Theor Appl Genet (2004) 108:1309–1321
DOI 10.1007/s00122-003-1540-5

ORIGINAL PAPER

A. Baranger · G. Aubert · G. Arnau · A. L. Lainé · G. Deniot · J. Potier · C. Weinachter · I. Lejeune-Hénaut · J. Lallemand · J. Burstin

Genetic diversity within *Pisum sativum* using protein- and PCR-based markers

Received: 5 September 2003 / Accepted: 10 November 2003 / Published online: 15 January 2004

Abstract A collection of 148 *Pisum* accessions, mostly from Western Europe, and including both primitive germplasm and cultivated types, was structured using 121 protein- and PCR-based markers. This molecular marker-based classification allowed us to trace back major lineages of pea breeding in Western Europe over the last decades, and to follow the main breeding objectives: increase of seed weight, introduction of the *afila* foliage type and white flowers, and improvement of frost tolerance for winter-sown peas. The classification was largely consistent with the available pedigree data, and clearly resolved the different main varietal types according to their end-uses (fodder, food and feed peas) from exotic types and wild forms. Fodder types were further separated into two sub-groups. Feed peas, corresponding to either spring-sown or winter-sown types, were also separated, with two apparently different gene pools for winter-sown peas. The garden pea group was the most difficult to structure, probably due to a continuum in breeding of feed peas from garden types. The classification also stressed the paradox between the narrowness of the genetic basis of recent cultivars and the very large diversity available within *P. sativum*. A sub-collection of 43 accessions representing 96% of the whole allelic variability is proposed as a starting point for the construction of a core collection.

Communicated by C. Möllers

A. Baranger (✉) · G. Deniot
Domaine de la Motte,
UMR INRA-ENSAR APBV,
BP35327, 35653 Le Rheu, France
e-mail: baranger@rennes.inra.fr
Tel.: +33-02-23485128
Fax: +33-02-23485120

G. Aubert · J. Potier · C. Weinachter · J. Burstin
INRA URGAP BV1540,
21034 Dijon, Cedex, France

A. L. Lainé · I. Lejeune-Hénaut
Domaine Brunehaut,
INRA GAP,
80200 Peronne, France

G. Arnau · J. Lallemand
Domaine du Magneraud,
GEVES SEV,
Saint Pierre d'Amilly, BP52, 17700 Surgères, France

Introduction

The development of cultivated species and the breeding of new varieties have always relied on the availability of biological diversity, issuing from the long-term evolution of species. Modern plant breeding methods focusing on wide adaptation and high crop yield and intensive selection on crop species have raised the question of the amount of genetic variation still available in breeding pools for sustainable improvement (Hodgkin 1995). Together with a need for biodiversity conservation, the need for an assessment of crop diversity and a better understanding of the impact of breeding on this diversity has emerged. Molecular markers have allowed the study of diversity through DNA sequence variation, thus facilitating (1) the understanding of crop species domestication (White and Doebley 1998), (2) the deciphering of elite cultivar breeding history (Dubreuil and Charcosset 1999; Narvel et al. 2000; Russell et al. 2000), (3) the assessment of genetic diversity within germplasm and/or cultivated types for various species (Prasad et al. 2000; Liu et al. 2001; Métais et al. 2002; Steiger et al. 2002) and (4) the identification of cultivars (Rongwen et al. 1995; Russell et al. 1997). Recently, molecular markers have been used to examine the effect of modern plant breeding methods on genetic diversity in barley (Russell et al. 2000; Koebner et al. 2003), wheat (Christiansen et al. 2002; Soleimani et al. 2002), and maize (Lu and Bernardo 2001). These studies concluded that the level of genetic variability within the cultivated pools had been maintained during modern selection, either through the differentiation of heterotic groups or through the maintenance of independent breeding programs.

The genetic diversity within recently released pea (*Pisum sativum* L.) cultivars in Western Europe has often been described as very narrow, especially in the spring-sown feed pea breeding pool. This has been accompanied by increasing difficulties in the registration process, both to discriminate new varieties from older ones, and to make significant progress in productivity. Pea breeding is facing new challenges to increase its acreage and to develop diversified products: to find resistance to diseases and frost, and enhance seed quality. To these ends, exploitable resources should be identified within the *Pisum* genus, which is now considered monospecific (Blixt 1972) since all former species can inter-cross. Ellis et al. (1998) suggested that the *Pisum* genus includes three main groups (1) *P. fulvum*, (2) *P. abyssinicum*, a distinctive Ethiopian form domesticated independently from *P. sativum* and (3) all the other *Pisum* subsp. including two wild groups, i.e. *P. humile* and *P. elatius*, both considered as the basis for domestication of cultivars, and *P. sativum* subsp. *sativum*, which comprises wild and cultivated forms, among which morphologically distinct types, dedicated to different end-uses, have been selected (Smartt 1990). Fodder peas, from former subsp. *arvense*, display a bushy and indeterminate habit, and were grown primarily for feeding livestock. Garden peas, from former subsp. *hortense*, include various types, used as dry split peas, green immature seeds, or immature pods ('mangetout peas') for human consumption. They also include different morphological types: vining plants with very long internodes or dwarf plants with more determinate habits. Feed peas, harvested as dry seeds for animal feed, were selected from garden peas in the 1970s. They have progressively incorporated different features desirable for feeding, combine harvesting, and for increased yield: a dwarf and determinate habit, the *afila* mutation transforming the leaflets into tendrils for a better standability and index ratio, white flowers indicating a low level of tannins in the seeds, and larger seeds. Nowadays, the feed pea crop predominates in Western Europe for pig and poultry feeding. It is also a major crop in North America, China and Australia. Different forms of garden peas are still sown for human consumption, mainly for freezing and canning. Fodder peas have almost disappeared from the cropping systems.

Recent diversity studies in pea have focused either on biosystematic studies within the *Pisum* genus (Hoey et al. 1996; Ellis et al. 1998), or on the assessment of different molecular markers to investigate the genetic diversity within *Pisum* (Lu et al. 1996; Posvec and Griga 2000; Burstin et al. 2001; Simioniuc et al. 2002). However, all these studies have focused either on rather small numbers of accessions, or on specific groups of pea genotypes. In the present study, we assessed the genetic diversity among a large sample of food, fodder and feed cultivars, as well as some wild accessions. Our objectives were (1) to investigate the genetic relationships between the different cultivated types, since very little information on cultivar genealogies is available, (2) to assess the genetic diversity present in cultivated *P. sativum* gene pools and the effect of recent breeding on this diversity and (3) to identify new sources of variability for future West European pea breeding.

Materials and methods

Plant material

One hundred and forty-eight *Pisum* accessions (Table 1) including cultivars, breeding lines, local varieties, genetic stocks and primitive cultivated forms were analyzed. The seeds were supplied by several institutes involved in legume genetics research and/or genetic resource conservation (Table 1); 93 were supplied by or via the Institut National pour la Recherche Agronomique and the Groupe d'Etude et de Contrôle des Variétés et des Semences (France), 27 by the John Innes Center (UK), 14 by the Vavilov Institute (Russia), 7 by the Chinese Academy of Agricultural Sciences (China), 5 by the University of Valladolid (Spain), 2 by the International Programme for Genetic Resources (Bulgaria), 2 by the United States Department of Agriculture Plant Introduction Station in Pullman (USA). Most accessions (114) were landraces, breeding lines or cultivars: 74 originated from Western European countries (France, Germany, Netherlands, United Kingdom and Spain), 6 from Northern European countries (Sweden and Denmark), 19 from Eastern Europe (Russia, Czech Republic, Bulgaria, Hungary, Lithuania, Latvia, Ukraine), 7 from the United States, and 8 from China. They included plant material characterized both by different end-uses (food, feed or fodder), and by different sowing types (spring and winter peas). These two latter categories are classified according to winter hardiness. The remaining accessions included germplasm from other parts of the world including the potential secondary diversification areas (Afghanistan, Ethiopia, Sudan) and accessions from *P. humile, P. elatius, P. abyssinicum* and *P. fulvum*. Pedigree information was mainly restricted to some recent cultivars (Table 1).

Molecular marker assays

Plants from all accessions were grown in a greenhouse. DNA extractions were performed from young leaves as described by Doyle and Doyle (1990) or by a modified Dellaporta method (1983). Mature seeds were harvested and proteins extracted for the isozyme and storage protein assays.

