

# A New and Improved Automated Technology for Early Sex Determination of *Ginkgo biloba*

Vincent Echenard, François Lefort, Gautier Calmin, Robert Perroulaz, and Lassaad Belhagri

**Abstract.** Random amplified polymorphic DNA (RAPD) technique with male associated decamer primer S1478 was used to amplify DNA from 72 leaf samples collected from *Ginkgo biloba* trees with known sexual determinism in the canton of Geneva, Switzerland. This marker was found to be male-specific and was lacking in all female plants. Automated random polymorphic DNA analysis (ARPA), a new automated technology developed in the frame of this work, proved highly effective in distinguishing males and females with 100% efficiency and successful in male and female discrimination from a collection of young seedlings derived from a sexual cross. Our findings provide unambiguous evidence that ARPA combined with the male-associated decamer primer S1478 could be considered an efficient, rapid, and easy method to make an early sex determination in the dioecious tree *Ginkgo biloba*.

The maidenhair tree *Ginkgo biloba* is a widely popular deciduous and dioecious gymnosperm species that is native to China and has been cultivated for well over a millennium. *Ginkgo biloba* has been described as a “living fossil” because it is known to have existed early in the Jurassic period and is believed to be the sole survivor of the ancient family of Ginkgoaceae (Carrier et al. 1998; Zhou and Zheng 2003). In Asia, *G. biloba* is used in traditional medicine and its seeds, also known as Ginkgo nuts, are considered a delicacy and are also a popular cuisine item (Kiple and Ornelas 2000). Asian people systematically planted the tree and many living ginkgoes are known to be more than five centuries old in China and for approximately 1,000 years in Japan. Ginkgo was introduced to Europe in the 18th century where it was very popular in large cities like London and Paris (Rohr 1989). The oldest European ginkgo would have been sown in 1730 in the Utrecht Botanical Garden. Most of the earlier trees raised in Europe appear to have been males. The first recorded female tree was found in Bourdigney near Geneva in 1814 of which scions were grafted on a male tree in the Botanic garden of Montpellier where the first perfect seed has grown (Michel 1985).

*G. biloba* had an ability to survive pests, drought, storms, ice, and city soils (Aoki 1997; Handa et al. 1997; Honda 1997; Kim et al. 1997). Because it does not have invasive roots and requires minimum maintenance, it could be the perfect urban tree with a large potential planting range in temperate and subtropical climates in both hemispheres. The ginkgo is dioecious. That simply means that there are separate male and female plants (Laurain 2000). Female trees produce seeds that emit a noxious, foul odor on falling to the ground. The smell’s description ranges from “rancid butter” to “vomit” and therefore female trees are considered to be undesirable (Wada and Haga 1997). The slippery pulp can also be a liability. These features caused city governments to actually remove and ban the female from being planted. Male ginkgoes do not produce a fruit and are selected as the main cultivars used to transplant in urban communities. This male cultivar of Ginkgo casts dense shade and the dense crown makes it suitable as a screen or noise buffer. It makes a durable

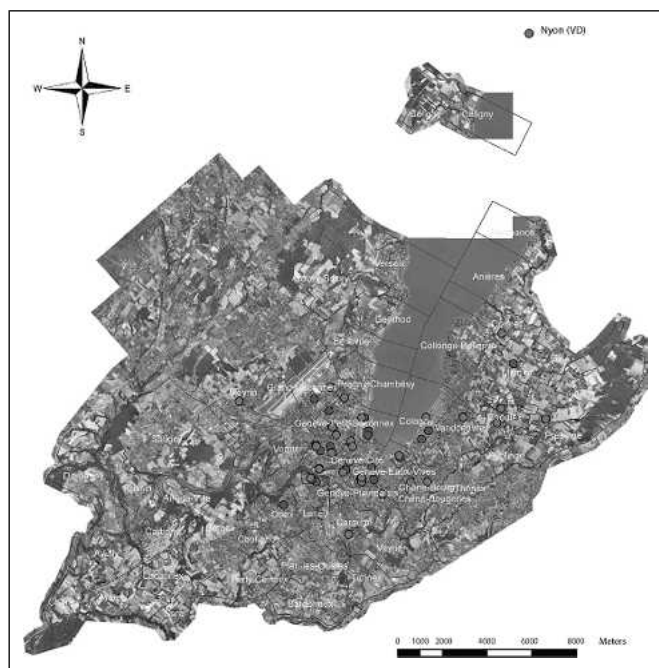
street tree where there is enough overhead vertical space to accommodate the large size.

The availability of male and female associated random amplified polymorphic DNA (RAPD) markers such as the decamer primer S1478 (Jiang et al. 2003) prompted us to validate a reliable marker by testing it over a large collection of trees in the Canton of Geneva that originates from China as well as Japan. Jiang et al. (2003) screened 1200 random decamers, which generated 8,372 RAPD primers among which they found two male-linked markers RAPD markers. One of these markers, S1478, was validated for sex determination in Ginkgo trees from two Chinese regions. The need for a more rapid and reproducible method of early sexual determinism in *G. biloba* led us to develop an automated version of RAPD, which previously relied on manual agarose gel electrophoresis and DNA detection by ethidium bromide staining. Automated random polymorphic DNA analysis (ARPA) has several advantages over the former method, thus increasing its usefulness as a technique for assessing the sexual determinism in *G. biloba*. The new method was found to be highly reproducible at all levels of replication tested. In this study, a capillary electrophoresis system was used to resolve large (up to 682 bp) ARPA–polymerase chain reaction size fragments from plant genomic DNA, representing a new application for this automated system. Therefore, ARPA was shown to be an efficient, easy, and cost-effective means for processing a large collection of *G. biloba* seeds for early sex determination.

