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The impact of forest disturbance on the genetic diversity and population structure of a late-successional moss

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SUMMARY

Human-induced disturbances threaten the genetic variation of wild plant populations. The genetic diversity and spatial population structure of the moss *Isothecium myosuroides*, a late-successional forest species, was investigated in subtropical cloud forests (La Gomera, Canary Islands) using inter-simple sequence repeat (ISSR) markers. Inter- and intrapopulation genetic variability was assessed in two ancient and four disturbed forest stands, which were classified according to their vegetation, forest age and type of disturbance. ISSR analysis of 144 epiphyte colonies with eight primers resulted in 211 reliably amplified bands. Our findings show that in disturbed forest stands, the population structure is increased, and the genetic diversity decreased compared with the levels observed in ancient forests. Although ancient and disturbed stands were located relatively close to each other, the (re-) established epiphyte populations did not reach their original genetic condition, 40 years following disturbance. Strong differentiation among populations of *I. myosuroides* at several spatial scales and differences in genetic diversity are mainly related to the local environmental conditions and the availability of suitable microhabitats in anthropogenically disturbed forest stands.

KEYWORDS: Canary Islands, cloud forest, epiphytic bryophyte, exotic tree plantation, forest disturbance, habitat suitability, ISSR

INTRODUCTION

The anthropogenic use of natural resources to meet the requirements of the growing human population has led to an accelerating loss of biodiversity. Deforestation can significantly reduce the size (e.g. area and patch number) and the environmental complexity of a natural habitat. Under such circumstances, population size often decreases, which can have negative effects on genetic variation and, subsequently, the ability to adapt to changing environments (Baucom, Estill & Cruzan, 2005; Frankham, 2005). Since genetic structure regulates within-populations fitness (Lowe *et al.*, 2005; Trénel *et al.*, 2008), understanding how forest destruction alters the genetic diversity of populations is vital to ensure the long-term survival of bryophytes (Pharo & Zartman, 2007). We present here a study of deforestation effects on the fine-scale population structure of *Isothecium myosuroides* in oceanic cloud forests.

Epiphytic bryophytes constitute a diverse and highly productive group of organisms in many subtropical and tropical forest ecosystems (Pereira & Cavalcanti, 2007; Patiño, González-Mancebo & Fernández-Palacios, 2009a; Patiño *et al.*, 2009b). Bryophytes have features that make them ideal for investigating the genetic impacts of forest destruction caused by human disturbance. For instance, many epiphytes are sensitive to habitat degradation, as a consequence of their poikilohydric nature and the physical and chemical characteristics of the host tree (Barkman, 1958). The forest age, which is associated with the number and availability of microhabitats, thus influences the probability of colonisation by bryophytes (Snäll, Ehrlén & Rydin, 2005; Pereira & Cavalcanti, 2007; Hutsemekers, Dopagne & Vanderpoorten, 2008).

Several studies have shown a relationship between population history (related to human-induced disturbances) and the levels of genetic variation in temperate and boreal forests (Cronberg *et al.*, 2005; Wang *et al.*, 2006; Spagnuolo *et al.*, 2007a). By contrast, very few molecular studies have analysed epiphytes or have been carried out in disturbed (sub-) tropical forests (e.g. Zartman, McDaniel & Shaw, 2006). Furthermore, most available studies have exclusively analysed well-established populations that remain in old forest patches (for exceptions see Cronberg *et al.*, 2005; Wang *et al.*, 2006). As a result, genetic factors have rarely

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been integrated into forest restoration or management programmes designed to protect viable bryophyte populations (Pohjamo, Korpelainen & Kalinauskaité, 2008).

Following deforestation, it is predicted that a restoration phase will occur (Snäll *et al.*, 2005). During this phase, diaspores of locally extinct species might gradually arrive, enabling re-establishment and increasing genetic diversity (Cronberg, 2002; Hassel *et al.*, 2005). Newly re-established populations usually originate from a few founders and show a limited number of haplotypes (founder event; Hartl & Clark, 1997). As new individuals (e.g. diaspores) immigrate into the population over time, genetic variation increases and the footprints of founder events are progressively removed (Hanski, 2001). However, this may be an extremely long-term process, especially with regard to the occurrence of rare alleles (Caujapé-Castells & Pedrola-Monfort, 2004).

Bryophytes have smaller and more easily dispersed diaspores than most vascular plants (Muñoz *et al.*, 2004). Some studies have reported long-distance dispersal events (Miller & McDaniel, 2004), but large spatial distances between populations are not always translated into a marked genetic separation (Cronberg, 2002; Gunnarsson, Hassel & Söderström, 2005). Bryophytes can show aggregated distribution patterns (Snäll *et al.*, 2005) and re-established populations can retain strong genetic divergence if the levels of gene flow are low (Spagnuolo *et al.*, 2007b; Pohjamo *et al.*, 2008). This idea has even been supported on a local spatial scale (Gunnarsson, Lönn & Shaw, 2006). It has been proposed that spore-mediated gene flow might be restricted due to limited dispersal ability (Derda & Wyatt, 1999; Pohjamo *et al.*, 2006) or unsuitable habitat for spores to germinate (Hassel *et al.*, 2005), thus leading to the aggregated distribution pattern.

The present study examines how the genetic variation of an epiphytic bryophyte species responds to deforestation across cloud forest landscapes in an oceanic archipelago. Species on the relatively isolated islands usually have a limited population size and, consequently, are especially vulnerable to human disturbances (Gillespie, Claridge & Roderick, 2008). Furthermore, different levels of biodiversity in forests on oceanic islands (e.g. species and genetic diversity) are currently threatened around the globe (Caujapé-Castells *et al.*, 2010). As very little is known about the effect of disturbances on the genetic diversity of bryophytes within landscapes, our study aims to formulate sustainable management practices for bryophytes in subtropical oceanic cloud forests.

