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Expanding informativeness of microsatellite motifs through the analysis of heteroduplexes: a case applied to *Solanum tuberosum*

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Abstract The incorporation of heteroduplex analysis into conventional strategies for the study of polymorphisms at microsatellite loci has allowed us to obtain information useful in determining genetic diversity and relationships among organisms. We have chosen, as a model for the testing of this strategy, several *Solanum tuberosum* varieties cultivated on Tenerife Island (Canary Archipelago) and a $(TCT)_n$ microsatellite located in intron I from the gene for granule-bound starch synthase. The data obtained confirm the high degree of agreement between molecular and farmer taxonomy.

Key words Microsatellite · Heteroduplex · Homoplasy · *Solanum tuberosum* · GBSS gene

Introduction

Microsatellites or simple sequence repeats (SSRs) are tandemly repeated, short nucleotide sequences that have been shown to be highly polymorphic and abundant in higher eukaryotes (Tautz and Renz 1984; Bell and Ecker 1994; Wang et al. 1994). They appear to be randomly distributed throughout the genome and occur both intragenically and intergenically (Weber 1990). Microsatellites are among the fastest evolving DNA sequences with mutational rates estimated to vary between 2.5×10^{-5} and 10^{-2} mutations per generation (Kashi et al. 1990; Hederson and Petes 1992; Weber and Wong 1993). During recent years SSRs have attracted considerable attention because of their abundance, the simplicity of the experimental procedures for their analysis and their considerable variation among different organisms. These distinctive characteristics can be used to identify evolutionary relationships among closely related species (Crawford et al. 1998) and between varieties or populations of the same specie (Provan et al. 1996; Krieger and Keller 1997).

In routine studies, microsatellite sequences are amplified by polymerase chain reaction (PCR), and alleles are identified by size. PCR-generated DNA fragments containing allelic microsatellite motifs may have the same length but different nucleotide sequences (size homoplasy: Estoup et al. 1995; Fitzsimmons et al. 1995; Grimaldi and Crouau-Roy 1997; Angers and Bernatchez 1997), and this situation can arise from independent mutational events that have converged in an allelic size-class. One consequence of homoplasy is a clear underestimation of genetic distances, especially between distantly related populations, since the range of repeat numbers found at microsatellite loci appears to be constrained (Bowcock et al. 1994; Goldstein et al. 1995; Gorza et al. 1995; Lehmann et al. 1996). This fact calls into question whether allelic size is a reliable indicator of phylogenetic affinity. Recently, Ortí et al. (1997) described an alternative assay for scoring microsatellite loci that minimised size homoplasy. They used single-strand conformational polymorphism (SSCP) for the identification of alleles and gel isolation for direct sequencing analysis. In order to reveal this occult polymorphism we have incorporated the analysis of heteroduplexes, as an optimised approach, to the conventional genotyping with microsatellites. Heteroduplexes are double-stranded DNA molecules formed between two different alleles and, therefore, they contain mismatches. Heteroduplexes can be detected because they migrate differently to the corresponding homoduplexes in an electrophoresis gel (White et al. 1992). The greater the number of mismatches, the greater the relative gel retardation of the heteroduplex molecules, and unstable mismatches contribute more to the gel retardation than stable mismatches (Aboul-ella et al. 1985; Ke and Wartell 1993, 1996). The strategic labelling of 'probe' sequences has given rise to a modification of heteroduplex analysis termed directed-heteroduplex analysis (DHDA, Zimmerman et al. 1993), which has allowed a more accurate testing of genetic polymorphism as well

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as the standardisation and automat of this class of genotyping (Zimmerman et al. 1995; Argüello et al. 1998). These approaches have been used successfully in the assessment of haplotypes and estimation of genetic distances and phylogenetic relationships (Campbell et al. 1995; Rodrigues et al. 1995; Martinelli et al. 1996; Moreira et al. 1996). At present, practically any difference in genetic composition between two amplified allelic DNA fragments with the same length can be revealed by heteroduplex analysis (Van den Akker et al. 1992; Zimmerman et al. 1993; Oka et al. 1994; Gu et al. 1998). Furthermore, heteroduplex fragments can serve for separating alleles from any heterozygous genetic locus without cloning (Arguello et al. 1997). Once a heteroduplex fragment is isolated from an electrophoresis gel it can be employed for directly sequencing two different alleles with the same primers used in amplification reactions (Tamura et al. 1993; Arguello et al. 1997).