Isozyme assays were performed as described by Bourgoin-Grenèche and Lallemand (1993) for six enzyme systems: phosphoglucomutase (PGM), alpha-amylase (AMY), 6-phosphogluconate dehydrogenase (6-PGD), isocitrate dehydrogenase (IDH), glutamate oxaloacetate transaminase (GOT) and shikimate dehydrogenase (SDH). Storage protein assays were performed as described in Bourgoin-Grenèche and Lallemand (1993). The complex banding patterns were scored as profiles in four zones of acrylamide gels (A, B, C, D from the high molecular weight to the low molecular weight bands). RAPD assays were performed according to Laucou et al. (1998). Twenty 10-mer oligonucleotide primers (A02, A08, A10, A15, A16, B07, C17, E16, F08, H06, J12, M16, N13, O07, P04, P11, Q20, R12, W18, X01), purchased from Operon Technologies Inc. (Alameda, Calif., USA) were chosen after a survey in our laboratories for their reproducibility (data not shown). ISSR assays were performed as described in Arnau et al. (2002). Genomic DNAs were used as templates for amplification with two 17-mer primers, both based on dinucleotide SSR repeats, anchored at the 5′ end by partially degenerate selective nucleotides, ISSR1 [5′-VBV$(AC)_7$-3′, where V=nonT and B=nonA], and ISSRa [5′-GCV$(TC)_7$-3′]. These primers were previously identified as revealing a high level of polymorphism on a sub-sample of pea cultivars (Lallemand and Arnau 2000). SSRs were performed as described in Burstin et al. (2001), using 13 primer pairs revealing SSR variation in GenBank registered genes (AA427337, AA430902, AF016458, AF029243, PATRG31,

Table 1 Geographical origins, cultivated status, end-use type, sowing type, morphological description and pedigree of the 148 *Pisum* accessions, following their order of appearance in the molecular-based classification (see Fig. 1). All accessions are *Pisum sativum* spp *sativum* except for JI1006 (*Pisum fulvum*), JI241 (*Pisum humile*), JI1089, JI261 (*Pisum elatius*), L808 (*Pisum abyssinicum*). *Cv* Cultivar, *Bl* breeding line, *Lv* local variety, *Gmp* germplasm, *Fd* fodder, *Gd* garden, *Ws* winter sown, *Ss* spring sown, *Nl* normal leaf, *af afila*, *rg* rogue, *P* purple, *W* white, *O* orange, *Sm* smooth, *Wr* wrinkled, *G* green, *Y* yellow, *C* clear, *B* black or brown, *XS* very small, *S* small, *M* medium, *L* large, *XL* very large

Name groups	Country of origin	Breeding company or donor	Registration year	Cultivation status	End-use	Sowing type	Foliage	Flower color	Seed type	Cotyledon color	Hilum color	Seed size	Pedigree
Group I													
Winkossa	Germany	Sperling	1978	Cv	Gd	Ws	Nl	W	Wr	G	C	S	Austrian Winter×dry pea
Winterberger[a]	–	–	–	Cv	Fd	Ws	Nl	P	Sm	Y	B	S	–
Fenn[a]	USA	CSSA	1971	Cv	Fd	Ws	Nl	P	Sm	Y	B	S	Selection from Austrian Winter
B468[a]	–	–	–	Gmp	Fd	–	Nl	P	Sm	–	B	S	–
B460[a]	–	–	–	Gmp	Fd	–	Nl	P	Sm	–	B	S	–
B461	–	–	–	Gmp	Fd	–	Nl	P	Sm	–	B	M	–
B195	–	–	–	Gmp	Fd	–	Nl	P	Sm	Y	B	S	–
Group II													
Glacier[a]	USA	–	–	Cv	Gd	Ss	Nl	W	Wr	Y	C	M	–
CCA[a]	Bulgaria	IPGR	–	Cv	Dry	Ws	Nl	P	Sm	Y	B	S	–
Hol11	Hungary	–	–	–	Fd	–	–	–	–	–	–	–	–
Melrose	USA	CSSA	1977	Cv	Fd	Ws	Nl	P	Sm	Y	B	XS	Selection from Perfection edible× Austrian Winter
Champagne	France	–	–	Cv	Fd	Ws	Nl	P	Sm	Y	B	XS	–
Piver	France	Lafite	1964	Cv	Fd	Ws	Nl	P	Sm	Y	B	XS	–
B287	–	–	–	Gmp	Fd	–	Nl	P	Sm		B	M	–
EP	–	–	–	Gmp	Fd	–	Nl	P	Sm	Y	B	M	–
DP	–	–	–	Gmp	Fd	–	Nl	P	Sm	Y	B	S	–
Austrian Winter[a]	USA	–	–	Cv	Fd	Ws	Nl	P	Sm	Y	B	XS	–
Group III													
JI96[a]	Afghanistan	John Innes C.	–	Gmp	–	–	Nl	P	Sm	G	C	XS	–
JI241 *(P.humile)*	–	John Innes C.	–	Gmp	–	–	Nl	P	Sm	G	C	XS	–
JI103[a]	Afghanistan	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	B	XS	–
JI281[a]	Ethiopia	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	C	S	–
JI190	Sudan	John Innes C.	–	Gmp	–	–	Nl	P	Sm	G	C	S	–
JI1006[a]	–	John Innes C.	–	Gmp	–	–	Nl	O	Sm	Y	B	XS	–
L808[a]	–	–	–	Gmp	–	–	Nl	P	Sm	Y	C	S	–
JI1089[a]	Syria	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	B	S	–
JI261[a]	–	John Innes C.	–	Gmp	–	–	Nl	P	Sm	G	B	XS	–
GSP[a]	–	–	–	–	–	–	–	–	–	–	–	–	–
Group IV													
Pscaucgeor[a]	Georgia	–	–	Gmp	–	–	Nl	P	Sm	Y	B	XS	–
JI45	–	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	B	XS	–
JI146	Georgia	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	B	XS	–
Group V													
ZP126	Spain	Univ. of Valladolid	–	Lv	Gd	Ss	Nl	W	Sm	Y	C	M	–
ZP103	Spain	Univ. of Valladolid	–	Lv	Gd	Ss	Nl	W	Sm	Y	C	L	–
ZP142	Spain	Univ. of Valladolid	–	Lv	Gd	Ss	Nl	W	Sm	G	C	L	–
ZP141	Spain	Univ. of Valladolid	–	Lv	Gd	Ss	Nl	W	Sm	G	C	L	–
JI252	Ethiopia	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	B	XS	–
G1503, Yangwan	China	CAAS	–	Lv	Gd	Ss	Nl	W	Sm	–	C	M	–
G0770, Bendi[a]	China	CAAS	–	Lv	Gd	Ss	Nl	W	Sm	Y	C	L	–
G0553, Wandou[a]	China	CAAS	–	Lv	Gd	Ss	Nl	?	Sm	Y	C	M	–
JI1491	China	John Innes C.	–	Gmp	–	–	Nl	W	Sm	Y	B	M	–
G1265, Channhe	China	CAAS	–	Lv	Gd	Ss	Nl	P	Sm	Y	B	M	–
G1529, Hecai	China	CAAS	–	Lv	Gd	Ss	Nl	P	?	Y	C	L	–
G1409, Er Blanch	China	CAAS	–	Lv	Gd	Ss	Nl	W	Sm	Y	C	L	–
G0299, Wandou[a]	China	CAAS	–	Lv	Gd	Ss	Nl	P	Sm	Y	C	L	–
Group VI sgI													
JI124	Sweden	John Innes C.	–	Gmp	–	–	Nl	W	?	Y	B	XL	–
JI1860 (E344)	Sweden	John Innes C.	–	Gmp	–	–	Nl	P	?	Y	B	XL	–
K4088[a]	Ukraine	Vavilov Inst.	–	Lv	Dry	Ss	Nl	W	Sm	Y	C	S	–
K5127	Russia	Vavilov Inst.	–	Lv	Dry	Ss	Nl	W	Sm	Y	C	S	–
K4834[a]	Lithuania	Vavilov Inst.	–	Cv	Dry	Ss	Nl	W	Sm	Y	C	M	–
K30[a]	Russia	Vavilov Inst.	–	Lv	Dry	Ss	Nl	W	Sm	Y	C	M	–
K4885[a]	Russia	Vavilov Inst.	–	Cv	Dry	Ss	Nl	W	Sm	Y	C	M	–
JI196	Mongolia	John Innes C.	–	Gmp	–	–	Nl	W	Sm	Y	C	M	–
K4819[a]	Latvia	Vavilov Inst.	–	Lv	Dry	Ss	Nl	W	Sm	Y	C	M	–