## MATERIALS AND METHODS

### Plant Material

A total of 72 *G. biloba* trees of identified sex collected from Geneva, Carouge, Chêne-Bougeries, Choulex, Cologny, Corsier, Grand-Lancy, Grand-Saconnex, Jussy, Meinier, Meyrin, Nyon, Onex, Petit-Lancy, Petit-Saconnex, Vandoeuvre (Canton, Geneva) were used in this study. These are trees planted in different streets in the listed localities (Figure 1) and were located before the flowering period. A picture of each tree was taken on four occasions in each season from 11 November 2006



**Figure 1.** Distribution of the different regions in the Canton of Geneva on which *G. biloba* trees were sampled. Blue and rose spots refer, respectively, to male and female trees (1 km = 0.62 mi).

until 7 September 2007 and compiled in a database. Trees were then checked during flowering for the sexual type of the flower (April 2007). Only trees for which the sex was determined without ambiguity were retained for leaf sampling, which took place in June 2007. GPS Swiss coordinates are given in Figure 2. Most trees are of Chinese origin, whereas five trees located in one park of Geneva City come from Japan. Plant material consisted of young leaves randomly sampled from the low branches of the low canopy of adult trees. Such a choice was determined by the difficulty to sample leaves on adult trees, which may be very tall. Three leaves were sampled per tree and kept frozen in nitrogen liquid until used. Seedlings of *G. biloba* used in this study were obtained from seeds collected from a female tree located in “Parc des Eaux-Vives” (Geneva, Satellite Swiss coordinates X: 501,956.004, Y: 118,199.472), which were sown in individual pots of horticultural substrate and then labeled for differentiating from one another.

### DNA Isolation

DNA was purified from leaves with the use of the protocol described by Lefort and Douglas (1999) according to the manufacturer’s specifications. Quality was checked by visualization under ultraviolet light after electrophoresis separation with a molecular mass standard (1 Kb Ladder Plus DNA Marker; Invitrogen, Basel, Switzerland) in 2% agarose gel (Sigma, Buchs, Switzerland) in 1XTBE subjected to 100 V for 1 hr, and stained

**Table 1.** Sampled trees: sample number, sex based on flower morphology, circumference on 15 January 2007, GPS coordinates, and sampling dates (1 m = 3.28 ft).

Sample number	Sex	Location	Corporation	Circumference (m) on 15 January 2007	Satellite coordinate X (m)	Satellite coordinate Y (m)	Sampling date
1	Male	Rosemont, 11	Chêne-Bougeries	1.91	502427.078	117107.687	19 April 2007
2	Male	Route de Chêne, 131	Chêne-Bougeries	2.73	503180.284	117124.230	19 April 2007
3	Male	Parc Bertrand	Genève	1.12	501168.857	116572.275	19 April 2007
4	Male	Parc des Bastions	Genève	2.34	500249.313	117130.946	19 April 2007
5	Male	Parc des Bastions	Genève	2.20	500240.375	117112.005	19 April 2007
6	Male	Parc des Bastions	Genève	2.13	500224.768	117300.774	19 April 2007
7	Male	Parc des Bastions	Genève	2.87	500235.906	117302.264	19 April 2007
8	Male	Parc de Battelle	Carouge	1.51	499646.597	114762.742	19 April 2007
9	Male	Cycle de la Golette	Meyrin	1.33	494765.104	120717.605	19 April 2007
10	Male	Cycle de la Golette	Meyrin	0.84	494766.338	120713.831	19 April 2007
11	Male	Rue du Moillebeau	Genève	1.96	498892.509	119582.782	19 April 2007
12	Male	Parc Trembley	Genève	1.48	498935.706	119557.781	19 April 2007
13	Male	Parc Châtelaïne	Genève	3.46	498206.596	118700.383	19 April 2007
14	Male	Route de Pressy, 16	Vandoeuvre	1.89	504781.494	119997.565	20 April 2007
15	Male	Chemin du Bonheur	Choulex	1.32	506285.127	119751.436	20 April 2007
16	Male	Église de Choulex	Choulex	1.67	506477.043	120392.574	20 April 2007
17	Male	Route de la Tour-Carrée	Coligny	2.37	503116.189	119996.154	20 April 2007
18	Male	Parc des Eaux-Vives	Genève	2.60	501910.562	118282.329	20 April 2007
19	Male	Jardin Botanique	Genève	1.57	500243.074	119979.878	19 April 2007
20	Male	Parc de Beaulieu	Genève	1.55	499753.027	118936.416	19 April 2007
21	Male	OMS	Genève	1.27	499460.573	120873.058	19 April 2007
22	Male	OMS	Genève	1.22	499465.871	120879.953	19 April 2007
23	Male	Parc des Croupettes	Genève	1.92	499837.210	118685.191	19 April 2007
24	Male	Quai de Nyon	Nyon	3.12	507721.000	137163.000	19 April 2007
25	Male	Parc du Seujet	Genève	2.02	499675.030	118022.716	19 April 2007
26	Male	Cimetière des Rois	Genève	1.19	499401.874	117543.673	19 April 2007
27	Male	Mairie de Lancy	Grand-Lancy	1.38	498440.311	115603.535	19 April 2007
28	Male	Mairie du Grand Saconnex	Grand-Saconnex	2.52	498830.482	121343.549	19 April 2007
29	Male	Route de Thonon, 245	Corsier	1.70	506509.175	123745.520	20 April 2007