We propose that the study of PCR-amplified microsatellite loci at both the homo- and heteroduplex level can be a powerful tool for inferring genetic relationships among polyploid individuals belonging to different varieties of the same species. In the investigation presented here, we have chosen some of the different varieties of *Solanum tuberosum* that are currently planted on Tenerife Island (Canary Archipelago; Alvarez and Gil 1996). Some are imported yearly as tuber seeds from Europe, mainly from Great Britain (i.e. 'King Edward'), and these occupy the largest part of the fields dedicated to potato cultivation on the island due to their high productivity. Others are traditional local varieties which were introduced to Tenerife Island during the past centuries (Gil 1997) from Europe (i.e. 'Palmera', 'Peluca') as well as from America (i.e. 'Negra' and 'Borralla'). These last two varieties probably arrived from Peru (Lobo-Cabrera 1988) independently of the introduction of *Solanum tuberosum* into the European Continent, which occurred during the last quarter of the sixteenth century (Hawkes 1992), as after the discovery of the Americas, the Canary Archipelago was an obligatory stop-over for ships on the Spain-America route due to its geographical situation. These Canary potato varieties are still present in a relict form and, despite their low productivity, are highly appreciated for local consumption. The identification of these local varieties by farmers is based on morphological (skin and flesh colour, tuber eyes) and cultivation characteristics. Has this popular classification system a genetic basis? To resolve the dilemma – molecular taxonomy versus folkloric classification – we decided to study genetic polymorphism at a microsatellite locus in five potato cultivars of *Solanum tuberosum* from Tenerife Island employing the strategy outlined above.

Materials and methods

Plant material and DNA isolation

Studies were carried out on four local *S. tuberosum* varieties from the Canary Islands with varying degrees of relatedness (Zubeldia et al. 1955), three varieties being tetraploid ('Peluca negra', 'Palmera negra' 'and 'Bor-ralla') and one triploid ('Negra'), and a tetraploid British variety ('King Edward'). The number of samples analysed from each variety were 20 from 'Negra', 8 from 'Peluca negra' and 'King Edward' and 7 from 'Palmera negra' and 'Borralla'. Tubers belonging to the same varieties were collected from different cultivars. Genomic DNA was isolated using a miniprep procedure based on the method described by Edwards et al. (1991). The surface of the potato tubers was cleaned by polishing with a paper soaked in ethanol. Small pieces of tuber peel (50 mg) were excised with a scalpel, avoiding underlying starch, deposited into a microfuge tube and frozen at -70 ºC for at least 1 h. Tissue samples were ground while frozen, dispersed in 400 µl of extraction buffer (200 m*M* TRIS-HCl, pH 8.0, 250 m*M* NaCl, 25 m*M* EDTA, 0.5% SDS) by vigorous vortexing for 10 s and incubated at room temperature for 5 min. Cellular debris was eliminated by centrifugation at 13 000 *g* for 10 min at room temperature, and the supernatant (approx. 300 µl) was then collected and mixed with an equal volume of isopropanol. After a standing phase of 15 min and a centrifugation at 13 000 *g* for 10 min, both at room temperature, the resulting pellet was washed twice with 80% ethanol (5 min each time) and dried at 50 ºC (10 min). Nucleic acids were recovered by incubation in 100 µl of sterile TE buffer (10 m*M* TRIS-HCl, pH 8.0, 1 m*M* EDTA) at 50 ºC for 1 h, with occasional gentle agitation. Finally, the sample was centrifuged at 13 000 *g* for 5 min at room temperature, and 80 µl of the supernatant was transferred to a new microfuge tube.

