Introgression analysis of an interspecific hybrid population in peanuts (*Arachis hypogaea* L.) using RFLP and RAPD markers

G.M. Garcia, H.T. Stalker, and G. Kochert

Abstract: Forty-six introgression lines ($F_{10}C_9$) from a cross between *Arachis hypogaea* L. ($2n = 4x = 40$) and *A. cardenasii* Krapov. & W.C. Gregory ($2n = 2x = 20$) were analyzed for the introgression of *A. cardenasii* chromosome segments. Seventy-three RFLP probes and 70 RAPD primers, expressing from one to four *A. cardenasii*-specific bands, were used to evaluate the set of introgression lines. Thirty-four RFLP probes and 45 RAPD primers identified putative *A. cardenasii* introgressed chromosome segments in one or more lines. Introgressed segments were detected by RFLP analysis in 10 of the 11 linkage groups; the smallest introgressed fragments were detected by single RFLP markers and the largest were detected by three or four adjacent markers and represented introgressed segments of 30–40 cM. Similar results were obtained with RAPD markers, although markers detecting introgressed fragments could not be placed on the peanut linkage map. Introgression into both *A. hypogaea* genomes was detected and its implication in breeding for disease resistance is discussed.

Key words: peanut, *Arachis hypogaea*, *Arachis cardenasii*, RFLPs, RAPDs, introgression, reciprocal recombination, translocation, alien gene transfer, wide cross.

Résumé : Quarante-six lignées d'introgression ($F_{10}C_9$) issues d'un croisement entre *Arachis hypogaea* L. ($2n = 2x = 40$) et *A. cardenasii* Krapov & W.C. Gregory ($2n = 2x = 20$) ont été analysées afin d'évaluer l'introgression de segments chromosomiques du *A. cardenasii*. Soixante-treize sondes RFLP et 70 amorces RAPD, montrant entre un et quatre fragments spécifiques au *A. cardenasii*, ont été employées afin d'examiner l'ensemble des lignées. Trente-quatre sondes RFLP et 45 amorces RAPD ont détecté des segments de chromosomes du *A. cardenasii* introgressés chez une ou plusieurs lignées. Des segments introgressés ont été décelés par analyse RFLP dans 10 des 11 groupes de liaison; les plus petits segments n'étant détectés que par un seul RFLP alors que les plus grands étaient identifiés par trois ou quatre marqueurs adjacents et représentaient des segments mesurant 30–40 cM. Des résultats semblables ont été obtenus avec les marqueurs RAPD, bien que les marqueurs détectant ces fragments introgressés n'aient pu être situés sur la carte génétique de l'arachide. L'introgression dans les deux génomes du *A. hypogaea* a été observée et ces résultats sont discutés en relation avec l'amélioration génétique de la résistance aux maladies.

Mots clés : arachide, *Arachis hypogaea*, *Arachis cardenasii*, RFLPs, RAPDs, introgession, recombinaison réciproque, translocation, transfert de gènes étrangers, croisements interspécifiques.

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Introduction

The cultivated peanut, *Arachis hypogaea* L. ($2n = 4x = 40$) is an allotetraploid native to South America containing two genomes that originated from different diploid wild ancestors (Smartt and Stalker 1982). Krapovickas and Gregory (1994) divided the genus into nine sections based on morphology, geographic distribution, and cross-compatibility. The section *Arachis* contains *A. hypogaea* and about 25 cross-compatible diploid annual and perennial species.

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Although progress has been made to increase yield in cultivars, satisfactory resistance to most diseases and insect pests has not been achieved because of limited genetic resources within the *A. hypogaea* gene pool. In contrast, very high levels of resistance have been reported in wild species of section *Arachis* for many disease and insect pests (Stalker and Moss 1987). However, introgressing genes into *A. hypogaea* is difficult because of genomic and ploidy barriers. Direct hybridization between the cultivated and diploid wild species results in sterile triploid hybrids. The chromosome number of triploids can be doubled with colchicine to produce hexaploids that, in turn, can be either backcrossed to the cultivated parent or selfed until the chromosome number spontaneously drops to the tetraploid level (Stalker and Moss 1987). During this process it is hoped that wild species chromosome segments will be introgressed into *A. hypogaea*. However, since chromosome pairing will likely be preferential, pairing among genomes of different species followed by recombination may be limited. Cytological analyses of triploid hybrids have shown that homoeologous intergenomic pairing occurs and is manifested by more than the expected 10 bivalents and by multivalents; thus, a high degree of genetic exchange between chromosomes of wild and cultivated species is believed to occur in these triploids (Singh and Moss 1984).

Multivalent associations observed in hexaploids and their backcross progenies also suggest inter- and intragenomic homoeologous pairing between chromosomes of *A. hypogaea* and wild species. Other evidence for intergenomic pairing comes from crosses between *A. hypogaea* with (*a*) induced autotetraploids of the diploid wild species (Singh 1986*a*) and (*b*) synthetic amphidiploids (Singh 1986*b*). The frequency and chromosomal location of introgressed segments during hybridization generations, however, has not been determined.

A tetraploid interspecific hybrid population was obtained by selfing hexaploids of a cross between cultivated peanut and *A. cardenasii* Krapov. & W.C. Gregory, a diploid wild species (Stalker et al. 1979). Lines with high yield (Guok et al. 1986), high levels of resistance to *Cercospora arachidicola* Hori (Stalker 1984; Stalker and Beute 1993), moderate levels of resistance to *Cercosporidium personatum* (Berk et Curt.) Deighton (Stalker and Beute 1993), and resistance to several insects (Stalker and Campbell 1983) were selected from this population.

In *A. hypogaea*, very little genetic polymorphism has been detected with molecular markers, including isozymes (Grieshammer and Wynne 1990; Lacks and Stalker 1993), RFLPs (Kochert et al. 1991; Paik-Ro et al. 1992), and RAPDs (Halward et al. 1991, 1992; Lanham et al. 1992). However, a large amount of variability has been observed among diploid *Arachis* species (Kochert et al. 1991; Halward et al. 1991, 1992; Paik-Ro et al. 1992). By comparing the RFLP patterns of the wild diploid species with those of *A. hypogaea*, Kochert et al. (1991) were able to distinguish the two genomes present in tetraploid cultivated peanut. These authors also suggested, on the basis of band sharing analysis, that *A. ipaensis* Krapov. & W.C. Gregory could have contributed one genome to cultivated peanut, with the other genome coming from *A. duranensis* Krapov. & W.C. Gregory or *A. spegazzinii* (since renamed as *A. duranensis* by Krapovickas and Gregory (1994)). To exploit this variability, species-specific markers presumably could be used to tag and follow introgression of chromosome segments (Tanksley et al. 1989). Associations between markers detecting introgressed chromosome segments and traits controlled by genes on introgressed segments could then provide the basis for marker-assisted selection experiments.