To investigate the effects of forest disturbance at the regional and local spatial scale, we selected populations of the late-successional moss *I. myosuroides* Brid. (Lembophyllaceae) to analyse in two localities on La Gomera (Canary Islands). Based on variations in inter-simple sequence repeats (ISSRs), this study examines the relationship between genetic variation and three types of forest stands: ancient cloud evergreen forests, disturbed cloud evergreen forests, and exotic tree plantations. Our specific aims were to investigate: (1) the differences in population genetic structure between ancient and recolonised disturbed forests; (2) whether geographical distance is a key factor in explaining this genetic separation; and (3) how genetic diversity is distributed between and within

Table 1. *Description of the* I. myosuroides *population and characteristics of the studied areas.*

		Forest stand characteristics					Bryophyte populations characteristics				
Sites	Labels	Altitude (m a.s.l.)	Circumference (cm)	Forest age (year)	Forest height (m)	Canopy cover (%)	*n*	Host species	Growth form	Sporophyte occurrence (%)	Trunk frequency
El Cedro							96				
Ancient forests	C–U1	940	84.3±19.4	>300	30	85	12	Ln, Mf	We, Sd	41.67	93.3±11.5
undisturbed (C–U)	C–U2	950	85.2±18.4	>300	25	90	12	Ln, Mf	We, Sd	66.67	90.2±8.6
Disturbed forests	C–A1	950	78.4±24.3	40	15	70	12	Ln, Mf	We, Sd	33.34	42.8±6.2
clearcut (C–A)	C–A2	955	71.9±18.3	40	12	75	12	Ln, Mf	We, Sd	8.34	53.3±19.6
Disturbed forests	C–B1	960	55.5±14.7	40	10	70	12	Ln, Mf	We	16.67	34.3±6.8
clearcut (C–B)	C–B2	965	62.8±13.9	40	11	60	12	Ln, Mf	We, Sd	8.34	33.5±7.7
Disturbed forests	C–C1	950	51.9±19.7	40	10	75	12	Ln, Mf	We, Sd	0	36.5±4.2
clearcut (C–C)	C–C2	960	58.7±16.8	40	12.5	70	12	Ln, Mf	We	8.34	30.7±1.6
Enchereda							24				
Ancient forests	E–U1	800	35.5±12.6	>300	7	40	6	Ea, Mf	Sd, Pe	66.67	85.1±4.5
undisturbed (E–U)	E–U2	826	36.9±10.7	>300	8	50	6	Ea, Mf	Sd, Pe	100	88.2±9.2
Disturbed forests exotic tree	E–A1	725	95.1±25.5	40	18	70	6	Pr	Sd, Pe	16.67	20.3±5.7
plantation (E–A)	E–A2	780	91.6±33.3	40	25	80	6	Pr	Sd	16.67	18.7±7.1

Circumference shows the mean circumference at breast height (±SD) of the tree sampled; *n*: number of wefts recollected. Ln: *Laurus novocanariensis*; Mf: *Myrica faya*; Ea: *Erica arborea*; Pr: *Pinus radiata*; We: weft; Sd: subdendroid; Pe: pendant. Sporophyte occurrence number of colonies with mature sporophytes expressed as a % of total number of colonies with immature, mature or old sporophytes. Trunk frequency: the proportion of trunks with presence of *I. myosuroides* within each 0.1-ha plot; it is shown the mean values of each subpopulation. To check the maxima distances between the ancient forests and each disturbed forest areas considering each geographical area separately (El Cedro versus Enchereda, see Fig. 1).

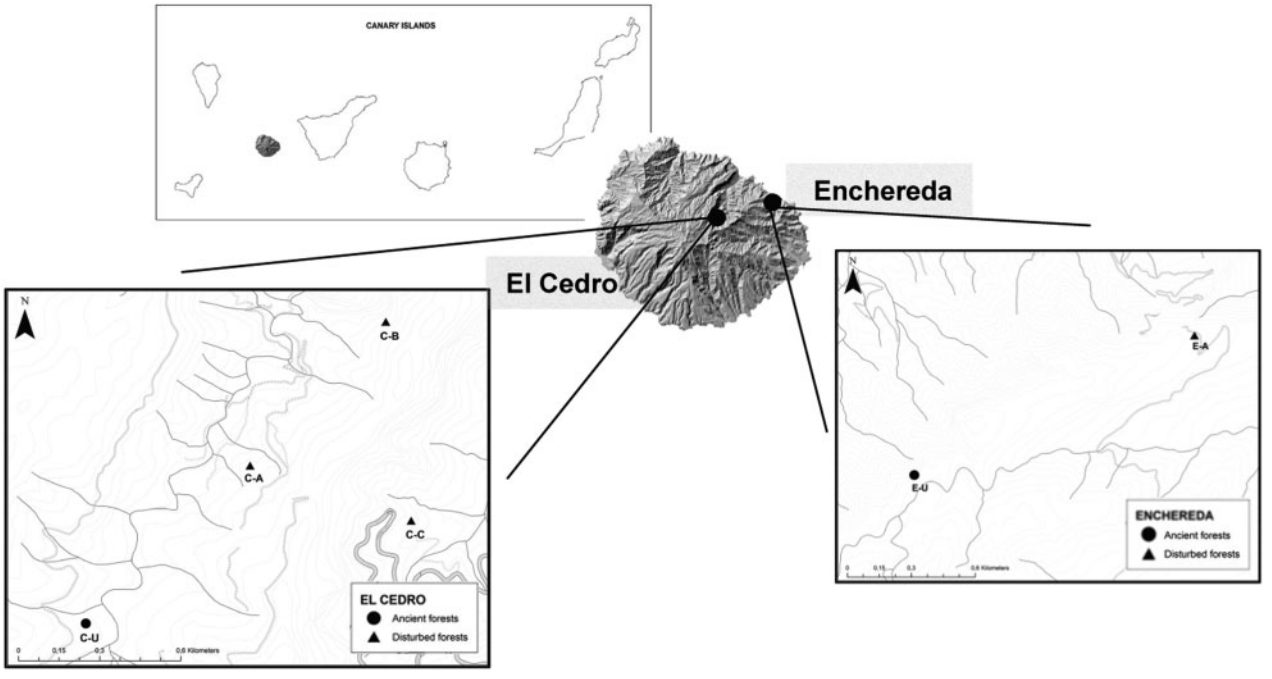

Figure 1. Locations of the two study areas, El Cedro and Encherda, in La Gomera, Canary Islands, Spain, used to investigate populations of *I. myosuroides*. In each area, location of the populations and type of forest are indicated.

populations that inhabit forest stands of different age and disturbance degree.

Materials and Methods

Study system

I. myosuroides is a dioicous, robust, pleurocarpous moss, with spores of a diameter of 16–24 μm, and with growth forms that range from weft to subdendroid or even to pendant in the moistest environments (Table 1). Specialised vegetative propagules are not known, but gametophyte fragments may act as propagules. Although its reproductive effort has not been measured, *I. myosuroides* may be classified as a perennial stayer (González-Mancebo *et al.*, 2008). Its distribution covers Europe, and the west and east coasts of North America (Ryall *et al.*, 2005), and reaches its southern limit in Cape Verde.