Table 1 (continued)

Name groups	Country of origin	Breeding company or donor	Registration year	Cultivation status	End-use	Sowing type	Foliage	Flower color	Seed type	Cotyledon color	Hilum color	Seed size	Pedigree
Torsdag[a]	Sweden	–	–	Cv	Dry	Ss	Nl	W	Sm	Y	C	L	–
K1666[a]	Russia	Vavilov Inst.	–	Lv	Dry	Ss	Nl	W	Sm	Y	C	S	–
JI1846[a]	Egypt	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	C	S	–
Group VI sgII													
Vendevil	France	Vilmorin	1981	Cv	Dry	Ws	Nl	W	Sm	G	C	S	Vilmorin line× Austrian Winter
Froidure	France	Cambier	1990	Cv	Dry	Ws	Nl	W	Sm	G	C	S	Vendevil×Printivert
Victor	USA	Pioneer	1992	Cv	Dry	Ws	Rg	W	Sm	Y	C	M	Progretta×(Frimas× Print)
Cheyenne	France	GAE-Recherche	1997	Cv	Dry	Ws	Af	W	Sm	Y	C	M	–
Virgo	France	–	–	Cv	Dry	Ss	Af	W	Sm	Y	C	M	–
Mir[a]	Germany	–	–	Cv	Dry	Ss	Nl	P	Sm	Y	B	M	–
Bohatyr	Czech Rep.	–	1980	Cv	Dry	Ss	Nl	W	Sm	Y	C	L	Unicum×(Pyram× Dick Trom)
Karnobat	Bulgaria	–	–	Cv	Dry	Ws	Nl	P	Sm	Y	B	M	–
CF100 (GP)	France	INRA	–	Bl	Dry	Ws	Nl	W	Sm	Y	C	XS	[(Amac×Ceb406)× (Hol11×Stehgolt)]× [(Pol3707×595)× (B157×136)]
Aravis	France	Danisco	1995	Cv	Dry	Ws	Nl	W	Sm	Y	B	M	–
Group VI sgIII													
Puget	UK	Brotherton	–	Cv	Gd	Ss	Nl	W	Wr	G	C	M	–
Mini	USA	Asgrow	1975	Cv	Gd	Ss	Nl	W	Wr	G	C	S	Nugget×Lig Asgrow
K4626[a]	Lithuania	Vavilov Inst.	–	Lv	Fd	Ss	Nl	P	Sm	Y	B	M	–
JI296	France	John Innes C.	–	Cv	Gd	Ss	Nl	W	Wr	–	–	–	–
WavF502	Germany	–	–	Bl	Gd	Ss	Nl	W	Wr	G	C	M	–
WavF750	Germany	–	–	Bl	Gd	Ss	Nl	W	Wr	G	C	M	–
JI174	Ethiopia	John Innes C.	–	Gmp	–	–	Nl	P	Sm	G	C	M	–
Vitalis	Holland	Sluis and Groot	1963	Cv	Gd	Ss	Nl	W	Wr	G	C	S	–
Multifreezer	–	–	–	Cv	Gd	Ss	Nl	W	Wr	Y	C	M	–
Kelvedon Wonder	UK	Hurst	1952	Cv	Gd	Ss	Nl	W	Wr	G	C	L	Lincoln×Petite Merveille
Erygel	France	INRA	1972	Cv	Gd	Ss	Nl	W	Wr	Y	C	M	Mexique3×Lancet
Progreta	–	Processors	–	Cv	Dry	Ss	Rg	W	Sm	G	C	L	Maro rogue
Maro	Holland	Cebeco	–	Cv	Dry	Ss	Nl	W	Sm	G	C	XL	–
JI813[a]	–	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	C	XL	–
Countess (V45)	UK	Booker seeds	–	Cv	Dry	Ss	Af	W	Sm	Y	C	L	Ceb204×Filby
Ballet[a]	UK	Nickerson	1988	Cv	Dry	Ss	Af	W	Sm	G	C	M	Selection from Filby
Group VI sgIV													
ZP130	Spain	Univ. of Valladolid	–	Lv	–	Ss	Nl	P	Sm	Y	C	M	–
K4170	Latvia	Vavilov Inst.	–	Lv	Fd	Ss	Nl	P	Sm	Y	–	S	–
Juwel[a]	Germany	Van Waveren	1955	Cv	Gd	Ss	Nl	W	Wr	G	C	M	Selection from Horal
Sommette	Holland	Sluis and Groot	1972	Cv	Gd	Ss	Nl	W	Sm	G	C	L	Aurora×Express Alaska
JI833	Sweden	John Innes C.	–	Gmp	–	–	Rg	W	–	Y	C	M	–
K8349	Russia	Vavilov Inst.	–	Cv	Dry	Ss	Af	W	Sm	Y	C	L	–
JI181	Nepal	John Innes C.	–	Gmp	–	–	Nl	P	Sm	G	C	S	–
Uladovskij10[a]	Ukraine	–	–	Cv	Gd	Ss	Nl	W	Sm	G	C	M	–
JI1184[a]	–	–	–	Cv	–	–	Nl	P	Sm	Y	B	S	–
Gradus	UK	Thomas Laxton	1952	Cv	Gd	Ss	Nl	W	Wr	Y	C	L	Express Alaska×Duc d'Albany
Lincoln	–	SOC	1952	Cv	Gd	Ss	Nl	W	Wr	Y	C	M	Selection from Senateur
Clauselan	France	Clause	1961	Cv	Gd	Ss	Nl	W	Sm	Y	C	L	Serpette Nain Cent Pour Un×Gloire de Quimper
K4252	Germany	Vavilov Inst.	–	Lv	Dry	Ss	Nl	W	Sm	G	C	L	–
JI805	–	John Innes C.	–	Gmp	–	–	Nl	W	Sm	Y	C	L	–
Surgevil[a]	France	Vilmorin	1975	Cv	Gd	Ss	Nl	W	Wr	G	C	M	Vervil×Vilmorin line
JI1594	Ethiopia	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	C	M	–
Wiscmerit	–	–	–	Cv	Gd	Ss	Nl	W	Wr	G	C	L	–
Costa Rica	Costa Rica	–	–	Gmp	Gd	Ss	Nl	W	Sm	Y	B	XL	–
Colmo	Holland	Nunhem'Zaden	1972	Cv	Gd	Ss	Nl	W	Sm	G	C	M	Profusion×Horal× Korora
Vercas[a]	France	Blondeau	1964	Cv	Gd	Ss	Nl	W	Sm	G	C	XL	Strubes Vert× (Stijftroo×Brunswiga)
Starcovert[a]	France	Blondeau	1957	Cv	Gd	Ss	Nl	W	Sm	G	C	M	Clamart Trois Gousses×Brunswiga
Amino	France	Blondeau	1977	Cv	Dry	Ss	Nl	W	Sm	Y	C	L	Stijfstroo×Goldkugel
Pilet	France	Blondeau	1971	Cv	Gd	Ss	Nl	W	Sm	G	C	L	Supcovert×Kelva
Cameor	France	Seminor	1973	Cv	Gd	Ss	Nl	W	Sm	Y	C	M	–
JI1431	Ethiopia	John Innes C.	–	Gmp	–	–	Nl	P	Sm	Y	C	S	–
Alaskor	France	Seminor	1978	Cv	Gd	Ss	Nl	W	Sm	G		M	(Alaska×Amac)× Cameor
K4356[a]	Russia	Vavilov Inst.	–	Lv	Dry	Ss	Nl	W	Sm	Y	C	S	–
Cennia	Germany	Gaters	–	Cv	Gd	Ss	Nl	W	Wr	G	C	M	Selection from Alaska Sweet
K4269	Lithuania	Vavilov Inst.	–	Lv	Dry	Ss	Nl	W	Sm	G	C	M	–