In this study, a set of 46 introgression lines derived from an *A. hypogaea* × *A. cardenasii* hybrid were evaluated to test the suitability of RFLPs and RAPDs for detecting the amount of introgression from a wild diploid *Arachis* species into *A. hypogaea*. Seventy-three RFLP probes of known map location and a set of 70 RAPD primers were used in the analyses.

Materials and methods

The 46 introgression lines used in this study (Table 1) originated from an interspecific hybrid made by Smartt and Gregory (1967) between *A. hypogaea* subsp. *fastigiata* var. *fastigiata* (PI 261942 or 261943) and *A. cardenasii* (10017 GKP, PI 262141). The original plants used in the cross were not available, so single plants grown from the same *A. hypogaea* and *A. cardenasii* accessions were analyzed. The *A. cardenasii* parent is a diploid species of section *Arachis* collected from Robore, Bolivia. It expresses high levels of resistance to leaf spots, insect pests (Stalker and Moss 1987), and nematodes (Nelson et al. 1989). The triploid F_1 hybrid was treated with colchicine and fertility was restored at the hexaploid ($2n = 6x = 60$) chromosome level. Hexaploids were self-fertilized for five generations at which time cytological observation revealed that progeny had 40 chromosomes (Stalker et al. 1979). Morphologically, they ranged from almost identical to the *A. hypogaea* parent to others that were more similar to the wild species. The introgression lines were examined after nine generations of selfing ($F_{10}C_9$).

RFLP analysis

Seventy-three RFLP probes of known map position (Halward et al. 1993) were selected to provide good coverage of all known linkage groups. The probes detected polymorphic restriction fragments between *A. cardenasii* and *A. hypogaea* and could thus be used to detect introgressed chromosome segments. Survey filters with genomic DNA from *A. duranensis* (10038 GKP) (previously named *A. spegazzinii*), *A. duranensis* (36006 KSBScCo), *A. stenosperma* Krapov. & W.C. Gregory (410 HLK), *A. ipaensis* Krapov. & W.C. Gregory (30076 GKBSPSc), *A. helodes* Martius ex Krapov. & Rigoni (6331 VSGr), and *A. villosa* Benth. (22585 Bu) were screened with the same set of RFLP probes in attempts to identify which of the two *A. hypogaea* genomes was being introgressed. The peanut RFLP map (Halward et al. 1993) was produced in a F_2 population derived from a cross between *A. cardenasii* and *A. stenosperma*. For this study we assumed that the genomes present in *A. hypogaea* were colinear with the wild species used to produce the map.

Genomic DNA extraction, restriction endonuclease digestion, and Southern analysis were as described by Kochert

Table 1. Introgression lines used for RFLP and RAPD analysis and their reaction to several diseases and insects.

Code	Pedigree	Characteristics[a]
GA 1	PI 261942 × 10017-GP NCWS-1	Early leaf spot resistance
GA 2	PI 261942 × 10017-GP NCWS-2	Early leaf spot and nematode resistance
GA 3	PI 261942 × 10017-GP NCWS-3	Early leaf spot resistance
GA 4	PI 261942 × 10017-GP NCWS-4	Early leaf spot resistance
GA 5	PI 261942 × 10017-CS35	Nematode resistance
GA 6	PI 261942 × 10017-CS2	Nematode and rust resistance
GA 7	PI 261942 × 10017-IC 1-2	Nematode and early leaf spot resistance
GA 8	PI 261942 × 10017-IC 1-11	Late leaf spot resistance
GA 9	PI 261942 × 10017-IC 1-19	Nematode and insect resistance
GA 10	PI 261942 × 10017-IC 1-30	Insect resistance
GA 11	PI 261942 × 10017-NC18458	High yield, large seeds
GA 12	PI 261942 × 10017-NC18435	High yield, large seeds
GA 13	PI 261942 × 10017-NC18451	High yield, large seeds
GA 14	PI 261942 × 10017-CS9	
GA 15	PI 261942 × 10017-CS36	
GA 16	PI 261942 × 10017-CS14	
GA 17	PI 261942 × 10017-CS3	
GA 18	PI 261942 × 10017-CS6	
GA 19	PI 261942 × 10017-CS13	
GA 20	PI 261942 × 10017-CS39	Early leaf spot resistance
GA 21	PI 261942 × 10017-1	
GA 22	PI 261942 × 10017-9	Early leaf spot resistance
GA 23	PI 261942 × 10017-20	Late leaf spot resistance
GA 26	PI 261942 × 10017-42B	Early leaf spot resistance
GA 27	PI 261942 × 10017-86B	Early leaf spot and nematode resistance
GA 28	PI 261942 × 10017-87A	Early leaf spot resistance
GA 29	PI 261942 × 10017-CS4	
GA 30	PI 261942 × 10017-CS7	
GA 31	PI 261942 × 10017-CS8	
GA 32	PI 261942 × 10017-CS11	
GA 33	PI 261942 × 10017-CS12	
GA 38	PI 261942 × 10017-CS20	
GA 40	PI 261942 × 10017-CS22	
GA 42	PI 261942 × 10017-CS30	
GA 46	PI 261942 × 10017-CS37	
GA 47	PI 261942 × 10017-CS38	
GA 49	PI 261942 × 10017-CS46	
GA 54	PI 261942 × 10017-CS55	
GA 56	PI 261942 × 10017-CS62	
GA 57	PI 261942 × 10017-CS128	
GA 58	PI 261942 × 10017-IC 2-2	Insect resistance
GA 59	PI 261942 × 10017-IC 2-5	Insect resistance
GA 60	PI 261942 × 10017-IC 2-8	Insect and early and late leaf spot resistance
GA 61	PI 261942 × 10017-IC 2-12	Insect resistance
GA 62	PI 261942 × 10017-IC 2-13	Insect resistance
GA 63	PI 261942 × 10017-IC 2-25	Insect resistance
GA 64	PI 261942 × 10017-IC 2-30	Insect and early leaf spot resistance

[a]Insect resistance includes leafhopper, corn earworm, and southern corn rootworm; nematode resistance is for *Meloidogyne arenaria*; lines without resistance traits were randomly selected and without detected resistance traits.

et al. (1991). DNA was isolated from fresh leaves removed from single plants grown in a greenhouse at North Carolina State University. Graphical genotypes were produced using the HYPERGENE program developed by Dr. Nevin Young of the University of Minnesota.

RAPD primer screening and PCR conditions

Initially, 270 primers, each 10 nucleotides long, were used to screen the parents of the introgression lines. Seventy primers, each showing one to four *A. cardenasii*-specific bands, were selected to evaluate the set of 46 introgression

Table 2. Introgression lines in which introgression of *A. cardenasii* germ plasm was identified using RFLP analysis.