Although depauperate populations have been detected in disturbed Canarian cloud evergreen forests (Patiño *et al.*, 2009a), healthy populations are mainly concentrated in well-conserved forest stands (González-Mancebo *et al.*, 2008). In the cloud forests, this facultative epiphyte, which may also grow on rocks and soils, is commonly found on *Erica arborea* L., *Laurus novocanariensis* Rivas-Mart. *et al.* and *Myrica faya* Aiton often becoming the dominant species on these hosts (González-Mancebo *et al.*, 2008). In the study area, it is possible to find populations of *I. myosuroides* in a matrix of disturbed and undisturbed forest stands, making it ideal for evaluating the effects of local forest degradation on the genetic structure of the population.

Study area

Macaronesian cloud evergreen forests are one of the most biologically diverse ecosystems in Europe (Médail & Quézel, 1997), and fall within the Mediterranean basin biodiversity hotspot (Myers *et al.*, 2000). One of the most protected Macaronesian forests is found on La Gomera Island (Canary Islands; Fernández-López, 2009), where bryophyte communities, including epiphytes, constitute a diverse and highly productive plant group (González-Mancebo *et al.*, 2008; Patiño *et al.*, 2009b). Some forest areas of this island have been modified by timber harvesting, agriculture and planting of exotic species (Fernández-López, 2009). Because Canarian cloud evergreen forests today occupy $\sim$164 km^2, which is less than 20% of the potential area (Fernández-López, 2001), genetic studies on the responses of forest species to human disturbances are urgently required.

The present study was conducted in two forest areas on La Gomera Island (Fig. 1), El Cedro, and Enchereda. El Cedro (Table 1) has a mean altitude of 954 m a.s.l., a mean annual rainfall of 723 mm and additional moderate fog precipitation with 620 mm of throughfall precipitation (Fernández-López, 2009). A significant sector of El Cedro is included within the Garajonay National Park and is dominated by ancient forests where the predominant tree species are *L. novocanariensis*, *M. faya*, and *Persea indica* (L.) C.K.Spreng. Yet, in the past, nearby areas were subjected to intensive, stand-level clear cutting, followed by a period of agriculture. The abandonment of agriculture in the 1970s stimulated a natural process of forest regeneration. Hence, a matrix of secondary forest patches currently surrounds the area of El Cedro.

Enchereda (Table 1) has a mean altitude of 783 m a.s.l. Although there is no climatic data for this area, El Bailadero (a topographically and environmentally similar area close to Enchereda) has a mean annual rainfall of 665 mm but with a high throughfall precipitation of 1068 mm (Fernández-López, 2009). Most of the Enchereda area is included within Majona Natural Park. The predominant tree species are *E. arborea*, *E. platycodon* (Webb & Berthel.) Rivas-Mart. *et al.*, *Ilex canariensis* Poir. and *L. novocanariensis*. Areas adjacent to these forests were subjected to clear cutting, and the original vegetation was substituted by plantations of *Pinus radiata* D.Don in around 1970, covering an area of ~ 3 km^2.

I. myosuroides was collected on different host tree species in six forest stands (Fig. 1; Table 1): two ancient stands more than 250 years old (one in El Cedro and the other in Enchereda); three naturally regenerated stands in El Cedro, all about 40 years' old (clearcut; Table 1); and one stand of pine forest in Enchereda of about 40 years (exotic tree plantation). Geographical distances between forest stands were variable. In El Cedro, pairwise distances ranged from ~ 600 to 1750 m. In Enchereda, they varied from ~ 450 to 1050 m. The mean distance between the populations in El Cedro was 856 m (± 454), and 688 m (± 272) in Enchereda, while the distance between the populations of El Cedro and Enchereda was 7208 m (± 789). In each stand, the geographical separation between subpopulations ranged from ~ 100 to 400 m.

Sampling design

A total of 32 0.1-ha plots (20×50 m) in the six forest stands were analysed in the study. Within each of the forest stands, six 0.1-ha study plots were investigated in El Cedro (in total 24 plots). First, in each forest stand, two transects were placed in a NE to SW alignment following the prevailing wind direction and were separated 100 m from each other. Second, three 0.1-ha plots were systematically positioned every 20 m along each transect; for purposes of our analyses, we considered the forest stand as one population and individual transects as subpopulations. In the case of Enchereda, this sampling method was modified due to the problems of accumulating larger sample sizes, especially in pine plantations; thus, we carried out two 0.1-ha plots per transect (in total eight plots), and transects within each Enchereda forest stand were separated from each other by approximately 400 m.

Within each 0.1-ha plot, we first considered all the host trees placed at each corner, which had a circumference at breast height similar to the mean circumference at breast height for the stand. To increase the possibility of sampling genetically distinct individuals, only four trees per 0.1-ha plot (one tree in each corner) was randomly selected for sampling. Each colony was sampled only once per tree, the sample height varied from 0.5 to 1.5 m above the ground. Hence, molecular analyses were performed on four colonies (i.e. samples) per 0.1-ha study plot and on, at least, 24 colonies per forest stand (Table 1). In the case of Enchereda, as mentioned above, this sampling effort was reduced by half.

In total, 144 colonies of 100 cm^2 were collected (Table 1). Characteristics for each subpopulation were registered in the field (Table 1), including: sporophyte occurrence [i.e. the number (mean number of the three 0.1-ha plots along each transect) of colonies that showed the presence of young or old sporophyte structures divided by the total number of colonies present in all the trunks]; and trunk frequency [i.e. the number (mean number of the three 0.1-ha plots along each transect) of trunks with presence of *I. myosuroides* divided by the total number of trunks].

DNA extraction, polymerase chain reaction (PCR) amplification and haplotype assessment

All samples were stored at 14–18°C until the laboratory work was carried out. All the colonies were carefully inspected under a dissecting microscope to ensure their purity. Genomic DNA was extracted from individual colonies using the sodium dodecyl sulphate protein precipitation method described by Milligan (1998). The purified DNA was stored at 4°C. Five voucher specimens of each population are held in the botanical herbarium at La Laguna University.