(continued)

Table 1. (continued)

Sample number	Sex	Location	Corporation	Circumference (m) on 15 January 2007	Satellite coordinate X (m)	Satellite coordinate Y (m)	Sampling date
30	Male	Parc Trembley	Genève	0.98	499100.651	119219.240	19 April 2007
31	Male	Center de Lullier	Jussy	0.99	508481.722	119923.273	20 April 2007
32	Male	Cimetière St. Georges	Petit-Lancy	1.09	497946.853	117284.458	19 April 2007
33	Male	Cimetière St. Georges	Petit-Lancy	0.46	497952.573	117287.705	19 April 2007
34	Male	Parc de St-Jean	Genève	1.01	498335.260	117687.124	19 April 2007
35	Male	Rue de Lamartine, 7	Genève	0.79	498899.845	118435.259	19 April 2007
36	Male	Rue de Lamartine, 8	Genève	0.70	498899.119	118409.928	19 April 2007
37	Male	Center de Lullier (cv. Horizontalis)	Jussy	0.76	508468.108	119922.504	20 April 2007
38	Female	Center de Lullier (cv. Variegata)	Jussy	0.31	508475.014	119924.052	20 April 2007
39	Female	Square Rodolphe-Toepfer	Genève	2.40	500775.389	117228.107	19 April 2007
40	Female	Square Rodolphe-Toepfer	Genève	2.41	500783.773	117218.338	19 April 2007
41	Female	Parc Bertrand, Genève	Genève	1.08	501170.986	116575.952	19 April 2007
42	Female	Parc des Bastions	Genève	0.80	500186.569	117255.623	19 April 2007
43	Female	Parc des Bastions	Genève	1.37	500231.862	117292.829	19 April 2007
44	Female	Parc des Bastions	Genève	2.01	500234.700	117299.923	19 April 2007
45	Female	Cimetière des Rois	Genève	1.41	499518.314	117637.221	19 April 2007
46	Female	Cimetière St. Georges	Petit-Lancy	1.00	498201.302	117126.888	19 April 2007
47	Female	Cimetière St. Georges	Petit-Lancy	1.18	498013.183	117223.430	19 April 2007
48	Female	Avenue du Bois de la Chapelle, 75	Onex	1.52	496749.453	116077.343	19 April 2007
49	Female	Cycle de la Golette	Meyrin	1.42	494769.676	120716.807	19 April 2007
50	Female	Parc Châtelaine	Genève	1.75	498196.235	118771.783	19 April 2007
51	Female	Rue de la Capite, 47	Cologny	2.14	503222.831	119401.071	20 April 2007
52	Female	Route de Pressy, 16	Vandoeuvre	1.73	504783.032	120010.795	20 April 2007
53	Female	Eglise de Choulex	Choulex	1.29	506459.353	120410.726	20 April 2007
54	Female	École de Meinier	Meinier	1.02	507025.828	122394.806	20 April 2007
55	Female	Route de la Tour-Carrée	Cologny	1.49	503120.314	119998.871	20 April 2007
56	Female	Parc des Eaux-Vives	Genève	2.53	501956.004	118199.472	20 April 2007
57	Female	Perle du Lac	Genève	1.46	500531.590	119394.281	19 April 2007
58	Female	Perle du Lac	Genève	1.72	500534.784	119385.934	19 April 2007
59	Female	Parc du Château-Banquet, 12	Genève	1.26	500510.572	119212.345	19 April 2007
60	Female	UNICEF	Genève	0.94	500369.774	119973.753	19 April 2007
61	Female	Rue de Beaulieu	Genève	2.26	499763.977	118876.294	19 April 2007
62	Female	Parc de Beaulieu	Genève	1.40	494752.519	118930.247	19 April 2007
63	Female	Parc de Beaulieu	Genève	1.11	499750.705	118932.642	19 April 2007
64	Female	OMS	Genève	1.13	499467.032	120873.058	19 April 2007
65	Female	Cimetière du Petit Saconnex	Petit-Saconnex	0.92	498765.955	120285.023	19 April 2007
66	Female	Chemin du Préjus	Grand-Saconnex	0.97	498129.610	120817.300	19 April 2007
67	Female	Avenue Wendt, 59	Genève	1.45	498825.823	118688.622	19 April 2007
68	Female	Rue de Bourgogne, 20	Genève	1.05	498384.009	118508.079	19 April 2007
69	Female	Muséum de Cologny	Cologny	1.39	502919.683	119022.523	20 April 2007
70	Female	Jardin Anglais	Genève	3.47	500683.525	117763.123	20 April 2007
71	Female	Center de Lullier	Jussy	1.07	508460.647	119919.274	20 April 2007
72	Female	Parc St.-Jean	Genève	1.17	498342.949	117724.987	19 April 2007

with Gel Red (Chemie Brunschwig AG, Basel, Switzerland) according to the manufacturer's recommendations. Concentrations were assayed with a NanoDrop ND-1000 ultraviolet spectrophotometer (Witec AG, Luzern, Switzerland). Performance of the different DNA samples used in this study in polymerase chain reaction (PCR) amplification have validated using the *Ginkgo* male and female primers VE-US-F (5' CTG GTG CTG ATG ATC ATG CCA AAT GGT G 3') and VE-US-R (5' CTG GTG CTG AGA ACT ACC TCT TTA TTC CAT GC 3'). All DNA samples proved successful in PCR amplification with the expected size of the amplicon (data not shown).