Table 1 (continued)

Name groups	Country of origin	Breeding company or donor	Registration year	Cultivation status	End-use	Sowing type	Foliage	Flower color	Seed type	Cotyledon color	Hilum color	Seed size	Pedigree
Preclamex	France	France-Graines	1972	Cv	Gd	Ss	Nl	W	Sm	Y	C	M	Roi Des Fins Verts×Clamart Trois Gousses
Finette	Holland	–	–	Cv	Gd	Ss	Nl	W	Sm	G	C	M	–
Aldot	USA	Roger Brothers	1968	Cv	Gd	Ss	Nl	W	Sm	G	C	M	–
Cador	France	Clause	1971	Cv	Gd	Ss	Nl	W	Sm	G	C	S	Hybris×Small Sieve Alaska
C667	France	INRA	–	Bl	Gd	Ss	Nl	W	Wr	G	C	S	Aldot×Alaska Sweet
Group VII													
Rafale	France	INRA	1991	Cv	Dry	Ws	Af	W	Sm	Y	C	M	(Finale×Frimas)×Baf
CP	France	INRA	–	Bl	Dry	Ws	Af	W	Sm	Y	C	S	Amac×C247
Kazar	France	Clause	1988	Cv	Dry	Ws	Nl	W	Sm	G	C	S	Vendevil×(Frimas×Novasem)
Mercure	France	–	–	Bl	Dry	Ws	Af	W	Sm	Y	C	S	(Filby×Frisson)×L641
Dove	France	INRA	–	Cv	Dry	Ws	Af	W	Sm	G	C	M	–
CD5MM	France	INRA	–	Bl	Dry	Ss	Af	W	Sm	Y	C	L	–
C437	France	INRA	–	Bl	Dry	Ws	Af	W	Sm	Y	C	S	–
Frilène	France	INRA	1987	Cv	Dry	Ws	Nl	W	Sm	Y	C	M	Frisson×Finale
CE101 (FP)	France	INRA	–	Bl	Dry	Ws	Nl	W	Sm	Y	C	S	[(Hol11×(136×Hativer)]×(Amac×Ceb406)x(Hol11×Stehgolt)
C413 (AP)	France	INRA	–	Bl	Dry	Ws	Nl	W	Sm	Y	C	S	(Frimas×Frijaune)×Amac
Frogel[a]	France	INRA	1974	Cv	Gd	Ws	Nl	W	Wr	G	C	S	Champagne×Vitalis
Frisson	France	INRA	1979	Cv	Dry	Ws	Nl	W	Sm	Y	B	S	Champagne×Vitalis
Amac	France	INRA	1987	Cv	Dry	Ws	Nl	W	Sm	Y	C	M	Frisson×Finale
Messire	France	Serasem	1989	Cv	Dry	Ss	Rg	W	Sm	Y	C	L	(Frimas×Rondo)×Progretta
C776	France	INRA	–	Bl	Dry	Ss	Af	W	Sm	G	C	L	–
Group VIII sgI													
Paloma	Holland	Cebeco	1976	Cv	Dry	Ss	Nl	W	Sm	Y	C	L	–
Alex	France	Blondeau	1989	Cv	Dry	Ss	Af	W	Sm	Y	C	XL	Filby×Flavanda
Optima	France	–	1998	Cv	Dry	Ss	Af	W	Sm	Y	C	L	–
Eiffel[a]	France	Danisco	1993	Cv	Dry	Ss	Af	W	Sm	Y	C	L	Solara×(Bohatyr×MD 420065)
Baccara[a]	France	F.Desprez	1991	Cv	Dry	Ss	Af	W	Sm	Y	C	L	(USA811197×Finale)×Finale
Athos	France	Nickerson	1997	Cv	Dry	Ss	Af	W	Sm	Y	C	XL	–
Badminton	France	F.Desprez	1996	Cv	Dry	Ss	Af	W	Sm	Y	C	L	–
Figaro (C744)	France	INRA	–	Bl	Dry	Ss	Af	W	Sm	G	C	L	C661×C551
Group VIII sgII													
K8290[a]	Russia	Vavilov Inst.	–	Cv	Dry	Ss	Af	W	Sm	Y	C	L	–
PF31905[a]	Germany	Pajgergfonden	–	Bl	Dry	Ss	Af	W	Sm	Y	C	L	–
Katrin	Germany	Petersen	–	Cv	Dry	Ss	Nl	W	Sm	Y	C	XL	–
Rondo	Holland	Cebeco	1952	Cv	Gd	Ss	Nl	W	Sm	G	C	L	Unica×[Coronax(Victoria×Schokker)]
Danto	Denmark	Daehnfeldt	1987	Cv	Dry	Ss	Af	W	Sm	G	C	L	Selection from Allround
BP	France	INRA	–	Bl	Dry	Ws	Af	W	Sm	Y	C	S	C320×C247
Terese	Denmark	Pajbjergfonden	1988	Cv	Dry	Ss	Af	W	Sm	Y	C	L	Finale×Filby
Solara	Holland	Cebeco	1986	Cv	Dry	Ss	Af	W	Sm	G	C	XL	Finale×Ceb238-6
Finale	Holland	Cebeco	1976	Cv	Dry	Ss	Nl	W	Sm	G	C	L	Dick-Trom×Cebeco 61-207
PF35323	Germany	Pajgergfonden	–	Bl	Dry	Ss	Nl	W	Sm	Y	C	M	–
C661	France	INRA	–	Bl	Dry	Ss	Af	W	Sm	G	C	L	Finale *afila*

[a] Accessions belonging to the subset representing 96% of the allelic richness of the collection

PSADH1, PSAJ3318, PSAS, PSBlox2, PSGAPA-1, PSU51918, PSU81288, PSY14558). STS assays were performed as described in Gilpin et al. (1997), using nine primer combinations allowing the PCR amplification of genes of known function followed by digestion with the indicated restriction enzyme (K6-*Alu*I, L109-*Sau*3A, M27-*Rsa*I, P202-*Taq*I, P393-*Sty*I, P482-*Mse*I, P628-*Hinf*I, Q363-*Alu*I, Q500-*Hinf*I). All molecular data were scored visually. Altogether, 121 markers were examined. The chromosomal locations of 47 markers that were mapped in other studies and corresponding references are listed in Table 2.

Morphological description of the accessions

In order to characterize the plant morphology diversity, several traits (flower color, leaf type, color of cotyledons, thousand-seed weight, shape of seeds, hilum color) were scored at different developmental stages. The different genotypes were described for their date of sowing (winter types, spring types), their end-use (food, feed, fodder), and, for cultivars, their year of registration when available.

Statistical analyses

The squared modified Roger's distance was computed between all pairs of genotypes (Rogers 1972) for codominant markers sepa-

Table 2 Map position of the 47 mapped markers on the seven *Pisum sativum* genetic linkage groups (Gilpin et al. 1997; Laucou et al. 1998; Weeden et al. 1998; Pilet-Nayel et al. 2002; K. Loridon, personal communication; S. Prioul, personal communication)

	LG I	LG II	LG III	LG IV	LG V	LG VI	LG VII	Total
RAPD	4	3	6	3	0	3	2	21
SSR	3	1	4	1	1	0	0	10
STS	1	1	3	3	0	0	1	9
Isozymes	1	1	0	0	1	0	4	7
Total	9	6	13	7	2	3	7	47

Table 3 Number of systems, number of markers, mean number of alleles per locus, number of alleles, mean allelic frequencies and percentages of rare alleles for each marker type

	Systems	Markers	Mean alleles/ locus	Alleles	Mean allelic frequency. (range)	Percentage of rare alleles (f <0.05)
RAPD	20	38	2		0.61 (0.03–0.99)	3.0
ISSR	2	51	2		0.23 (0.01–0.88)	28.0
SSR	13	13	5.6	2–11	0.18 (0.01–0.99)	50.0
STS	9	9	3.6	2–5	0.28 (0.01–0.76)	38.7
Isozymes	6	8	2.2	2–3	0.44 (0.01–0.90)	11.8
Storage proteins	4	4	6.5	2–11	0.15 (0.01–0.67)	44.0

rately and together. The Jaccard distance was computed between all pairs of genotypes (Jaccard 1908) for dominant markers separately, for dominant markers together, and for all markers pooled. Correlations between distances were tested according to Mantel's test (Mantel 1967). A Ward hierarchical ascendant classification (Ward 1963) was then performed on the total molecular distance matrix and a dendrogram was built to represent the structure of the genetic diversity within our collection. The calculation of genetic distances, the hierarchical classification and the drawing of the dendrogram were performed using the LCDMV Software (Dubreuil et al. 2003) running on SAS-IML v6.12 (SAS Institute, Cary, N.C.). Associations among genotypes were also investigated by a principal component analysis (PCA) on the binary data matrix of the total set of markers (SAS Institute 1988). To relate this molecular structure with morphological data, analyses of variance were performed on the first axes of the PCA, using morphological and cultivar types as explicative variables. Finally, the genetic redundancy of the collection and a subset of genotypes displaying a large part of the allelic richness present in the collection were defined using the MSTRAT program, which generates through an iterative maximization procedure large numbers of possible core collections and selects those with the highest allelic richness (Gouesnard et al. 2001).