ISSRs were used to analyse population genetic structure and genetic diversity. This method has numerous advantages including low development costs, no requirement for DNA sequence information and high reproducibility (Nybom, 2004). Standardised laboratory procedures have been developed for a range of plants (e.g. Hassel and Gunnarsson, 2003), and these techniques have been successfully applied in population studies of both vascular plants and bryophytes (Ge *et al.*, 2005; Hassel *et al.*, 2005; Spagnuolo *et al.*, 2007b). A total of eleven ISSR primers (purchased from MWG-Biotech, Ebersberg, Germany) were initially screened: CA4, ISSR4, OW1, OW2, OW3, OW4, OW5, OW12, IN5, YNZ, and YN73. Of the primers tested, the first eight were selected for the present ISSR analysis based on the high number of reproducible polymorphic bands (Appendix).

The amplification reaction mixture was carried out in a final volume of 20 μl containing 1 μl of DNA-extract (diluted 10 times), 0.8 units Taq polymerase (Oncor Appligene, Gaithersburg), 2 mM $MgCl_2$, 200 μM of each of the four deoxyribonucleotide triphosphate, 400 μM of ISSR primer and the buffer supplied by the manufacturer of the enzyme. Two per cent of BLOTTO (10% skimmed milk powder and 0.2% $NaNO_3$) was added to the reaction mix. BLOTTO is reported to attenuate PCR inhibition in the presence of plant compounds (de Boer *et al.*, 1995). All the PCR reactions were performed in an Eppendorf Mastercycler using the following conditions: 3 min at 94°C followed by 30 cycles of 15 s at 94°C, 30 s at 45°C and 1 min at 72°C, followed by a final extension step of

7 min at 72°C. 5 μl of the amplification reaction were separated on 6% polyacrylamide gels. The DNA bands were visualised by silver staining and gels were photographed above a light source. The sizes of the ISSR bands were estimated using a 100-bp ladder (Fermentas, Burlington, ON, Canada) as a molecular size marker.

The presence or absence of polymorphic ISSR marker bands was manually scored (1=present, 0=absent), using the software SigmaGel version 1.0 (Jandel Scientific Co., San Rafael, CA, USA). Only reproducible, well-resolved bands were included in the study. The scoring was repeated twice by the same investigator. Some samples, whose band pattern was unclear, were subjected to a second round of DNA extraction, PCR and electrophoresis, which was then compared to previous runs to ensure that bands were being amplified consistently. In every case, banding patterns on the first and second gels were identical.

Genetic diversity analyses

Because there was a variation in sample sizes between populations (Table 1), which may affect measures of allelic richness and bottlenecks (Leberg, 2002), we compared the observed band number with the predicted band number using bootstrap analysis with Species Diversity and Richness software (Pisces Conservation Ltd, 2002). Whether the addition of more samples would increase haplotypic resolution was also surveyed by generating a plot of the number of samples against the number (mean) of haplotypes per primer, and determining whether the relationship had reached a plateau.

The coefficient of gene differentiation (G_{ST}) among (sub-) populations measured using dominant markers such as ISSR is generally upwardly biased when the sample size is small (Isabel *et al.*, 1999). Thus, an analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was used to partition the genetic variance within- and among-populations at the regional level. These analyses were implemented using ARLEQUIN software (Schneider, Roessli & Excoffier, 2000). The number of permutations for significant testing was set at 10 000 for each analysis. The interpopulation genetic distance was also estimated using the exact test of population differentiation (Raymond & Rousset, 1995). Here the hypothesis of a random distribution of k different haplotypes among r populations was tested by an $r \times k$ contingency table approach, which is analogous to Fisher's exact test on a 2×2 contingency table (Schneider *et al.*, 2000).

In order to test for isolation by distance among all the (sub-) populations of *I. myosuroides*, a Mantel test (Mantel, 1967) was also carried out in ARLEQUIN (Schneider *et al.*, 2000). Both study areas were analysed together and separately and the significances of the resulting correlation coefficients between geographical and genetic distances were determined by 1000 permutations. Pairwise geographical distances were measured between (sub-) populations. Such calculations were performed using topographical maps and numbers were rounded to the nearest 10 m.

To test the molecular relationships among the analysed (sub-) populations, we used non-metric multi-dimensional scaling (NMDS) performed using PRIMER 6 (Clarke & Corley, 2006). NMDS is a robust and suitable method for ecological data because it makes no assumptions about the underlying data (Clarke & Corley, 2006). This analysis is an ordination method that uses ranked distances (the Bray-Curtis distance was applied). NMDS was performed on the presence/absence data matrix obtained from the scoring of ISSR banding from each subpopulation. The consistency of the dimensional approximations among subpopulations was checked with a cluster analysis, where superimposed clusters were defined by levels of similarity among subpopulations.

The ISSR data were analysed based on the assumptions that populations were in Hardy–Weinberg equilibrium. We estimated the following genetic diversity parameters for each (sub-) population using POPGENE v. 1.31 (Yeh, Yang & Boyle, 1999): the observed number of alleles per locus (A_o), the effective number of alleles per locus (A_E), Nei's genetic diversity (h), and the percentage of polymorphic loci (% PL).

Because allelic diversity is generally lost at a much faster rate than gene diversity in a bottleneck situation, bottlenecked populations are expected to have a heterozygote excess (Luikart & Cornuet, 1998). We applied a sign test for revealing populations that recently have passed through a bottleneck (Luikart & Cornuet, 1998) from allele frequency data at polymorphic loci (per sample per population) under assumption of the infinite allele model (IAM) and the stepwise mutation model (SMM). We used the program Bottleneck version 1.2.02 (Piry, Luikart & Cornuetdata 1999). To be statistically conservative, one should only use the SMM when analysing simple sequence repeats (e.g. ISSRs); but, since the true model of mutation for most loci is intermediate between IAM and SMM, using both models is recommended (Luikart and Cornuet, 1998).

The influence of forest age (years), tree diameter (cm), canopy cover (%), forest height (m), altitude (m a.s.l.) and disturbance type on genetic diversity parameters of all the samples of *I. myosuroides* (per 0.1-ha plot per subpopulation) were examined using linear regression; the mean values of each variable for each subpopulation, as well as the sample size of each subpopulation are shown in Table 1. Three categories were considered in the disturbance type: ancient (1), clearcut (2), and exotic tree plantation (3); the numbers between brackets indicate how each was coded for the regression analyses. These analyses were carried out using SPSS 15.0 for Windows (2006).