Linkage group	RFLP probe	Introgression line
1a	*Xuga.cr193*	GA 1, 32, 33, 60
	Xuga.cs046	GA 20
1b	*Xuga.cr074*	GA 7
	Xuga.cr243	GA 7
	Xuga.cs045	GA 7
	Xuga.cr113	GA 7
	Xuga.cr239	GA 28, 31, 38, 40, 59, 61
	Xuga.cs047	GA 59, 60, 62, 64
	Xuga.cs040	GA 59, 60, 62, 64
3	*Xuga.cr274*	GA 20
	Xuga.cs044	GA 2, 8, 14, 20, 23
4	*Xuga.cr132*	GA 5, 7
	Xuga.cr015	GA 15, 58, 60, 62, 63, 64
	Xuga.cr197	GA 3, 7, 8, 15, 23, 58, 59, 60, 61, 62, 63, 64
	Xuga.cs026	GA 3, 7, 21, 23, 58, 59, 60, 61, 62, 63, 64
	Xuga.cr244	GA 3, 7, 8, 15, 16, 17, 18, 19, 20, 21, 23, 28, 30, 42, 46, 47, 49, 54, 56, 57, 58, 59, 60, 61, 62, 63, 64
	Xuga.cr284	GA 20
5a	*Xuga.cr251*	GA 4, 7, 9, 23, 58, 60, 63, 64
6	*Xuga.cr277*	GA 7, 9, 42, 64
	Xuga.cr138	GA 54
	Xuga.cs065	GA 7
	Xuga.cr090	GA 6, 7, 23
	Xuga.cr207	GA 59, 60, 62, 64
	Xuga.cr101	GA 6, 7, 21, 23, 58, 59, 60, 62, 63, 64
	Xuga.cr116	GA 6, 7, 8, 21, 23, 58, 59, 60, 62, 63, 64
	Xuga.cr073	GA 6, 7, 23, 62, 63
7	*Xuga.cr256*	GA 4, 6, 7, 23, 63, 64
	Xuga.cs033	GA 4, 6, 7, 9, 47, 63, 64
8	*Xuga.cr228*	GA 2, 21, 23, 58, 59, 60, 62, 63, 64
	Xuga.cr159	GA 2, 59, 60, 62, 64
9	*Xuga.cs023*	GA 6, 7, 23
10	*Xuga.cr272*	GA 15, 23
11	*Xuga.cr086*	GA 1, 10, 11, 12, 13, 15, 32, 33, 38, 40, 59
	Xuga.cs028	GA 15

lines. Owing to the dominant nature of these markers, only primers that generated bands in the wild species parent and not in *A. hypogaea* could be used to detect introgression.

Reaction mixtures for PCR (15 μL) were carried out according to Williams et al. (1990) with a modified reaction mix (10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 100 mM each of dATP, dCTP, dGTP, and dTTP, 20 ng of primer, 1.5 μL of nonacetylated bovine serum albumin, 10–15 ng of genomic DNA, and 1 unit of *Thermus aquaticus* DNA polymerase (Taq)). The reaction mixture was overlaid with approximately 50 μL of light mineral oil. Amplification was performed in polyvinyl chloride microtest plates (Falcon) placed in a 96-well thermal cycler (M.J. Research, Inc.) under the following temperature conditions: 92°C for 1 min, 35°C for 1 min, 72°C for 2 min, for 40 cycles. Amplification products were analyzed by electrophoresis in 1.5% agarose gels run with 1× TBE buffer at 100 V for 5 h, stained with ethidium bromide, and visualized by illumination with uv light. Primers were obtained from Operon Technologies Inc., Alameda, Calif.

Scoring for introgression

RFLPs

When the banding pattern of the introgression lines was similar to the donor *A. cardenasii* parent, the result was considered "positive" and indicated introgression of the wild donor parent chromosome segment at that RFLP locus. Flanking RFLP markers were then checked to determine the approximate size of the introgressed segment. When adjacent markers showed different genotypes, it was assumed that the recombination event occurred midway between the markers.

RAPDs

Bands present in the wild progenitor that were detected in any of the introgression line plants were scored as "positive" (suggesting introgression), while those showing the *A. hypogaea* pattern were scored as "negative". Bands

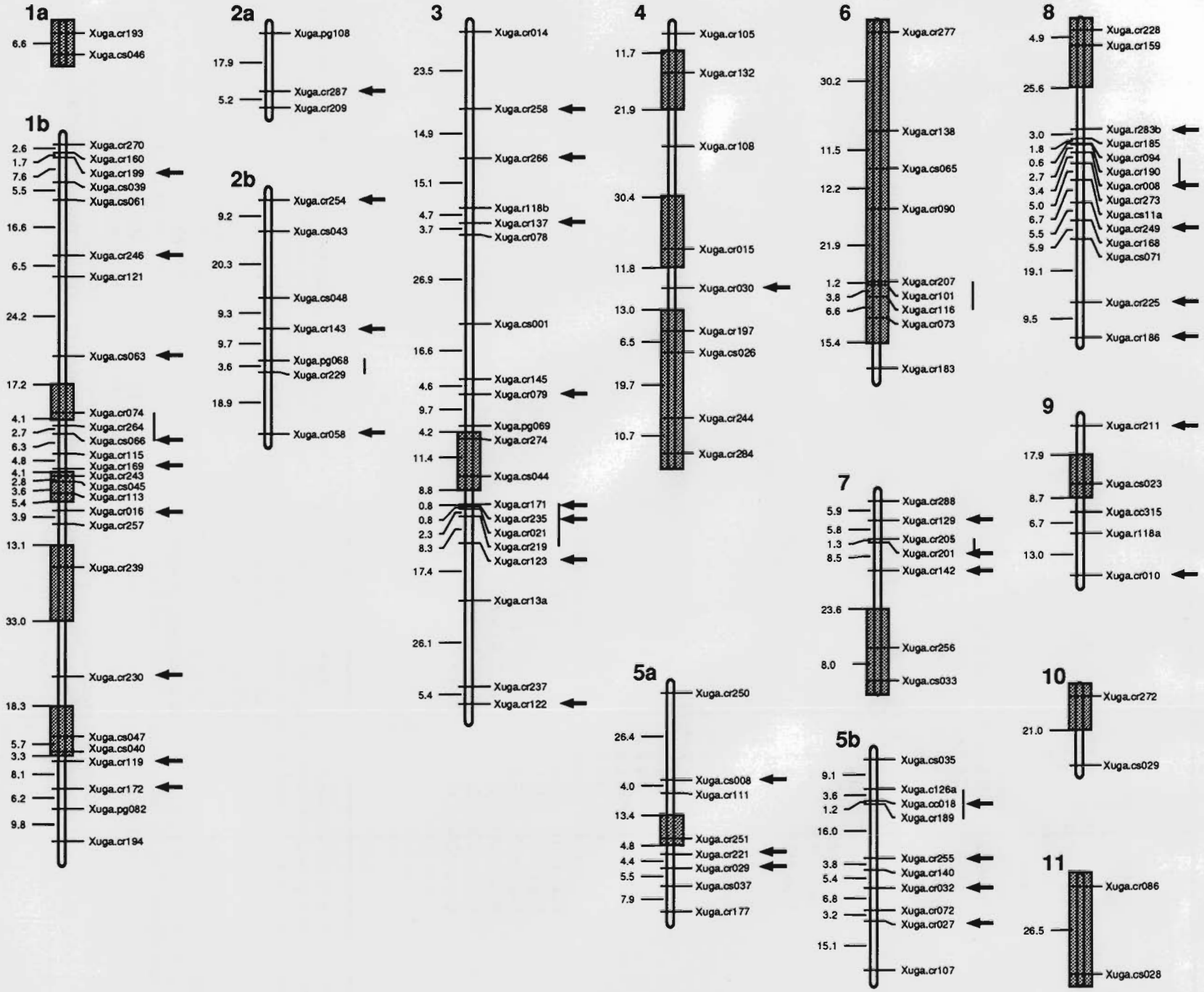
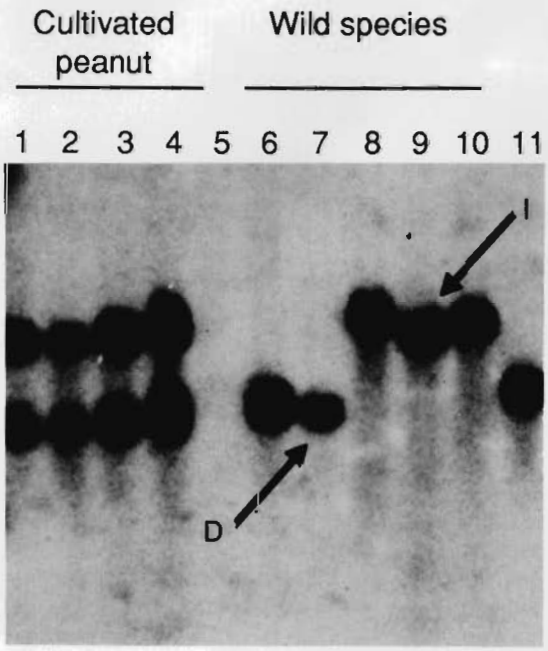