Results

Levels of polymorphism for the different markers

Altogether, the 54 primer combinations or systems revealed 121 polymorphic patterns, all revealing at least one difference among the 148 accessions. The SSRs, STSs and isozymes displayed only one or two reproducible, multi-allelic, and easy-to-score loci. The 20 RAPD oligonucleotides were previously chosen for their ability to display at least one reproducible polymorphic band per primer. After excluding faint bands, 18 other RAPD bands displayed with the same primers were also scored, leading to a total of 38 RAPD polymorphic bands scored. Similarly, ISSR primers were pre-selected for their ability to reveal polymorphisms in the pea (Lallemand and Arnau 2000). The two ISSR primer combinations allowed the identification of 51 polymorphic bands: 31 bands ranging from 325 to 720 bp with ISSRa, and 20 bands ranging from 290 to 670 bp with ISSR1. The level of polymorphism detected with the different markers is presented in Table 3. The storage protein and microsatellite markers showed the highest number of alleles per locus, whereas the STS and isozyme markers possessed fewer. The highest levels of polymorphism among the 148 pea lines were obtained for storage protein B zone (11 different banding patterns), and for SSR loci *PSADH1* and *PSGAPA-1*, each displaying 11 alleles. These two markers already showed the highest polymorphism among 12 genotypes (Burstin et al. 2001). SSRs and storage proteins also displayed the lowest mean allelic frequencies and highest percentages of rare alleles. The mean allelic frequency was highest for the RAPDs, for which the dominant allele (band present) was often largely shared, as compared to ISSR markers, where dominant alleles (band present) were less frequent, and which showed a higher percentage of rare alleles (Table 3).

Classification of the 148 *Pisum* genotypes according to molecular data

The classification obtained from the entire molecular data revealed eight groups (Fig. 1), consistent with geographical origins and known cultivated types (Table 1). Group I comprised mainly fodder peas, including cultivated varieties such as 'Winterberger' or 'Fenn', together with germplasm accessions. Group II comprised mostly winter fodder accessions, including varieties such as 'Austrian Winter', 'Champagne', 'Melrose' or 'Piver' and closely related accessions that show strong morphological similarities to these cultivars. Groups I and II were clearly resolved from the other accessions, and included most of the fodder peas. Most of these have purple flowers, leafy

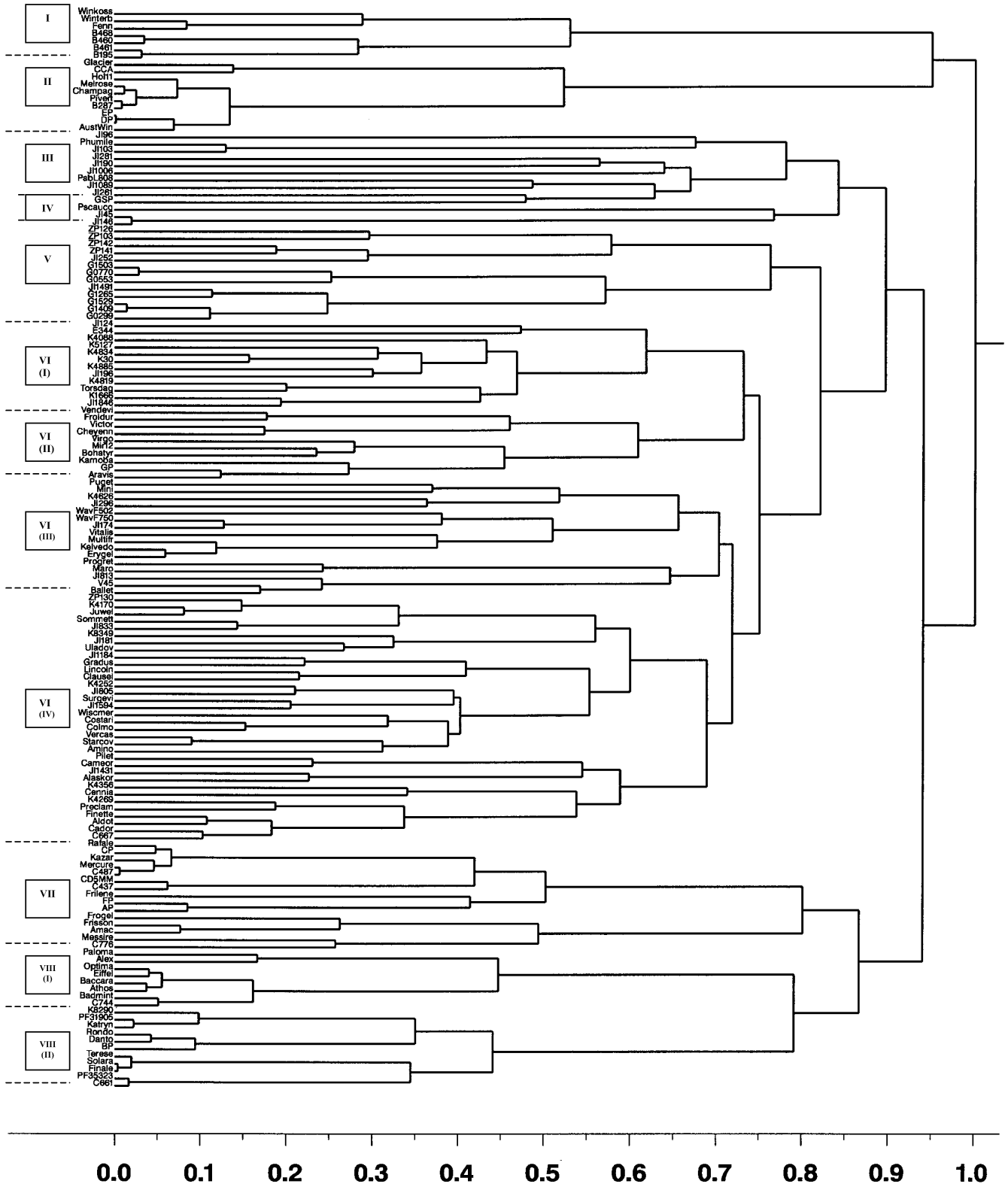

Fig. 1 Classification of 148 *Pisum* accessions based on molecular data obtained from 121 DNA and protein markers. Jaccard's distances were computed for all pairs of accessions and a Ward hierarchical classification procedure was used to classify the accessions within groups. Groups (subgroups) are indicated on the *left*, limits between groups appear as *dotted lines* (see Table 1)

Table 4 Correlations between distances within 148 *Pisum* accessions computed for the different marker types with the global distance computed from all markers. MRD (Rogers 1972) was used for codominant markers (the STSs, SSRs and the allozymes). The Jaccard (1908) distance was used for dominant markers (RAPDs and ISSRs) and for the global distance