RESULTS

The frequency of *I. myosuroides* was higher in ancient forest stands from El Cedro and Enchereda (C–U and E–U) than that in disturbed forest stands from both geographical areas

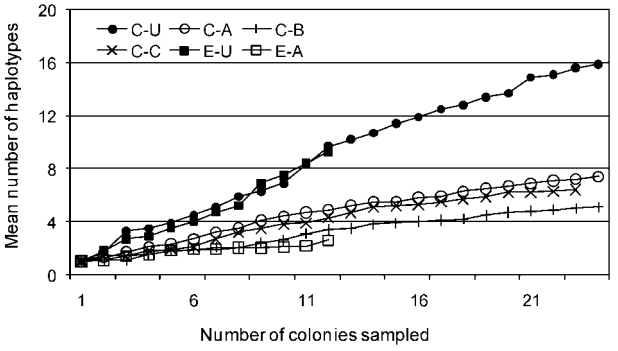

Figure 2. The relationship between the number of haplotypes (mean) per primer and the sample size for each population of *I. myosuroides*.

(Table 1). We also observed a pattern for sporophyte occurrence, with many more sterile colonies in disturbed forest than in ancient forest stands where more colonies had capsules (Table 1).

Analysis of ISSR banding patterns

The eight primers screened produced 211 reliable ISSR bands in the 144 colonies of the six forest areas studied (Appendix). The average number of loci per primer was 26.4 ($\pm$5.0). Of these reliable loci, 202 (95.7%) were polymorphic (Appendix). Plots of sample size (Table 1) against the number of unambiguously scored bands were analysed using the non-parametric bootstrap estimator. Estimated values were relatively similar to those observed for all subpopulations (from 185.7 to 103.5 bands); the overall mean percentage of completeness ranged from 99.8 to 85.2 (96.7$\pm$5.7). The addition of more samples increased the haplotypic resolution in ancient forest populations, thus their number of haplotypes should be considered as a minimum estimate. By contrast, the resolution seemed to be satisfactory in the disturbed stands where the number of haplotypes seems to have reached a plateau (Fig. 2). In tropical forests, Zartman *et al.* (2006) found that the genetic diversity of a liverwort saturated at around 10 individuals per sample unit. Here we surveyed from 12 to 24 individuals per sample unit (forest stand).

Population structure and genetic differentiation

The partitioning of genetic variation was significant at all three hierarchical levels (Table 2). The highest genetic variation was found within populations (36.1%; Table 2), followed by the genetic variation harboured among populations within each forest area (32.6%). The partitioning of genetic variation was also significantly different between the two forest areas (31.3%), although with a lower significance level (Table 2). The six populations are significantly differentiated from one another ($p<0.001$) according to Raymond and Rousset's test of population differentiation.

Results for the Mantel test analysing data from all populations indicated that the genetic distance estimated using the exact test of population differentiation can be explained by interpopulation physical distances ($r^2=0.761$; $p<0.001$). However, when such an analysis was carried out using the El Cedro and Enchereda data separately, the results were different. In the El Cedro analysis, the Mantel test showed significant correlation between genetic and geographical distances ($r^2=0.717$; $p=0.001$). In contrast, we failed to detect a similar correlation for Enchereda populations ($r^2=0.639$; $p=0.172$).

NMDS ordination analysis based on the scoring of ISSR banding provided visual representations of genetic proximities among all the colonies recorded, defining (sub-) populations from the same geographical area (El Cedro versus Enchereda; Fig. 3). In both cases, the subpopulations within each population (e.g. C–U1 versus C–U2) were mostly clustered. Although the most geographically distant population in disturbed forests of the El Cedro (C–B) appear somewhat closely related to ancient forest populations (C–U), the populations most strongly separated by greater geographical distances tended to appear more distant in the ordination analysis, for instance C–C with respect to C–U and C–A (Fig. 3).

Genetic diversity

The different molecular indices per (sub-) population of *I. myosuroides* averaged across all loci are given in Table 3. Nei's genetic diversity (h) per population ranged from 0.119 to 0.183, and per subpopulation from 0.076 to 0.156. The percentage of polymorphic loci within populations ranged from 34 to 57% and within subpopulation from 22 to 48% (Appendix), with an average of 32%. Although the population that occupied the ancient forest from El Cedro (C–U) was genetically more diverse than a comparable population from Enchereda (E–U), their Nei's

Table 2. *AMOVA of the El Cedro and Enchereda forest areas.*

			Variation		
Source of variation	d.f.	Sum of squares	Variance	% variation	p
Among forest areas	5	777.5	14.2	31.3	<0.050
Among populations within each forest area	6	1313.8	14.8	32.6	<0.001
Within each population	108	1874.0	16.4	36.1	<0.001
Total	119	3965.3	45.5		

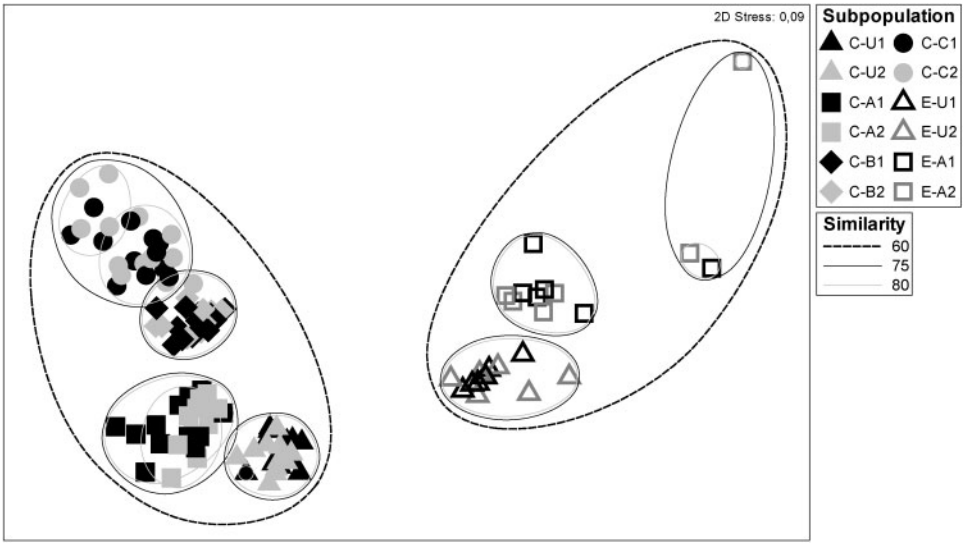

Figure 3. NMDS performed on the individuals of the six populations, analysing ISSR banding data of the 12 subpopulations. Clusters formed at the three arbitrary levels of similarity (60, 75, and 80%) are superimposed on the two-dimensional NMDS, both obtained from Bray–Curtis similarities. See Table 1 for definitions of abbreviated site codes.

diversity and polymorphic loci values per (sub-) population were quite similar (Table 3). The lowest genetic diversity and polymorphic loci values were found in the exotic tree plantation of Enchereda (E–A), and in the most distant clearcut from El Cedro (C–B). Regardless of whether two study areas were analysed together or separately, it is possible to determine that ancient forests presented higher values for all the molecular indices than the respective disturbed forest stands (Table 3).