### Primers and Polymerase Chain Reaction Assays

RAPD amplifications of *Ginkgo* DNA leaf samples were carried out using previously described RAPD male-associated marker S1478 (Jiang et al. 2003). The reaction mixture contained 1× PCR buffer (75 mM Tris-HCl [pH 9.0], 50 mM KCl, 20 mM [NH<sub>4</sub>]<sub>2</sub>SO<sub>4</sub>), 0.1 mM dNTPs, 0.25 mM of the primer S1478, 1.5 mM MgCl<sub>2</sub>, 1 U of Taq polymerase (Biotools, Madrid, Spain), and 1 μL (35.10<sup>-6</sup> fl oz) of leaf DNA in a total volume of 50 μL (176.10<sup>-5</sup> fl oz). Amplifications were carried out in a Master Gradient thermocycler (Eppendorf, Hamburg, Germany) according to the following amplification program: an initial de-

naturation step of 95°C (203°F) for 5 min followed by 39 cycles including denaturation for 60 sec at 94°C (201.2°F), annealing for 60 sec at 40°C (104°F), and extension for 2 min at 72°C (161.6°F). Amplification was terminated by a final extension step of 7 min at 72°C (161.6°F). Amplicons were checked by gel electrophoresis as previously described.

### Automated Random Polymorphic DNA Analysis

ARPA-PCR was performed following the method of Fisher and Triplett (1999) with few modifications. Reaction mixtures were as previously described. The primer used S1478, 5' CTGGTGC-TGA 3' (male-associated decamer primer; Jiang et al. 2003) and was 5' end labeled with the phosphoramidite dye 5-FAM. Labeled PCR products have then been analyzed according to Fisher and Triplett (1999) by Microsynth GMBH (Balgach, Switzerland). The data were analyzed by using the GeneMarker software program (Soft Genetics distributed by GATC Biotech AG., Konstanz, Germany). The program output is a series of peaks (an electropherogram), the sizes of which are estimated by comparison to fragments in the internal size standard (GeneScan®-1000 [ROX]<sup>TM</sup>, Applied Biosystems, Rotkreuz, Switzerland).

### Random Amplified Polymorphic DNA Amplicon Cloning

RAPD PCR products were cloned using the TOPO cloning kit (Invitrogen, Basel, Switzerland) and sequenced directly in both sense and antisense directions (Fasteris SA, Geneva, Switzerland). All isolates were sequenced twice and a consensus sequence was created from the duplicates. DNA sequences have been deposited in GenBank (Bethesda, MD) (F1136224, F1136225). DNA sequence of the other recovered amplicons that are common to males and females have been also registered under the accession numbers (F1136226, F1136227).

## RESULTS

### Validation of Decamer Primer S1478 as Male-Associated

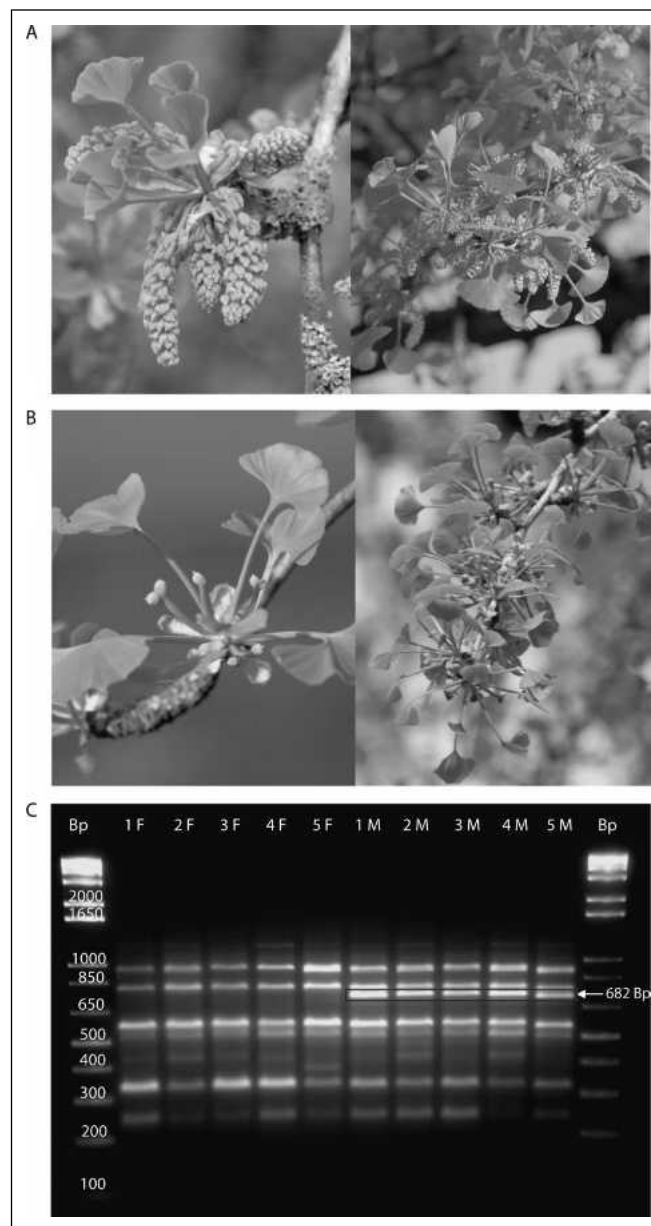
To test if the S1478 male-associated RAPD marker could be reliably used for sex determination in *G. biloba*, 72 trees with known sexual determinism (37 male trees and 35 female trees) have been identified all around the Canton of Geneva territory (Figure 3) and leaf samples were collected from these trees in June 2007. Sexual determinism has been verified in individual trees in the flowering season to confirm the sex in individual trees. Amplification reactions carried out with DNA from ten male and ten female plants (originating from China) and using the decamer primer S1478 showed an approximately 680 bp sized band that was present in males and absent from all females tested (Figure 3).