PCR amplification and analysis of a microsatellite locus

A $(TCT)_n$ microsatellite, located in intron I from the gene for granule-bound starch synthase of *S. tuberosum* (GBSS gene; Van der Leij et al. 1991), was amplified employing as primers the oligonucleotides of sequence 5'-AATCGGTGATAAATGTGAATGC-3' and 5'-ATGCTTGCCATGTGATGTGT-3', which were designed by Provan et al. (1996). PCR was carried out in a total reaction volume of 20 µl containing 16 m*M* $(NH_4)_2SO_4$, 67 mM TRIS-HCl (pH 8.8), 0.01% Tween-20, 2.5 m*M* $MgCl_2$, 0.2 m*M* dNTPs, 1 µ*M* each primer, 0.2 U of *Taq* DNA polymerase (Bioline, UK) and 2 µl of the template DNA preparation (approx. 50–100 ng). Amplifications were performed in a PTC-100™ thermal cycler (MJ Research, Mass.) using the following cycling profile: initial denaturation step at 94 ºC (2 min); followed by 25 cycles at 94 ºC (30 s), 58 ºC (30 s) and 72 ºC (40 s); and one final elongation step at 72 ºC (5 min). The products of the amplification reactions carried out under the conditions described above were essentially homoduplex DNA molecules. The generation of a mixture of homo- and heteroduplex DNA molecules was ensured by increasing the number of amplification cycles up to 30 and, before the final elongation step at 72 ºC, denaturing the PCR products at 94 ºC for 2 min and allowing renaturation at 20 ºC for 30 min.

For the re-amplification of specific subgroups of allelic microsatellites, the desired PCR products were recovered from the gel with a scalpel and transferred separately to microfuge tubes. The plugs of gel were triturated, mixed with 50 µl of 0.3 *M* sodium acetate (pH 6.8), 1 m*M* EDTA, and incubated at 37 ºC overnight. Following a centrifugation step at 13 000 *g* for 10 min at room temperature, 10 µl of the supernatant was collected and diluted 10^3- and 10^4-fold with TE buffer. These diluted samples were mixed in all combinations and used as template DNA (2 µl) in PCR reactions. Different proportions of samples were tested in these mixtures until a balanced amplification of individual allelic microsatellites was achieved.

Duplexes were separated on 8% non-denaturing polyacrylamide gels (19:1 acrylamide: bisacrylamide) run in 1 × TBE buffer (89 m*M* TRIS-borate, pH 8.3, 2 m*M* EDTA) at 300 V (constant) for 1.5 h in a Xcell II Mini-Cell electrophoresis unit (NOVEX, San Diego, Calif.). Gels were stained with ethidium bromide (1 µg/µl) for 15 min, destained in distilled water for 5 min and photographed under UV light.