The mean allelic richness per locus was higher in the ancient forest stands (1.573 in El Cedro versus 1.524 in Enchereda) than in disturbed forest stands (from 1.442 to 1.369). Of the alleles scored, 16 were exclusive to the population in the El Cedro ancient forest stand (C–U), and nine to the Enchereda ancient forest population (E–U). We detected nine alleles shared exclusively between the populations that occur in both ancient forest stands (C–U, E–U). It was found two alleles shared between the populations of Enchereda (E–U and E–A). The remaining alleles were shared by different combinations of the six populations analysed. Whereas none of the populations that occur in ancient forest stands showed evidence of a recent bottleneck, the genetic signature of a bottleneck was detected in the populations of *I. myosuroides* that inhabit disturbed forest stands (Table 4). Under the strict SMM scenario, a low *p* value indicated that the population in the pine forest of Enchereda had gone through a bottleneck (Table 4).

Table 3. *Within-population genetic diversity of* I. myosuroides *detected by ISSRs.*

Population	A_O	A_E	*h*	%PL
C–U(24)	1.573 ± 0.496	1.308 ± 0.362	0.183 ± 0.194	57.28
C–U1(12)	1.476 ± 0.501	1.261 ± 0.347	0.156 ± 0.188	47.57
C–U2(12)	1.437 ± 0.497	1.259 ± 0.319	0.155 ± 0.192	43.69
C–A(24)	1.524 ± 0.496	1.299 ± 0.373	0.174 ± 0.198	52.43
C–A1(12)	1.447 ± 0.498	1.257 ± 0.337	0.145 ± 0.189	44.66
C–A2(12)	1.311 ± 0.464	1.192 ± 0.332	0.111 ± 0.181	32.07
C–B(24)	1.369 ± 0.484	1.222 ± 0.348	0.128 ± 0.189	36.89
C–B1(12)	1.286 ± 0.453	1.169 ± 0.309	0.099 ± 0.171	28.64
C–B2(12)	**1.277 ± 0.448**	**1.189 ± 0.343**	**0.106 ± 0.184**	**27.67**
C–C(24)	1.432 ± 0.497	1.239 ± 0.344	0.142 ± 0.188	43.20
C–C1(12)	1.211 ± 0.464	1.186 ± 0.327	0.109 ± 0.179	31.07
C–C2(12)	1.349 ± 0.478	1.215 ± 0.345	0.125 ± 0.187	34.95
E–U(12)	1.529 ± 0.452	1.297 ± 0.396	0.176 ± 0.173	52.86
E–U1(6)	1.449 ± 0.429	1.256 ± 0.286	0.147 ± 0.162	45.58
E–U2(6)	1.367 ± 0.443	1.224 ± 0.303	0.139 ± 0.171	39.93
E–A(12)	1.388 ± 0.488	1.213 ± 0.319	0.119 ± 0.179	33.83
E–A1(6)	**1.218 ± 0.414**	**1.126 ± 0.265**	**0.076 ± 0.152**	**21.84**
E–A2(6)	**1.321 ± 0.467**	**1.199 ± 0.326**	**0.079 ± 0.181**	**22.04**

A_O=observed number of alleles; A_E=effective number of alleles; *h*=Nei's genetic diversity; %PL=percentage of polymorphic loci. The three lowest values for each genetic variation parameter are marked in bold. Number of samples for each (sub-) population is represented in brackets. Please see Table 1 for definitions of abbreviated site codes.

Table 4. *Results from bottleneck analysis (sign test) for each population of* I. myosuroides *under the IAM, and the SMM.*

		IAM		SMM	
Populations	*n*	H_d/H_e	*p*	H_d/H_e	*p*
El Cedro					
C–U	24	75/43	**0.000**	69/49	**0.015**
C–A	24	36/72	**0.000**	43/65	**0.008**
C–B	24	20/56	**0.000**	26/50	**0.001**
C–C	24	28/61	**0.000**	33/56	**0.003**
Enchereda					
E–U	12	45/33	**0.003**	45/33	0.228
E–A	12	35/45	**0.008**	35/45	0.297

H_d/H_e=number of loci with heterozygote deficiency and excess, respectively; Significant *p* values are shown in bold. *n*=number of colonies sampled.

Effects of forest characteristics on genetic diversity

When the El Cedro and Enchereda areas are surveyed together, only Nei's genetic diversity and percentage of polymorphic loci were significantly correlated with forest age and disturbance type (Table 5). When these forest areas are analysed separately, the results differ. In the El Cedro area, the effective number of alleles, Nei's genetic diversity and the percentage of polymorphic loci were significantly related to all the variables considered, except altitude (Table 5). In Enchereda, forest age and canopy cover showed a significant relationship with genetic diversity and the percentage of polymorphic loci, whereas disturbance type had a significant effect on the genetic diversity of *I. myosurodes* (Table 5).

Discussion

An assessment of the complex interplay of historical events, life-traits and environmental conditions may provide the background knowledge necessary to understand the effects of human-induced disturbances on the genetic diversity of plant populations (Lowe *et al.*, 2005; Frankham, 2005; Trénel *et al.*, 2008). The present study indicates that geographic distances alone cannot explain the genetic variation observed among populations. Thus, the type of forest, as well as the level of disturbance, seems to affect the genetic diversity and population structure of *I. myosuroides*, an epiphyte moss inhabiting subtropical island cloud forests.

Geographic effects on spatial population structure

The results for the AMOVA analyses showed some differences in the partitioning of the genetic variation according to the spatial scale considered (Table 2). Although these differences were not great, the fact that a significant percentage of genetic variation was observed at the forest area level (31.2%) points to the high population structure for *I. myosuroides* across the two study areas. The isolation-by-distance model was supported by the Mantel test, which found that genetic distances were correlated with geographical distances. The haplotypic clusters revealed by the NMDS analysis and the significant global population differentiation (Raymond and Rousset's test) are consistent with such a geographical differentiation among populations, possibly indicating restricted gene flow among population (Slatkin, 1987; Baucom *et al.*, 2005).