	RAPD	ISSR	STS	SSR	Allozymes	Dominant	Codominant
Global distance	0.72	0.26	0.50	0.66	0.46	0.72	0.80

foliage, long internodes, very indeterminate flowering, smooth seeds and yellow cotyledons. However, there were two exceptions: 'Winkossa' and 'Glacier', which are white-flowered and wrinkle-seeded garden pea cultivars. 'Winkossa' is known to derive from a cross including 'Austrian Winter', and was bred for adaptation of the garden pea to winter sowings. Group III included *P. fulvum* (JI1006) and accessions from wild groups from the *Pisum* germplasm (*P. humile, P. abyssinicum* and *P. elatius)*, as well as *P. sativum* exotic germplasm accessions from secondary centers of diversity (Afghanistan, Ethiopia, Sudan). Although clustering together, the tree showed that some of these accessions were distant from one another. All these accessions carry ancient traits such as colored flowers, leafy foliage and indeterminate flowering. Accessions from Georgia and Caucasia clustered together into group IV. Group V gathered mostly spring-sown garden pea local varieties from China and Spain. A surprising exception was JI252, a germplasm accession originating from Ethiopia. Group VI included feed and garden peas and was composed of four subgroups. The first subgroup included mainly local varieties and accessions originating from Eastern Europe, provided by the Vavilov Institute, together with an accession from Mongolia, two accessions and a cultivar from Sweden. Surprisingly, an accession from Egypt (JI1846) clustered in this subgroup. This accession is referred to as *P. hibernicum* in the John Innes Centre collection. The second subgroup included mainly winter feed peas from Western Europe, together with spring feed peas from Central Europe. In the third and fourth subgroups were found mainly spring-sown garden peas (including split peas, sugary garden peas and 'mangetout' peas) and feed peas from Western Europe. The available pedigree information was consistent with the molecular classification: 'Progreta' is closely related to 'Maro', known as its rogue near-isogenic line. 'Countess' also derives from 'Maro' according to Simioniuc et al. (2002). 'Countess' and 'Ballet', both selected from 'Filby', clustered together. Group VII consisted of winter feed pea cultivars or breeding lines, mainly from French breeding programs. Some accessions in this group share common progenitors: 'Amac', 'Frimas', 'Frisson', 'Champagne' and 'Austrian Winter' via 'Vendevil'. 'Messire' was one of the first attempts to produce a winter feed pea, but due to limited frost resistance, it was registered as a spring type (R. Cousin, personal communication). 'Frogel', a garden pea cultivar, also results from one of the rare attempts to grow peas in winter. Winter garden peas were expected to circumvent the productivity loss due to the development of very early types for 'primeurs' (R. Cousin, personal communication). Two other spring feed breeding lines clustered into this group; it is possible that they derive from crosses including both spring and winter gene pools. Group VIII consisted mainly of feed spring cultivars and breeding lines from Western Europe. A first subgroup clustered French breeding releases from the 1990s ('Baccara', 'Athos', 'Badminton', 'Eiffel', 'Optima') and an earlier release ('Paloma'). A second subgroup included cultivars from Central Europe, and cultivars or breeding lines that were developed to introduce the *afila* mutation into feed pea breeding ('Solara', C661).

As expected, the highest Jaccard distances computed with all markers were observed for pairs including either JI1006 (*P. fulvum*), JI261 (*P. elatius*), JI103, JI190 or PabL808 (*P. abyssinicum*) together with another germplasm or cultivated accession. The lowest distances were observed for four couples, ie JI45/JI146, both from Caucasia, 'Progreta'/'Maro' ('Progreta' being a rogue line deriving from 'Maro'), C413/CE101, both coming from the Institut National pour la Recherche Agronomique winter pea breeding programs, and ZP141/ZP142, both accessions from the Asturias province in Spain (A. Monreal, personal communication).

Structure of the genetic variability as revealed by the different marker types

The different marker types on their own, dominant markers together, or codominant markers together, were less powerful in revealing the classification than considering all markers together (data not shown). Nevertheless, the genetic structure revealed by the different types of markers was congruent, suggesting a classification robust to the changes of samples of markers used. Dominant markers on their own, or codominant markers on their own, were efficient in clustering the different subspecies and wild groups together (group III), fodder types (groups I and II), and most of the feed pea groups (groups VII, VIII). The structure revealed by RAPDs analysis alone was very close to that obtained with all markers, whereas ISSR analysis alone did not distinguish as efficiently the feed and garden pea groups. Among the codominant markers, the structure obtained with SSRs alone was the closest to that obtained with all markers.

The correlations between the distances computed for the different types of markers and the global distance computed with all markers were variable (Table 4). Among dominant markers, the RAPD-based distance was better correlated to the global distance than the ISSR-

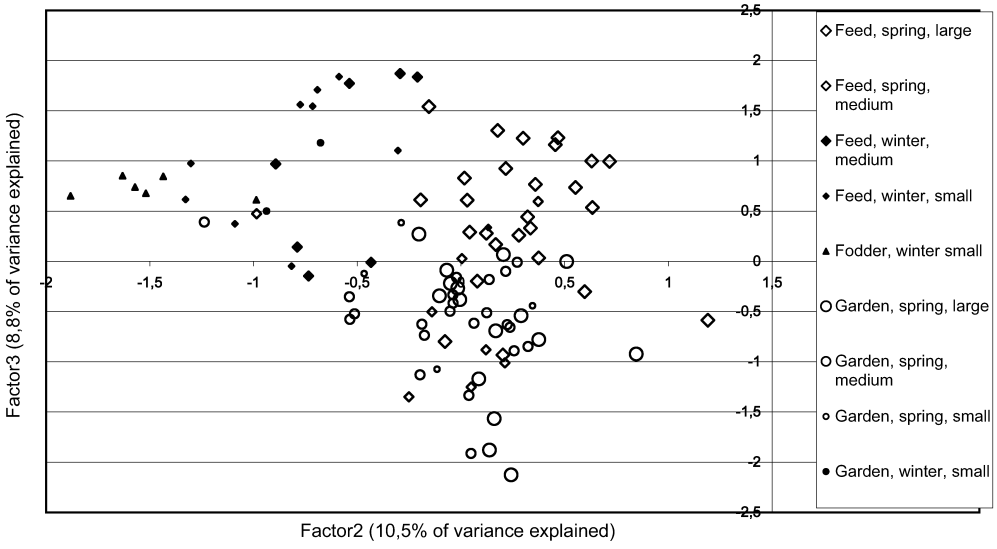

Fig. 2 Associations among 148 *Pisum* accessions characterized by their sowing types, end-uses types, and seed size, as revealed by a principal component analysis on the molecular data obtained with 121 molecular markers

based distance. RAPD data from 38 markers therefore seemed more informative than ISSR data from 51 markers. Despite the low number of markers involved, the correlations of distances computed for the different codominant marker types (isozymes, SSRs and STSs) with the global distance were all significant. Among the codominant markers, the SSR-based distance seemed to be the most informative, with the highest correlation with the global distance. Finally, RAPDs (38 bands) were as informative as SSRs (13 loci), RAPDs and SSRs being equally correlated to the distance obtained with all markers.

Genetic diversity among the different gene pools—proposal of a core subset of genotypes

Seventy-five alleles out of 235 (i.e. 32%) showed a frequency below 0.05 within the 148 accessions. High numbers of rare alleles (35, 24, 20, 17) were found in group III, including exclusively exotic germplasm accessions, in group VI subgroup I, displaying accessions from Northern and Eastern Europe, in group VI subgroup IV, mainly in germplasm and local varieties accessions, and in group V, displaying local varieties from China and Spain. There were 18, 5, 4 and 5 rare alleles specific to these groups respectively. From the exotic germplasm group III, 17 rare alleles were shared with group VI subgroup I (8 alleles), group VI subgroup IV (8 alleles), group I and group V (5 alleles each). None of these was shared with the recent Western European feed pea cultivars from group VIII, and only 2 were shared with Western European winter feed peas from group VII. Similarly, none of the 19 rare alleles from group VI subgroup I and none of the 12 rare alleles from group V were shared with either of the feed pea cultivated groups VII and VIII. This suggests a significant loss of alleles in the course of recent Western European feed pea breeding. Finally, the 15 rare alleles found in fodder peas in groups I and II were all shared with other groups displaying germplasm accessions (group III, group VI subgroup I), and also with groups including winter-sown varieties or breeding lines (group VI subgroup II and group VII). This probably indicates the partial use of these fodder peas to introduce frost resistance into winter-sown feed peas. Other groups displayed fewer than ten rare alleles, the lowest being found in the feed pea groups VII, VIII subgroups I and II (4, 2 and 3 rare alleles respectively, all shared with other groups).