Results and discussion

Generation and identification of heteroduplex DNA molecules

The formation of heteroduplexes of DNA molecules can arise during the PCR or can occur as a post-PCR event (White et al. 1992; Jensen and Straus 1993) by cross-hybridisation between different alleles from heterozygous individuals. The first approach implies sub-optimum conditions for an adequate amplification, such as a low primer concentration or a higher number of amplification cycles, and hence is more liable to produce unspecific products, artefactual bands and smears. The procedure described in the Materials and methods section falls within the framework of the second approach and permits a reproducible and informative pattern of electromorphs, including homo- and heteroduplex molecules (Fig. 1a), to be obtained. After 30 PCR cycles the concentration of primer reached a level so low that it would not efficiently compete with template reannealing when the activity of the *Taq* polymerase was greatly reduced (post-PCR step at 20 ºC). The amplification reaction under these conditions rendered sufficient product for visualisation of complex electrophoretic patterns with up to 16 bands (var 'Borralla', Fig. 1a). Sometimes, and especially with very fresh samples, the yield of DNA extraction was so high that the primers were soon depleted in the amplification mixture and heteroduplexes were produced during PCR. Therefore, the number of amplification cycles was reduced to 25 when the exclusive visualisation of homoduplexes was required (Fig. 1b). The nature and origin of some of these hybrid duplexes were demonstrated through the re-amplification of defined subgroups of homoduplexes. This analysis was carried out using the triploid variety 'Negra'; three PCR fragments of different lengths were visualised (Fig. 1b) and, therefore, homoplasy can be disregarded. This allele dosage implied that six different heteroduplexes could be assembled. The results of this study are shown in Fig. 2 and summarised in Table 1. As expected, heteroduplexes were generated in pairs only if at least two different microsatellites were present in the assays. In this case, one heteroduplex of pair H7 and H8, for example, is formed by pairing the sense strand of M4 and antisense strand of M5 and the other heteroduplex by the assemblage of the antisense strand of M4 and the sense strand of M5. The maximum number of heteroduplexes which can be formed are 2 x ($C_{n,2}$), n being the number of alleles. Variety 'Borralla', with four homoduplexes, can form 2 x ($C_{4,2}$) = 12 heteroduplexes (lane 4, Fig. 1a).

Heteroduplexes can be detected on polyacrylamide gels because they migrate more slowly than their corresponding homoduplexes. This shift is enhanced by differences in the length of the strands composing the heteroduplex (Van der Akker et al. 1992), a circumstance that usually occurs when different microsatellite alleles are amplified in the same reaction. The relative electrophoretic mobility of the heteroduplexes is moderately sensitive to the separation conditions. In our assays, the proportion between acrylamide and bisacrylamide was the most influential factor on the global pattern of electromorphs obtained from an individual sample.

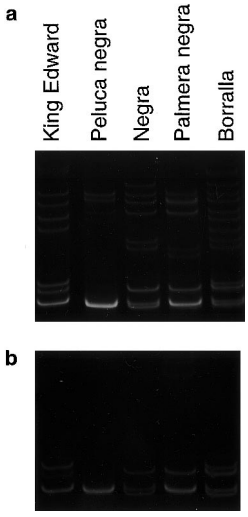

Fig. 1 a Homo- and heteroduplex band patterns. **b** Homoduplex band patterns

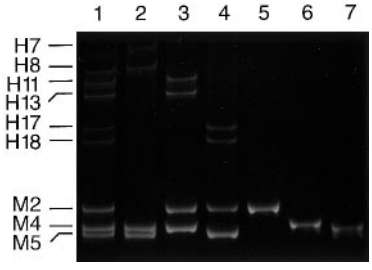

Fig. 2 Heteroduplexes generated after re-amplification of different subgroups of homoduplexes from var 'Negra'. *Numbers above lanes* are the same as these in Table 1, thereby enabling the comparison of these results with data included in Table 1

Table 1 Summary of the results from Fig. 2

Lane	Homoduplex[a]	Heteroduplex
1	M2, M4, M5	H7, H8, H11, H13, H17, H18
2	M4, M5	H7, H8
3	M2, M4	H11, H13
4	M2, M5	H17, H18
5	M2	–
6	M4	–
7	M5	–

[a] Allele M4 was later renamed M4a

Composition of GBSS alleles deduced by heteroduplex analysis

The current approach for the analysis of microsatellite loci presupposes that allelic PCR fragments of the same length present identical nucleotide sequences. Nevertheless, several reports point out the possibility of homop-