In contrast, several studies found no difference among populations from relatively geographically distant regions (a few kilometres apart), which has supported the idea of long-distance spore dispersal (Cronberg, 2002; Zartman *et al.*, 2006), as well as retention of ancestral polymorphism or lack of genetic variation in the founders (Gunnarsson *et al.*, 2005; Hassel *et al.*, 2005). Our results must therefore be interpreted with care because such a geographical divergence might be a methodological artifact arising from the fact that both forested areas had unequal sample sizes (Table 1). In spite of this, ISSR tends to over-emphasise differences among close populations and to attribute a lower variation to populations separated by large geographical distances (Nybom, 2004). Our findings are consistent with other studies of bryophytes and vascular plants that found relatively high genetic variation at the landscape scale (e.g. Snäll *et al.*, 2004; Ge *et al.*, 2005; Gunnarsson *et al.*, 2006; Pohjamo *et al.*, 2008).

El Cedro and Enchereda forest areas present different climatic conditions, mainly with regard to fog precipitation (Fernández-López, 2009). Although we did not test if particular haplotypes and forest areas were related, certain morphological expressions were more frequent in particular areas; weft forms were more frequent in El Cedro, while in Enchereda subdendroid and pendent forms predominated (Table 1). In habitats with high humidity, dendroid and pendant are typical forms of growth (González-Mancebo *et al.*, 2008). To explore whether such morphological features have a molecular basis, further studies based on transplant or garden experiments are needed.

Stand-level disturbance effects on spatial population structure

In agreement with studies on bryophytes and vascular plants (Cronberg, 2002; Baucom *et al.*, 2005), our results showed

Table 5. *Statistics of linear regression, indicating the effects of forest age, structural forest features (tree diameter, forest height and canopy cover), altitude, and disturbance type on some genetic diversity parameters of* I. myosuroides.

	General (n=120)			El Cedro (n=96)			Enchereda (n=24)		
	A_E	h	%PL	A_E	h	%PL	A_E	h	%PL
Forest age (year)	0.114 (0.283)	**0.462 (0.015)**	**0.374 (0.035)**	**0.518 (0.044)**	**0.630 (0.019)**	**0.539 (0.038)**	0.088 (0.703)	**0.886 (0.029)**	**0.891 (0.018)**
Tree diameter (cm)	0.064 (0.826)	0.034 (0.565)	0.013 (0.725)	**0.785 (0.005)**	**0.757 (0.005)**	**0.732 (0.007)**	0.116 (0.659)	0.398 (0.072)	0.687 (0.050)
Forest height (m)	0.278 (0.078)	0.027 (0.612)	0.039 (0.535)	**0.697 (0.010)**	**0.787 (0.003)**	**0.738 (0.006)**	0.001 (0.994)	0.293 (0.110)	0.634 (0.054)
Canopy cover (%)	0.283 (0.075)	0.017 (0.686)	0.019 (0.667)	**0.660 (0.014)**	**0.706 (0.009)**	**0.629 (0.019)**	0.029 (0.829)	**0.922 (0.010)**	**0.817 (0.026)**
Altitude (m a.s.l.)	0.205 (0.140)	0.027 (0.613)	0.019 (0.671)	0.185 (0.075)	0.148 (0.086)	0.160 (0.081)	0.051 (0.774)	0.373 (0.066)	0.272 (0.185)
Disturbance type	0.275 (0.080)	**0.754 (0.001)**	**0.648 (0.002)**	**0.679 (0.012)**	**0.740 (0.006)**	**0.726 (0.007)**	0.088 (0.703)	**0.896 (0.022)**	0.671 (0.052)

The first column, General, includes data from El Cedro and Enchereda together, while in the second and third columns are shown the results from El Cedro and Enchereda separately. A_E=effective number of alleles; h=Nei's genetic diversity; %PL=percentage of polymorphic loci; n=number of samples (the data were aggregated at the sample level per 0.1-ha plot per subpopulation). The significant values for each genetic variation parameter are marked in bold.

that levels of genetic differentiation were higher in populations that recolonised secondary forest habitats (C–A, C–B, C–C, and E–A) than in populations inhabiting ancient forests (C–U and E–U). Genetic divergences among subpopulations were more pronounced when ancient forests were compared with disturbed forests (Fig. 2). We found significant correlation between geographical and genetic distances ($p<0.05$ in the Mantel test), although only for El Cedro forest populations; the lack of statistical significance in Enchereda could have been due to low sample size rather than a real lack of correlation between these measurements.

In some respects, the results presented here are seemingly contradictory. Certain pairwise populations, which were more closely related spatially (C–C and C–U; E–A and E–U), showed higher genetic divergence than other pairwise populations that were geographically more distant. Although such results might again be caused by founders having a dissimilar gene pool, we suggest three other possible scenarios.

First, since *I. myosuroides* is a broadly distributed moss species (Ryall *et al.*, 2005) and it has relatively narrow environmental requirements (at least in the Canaries; Patiño *et al.*, 2009a), a probable explanation is that increased interpopulation differentiation is related to a low availability of suitable microhabitats in secondary cloud forests and exotic pine stands. The restricted number of suitable habitats (e.g. host trees) might decrease the probability of diaspore germination and establishment, thus limiting the number of founders. Consequently, the variability of the gene pool within each new population is decreased (Wyatt, 1992; Hassel *et al.*, 2005; Gunnarsson *et al.*, 2006). Our observations on the fine-scale genetic variation of a late-successional bryophyte would indicate that habitat fitness after stand-replacing disturbances is a key factor influencing population structure (Snäll *et al.*, 2005; Zartman *et al.*, 2006; Hutsemekers *et al.*, 2008).

A second hypothesis is that many bryophytes, such as *I. myosuroides*, whose main dispersal mechanism we assume to be the spore, may present moderate dispersal ability, low recruitment and, hence, low rates of gene flow over time (the leptokurtic pattern; Derda & Wyatt, 1999; Spagnuolo *et al.*, 2007b). Late-successional bryophytes often disperse no further than a few tens of metres (Pharo & Zartman, 2007).