1a
6.6
Xuga.cr193
Xuga.cs046
1b
2.6
Xuga.cr270
1.7
Xuga.cr160
7.6
Xuga.cr199
5.5
Xuga.cs039
Xuga.cs061
16.6
6.5
Xuga.cr246
Xuga.cr121
24.2
Xuga.cs063
17.2
4.1
Xuga.cr074
2.7
Xuga.cr264
6.3
Xuga.cs066
4.8
Xuga.cr115
4.1
Xuga.cr169
2.8
Xuga.cr243
3.6
Xuga.cs045
5.4
Xuga.cr113
3.9
Xuga.cr016
Xuga.cr257
13.1
Xuga.cr239
33.0
Xuga.cr230
18.3
5.7
Xuga.cs047
3.3
Xuga.cs040
Xuga.cr119
8.1
Xuga.cr172
6.2
Xuga.pg082
9.8
Xuga.cr194
2a
17.9
Xuga.pg108
5.2
Xuga.cr287
Xuga.cr209
2b
9.2
Xuga.cr254
Xuga.cs043
20.3
9.3
Xuga.cs048
Xuga.cr143
9.7
3.6
Xuga.pg068
Xuga.cr229
18.9
Xuga.cr058
3
23.5
Xuga.cr014
Xuga.cr258
14.9
Xuga.cr266
15.1
4.7
Xuga.r118b
3.7
Xuga.cr137
Xuga.cr078
26.9
Xuga.cs001
16.6
4.6
Xuga.cr145
Xuga.cr079
9.7
4.2
Xuga.pg069
Xuga.cr274
11.4
Xuga.cs044
8.8
0.8
Xuga.cr171
0.8
Xuga.cr235
2.3
Xuga.cr021
8.3
Xuga.cr219
Xuga.cr123
17.4
Xuga.cr13a
26.1
5.4
Xuga.cr237
Xuga.cr122
4
11.7
Xuga.cr105
Xuga.cr132
21.9
Xuga.cr108
30.4
Xuga.cr015
11.8
Xuga.cr030
13.0
Xuga.cr197
6.5
Xuga.cs026
19.7
Xuga.cr244
10.7
Xuga.cr284
5a
26.4
Xuga.cr250
4.0
Xuga.cs008
Xuga.cr111
13.4
4.8
Xuga.cr251
4.4
Xuga.cr221
5.5
Xuga.cr029
Xuga.cs037
7.9
Xuga.cr177
6
30.2
Xuga.cr277
Xuga.cr138
11.5
Xuga.cs065
12.2
Xuga.cr090
21.9
1.2
Xuga.cr207
3.8
Xuga.cr101
6.6
Xuga.cr116
Xuga.cr073
15.4
Xuga.cr183
7
5.9
Xuga.cr288
Xuga.cr129
5.8
1.3
Xuga.cr205
Xuga.cr201
8.5
Xuga.cr142
23.6
Xuga.cr256
8.0
Xuga.cs033
5b
9.1
Xuga.cs035
3.6
Xuga.c126a
1.2
Xuga.cc018
Xuga.cr189
16.0
3.8
Xuga.cr255
Xuga.cr140
5.4
Xuga.cr032
6.8
3.2
Xuga.cr072
Xuga.cr027
15.1
Xuga.cr107
8
4.9
Xuga.cr228
Xuga.cr159
25.6
3.0
Xuga.r283b
1.8
Xuga.cr185
0.6
Xuga.cr094
2.7
Xuga.cr190
3.4
Xuga.cr008
5.0
Xuga.cr273
6.7
Xuga.cs11a
5.5
Xuga.cr249
5.9
Xuga.cr168
Xuga.cs071
19.1
Xuga.cr225
9.5
Xuga.cr186
9
Xuga.cr211
17.9
Xuga.cs023
8.7
Xuga.cc315
6.7
Xuga.r118a
13.0
Xuga.cr010
10
Xuga.cr272
21.0
Xuga.cs029
11
Xuga.cr086
26.5
Xuga.cs028

Fig. 1. Summary RFLP map of the peanut genome showing chromosomal segments introgressed from *A. cardenasii* into *A. hypogaea*. RFLP probes checked for introgression but not detecting *A. cardenasii* segments are marked with an arrow. Those which were positive for introgression in one or more of the introgression lines are contained within shaded boxes. Vertical lines to the right of the linkage groups indicate regions where the marker order is uncertain.