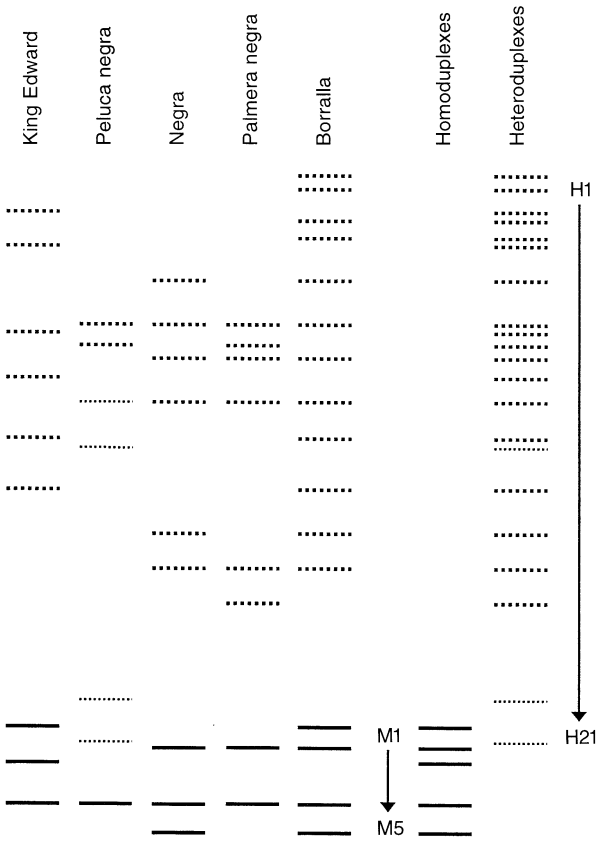

Fig. 3 Schematic representation of the homo- (*solid lines*) and heteroduplexes (*dashed lines*) electromorphs detected in all of the varieties analysed. *Thin dashed lines* represent heteroduplex molecules of low intensity. *M* Homoduplexes, *H* heteroduplexes, *a number* that increases in accordance with the electrophoretic mobility (*arrow*) of the DNA fragment

lasy (Estoup et al. 1995; Fitzsimmons et al. 1995; Grimaldi and Crouau-Roy 1997; Angers and Bernatchez 1997). The differences in genetic composition (nucleotide substitution, insertions and/or deletions) between two or more PCR products of the same length could be revealed by promoting the formation of hybrid molecules (heteroduplex pairs). A comparison of the results shown in Figs. 1 a and b allows the identification of five homoduplex and 21 heteroduplex electromorphs, which were designated as is indicated in Fig. 3. In our analysis we have assumed that if two varieties share a heteroduplex pair then they also share a homoduplex pair.

The two varieties ‘Negra’ and ‘Borralla’ produced a number of PCR fragments of different sizes that was equal to their ploidy (triploid and tetraploid, respectively), and therefore the homoplasy state can be disregarded. In ‘Borralla’, the presence of four GBSS alleles implies that 12 heteroduplex molecules could be formed (Fig. 3). Since the heteroduplexes H7, H8, H11, H13, H17 and H18 are present in ‘Borralla’ and ‘Negra’, the two varieties shared homoduplexes M2, M4, M5 (Table 1). Subsequent analysis demonstrated a homoplasy state for the allelic size-class M4, and hence we designated this first allele as M4a. The other heteroduplexes detected in

Table 2 Composition of GBSS alleles inferred by heteroduplex analysis

	Allele						
Variety	M1	M2	M3	M4a	M4b	M4c	M5
King Edward	+		+	+			
Peluca negra				+	+	+	
Negra		+		+			+
Palmera negra		+		+	+		
Borralla	+	+		+			+

‘Borralla’ result from M1 combining with the rest of the homoduplexes. The allele dosage inferred for vars ‘Borralla’ and ‘Negra’ are 1M1: 1M2: 1M4a: 1M5 and 1M2: 1M4a: 1M5, respectively.