Forty-three accessions represented 96% of the allelic diversity of the collection (Table 1). They belonged to all the groups, the most represented being group III and group VI subgroup I. Only one accession (‘Mir’) represented group VI subgroup II (winter feed pea cultivars), one (‘Frogel’) represented group VII (French winter feed pea cultivars), and 4 (‘Eiffel’, ‘Baccara’, K8290 and PF31905) represented group VIII. These 43 accessions possessed 237 alleles out of the 245 present in the 148 accessions tested, i.e. 96% of the total allelic diversity, and included all of the 75 rare alleles. They displayed all possible levels in end-uses, sowing-types and morphological characters (foliage type, flower color, seed type, cotyledon color, hilum color and seed size), except for rogue foliage.

Relationship between the genetic diversity observed and morphological diversity

The PCA performed on all molecular data structured the genetic variability according to cultivated types and end-uses, as already revealed by the hierarchical classification of the data (Fig. 2). The analysis of variance of the first axes of the PCA (Table 5), using morphological traits as explaining variables, related this structure with morphological traits: for example, the thousand-seed weight was significantly correlated with axis 1 and axis 2 of the PCA that represented 11.9% and 10.8% of the total variance,

Table 5 Percentages of variation explained by the first axes of the principal component analysis (PCA) on molecular data and level of significance and *F*-test value of the analysis of variance on the first axes coordinates using the end-use type, sowing type, foliage type, color of flower, shape of seed, cotyledon color, hilum color and seed weight as explaining variables

PCA axes	% Variance explained	End-use type	Sowing type	Foliage type	Flower color	Seed type	Cotyledon color	Hilum color	Seed weight
Axis 1	11.9	26.3***	27.6***	18.2***	57.0***	–	–	39.3***	30.0***
Axis 2	10.5	12.3***	115***	–	46.8***	–	–	–	3.5**
Axis 3	8.8	11.8***	43.0***	6.4**	16.6***	–	–	–	2.6*
Axis 4	7.9	3.6*	–	–	35.6***	–	–	–	–
Axis 5	7.2	27.5***	–	4.7*	3.2*	47.8***	4.4*	–	–

*$P<0.05$; **$P<0.01$; ***$P<0.001$

respectively. The first axis discriminated recent feed pea cultivars and relatives ('Athos', 'Solara', 'Eiffel', 'Optima', 'Finale', 'Baccara', negative values on axis 1) from primitive germplasm (JI1006, JI261, 'Austrian Winter', JI281, JI1089, JI96). The variations on this axis were associated with differences in flower color, foliage type and seed weight. The purple color of flowers, associated with the presence of tannins in seeds, is not found in recent feed pea cultivars. The *afila* leaf form is associated with the development of cultivars resistant to lodging, while recent feed pea cultivars present larger seeds. The second axis separated winter fodder peas ('Austrian Winter', 'Champagne', B287, 'Piver', 'Fenn') from primitive germplasm and an accession from Russia (JI1006, JI261, GSP, PabL808, K8349, JI96). This axis is associated with variations in flower color and to a lesser extent seed size. The third axis separated germplasm mainly from China (G0770, G0553, G0299, G1409, JI96, JI1491) from winter cultivars ('Frilene', 'Amac', Rafale, 'Frisson', CP). It is associated with variations in flower color, leaf type and to a lesser extent seed size. The fifth axis separated mainly garden wrinkled genotypes ('Multifreezer', JI1006, 'Finette', 'Lincoln', 'Surgevil', 'Gradus') from mainly Russian germplasm (K1666, 'Victor', K5127, K4252, K8290). The shape of the seeds, associated with sweet peas for human consumption, explained the variation on axis 5, as well as cotyledon color to a lesser extent. The year of registration, when available, was correlated with axes 3 and 5.

Discussion

A total of 121 markers of different types were used to analyze the genetic relationships among a collection of 148 *Pisum* genotypes. Forty-seven of the 121 markers were mapped and all linkage groups were represented (Table 2; Gilpin et al. 1997; Laucou et al. 1998; Weeden et al. 1998; Pilet-Nayel et al. 2002; Loridon, personal communication; Prioul, personal communication). The marker selection used was efficient in structuring the genetic variability within the collection, and the most informative classification was obtained when all markers were pooled together. The complementarity of the different marker types in structuring the genetic variability could be due to their different evolutionary features since they represented anonymous DNA (RAPDs, ISSRs), expressed DNA (SSRs, ESTs) and proteins (isozymes, storage proteins). They also detected different kinds of polymorphisms: the number of tandem repeats for SSRs, whose evolution is known to be rather rapid (Innan et al. 1997), and for storage proteins, point mutations or indels for RAPDs, isozymes, STSs, and ISSRs.

Our collection included few representatives of the different former *Pisum* subsp. *(humile, elatius, abyssinicum, fulvum*) and probably under-represented the genetic diversity from other regions of the world, but encompassed the available variability of past and present cultivated peas in Western Europe. The 148 accessions studied clustered into eight main groups, most of which corresponded to a main cultivated type (spring-sown or winter-sown), to a main end-use type (feed, food or fodder) or to a main geographical origin. Fodder types gathered in two distant groups (group I and II). *Pisum* subsp. *(humile, elatius, abyssinicum, fulvum*) and some primitive forms from Ethiopia, Afghanistan, and Sudan gathered together, apart from the cultivated accessions. The recent spring feed cultivars appeared in well-separated groups (group VII and VIII). Within groups, known genealogies often supported the aggregations. This classification is in agreement with other studies: Hoey et al. (1996) using allozymes and RAPDs and Ellis et al. (1998) using *Ty1-copia* retrotransposon-derived SSAP markers have also shown a clear distinction between wild ecotypes and cultivated peas. Within wild ecotypes, in our study as well as in those of Ellis et al. (1998) and Lu et al. (1996), accessions from Caucasus formed a distinct group, and *P. humile* JI241 clustered with accessions originating from Afghanistan. Within cultivated types, several studies have shown a distinction between fodder and feed peas using either RAPDs alone (Samec and Nasinec 1995) or RAPDs and AFLPs (Simioniuc et al. 2003). Our results add an additional classification within cultivated peas according to end-uses (garden, feed) or to variety types (winter or spring-sown). Our collection was clearly resolved in several groups corresponding to the different sowing types and end-uses, and very few rare alleles were shared between cultivated groups, suggesting a divergent selection in the different gene pools. A similar situation was found in barley where two sowing types (spring and winter barleys) and two end-uses (feed or malting) structured the diversity (Liu et al. 2001). In

beans, the genetic variability among cultivars was also structured according to their food type, the most recently selected pool of very fine French beans presenting the lowest level of diversity (Métais et al. 2002).

Interestingly, the structure of the collection based on molecular data allowed us to trace back some major lines of pea breeding in the last decades. As an example, we could trace back the selections for cultivars adapted to winter sowings in the 1980s. A large investment in the development of frost resistant cultivars has been based on the use of winter hardiness and frost resistance carried by fodder cultivars such as 'Austrian Winter', 'Champagne' or 'Winterberger'. 'Austrian Winter' was used in the United States as a source genotype to develop winter hardy cultivars ('Fenn', 'Melrose') used for winter silage (Slinkard and Murray 1972; Auld et al. 1978). Some garden pea cultivars, such as 'Winkossa' (group I) and 'Frogel' (group VII) were developed from 'Austrian Winter' and 'Champagne' respectively (R. Cousin, personal communication). The clustering of 'Winkossa' with fodder peas, and of 'Frogel' with winter feed peas highlighted the choices made in garden pea breeding towards winter hardiness. Finally, frost-resistant feed pea cultivars such as 'Frimas' and 'Frisson' (group VII) were derived from the cross 'Champagne'×'Vitalis', and have been subsequently largely used in the French winter pea breeding programs, leading to most of the group VII breeding lines and cultivars 'Kazar', 'Mercure', 'Rafale', 'Amac'. The cultivar 'Kazar', coming both from 'Champagne' (via 'Frimas') and 'Austrian Winter' (via 'Vendevil') may cumulate frost resistance from its two ancestor lines. Another example is the selection for plant morphology traits; 'Solara', C661 and later 'Térèse', which clustered together in group VIII, were all attempts to introduce the *afila* mutation into 'Finale' during the 1980s. Cultivars 'Ballet' and 'Countess' (group VI) were selected from crosses including the *afila* cultivar 'Filby'.