This step was followed by applying the method to the remaining 62 trees (Figure 4) and discriminated male and females. The sexualities of *G. biloba* trees deduced from the RAPD reactions were identical to the collector's records. The sex marker S1478-680 bp could be used to reliably detect the sexes of *G. biloba* trees from different areas and geographic origins because it amplified in samples from both origin, China and Japan.

### S1478 Male-Associated Polymerase Chain Reaction Amplicon Cloning and Sequencing

PCR products originating from RAPD amplifications with four male and female trees were purified and cloned into the pCR2.1

vector. Positive colonies were selected according to the electrophoretic mobility of the inserted fragment. For this purpose, PCRs with the selective primers were conducted using the colonies as a template. The mobility of the PCR product was compared with the corresponding RAPD fragment on the RAPD gel. At least two clones were selected and sequenced for each sex-linked RAPD marker. The sequence of the male-specific amplicons (682 bp in length) analyzed using Blast program (National



**Figure 3.** Morphologic and molecular differences between male and female *G. biloba* trees. (A) Male inflorescences. (B) Female inflorescences. (C) Random amplified polymorphic DNA (RAPD) pattern of male and female trees generated by primer S1478. Lane Bp, molecular weight marker; lanes 1F-5F, RAPD products from DNA of female plants collected from the city of Geneva; lanes 1M-5M, RAPD products from DNA of male plants collected from Chêne-Bougeries and Geneva. Arrowhead indicates male-related marker.

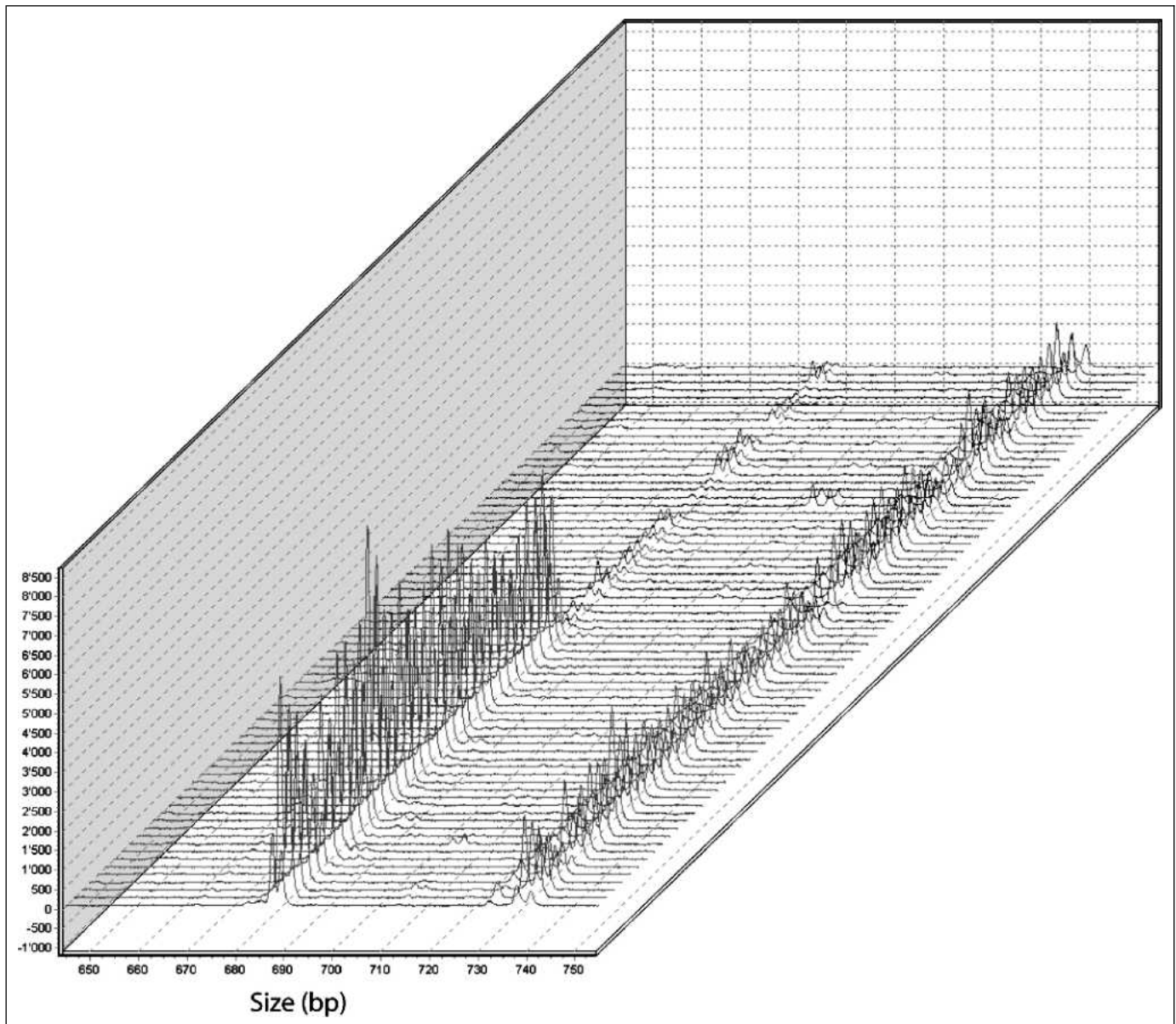


Figure 4. Automated random polymorphic DNA analysis profiles of male and female plants. Blue and rose pics refer, respectively, to male and female plants.