A third possible explanation is that the strong genetic structure might also be related to recent rapid spread (within the last $\sim$40 years), followed by relative isolation of the populations due to the distances among stands ($\sim$500 to 1750 m) and the interpolation of dense secondary cloud forests or exotic pine stands. Long-distance dispersal (i.e. stands separated by more than 100 m; see Ozinga *et al.*, 2004) and dense forests may hinder medium- and long-distance propagule dispersal (Green & Johnson, 1996; Spagnuolo *et al.*, 2007a).

Stand-level disturbance effects on genetic diversity

Our results showed that genetic diversity parameters of the moss *I. myosuroides* were higher in populations from ancient forest stands than in recolonised disturbed forest stands. This is consistent with some previous studies that have pointed to a lower molecular diversity of bryophytes and vascular plants in human-altered habitats (Wyatt, 1992; Cronberg *et al.*, 2005; Wang *et al.*, 2006; Baucom *et al.*, 2005; for an exception see Zartman *et al.*, 2006). Across both study areas, the lowest genetic diversity was found in *P. radiata* plantations from Enchereda. Since the differences in molecular indices between both ancient forests were weak (C–U and E–U), and there was a strong relationship between genetic diversity, forest age and human disturbance (Table 5), this result seems to be related to the history of each forest area.

Cleared stands from El Cedro (C–A, C–B, and C–C) followed a natural restoration process, whereas exotic pine plantations from Enchereda (E–A) suffered a complete and permanent alteration of their original forest structure and associated microclimatic gradients, at least during the last 40 years. Therefore, due to that stronger and more enduring environmental modification, it is probably that the (re-) colonisation or survival of *I. myosuroides* was lower in disturbed stands, especially in Enchereda. Furthermore, as the bottleneck tests were significant for the populations in secondary cloud forests and (especially) pine plantations (Table 4), we consider that reductions in population size due to recent anthropogenic disturbances might have affected their levels of genetic variation. Additionally, three observations support this assumption.

First, the deposition of *P. radiata* leaves on the ground (and the subsequent acidification) in exotic pine plantations might have limited the presence of *I. myosuroides* on soils and rocks, reducing diaspore arrival from additional sources. Leith, *et al.* (2008) showed that a low pH has negative effects on the survival of *I. myosuroides*. Second, we suggest that low funnelling ratios (i.e. stemflow) and the acidic bark of *P. radiata* (Crockford & Richardson, 1990) would favour other highly competitive species of the genera *Hypnum* Hedw. and *Homalothecium* Schimp. (Patiño, pers. obs.). This may be a negative factor for the establishment of populations of late-successional species (Hutsemekers *et al.*, 2008), such as *I. myosuroides*. Third, loss of habitat integrity might hinder successful reproduction (i.e. higher frequency of sporophytes in undisturbed habitats), particularly for late-successional species occurring in disturbed habitats (Cronberg *et al.*, 2003). However, due to the lack of robust data, further studies on the reproductive biology of bryophytes inhabiting cloud evergreen forests are essential.

The present study provides additional support for the hypothesis that some plant species are characterised by a pattern of short-distance dispersal (step-by-step) (Snäll *et al.*, 2004; Hassel *et al.*, 2005). These findings suggest that, at least in the case of Canarian *I. myosuroides* populations occurring in anthropogenic landscapes, the exchange of diaspores among populations seems unlikely due to the strong population structure detected. In summary, the low genetic diversity detected in disturbed forest stands might be partially related to the fact that young populations usually consist of a subsample of all the genotypes that exist

in the region (Hassel *et al.*, 2005). We hypothesise that if the process of environmental restoration continues and the availability of suitable habitats rises, it is possible that more founder events will happen, increasing diversity, decreasing differentiation and removing the footprints of recurrent bottlenecks and genetic drift due to anthropogenic disturbances (Hanski, 2001; Caujapé-Castells & Pedrola-Monfort, 2004; Baucom *et al.*, 2005; Trénel *et al.*, 2008).

Implications for forest management

Our results emphasise the importance of ancient forests for the maintenance of genetic diversity in anthropogenically disturbed landscapes. Populations of *I. myosuroides*, a late-successional moss in the study area, currently displays lower genetic variation and stronger population structure in secondary forests than in ancient cloud forests. Recently, Wang *et al.* (2006) showed similar outcomes in Asian spruce forests following harvesting. They compared the genetic diversity of a terricolous moss species in ancient, naturally regenerated and planted stands and found similar results. Cronberg *et al.* (2005) suggested that the positive relationship between forest age and genetic diversity of *Hylocomnium splendens* (Hedw.) Br. Eur. in boreal forests was related to its haploid diaspores. It was noted that a higher number of founders were needed to re-establish original molecular diversity in haploid organisms compared with diploid organisms.

Our findings indicate that detrimental effects due to forestry are of long-term concern, since most impacts on molecular diversity persist 40 years after disturbance. As current forestry regimes applied in many cloud forest regions, such as the Canary Islands, are based on very short cutting intervals (Patiño *et al.*, 2009a), we urgently recommend the conservation of ancient forests patches to restore the diversity of bryophyte species in forest landscapes which have undergone intense forest harvesting.

Loss of genetic variation seemed to be more severe if natural cloud forests were replaced by plantations of exotic tree species. This result has particular importance because most of the ~4000 ha of *P. radiata* plantations that exist in the Canary Islands have been planted in potential areas of cloud evergreen forests, which currently cover less than 20% of the pre-extension (Fernández-López, 2001). Promoting natural forest reestablishment through thinning treatments in cloud forested landscapes dominated by exotic tree species might be an effective management strategy, increasing the accessibility of potential habitats. This might favour the recolonisation of vanished species and accelerate the recovery of genetic variation in newly re-established populations following human-induced disturbances.

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Appendix

ISSR primer sequences and amplification products

Primer name	Sequence 5′→3′	Total bands	Number (%) polymorphic fragments
CA4	GAGAGAGAGAGAGAGYT	28	27 (96.4)
ISSR4	GACAGACAGACAGACA	30	27 (90.0)
OW1	GAGAGAGAGAGAGAGAA	28	28 (100)
OW2	GAGAGAGAGAGAGAGAC	20	19 (95.0)
OW3	GAGAGAGAGAGAGAGAT	30	28 (93.3)
OW4	GAGAGAGAGAGAGAGAYA	25	25 (100)
OW5	GAGAGAGAGAGAGAGAYC	32	30 (93.7)
OW12	GTGGTGGTGGTGGTG	18	18 (100)
Total		211	202 (93.7)

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