The tetraploid variety ‘Palmera negra’ exhibited only two homoduplex fragments of different signal strengths (M4 > M2; Fig. 1b), however 6 heteroduplexes could be detected (Fig. 3), which implies the presence of three different alleles. Two of these heteroduplexes are shared with ‘Negra’ and ‘Borralla’: heteroduplexes H11 and H13 formed by cross-hybridisation of M2 and M4a (Table 1). The other heteroduplexes (H8, H10, H18 and H19) must have been generated by the combination of M2 and M4a with another homoduplex, which must belong to the size-class M4 and, therefore, was named M4b. In conclusion, the allele dosage for this tetraploid variety should be 1M2: 2M4a: 1M4b or 1M2: 1M4a: 2M4b. Notice that the proposed allele dosage for this variety assumed a symmetric PCR amplification, which may not have occurred. The level of homoplasy was even more notable in ‘Peluca negra’, the other Canary variety of European origin, which rendered one homoduplex and 6 heteroduplex fragments (Fig. 3). ‘Peluca negra’ and ‘Palmera negra’ shared the heteroduplexes H8 and H10 and, therefore, they shared a pair of homoduplexes. These should be the electromorphs M4a and M4b since all the alleles detected in ‘Peluca negra’ belong to the M4 size-class and M4a and M4b are the unique homoduplexes of this class present in ‘Palmera negra’. The heteroduplexes H13, H15, H20 and H21 were detected exclusively in var ‘Peluca negra’, which implies the participation of new M4-class allele denominated as M4c. We could not discern which of these three alleles of the M4 size-class were present in two copies in var ‘Peluca negra’. The heteroduplexes H13 and H15, and especially H20 and H21, were difficult to detect (‘invisible’ heteroduplexes; Hatcher et al. 1993). Their clear visualisation required either an increase in the volume of sample loaded onto the electrophoresis gel or an increased exposure time of the photographic film.

The tetraploid ‘King Edward’ variety showed three homoduplex and 6 heteroduplex fragments (Fig. 3). ‘King Edward’ and ‘Borralla’ shared heteroduplexes H14 and H16 and, hence, these two varieties shared a pair of homoduplexes. These duplexes could only be M1 and M4a, the unique homoduplexes shared by ‘Borralla’ and ‘King Edward’ varieties. The homoduplex named

M3 is present exclusively in the British variety and, in combination with the other alleles, produces the rest of heteroduplexes that were not detected in the other varieties. Taking into account that the signal strength displayed by the homoduplexes detected was M4a > M1= M3, we estimate that the allele dosage of this variety is 1M1: 1M3: 2M4a.

A summary of the allele composition deduced for all the varieties studied through heteroduplex analysis is shown in Table 2. The M4a allele is present in all varieties, the M3 allele is present exclusively in the British variety and alleles M2, M4b and M5 are present in more than one of the local varieties. Note that heteroduplex analysis increases the informativeness of the microsatellite study, since alleles M4a, M4b and M4c are grouped in one size-class allele by the homoduplex classical approach.

Exactitude of farmer's assertions

The genetic differences among local potato varieties detected in this study correspond accurately with the current identification system of Tenerife farmers. Despite this great intervariety diversity, no differences were found when the intravariety diversity was examined: all samples belonging to a variety exhibited the same pattern of homo- and heteroduplex electromorphs. This result could be due to the sample size or to the fact that they truly reflect a genetic drift (autochthonous varieties are still present in a relict form) caused by agricultural practice. Present-day farmers are highly specialised, selecting only one variety to be planted in the field every year by tuber clonal propagation, in contrast with the diversity of tuber variety crops chosen by farmers in the past (Gil 1997). This old cultivation practice, similar to that utilised by farmers in some regions of the Peruvian Andes (Quiros et al. 1992), could explain the high degree of intervariety diversity found in Tenerife Island cultivars. The homogeneous procedure of cultivation of the current farmers should be in relation with the null intravariety polymorphism detected.

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References

Aboul-ella F, Koh D, Tinoco I, Martin FH (1985) Base-base mismatch. Thermodynamics of double helix formation for $dCA_3XA_3G + dCT_3YT_3G$ (X,Y = A,C,G,T). Nucleic Acids Res 13: 4811-4824

Alvarez CE, Gil J (1996) Inventario de las papas presentes en la isla de Tenerife y de los nombres que en ella reciben. Hoja Divulgativa nº 5. Servicio Insular de Agricultura del Cabildo Insular de Tenerife, Santa Cruz de Tenerife

Angers B, Bernatchez L (1997) Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. Mol Biol Evol 14: 230-238