Center for Biotechnology Information, Bethesda, MD) showed no hits to any known sequence in the database.

#### Automated Random Polymorphic DNA Analysis Development

ARPA-PCR has been thought as a new application following the method of Fisher and Triplett (1999). We used a male-associated decamer primer S1478, 5' CTGGTGCTGA 3' (Jiang et al. 2003), which was 5' end labeled with the phosphoramidite dye 5-FAM. Sample fragments were then discriminated by using the ABI 310 genetic analyzer (Perkin-Elmer, Waltham, MA), in which DNA is submitted to electrophoresis in a capillary tube filled with electrophoresis polymer rather than in a polyacrylamide gel. The samples were run under standard ABI 310 denaturing electrophoresis conditions for 1 hr each with the POP-4 polymer, and the data were analyzed by using the GeneScan 3.1

software program (Perkin-Elmer). Figure 4 shows successful analysis of sexual determinism in analyzed plants. As a result of the higher resolution of ARPA, the male-specific fragment was sized at 682 bp, whereas the fragment common to males and females displayed a length of 734 bp.

#### Automated Random Polymorphic DNA Analysis Performance on Seedlings Derived from a Sexual Cross

ARPA was used to assess the sexual determinism of a set of 21 2-year-old seedlings derived from seeds collected from a female tree. Initial experiments included testing of DNA performance in PCR using primers that perform well on male and female plants. This experiment showed an amplicon with expected accurate size. This control is thought to rule out false-negatives as a result of poor DNA quality. This analysis showed ten seedlings dis-

playing the male-specific amplified DNA fragment and 11 seedlings not displaying this male-specific fragment. These 11 seedlings are then considered female trees by inference from the molecular sexing results on adult trees. Three to four PCR replicates were performed yielding identical results for each sample. The ARPA profiles (electropherograms) for PCRs from this collection of seedlings (Figure 5) were highly reproducible as evidenced by the fact that the electropherograms representing the duplicate PCRs for the collection of seedlings are almost entirely overlapping (data not shown).

### DISCUSSION

Several techniques were used to distinguish between male and female trees in *G. biloba*, including biochemical tests (Zhong et

al. 1982). Later, these biochemical differences have been shown to be related to age and growth situation of trees (Jiang et al. 2003). Recently, Jiang et al. (2003) succeeded in distinguishing between male and female trees by a reliable RAPD-PCR approach using the male-specific RAPD marker S1478. This study aimed at validating S1478 as a male-associated marker in *G. biloba*. Using a large collection of male and female trees collected from the Canton of Geneva and that originated from China and Japan, we could provide strong evidence that this marker could reliably be used in this task.

The results obtained with ARPA should be cautiously interpreted. As a molecular technique that relies on total DNA extraction and PCR amplification, ARPA is subject to the usual problems associated with DNA quality for performance in PCR.