Fig. 2. Identification of the genomes of cultivated peanut by Southern blot analysis. DNA from four peanut cultivars and six diploid wild species was digested with *Hae*III and hybridized to probe *Xuga.cr244*. The two genomes of cultivated peanut can be seen in lanes 1–4. Identification of the bottom band (0.98 kb) of cultivated peanut as the "duranensis" genome and the top band (1.7 kb) as the "ipaensis" genome was made by comparison with the diploid wild species, which produces only one band. Lane 6, *A. duranensis* (10038 GKP; previously named *A. spegazzinii*); Lane 7, *A. duranensis* (D) (36006 KSBScC); Lane 8, *A. stenosperma* (410 HLK); Lane 9, *A. ipaensis* (I) (30076 GKBSPSc); Lane 10, *A. helodes* (6331 VSGr); Lane 11, *A. villosa* (22585 Bu).



showing putative introgressed fragments were excised from the gel and reamplified for use as hybridization probes. Target bands were stabbed with a pipette tip and the agarose plugs were placed in 25 μL of 20% TE buffer (D. Grattapaglia, personal communication). Five-μL aliquots were re-amplified with the same primer that had originally been used to generate the band. The products from the re-amplification were analyzed in 1.5% agarose in order to identify the desired band, which was then recovered from the gel slices by using GENECLEAN or Mermaid kits (Bio 101, Inc.) according to the manufacturer's instructions and labeled with ^{32}P using the random primer labeling method (Feinberg and Vogelstein 1984). Survey filters with genomic DNA from each of the parents of a mapping population and from the introgression line plants were then used in attempts to confirm the introgression by RFLP analysis and to map the introgressed segments detected by the RAPDs.

Results

Introgression analysis

RFLPs

Of the 73 RFLP markers analyzed, 34 (47%) detected *A. cardenasii* segments in one or more introgression line (Table 2, Fig. 1). Considering all the lines together, the total size of the introgressed segments represents approximately 360 cM (30%) of the diploid peanut genome.

No single introgression line contained all the introgressed segments. Three lines did not show any introgression with this set of probes, whereas line GA 7 showed the highest amount of introgression (176 cM, which is about 16% of the diploid genome). The linkage map shown in Fig. 1 depicts introgressed fragments detected by RFLP analysis when the information collected from all 46 introgression lines is combined.

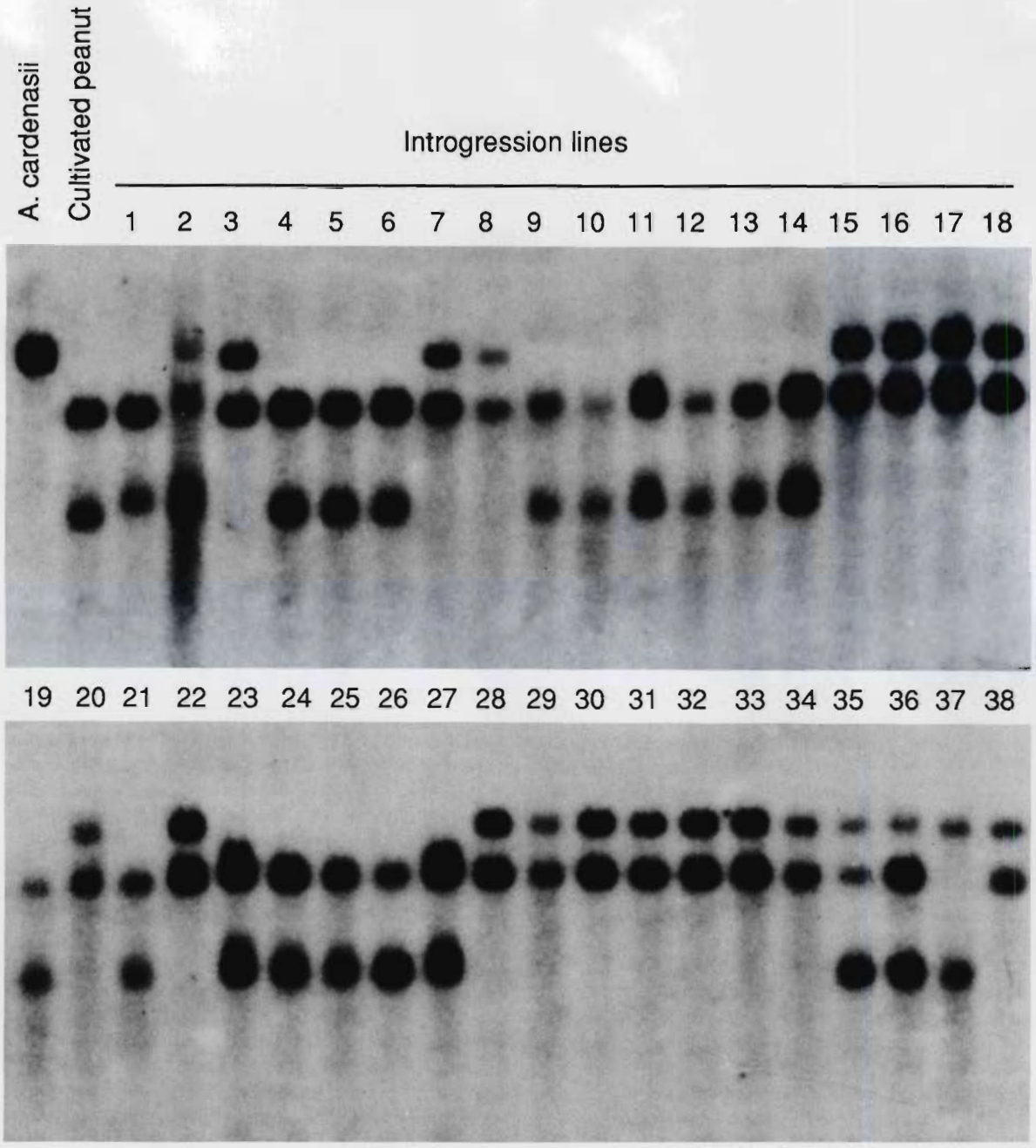
The smallest introgressed segments were those detected by a single RFLP marker, such as *Xuga.cr251*. The largest introgressed segments were detected in single lines by several adjacent RFLP markers, such as *Xuga.cr207–Xuga.cr073* on linkage group no. 6, which is at least 30 cM long. The positive RFLP probes showed significant variation in detecting *A. cardenasii* introgressed fragments. Probe *Xuga.cr244* detected introgression in 27 introgression lines, while 10 different probes detected introgression in a single line. Introgressed segments were detected in all linkage groups except no. 2, with group no. 6 showing the largest amount of introgression (eight markers covering almost the entire linkage group).

For many of the probes used in this study, the two genomes of cultivated peanut could be differentiated and identified (Fig. 2). The introgression of *A. cardenasii* chromosome segments into *A. hypogaea* showed three types of RFLP patterns: (*i*) *A. cardenasii* segments were introgressed into the "duranensis" genome; (*ii*) *A. cardenasii* segments were introgressed into the "ipaensis" genome; and (*iii*) *A. cardenasii* segments were present in addition to the segments from both the "duranensis" and "ipaensis" genomes (Fig. 3).