Arguello R, Pay AL, McDermott A, Ross J, Dunn P, Avakian H, Little A-M, Goldman J, Madrigal JA (1997) Complementary strand analysis: a new approach for allelic separation in complex polyallelic genetic systems. Nucleic Acids Res 11, 2236-2238

Argüello JR, Little AM, Pay AL, Gallardo D, Rojas I, Marsh SGE, Goldman JM, Madrigal JA (1998) Mutation detection and typing of polymorphic loci through double-strand conformation analysis. Nat Genet 18:192-194

Bell CJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19:137-144

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368: 455-457

Campbell NJ, Harris FC, Elphinstone MS, Baverstock PR (1995) Outgroup heteroduplex analysis using temperature gradient gel electrophoresis: high resolution, large scale, screening of DNA variation in the mithocondrial control region. Mol Ecol 4: 407-418

Crawford AM, Kappes SM, Paterson KA, de Gotari MJ, Dodds KG, Freking BA, Stone RT, Beattie CW (1998) Microsatellite evolution: testing the ascertainment bias hypothesis. J Mol Evol 46:256-260

Edwards K, Johnstone C, Thomson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res 19: 1349

Estoup A, Tailliez C, Cornuet J-M, Solignac M (1995) Size homoplasy and mutational processes of interrupted microsatellites in two species, *Apis mellifera* and *Bombus terrestris* (Apidae). Mol Biol Evol 12: 1074-1084

Fitzmmons NN, Moritz C, Moore SS (1995) Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Mol Biol Evol 12: 432-440

Gil J (1997) El cultivo tradicional de la papa en la isla de Tenerife.Asociación Granate. La Laguna

Goldstein DB, Ruiz-Linares A, Feldman MW, Cavalli-Sforza LL (1995) An evaluation of genetic distances for use with microsatellite loci. Genetics 139: 463-471

Gorza JC, Slatkin M, Freimer NB (1995) Microsatellite allele frequencies in human and chimpanzees with implications for constraints on allele size. Mol Biol Evol 12: 594-603

Grimaldi M-C, Crouau-Roy B (1997) Microsatellite allelic homoplasy due to variable flanking sequences. J Mol Evol 44: 336-340

Gu W, Aguirre GD, Ray K (1998) Detection of single-nucleotide polymorphism. Biotechniques 24: 836-837

Hatcher SL, Lambert QT, Teplitz RL, Carlson JR (1993) Heteroduplex formation: a potential source of genotyping error from PCR products. Prenat Diagn 13: 171-177

Hawkes J (1992) History of the potato in the potato crop. In: Harris PM (ed) The potato crop. Chapman and Hall, London

Hederson ST, Petes TD (1992) Instability of simple sequence DNA in *Saccharomyces cerevisiae*. Mol Cell Biol 12: 2749-2757

Jensen MA, Straus N (1993) Effect of PCR conditions on the formation of heteroduplex and single-stranded DNA products in the amplification of bacterial ribosomal spacer regions. PCR Methods Applic 3: 186-194

Kashi Y, Tikochinsky Y, Genislav E, Iragi F, Nave A, Beckmann JS, Gruenbaum Y, Soller M (1990) Large restriction fragments containing poly-TG are highly polymorphic in a variety of vertebrates. Nucleic Acids Res 18: 1129-1132

Ke SH, Wartell RM (1993) Influence of nearest neighbour sequence on the stability of base pair mismatches in long DNA; determination by temperature-gradient gel electrophoresis. Nucleic Acids Res 21: 5137-5143

Ke SH, Wartell RM (1996) The thermal stability of DNA fragments with tandem mismatches at a d(CXYG).d(CY'X'G) site. Nucleic Acids Res 24: 707-712

Krieger MJB, Keller L (1997) Polymorphism at dinucleotide microsatellite loci in fire ant *Solenopsis invicta* populations. Mol Ecol 6: 997-999

