (Wood & Del Moral 1987; Del Moral 1993; Kirman 2003). Adult plants are thus likely to facilitate seedling establishment via reduced water and nutrient stress under their canopies and in their litter.

Third, in early succession populations, seedling dispersal of *A. borbonica* is spatially aggregated around other conspecific trees and trees of other species which provide perching points for birds (Litrico 2004). Such nucleation is likely to be greatly enhanced by the dioecious mating system of the study species, which means that half of the trees present in a population produce all the established seedlings under or close to their canopy.

The effects of dispersal limitation, facilitation and nucleation can lead to spatial aggregation of genetically similar (nucleation under maternal parents) or dissimilar (nucleation under other species used as perching points by dispersal agents) individuals. The results of spatial autocorrelation analyses show that for a given spatial distance, individuals can be closely related while in others they can be genetically distinct. Such a patchy genetic structure, in association with spatial aggregation, could contribute to the high F_{IS} values as a result of crosses among related individuals and a Wahlund effect during sampling.

In conclusion, our study implicates a role for different ecological process and the mating system in the development of spatial genetic structure in early succession populations of *A. borbonica*. The fact that significant F_{IS} values were detected even in late succession populations suggests that the ecological processes which shape the genetic structure of pioneer populations of *A. borbonica* may have a lasting effect on the spatial distribution of genetic variation, as suggested for populations of other pioneer woody species (Herrera *et al.* 1994). We thus conclude by emphasizing the role of ecological processes involved in migration and colonization and the crucial impact of the initial dispersal and colonization events for the interpretation of spatial

patterns of genetic variation along gradients of primary succession, and thus in an age-structured metapopulation context.

Acknowledgements

We thank the CNRS, the Université de La Réunion and the Région de La Réunion for financial support. We thank Marie Maistre, Anabelle Dos Santos, Geneviève Debussche, the marker genetics teams at the Pôle de Protection des Plantes de La Réunion and at the CEFÉ-CNRS in Montpellier, particularly Emmanuelle Chapier and Emmanuel Jouen, for practical help. We thank Marie-Hélène Chevalier, Florence Nicole and Aurélie Bonin for help with software and Thierry Pailler for advice and encouragement. We are grateful to Philippe Jarne for very helpful comments on this study and two anonymous reviewers for detailed comments on previous versions.

References

- Austerlitz F, Jung-Muller B, Godelle B, Gouyon PH (1997) Evolution of coalescence times, genetic diversity and structure during colonization. *Theoretical Population Biology*, **51**, 148–164.
- Barrett SCH (1996) The reproductive biology and genetics of island plants. *Philosophical Transactions of the Royal Society of London Series B*, **351**, 725–733.
- Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) *genetix 4.02, logiciel sous Windows pour la génétique des populations*. Laboratoire Génome, Populations, Interactions; CNRS UMR 5000; Université Montpellier II, Montpellier (France).
- Bertness MD, Callaway R (1994) Positive interactions in communities. *Trends in Ecology and Evolution*, **9**, 191–193.
- Cadet T (1977) *Contribution à l'étude de la Végétation de L'île Rodrigue (Océan Indien)*. PhD Thesis, University of La Réunion.
- Callaway RM, Jones S, Ferren WRJ, Parikh A (1990) Ecology of a Mediterranean climate estuarine wetland at Carpinteria, California: plant distributions and soil salinity in the upper marsh. *Canadian Journal of Botany*, **68**, 1139–1146.
- Connell JH, Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist*, **111**, 1119–1144.
- Debussche M, Isenmann P (1994) Bird-dispersed seed rain and seedling establishment in patchy Mediterranean vegetation. *Oikos*, **69**, 414–426.
- Debussche M, Lepart J (1992) Establishment of woody plants in Mediterranean old fields: opportunity in space and time. *Landscape Ecology*, **6**, 133–145.
- Del Moral R (1993) Mechanisms of primary succession on volcanoes: a view from Mt St Helens. In: *Primary Succession on Land* (eds Miles J, Walton DWH), pp. 79–100. Blackwell Scientific, Oxford.
- Drake DR, Mueller-Dombois D (1993) Population development of rain forest trees on a chronosequence of Hawaiian lava flows. *Ecology*, **74**, 1012–1019.
- Giles BE, Goudet J (1997) Genetic differentiation in *Silene dioica* metapopulations: estimation of spatiotemporal effects in a successional plant species. *American Naturalist*, **149**, 507–526.
- Gray AJ (1993) The vascular plant pioneers of primary successions: persistence and phenotypic plasticity. In: *Primary Succession on*