In most cases, introgression appears to have occurred by the *A. cardenasii* chromosome segment having replaced either the "duranensis" or the "ipaensis" segment of *A. hypogaea* by reciprocal recombination. Introgression can thus occur into either genome; however, of 69 introgression events in which the two genomes could be clearly distinguished, 61 were introgressed into the "duranensis" genome and only eight into the "ipaensis" genome. There were also 19 cases where *A. cardenasii* restriction fragments were observed in addition to the fragments from both the "duranensis" and "ipaensis" genomes.

Several introgression lines (GA 58 – GA 64) showed similar patterns of introgression (shown in Fig. 4 as graphical genotypes). This suggests that some of the introgressed segments, such as those on linkage groups no. 4 and no. 6, are the products of a single introgression event that occurred during early generations after hybridization or that selection identified similar genotypes.

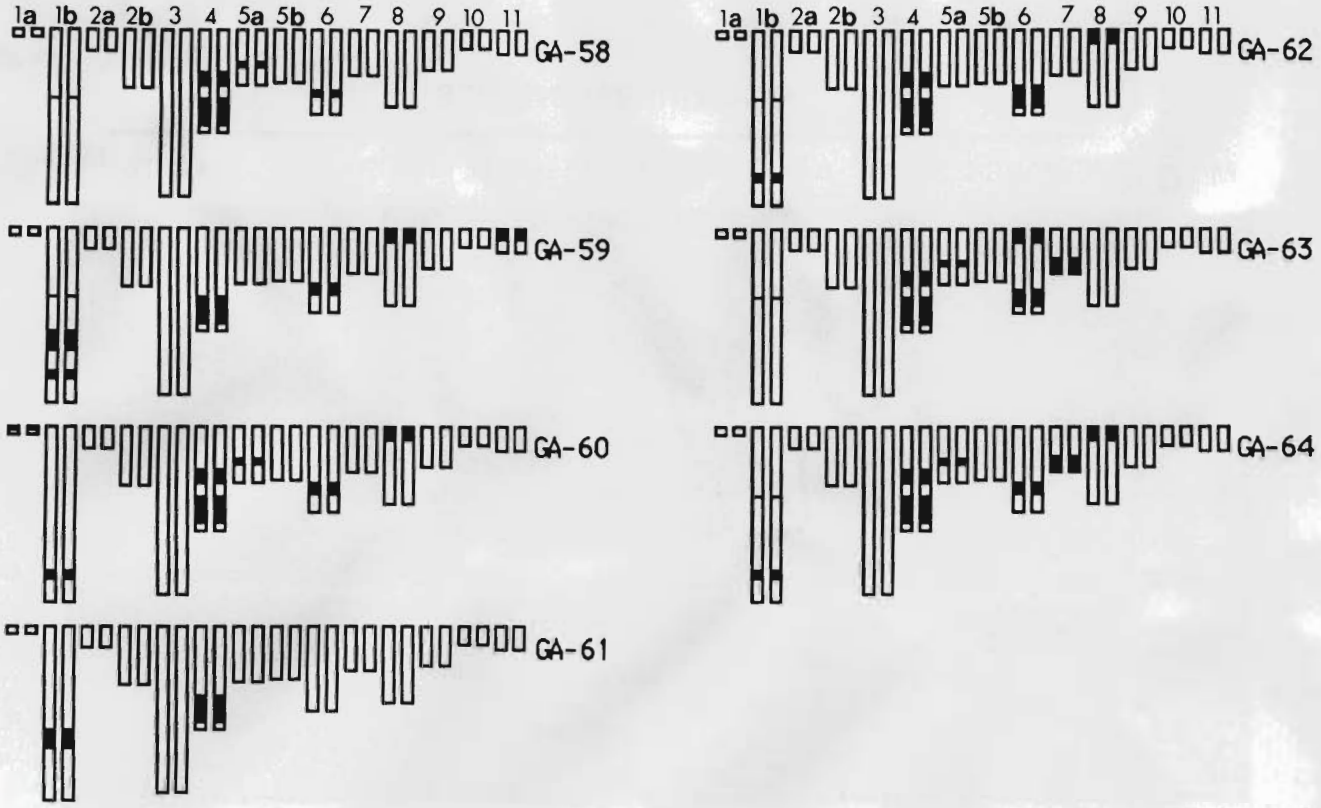
Fig. 3. RFLP analysis of introgression. DNA from several introgression lines was digested with *Hae*III and hybridized to probe *Xuga.cr244*. Introgression of the *A. cardenasii* chromosome segment (2.2 kb) has occurred into the "duranensis" genome of cultivated peanut (0.98 kb) in several introgression lines (such as 7, 8, 15–18). Introgression into the "ipaensis" genome of cultivated peanut (1.7 kb) has occurred in introgression line 37. Some introgression lines (GA 35, GA 36) contain *A. cardenasii* restriction fragments in addition to both genomes of cultivated peanut.



RAPDs

High levels of polymorphism were detected between *A. cardenasii* and *A. hypogaea*. The 270 primers screened resulted in 244 bands that were observed in *A. cardenasii* but not in cultivated peanut. From these, 70 primers that generated a total of 160 *A. cardenasii*-specific bands (an average of 2.4 species-specific bands per primer) were selected to evaluate the 46 introgression lines. Forty-five of the selected primers detected a total of 68 *A. cardenasii*-specific bands in the 46 introgression lines. As with the RFLP markers, some of the introgressed bands (26%) were detected in a single introgression line (Fig. 5*a*), while others (38%) were detected in several individuals (Fig. 5*b*). Ten *A. cardenasii* bands were shared by more than 10 individuals. Except for line GA 58, the same group of introgression lines showing common positive RFLP genotypes

Fig. 4. Graphical genotypes of several introgression lines to show similar patterns of introgression on linkage groups no. 4 and no. 6. Black bars indicate introgressed chromosome segments. The two genomes of cultivated peanut are not differentiated in this figure.



(GA 59 – GA 64) showed a common set of introgressed RAPD bands. Thus, large introgressed segments are being detected by both types of markers.

GA 38 was the only line that did not show introgressed RAPD bands, and GA 30 showed only one. On the other hand, six lines (GA 7, GA 21, GA 22, GA 60, GA 62, and GA 64) showed 20 or more introgressed bands. Similar results were observed with RFLPs; GA 30 and GA 38 showed one and two positive RFLP markers, respectively, while GA 7, GA 60, GA 62, and GA 64 (but not GA 21 or GA 22) were among the lines showing the highest percentage of introgression with RFLPs.