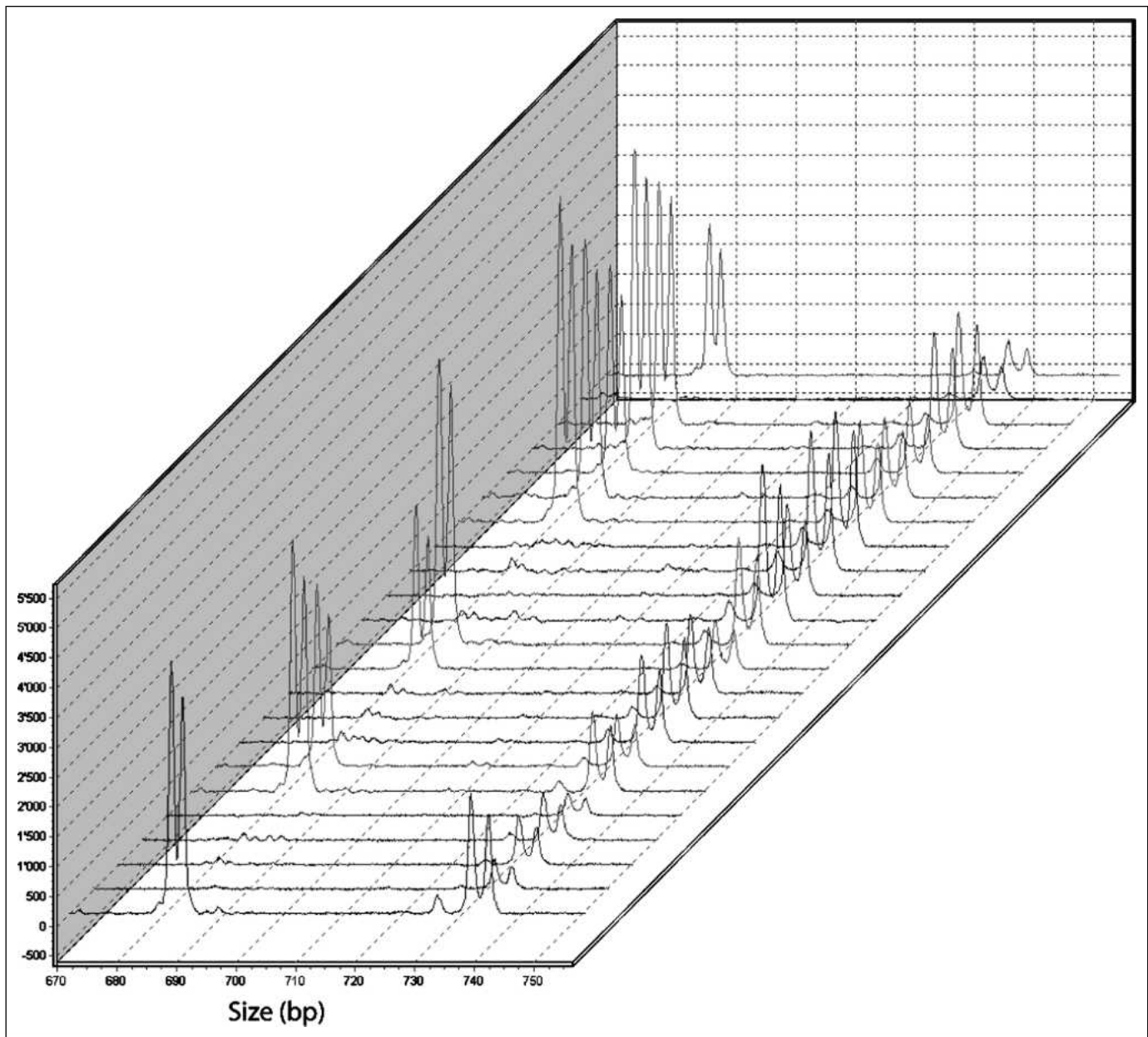


Figure 5. Automated random polymorphic DNA analysis profiles of male and female seedlings derived from a sexual cross. Blue and rose peaks refer, respectively, to male and female plants. Deep blue and red colors of peaks refer, respectively, to a previously identified male and female trees used as controls.

For these reasons, any conclusions regarding sexual determinism using ARPA profiles should be carefully made. Use of appropriate controls such as those used in this study allows accurate conclusions regarding sexual determinism to be correctly drawn.

Male plants could now be selected from seedlings issued from seeds. Decreasing prices in molecular biology consumables and technologies would result in affordable sexing services for larger numbers of plants at an early stage. Growing male plants in nurseries instead of a mix of both sexes will optimize the use of nursery surfaces and then contribute to lower the price of such trees, which on the other hand could be more desirable as urban trees once male-certified. Consequently, sexual reproduction and sewing of *Ginkgo* seeds could benefit this technique and allow the availability of a greater diversity in urban *Ginkgo* trees.

Early sexual determination gained from ARPA allows for effective selection of male *Ginkgo biloba* trees highly suited by city governments for planting in streets and allows practitioners to easily provide such plants with no need to grow female plants for over 30 years.

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#### LITERATURE CITED

- Aoki, T. 1997. Fungal association with *Ginkgo biloba*, p. 251–258. In: Hori, T., P. Del Tredici, R.W. Ridge, W. Tulecke, J. Tremouillaux-Guiller, and H. Tobe (Eds.). *Ginkgo biloba—A Global Treasure*. Springer-Verlag, Tokyo, Japan.
- Carrier, D.J., T.A. van Beek, R. van der Heijden, and R. Verpoorte. 1998. Distribution of ginkgolides and terpenoid biosynthetic activity in *Ginkgo biloba*. *Phytochemistry* 48:89–92.
- Fisher, M.M., and E.W. Triplett. 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Applied and Environmental Microbiology* 65:4630–4636.
- Handa, M., Y. Lizuka, and N. Fujiwara. 1997. *Ginkgo* landscapes, p. 259–283. In: Hori, T., P. Del Tredici, R.W. Ridge, W. Tulecke, J. Tremouillaux-Guiller, and H. Tobe (Eds.). *Ginkgo biloba—A Global Treasure*. Springer-Verlag, Tokyo, Japan.
- Honda, H. 1997. *Ginkgo* and insects, p. 259–283. In: Hori, T., P. Del Tredici, R.W. Ridge, W. Tulecke, J. Tremouillaux-Guiller, and H. Tobe (Eds.). *Ginkgo biloba—A Global Treasure*. Springer-Verlag, Tokyo, Japan.
- Jiang, L., R.L. You, M.X. Li, and C. Shi. 2003. Identification of a sex associated RAPD marker in *Ginkgo biloba*. *Acta Botanica Sinica* 45:742–747.
- Kim, Y.S., J.K. Lee, and G.C. Chung. 1997. Tolerance and susceptibility of *Ginkgo* to air pollution, p. 233–242. In: Hori, T., P. Del Tredici, R.W. Ridge, W. Tulecke, J. Tremouillaux-Guiller, and H. Tobe (Eds.). *Ginkgo biloba—A Global Treasure*. Springer-Verlag, Tokyo, Japan.
- Kiple, K.F., and K.C. Ornelas. 2000. *The Cambridge World History of Food*. Cambridge University Press, Cambridge, U.K. 1958 pp.
- Laurain, D. 2000. Cultivation of *Ginkgo biloba* on large scale, p. 63–79. In: Van Beek, T.A. (Ed.). *Ginkgo biloba*. Harwood Academic Publishers, Amsterdam, The Netherlands.
- Lefort, F., and G.C. Douglas. 1999. An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. *Annals of Science* 56:259–263.
- Michel, P.F. 1985. *Ginkgo biloba*: L'arbre qui a vaincu le temps. Editions du Félin, Paris. 108 pp.
- Rohr, R. 1989. Maidenhair tree (*Ginkgo biloba* L.), p. 574–590. In: Bajaj, Y.P.S. (Ed.). *Biotechnology in Agriculture and Forestry, Trees II*. Springer Verlag, Berlin, Germany.
- Wada, K., and M. Haga. 1997. Food poisoning by *Ginkgo biloba* seeds, p. 309–321. In: Hori, T., P. Del Tredici, R.W. Ridge, W. Tulecke, J. Tremouillaux-Guiller, and H. Tobe (Eds.). *Ginkgo biloba—A Global Treasure*. Springer-Verlag, Tokyo, Japan.
- Zhong, H.W., Z.H. Yang, G.L. Zhu, and Z.X. Cao. 1982. Peroxidase isozyme pattern as a biochemical test to distinguish the sex of individual plant in *Ginkgo biloba* L. *Scientia Silvae Sinica* 18:1–5.
- Zhou, Z.Y., and S.L. Zheng. 2003. Palaeobiology: The missing link in *Ginkgo* evolution—The modern maidenhair tree has barely changed since the days of the dinosaurs. *Nature* 423:821–822.