- Land (eds Miles J, Walton DWH), pp. 179–191. Blackwell Scientific, Oxford.
- Hardy OJ, Vekemans X (2002) SPAGED1: a versatile computer program to analyse spatial genetic structure at the individual and population levels. *Molecular Ecology Notes*, **2**, 618–620.
- Herrera J, Jordano P, Lopez-Soria L, Amat JA (1994) Recruitment of a mast-fruited, bird-dispersed tree: bridging frugivore activity and seedling establishment. *Ecological Monographs*, **64**, 315–344.
- Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.
- Husband BC, Barrett SCH (1996) A metapopulation perspective in plant population biology. *Journal of Ecology*, **84**, 461–469.
- Isabel N, Beaulieu J, Thériault P, Bousquet J (1999) Direct evidence for biased gene diversity estimated from dominant random amplified polymorphic DNA (RAPD) fingerprints. *Molecular Ecology*, **8**, 477–483.
- Kirman S (2003) *Cycles biogéochimiques et biodiversité en forêt tropicale humide: étude d'une succession primaire sur les coulées basaltiques (La Réunion Océan Indien)*. PhD Thesis, Université de Marseille-CEREGE, Aix en Provence.
- Kruvotovskii KV, Erofeeva SY, Aagaard JE, Strauss SH (1999) Simulation of effects of dominance on estimates of population genetic diversity and differentiation. *Journal of Heredity*, **90**, 499–502.
- Le Corre V, Kremer A (1998) Cumulative effects of founding events during colonization on genetic diversity and differentiation in an island and stepping stone model. *Journal of Evolutionary Biology*, **11**, 795–812.
- Lewontin RC (1972) The apportionment of human diversity. In: *Evolutionary Biology* (eds Dobzhansky TH, Hecht MK, Steere WC), pp. 381–397. Appleton-Century-Crofts, New York.
- Lichter J (2000) Colonisation constraints during primary succession on coastal Lake Michigan sand dunes. *Journal of Ecology*, **88**, 825–839.
- Litrico I (2001) Dynamique de la re-colonisation et variation des traits biologiques et du système de reproduction chez une espèce pionnière: *Le Cas d'Antirhea Borbonica Sur les Coulées de Lave À la Réunion* Diplôme d'Etudes Approfondies. University of Montpellier II. Montpellier, France.
- Litrico I (2004) *Evolution du Genre Sexuel et de la Diversité Génétique dans Une Succession Primaire: L'étude d'Antirhea borbonica (Rubiaceae) sur les Coulées de Lave à la Réunion*. PhD Thesis, Université de la Réunion.
- Litrico I, Gilles J, Sourrouille P, Vignes H (2004) Isolation of ten polymorphic microsatellite loci from *Antirhea borbonica* (Rubiaceae). *Molecular Ecology Notes*, **4**, 593–595.
- Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understorey shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, **82**, 1420–1425.
- Lynch M, Milligan G (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, **3**, 91–99.
- McCauley DE (1991) Genetic consequences of local population extinction and recolonisation. *Trends in Ecology and Evolution*, **6**, 5–8.
- McCauley DE, Olson MS, Taylor DR (2001) An association between chloroplast DNA haplotype and gender in a plant metapopulation. *Evolutionary Ecology*, **14**, 181–194.
- McCauley D, Raveill J, Antonovics J (1995) Local founding events as determinants of genetic structure in a plant metapopulation. *Heredity*, **75**, 630–636.
- Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceeding of the National Academy of Sciences of the United States of America*, **70**, 3321–3323.
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.
- Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution*, **19**, 153–170.
- Petit RJ, Bialozyt R, Garnier-géré P *et al.* (2004) Ecology and genetics of tree invasions: from recent introductions to Quaternary migrations. *Forest Ecology and Management*, **197**, 117–137.
- Petit RJ, Pineau E, Demesure B *et al.* (1997) Chloroplast DNA footprints of postglacial recolonization by oaks. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 9996–10001.
- Piry S, Alapetite A, Cornuet JM *et al.* (2004) GENECLASS2: a software for genetic assignment and first generation migrants detection. *Journal of Heredity*, **95**, 536–539.
- Prugnolle F (1999) *Dynamique des Populations et Biologie de la Reproduction d'Antirhea borbonica Gmel. (Rubiaceae) Dans un Paysage Fragmenté à l'île de la Réunion: Implications Pour la Biologie de la Conservation de la Forêt Tropicale Humide de Basse Altitude* Diplôme d'Etudes Approfondies, University of Paris.
- Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Genetics*, **94**, 9197–9201.
- Richardson DM, Higgins SI (1998) Pines as invaders in the southern hemisphere. In: *Ecology and Biogeography of Pinus* (ed. Richardson DM), pp. 450–473. Cambridge University Press, Cambridge, UK.
- Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.
- SAS Institute Inc (2001) *SAS/STAT Software Release 8.2*. Cary, NC. SAS Institute Inc.
- Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinction. *Theoretical Population Biology*, **12**, 253–262.
- Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.
- Strasberg D (1994) *Dynamique des Forêts Tropicales de l'île de la Réunion, Processus d'Invasion et de Régénération sur les Coulées Volcaniques*. PhD Thesis, University of Montpellier II.
- Strasberg D (1996) Diversity, size composition and spatial aggregation among trees on 1-Ha rain forest plot at La Réunion. *Biodiversity and Conservation*, **5**, 825–840.
- Thébaud C, Strasberg D (1997) Plant dispersal in fragmented landscapes: a field study of woody colonization in rainforest remnants of the Mascarene archipelago. In: *Tropical Forest Remnants: Ecology, Management, and Conservation of Fragmented Communities* (eds Laurence WF, Bierregaard RO), pp. 321–332. University of Chicago Press, Chicago.
- Vekemans X (2002) *AFLP-SURV version 1.0*. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgique.
- Vos P, Hogers R, Bleeker M *et al.* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.
- Wade MJ, McCauley DE (1988) Extinction and recolonisation: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.

- Weir BS, Cockerham CC (1984) Estimating F -statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction: Genetic correlations within founding groups. *Evolution*, **44**, 1717–1724.
- Wood DM, Del Moral R (1987) Mechanisms of early primary succession in subalpine habitats on Mount St Helens. *Ecology*, **68**, 780–790.
- Yeh FC, Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany*, 129–157.