Hybridization analysis of RAPD loci

Several amplification products were tested as hybridization probes to determine the map location of RAPD bands showing introgression. Of the 30 amplified bands tested, all except one ($AB_{17}420$) generated smears or complex patterns characteristic of repeated DNA (data not shown).

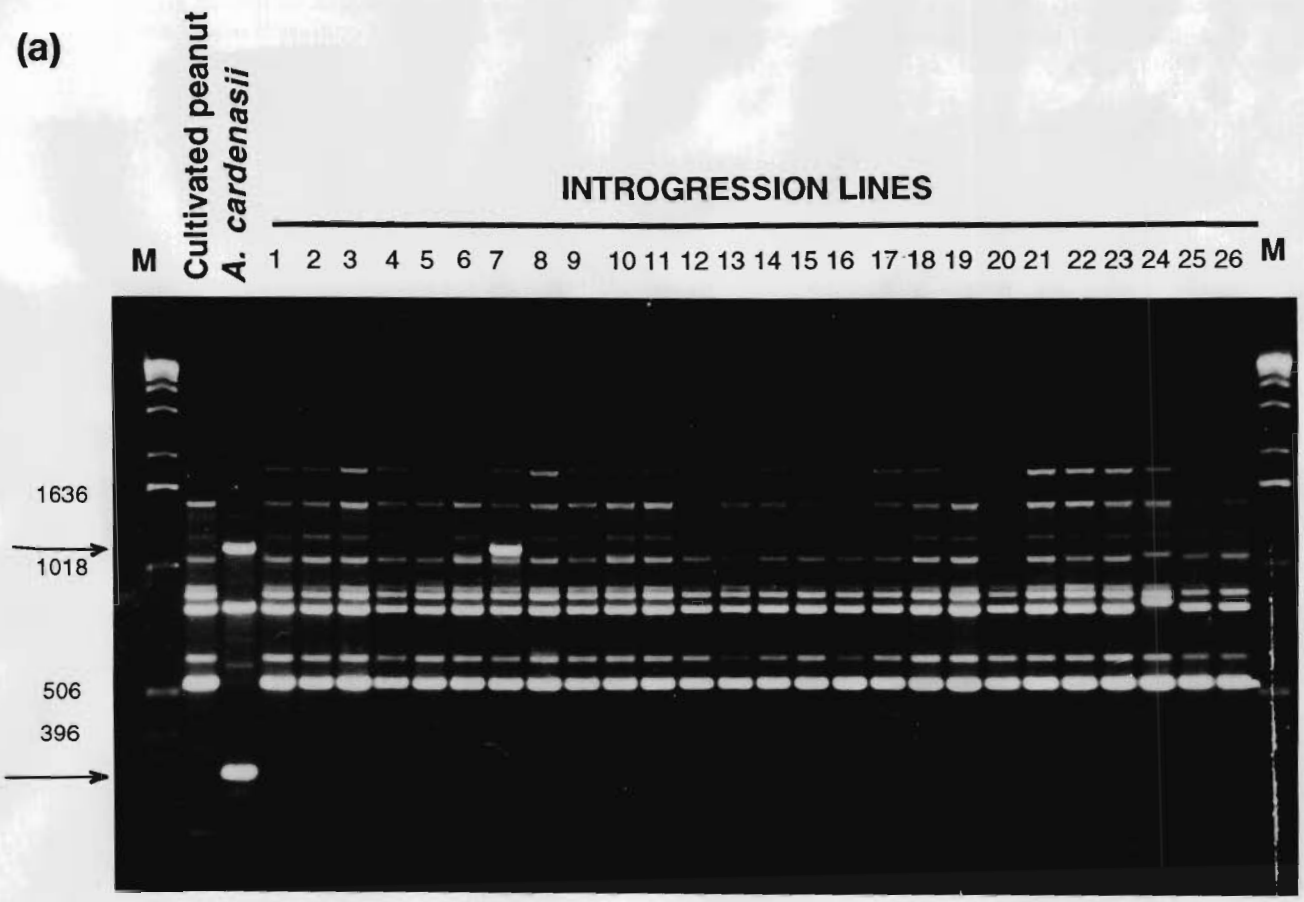
Discussion

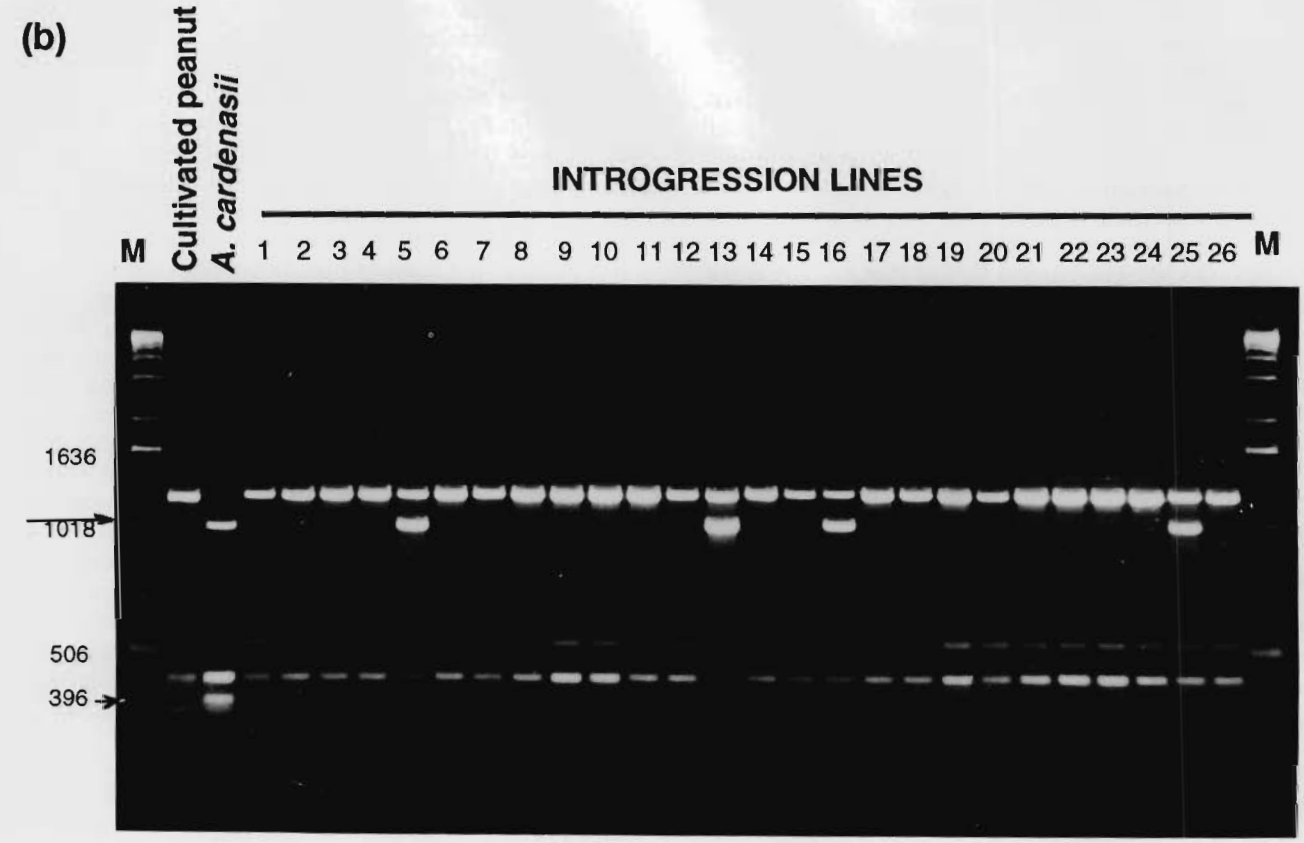
Few successes have been reported in the literature regarding introgression from the wild to the cultivated species of peanut, in large part because fertilization barriers and cross-incompatibilities limit gene transfer between species. When species in section *Arachis* have been used in hybridization programs directed at genetic improvement of *A. hypogaea*, fertile progenies generally have been obtained with little apparent genetic recombination (Stalker and Moss 1987). The use of molecular markers should enhance the ability of breeders to tag and follow the introgression of specific chromosome segments that are linked to desirable traits from wild species into improved cultivated lines.