Vincent Echenard

University of Applied Sciences of Western Switzerland  
Plants and Pathogens Group  
Research Institute Earth, Nature and Landscape  
150 route de Presinge Jussy  
Geneva 1254, Switzerland  
Geneva 1254, Switzerland

François Lefort (corresponding author)

University of Applied Sciences of Western Switzerland  
Plants and Pathogens Group  
Research Institute Earth, Nature and Landscape  
150 route de Presinge Jussy  
Geneva 1254, Switzerland  
francois.lefort@hesge.ch

Gautier Calmin

University of Applied Sciences of Western Switzerland  
Plants and Pathogens Group  
Research Institute Earth, Nature and Landscape  
50 route de Presinge Jussy  
Geneva 1254, Switzerland

Robert Perroulaz

University of Applied Sciences of Western Switzerland  
Agronomy Department  
Geneva 1254, Switzerland

Lassaad Belbahri

University of Applied Sciences of Western Switzerland  
Plants and Pathogens Group  
Research Institute Earth, Nature and Landscape  
50 route de Presinge Jussy  
Geneva 1254, Switzerland

**Résumé.** Une technique d'amplification polymorphe aléatoire d'ADN du gène S1478 associé au sexe mâle a été utilisée pour amplifier l'ADN de 72 échantillons de feuilles récoltés à partir de *Ginkgo biloba* dont le sexe était connu dans le canton de Genève en Suisse. Ce marqueur a été découvert comme étant spécifique au mâle et est absent de tous les plants femelles. L'analyse polymorphe aléatoire d'ADN automatisée, une nouvelle technologie d'automatisation développée dans ce but, s'est avérée être hautement effective pour distinguer les plants mâles des plants femelles, et ce à 100% d'efficacité et de succès dans la discrimination mâle versus femelle au sein d'une collection de jeunes semis obtenus à partir de croisements sexuels. Nos découvertes fournissent une preuve sans ambiguïté que l'analyse polymorphe

d'ADN combinée avec le gène S1478 peut être considérée comme une méthode efficace, rapide et facile pour déterminer dans les premiers stades le sexe de l'arbre dioïque *Ginkgo biloba*.

**Zusammenfassung.** Im Kanton von Genf, Schweiz, wurde von 72 Blattsammlungen von *Ginkgo biloba* mit bekannter sexueller Bestimmung zur Erweiterung ihrer DNS die zufällige, erweiterte, polymorphe DNS-Technik (RAPD) mit S1478 angewendet. Dieser Marker (S1478) ist männchen-spezifisch und fehlt in allen weiblichen Pflanzen. Automatische, randomisierte, polymorphe DNS-Analysen (ARPA), eine eigens im Rahmen dieser Arbeit entwickelte Technologie, zeigte sich als sehr effektiv in der Unterscheidung männlicher und weiblicher Pflanzen (100%) und erfolgreich bei der Unterscheidung männlicher und weiblicher Sämlinge, die aus einer sexuellen Kreuzung entstanden sind. Unsere Ergebnisse liefern den unstrittigen Beweis, dass ARPA, kombiniert mit S1478 als eine effiziente, schnelle und einfache Methode bei der

Geschlechtsbestimmung bei *Ginkgo biloba* in Betracht gezogen werden kann.

**Resumen.** Se empleó la técnica polimórfica al azar amplificada DNA (RAPD), con plantas masculinas asociadas S1478, para amplificar DNA de 72 muestras de hojas colectadas de árboles de *Ginkgo biloba* con determinismo sexual conocido en el cantón de Ginebra, Suiza. Se encontró que este marcador es específico para masculinos y no se encontró en plantas femeninas. El análisis polimórfico (ARPA), una nueva tecnología desarrollada en la estructura de este trabajo, probó altamente su efectividad para distinguir masculinos y femeninos con 100% de eficiencia y éxito en la discriminación de masculinos y femeninos de una colección de brinzales derivados de una cruce sexual. Nuestros hallazgos proveen evidencia inequívoca de que ARPA, combinada con el S1478, podría ser considerado un método eficiente, rápido y fácil para hacer una determinación sexual en árboles deciduos de *Ginkgo biloba*.