Lehmann T, Hawley WA, Collins FH (1996) An evaluation of evolutionary constraints on microsatellite loci using null alleles. Genetics 144: 1155-1163

Lobo-Cabrera M (1988) El comercio canario europeo bajo Felipe II. Gobierno de Canarias y Governo Regional da Madeira, Funchal

Martinelli G, Trabetti E, Farabegoli P, Buzzi M, Zaccaria A, Testoni N, Amabile M, Casartelli A, de Vivo A, Pignatti PF, Tura S (1996) 'Fingerprinting' of HLA-DQA by polymerase chain reaction and heteroduplex analysis. Mol Cell Probes 10: 123-127

Moreira MA, Almeida CA, Canavez F, Olicio R, Seuanez HN (1996) Heteroduplex mobility assays (HMAs) and analogous sequence analysis of a cytochrome b region indicate phylogenetic relationships of selected callitrichids. J Hered 87: 456-460

Oka T, Matsunaga H, Tokunaga K, Mitsunaga S, Juji T, Yamane A (1994) A simple method for detecting single base substitutions and its application to HLA-DPB1 typing. Nucleic Acids Res 9: 1541-1547

Ortí G, Pearse DE, Avise J C (1997) Phylogenetic assessment of length variation at a microsatellite locus. Proc Natl Acad Sci USA 94: 10745-10749

Provan J, Powell W, Waugh R (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). Theor Appl Genet 92: 1078-1084

Quiros CF, Ortega R, van Raamsdonk L, Herrera-Montoya M, Cisneros P, Schmidt E, Brush SB (1992) Increase of potato genetic resources in their center of diversity: the role of natural outcrossing and selection by the Andean farmer. Genet Resource Crop Evol 39: 107-113

Rodrigues F, Pena S, Tyler-Smith C (1995) PCR haplotypes for the human Y chromosome based on alphoid satellite DNA variants and heteroduplex analysis. Gene 165: 191-198

Tamura K, Yamamoto Y, Saeki Y, Furuyama J, Utsunomiya J (1993) Simple, rapid and accurate determination of deletion mutations by automated DNA sequencing of heteroduplex fragments of the adenomatous polyposis coli (APC) gene generated by PCR amplification. Hum Mutat 2: 478-484

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 17: 6463-6471

Van den Akker K, Braun JEF, Pals G, Lafleur MVM, Retèl J (1992) Single base mutations can be unequivocally and rapidly detected by analysis of DNA heteroduplexes, obtained with deletion-mutant instead of wild-type DNA. Nucleic Acids Res 20: 6745-6746

Van der Leij FR, Visser RGF, Ponstein AS, Jacobsen E, Feenstra WJ (1991) Sequence of the structural gene for granule-bound starch synthase of potato (*Solanum tuberosum* L.) and evidence for a single point deletion in the *amf* allele. Mol Gen Genet 228: 240-248

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88:1-6

Weber JL (1990) Informativeness of human $(dC\text{-}dA)_n*(dG\text{-}dT)_n$ polymorphisms. Genomics 7: 524-530

Weber JL, Wong C (1993) Mutation of human short tandem repeats. Hum Mol Genet 2: 1123-1128

White MB, Carvalho M, Derse D, O'Brien SJ, Dean M (1992) Detecting single base substitutions as heteroduplex polymorphisms. Genomics 12: 301-306

Zimmerman PA, Carrington MN, Nutman TB (1993) Exploiting structural differences among heteroduplex molecules to simply genotyping the DQA1 and DQB1 alleles in human lymphocyte typing. Nucleic Acids Res 19: 4541-4547

Zimmerman PA, Shapiro M, Tang J, Nutman TB, Unnasch TR (1995) Optimizing probe selection in directed heteroduplex analysis using HDProbe 1.1. Biotechniques 6: 972-977

Zubeldia A, López G, Sañudo A (1955) Estudio, descripción y clasificación de un grupo de variedades primitivas de patata cultivadas en las Islas Canarias. Bol Inst Nac Invest Agro 33: 287-325