In this study, 270 of the primers evaluated detected an average of 0.90 polymorphic bands/primer between *A. cardenasii* and *A. hypogaea*. The 244 unique wild species bands were found to be useful for studying introgression. The use of RAPD markers to detect alien gene introgression or to confirm the composition of interspecific hybrids has also been reported in several other crop species (Arnold et al. 1991; Baird et al. 1992; McCoy and Echt 1993).

RFLP markers detecting introgression of wild species chromosome segments have also been demonstrated in several species (Tanksley and Hewitt 1988; Paterson et al. 1990, 1991; Jena et al. 1992). A high percentage (47%) of the RFLP probes used in this study detected segments introgressed from *A. cardenasii* into one or more introgression lines. The total size of the introgressed segments represents approximately 30% of the diploid peanut genome covered by the *A. stenosperma* × *A. cardenasii* linkage map, with all but one of the linkage groups showing introgression.

Because all linkage groups showing introgression also showed nonintrogressed areas, the probable mechanism of introgression is chromosome recombination and not chromosome substitution. In the case of linkage group no. 6, where all except the most distal marker shows introgression when all lines are combined, the single line showing the largest introgressed chromosome fragment (GA 62) only


(a)
Cultivated peanut
A. cardenasii
INTROGRESSION LINES
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 M
1636
1018
506
396


(b)
Cultivated peanut
A. cardenasii
INTROGRESSION LINES
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 M
1636
1018
506
396

4

Fig. 5. Introgression analysis using RAPD markers. Genomic DNA from several introgression lines and their progenitor species was amplified using: (*a*) Primer O_7 (CAGCACTGAC). Two distinctive bands (1080 and 320 bp) were amplified from the wild progenitor (*A. cardenasii*) but not from the cultivated parent (arrows). Only introgression line GA 1 (lane 7) shows introgression of the 1080 base pair (bp) *A. cardenasii*-specific band; (*b*) Primer O_{11} (GACAGGAGGT). Two distinctive bands (1000 and 375 bp) were amplified from the wild progenitor but not from the cultivated one (arrows). Introgression lines GA 22, GA 21, GA 2, and GA 6 (lanes 5, 13, 16, and 25, respectively) show introgression of the 1000 bp. *A. cardenasii*-specific band. M, 1 kb ladder.

had four consecutive markers. Thus, either recombination occurred subsequently to a translocation event, or transfers resulted from several independent recombination events, which account for this large cumulative introgressed fragment.

The RFLP patterns in the introgression lines are consistent with introgression by reciprocal recombination involving both genomes. In 88% of the introgression events, an *A. cardenasii* allele replaced the A genome allele ("duranensis" genome), while in 12% of the cases an *A. cardenasii* allele replaced the B genome allele ("ipaensis" genome). This is consistent with earlier observations of multivalent formation that eventually could lead to homoeologous chromosome pairing and introgression. Most lines were apparently homozygous for either the *A. hypogaea* or the *A. cardenasii* allele, but some of the lines had RFLP patterns of both parents. These extra bands could represent cases where the line is still heterozygous for the introgressed segment (not very likely after nine generations of selfing) or the lines could have resulted from plants having a translocation with the *A. cardenasii* chromosome segment into the cultivated peanut genome. Further crosses and segregation analysis will be necessary to resolve these possibilities. Similar results were reported in a group of rice introgression lines by Jena et al. (1992).

The fact that the genome from a diploid wild species can pair and introgress into both genomes of the cultivated species has important implications in breeding for resistance. Smartt et al. (1978) previously suggested that only by introgression of resistance factors into both genomes could high levels of resistance be obtained. Introgression of both *A. hypogaea* genomes could explain the high levels of resistance found in selected lines for diseases (e.g., early and late leaf spot), which are under polygenic recessive genetic control (Nevill 1982). Alternatively, high levels of resistance could result from a change of gene expression in a new genetic background. In this study we did not detect introgression of the same chromosome segments from *A. cardenasii* into both genomes within single introgression lines. However, it is evident that introgression lines could be crossed and progeny selected in which both homoeologues are introgressed.

The percentage of *A. cardenasii*-specific bands generated by RAPD analysis that detected introgression was also high and showed the same pattern as the RFLP markers. Furthermore, both types of markers detected a common set of introgressed segments in the insect-resistant lines (GA 58–GA 64). These markers map to linkage groups no. 4 and no. 6 by RFLP analysis. In order to confirm the identity of the positive RAPD fragments (mainly to corroborate that they mapped to the same linkage group), more than 30 amplified fragments were re-amplified and used as RFLP probes. Strong signals, smears, and very complicated hybridization patterns indicated that the majority of the amplification products detected by RAPDs arose from highly repetitive DNA, which excludes their use for mapping purposes. Similar results were obtained by Devos and Gale (1992) in wheat and by Kazan et al. (1993) in *Stylosanthes* spp. These results are not unexpected, since the three genera have large genomes and a very high ratio of repetitive to unique DNA sequences, and RAPDs have been shown to amplify repeated regions of the genome (Williams et al. 1993).

The results from this study represent the first attempt to characterize alien gene introgression in 40-chromosome hybrid derivatives of peanut at the molecular level and provide the first step towards associating desirable genes from wild *Arachis* species with molecular markers. Both RFLP and RAPD markers were efficient in the detection of alien chromosome introgression. Because an RFLP linkage map already exists in peanut, it should be possible to combine the informativeness of RFLP markers in order to assign introgressed segments to specific linkage groups with the speed and efficiency possible using RAPD markers.

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