Surprisingly, group V clustered Spanish local varieties together with Chinese local varieties. All the Chinese local varieties clustered together, suggesting a limited level of genetic diversity within this gene pool. An interesting hypothesis would be that Spanish local varieties could have been transferred to the Far East through the so-called Manila Galleon marine route, from Spain to Philippines via Mexico, established from the second half of the16th century (M. Chauvet, personal communication). Spanish varieties may then have spread from the Philippines to China and other northeast Asian countries. From that point of view, the spreading of *Pisum* from Europe to Philippines and China may have been very different from the one of *Vigna* species, which seems to have taken place through tropical terrestrial routes (Santalla et al. 1998). To confirm this hypothesis, adding *Pisum* accessions from Central America and the Philippines may be useful. There is a unique accession originating from Costa Rica in our study, which does not aggregate with Spanish and Chinese accessions.

The genetic basis of European commercial feed spring pea cultivars is often said to be very narrow, due to genetic erosion subsequent to the industrialization of feed pea production for animal feeding (Lewis and Matthews 1984). Our results confirmed this statement. The main spring-sown varieties (the cultivars 'Athos', 'Baccara' and 'Badminton' covered 75% of the feed spring pea cultivated surfaces in France in the year 2000) all clustered together and displayed low genetic distances from each other. Accessions from the feed pea gene pools displayed a low number of rare alleles and were poorly represented in the subset of 43 genotypes. This confirms the high genetic redundancy in recent feed pea gene pools and suggests a loss of genetic diversity during recent spring feed pea selection. PCA of molecular data differentiated the most recent spring-sown feed pea cultivars from primitive and wild *Pisum* accessions and stressed the intense selection for *afila* foliage, white flowers, and seed size in recent feed pea cultivars. Seed size in the pea, as in many Mediterranean pulses, has responded to selection pressure (Smartt 1984); the largest seeds are found in the most highly selected cultivars. The correlation between molecular and morphological diversity indicated a linkage disequilibrium between molecular markers and the genes controlling these morphological traits (Burstin and Charcosset 1997), probably resulting from the very short history of feed pea breeding. The winter-sown feed pea genetic pools look somewhat different, with a gene pool mainly including French cultivars or breeding lines (group VII), close to spring feed pea cultivars (group VIII) and another gene pool (group VI subgroup II) including the main cultivated winter-sown varieties, 'Cheyenne' and 'Victor'. These varieties covered 63% of the winter feed pea cultivated surfaces in France in 2000. Winter feed pea breeding could probably gain from exchanges between these two gene pools, and from the further introduction of traits from fodder types where frost resistance (Lejeune-Hénaut et al. 1999) and resistance to *Mycosphaerella pinodes* are available (Prioul et al. 2003).

In the present study, molecular data were useful to gain an insight into the history of selection of recent feed pea cultivars. Our results showed that the intensive selection for yield in feed combining peas has lead to a decrease in the genetic diversity among cultivated varieties. However, a significant level of diversity was maintained within the *Pisum sativum* gene pool thanks to the diversity of end-uses and of breeding regions.

Acknowledgements The authors thank M. Ambrose (John Innes Centre, Norwich), S. Anguelova (International Programme for Genetic Resources, Plovdiv), A. Burghoffer (Institut National pour la Recherche Agronomique, Versailles), C. Coyne (United States Department of Agriculture, Pullman), A. Ramos Monreal (University of Valladolid), M. Vishnyakova (NI Vavilov Research Institute of Plant Industry, St Petersburg), and X. Zong (Chinese Academy of Agricultural Sciences, Beijing) for providing accessions with related information. They also thank M. Duparque, P. Marget and G. Morin for the description of the accessions, T. Bataillon for his help with the MSTRAT analysis, P. Dubreuil, M. Manzanares, M.L. Pilet-Nayel, R. Thompson and M. Trottet for helpful comments on the manuscript. Finally, we are very grateful to R. Cousin for spending time in tracing back the history of pea breeding

with us, and to M. Chauvet for sharing his knowledge on the history of pea and its uses.

References

Arnau G, Lallemand J, Bourgoin M (2002) Fast and reliable strawberry cultivar identification using inter simple sequence repeat (ISSR) amplification. Euphytica 129:69–79

Auld DL, Murray GA, O'Keeffe LE, Campbell AR, Markarian D (1978) Registration of Melrose field pea. Crop Sci 18:913

Bourgoin-Grenèche M, Lallemand J (1993) Electrophoresis and its application to the description of varieties: a presentation of the techniques used by GEVES. GEVES Editions, La Miniere

Blixt S (1972) Mutations genetics in *Pisum*. Agric Hortic Genet 30:1–293

Burstin J, Charcosset A (1997) Relationship between phenotypic and marker distances: theoretical and experimental investigations. Heredity 79:477–483

Burstin J, Deniot G, Potier J, Weinachter C, Aubert G, Baranger A (2001) Microsatellite polymorphism in *Pisum sativum*. Plant Breed 120:311–317

Christiansen MJ, Andersen SB, Ortiz R (2002) Diversity changes in an intensively bred wheat germplasm during the 20th century. Mol Breed 9:1–11

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA micropreparation: version II. Plant Mol Biol Rep 1:19–21

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dubreuil P, Charcosset A (1999) Relationships among maize inbred lines and populations from European and North American origins as estimated using RFLP markers. Theor Appl Genet 99:473–480

Dubreuil P, Dillmann C, Warburton M, Crossa J, Franco J, Baril C (2003) User's manual for the LCDMV software (calculation of molecular distances between varieties) for fingerprinting and genetic diversity studies http://www.cimmyt.org/abc/manual/controls.htm

Ellis THN, Poyser SJ, Knox MR, Vershinin AV, Ambrose MJ (1998) Polymorphism of insertion sites of *Ty1-copia* class retrotransposons and its use for linkage and diversity analysis in pea. Mol Gen Genet 260:9–19

Gilpin BJ, McCallum JA, Frew TJ, Timmerman-Vaughan GM (1997) A linkage map of the pea (*Pisum sativum* L.) genome containing cloned sequences of known function and expressed sequence tags (ESTs). Theor Appl Genet 95:1289–1299

Gouesnard B, Bataillon TM, Decoux G, Rozale C, Schoen DJ, David JL (2001) MSTRAT: an algorithm for building germplasm core collections by maximizing allelic or phenotypic richness. J Hered 9:93–94

Hodgkin T (1995) Some current issues in the conservation and use of plant genetic resources. In: Ayad WG, Hodgkin T, Jaradat A, Rao VR (eds) Molecular genetic techniques for plant genetic resources. Report of an IPGRI workshop, Rome, 9–11 October 1995

Hoey BK, Crowe KR, Jones VM, Polans MO (1996) A phylogenetic analysis of *Pisum* based on morphological characters, allozyme and RAPD markers. Theor Appl Genet 92:92–100

Innan I, Terauchi R, Miyashita NT (1997) Microsatellite polymorphism in natural populations of the wild plant *Arabidopsis thaliana*. Genetics 146:1441–1452

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaudoise Sci Nat 44:223–270

Koebner RMD, Donini P, Reeves JC, Cooke RJ, Law JR (2003) Temporal flux in the morphological and molecular diversity of UK barley. Theor Appl Genet 106:550–558

Lallemand J, Arnau G (2000) Marqueurs moléculaires (ISSR) pour l'identification des variétés de pois et la caractérisation des ressources génétiques: comparaison avec l'approche biochimique. In: Deuxièmes journées méthodologiques du GEVES, 24 November 2000, GEVES Editions, La Miniere, pp 21–22

Laucou V, Haurogné K, Ellis N, Rameau C (1998) Genetic mapping in pea. I. RAPD-based genetic linkage map of *Pisum sativum*. Theor Appl Genet 97:905–915

Lejeune-Hénaut I, Bourion V, Etévé G, Cunot E, Delhaye K, Desmyter C (1999) Floral initiation in field-grown forage peas is delayed to a greater extent by short photoperiods than in other types of European varieties. Euphytica 109:201–211

Lewis BG, Matthews P (1984) The world germplasm of *Pisum sativum*: could it be used more effectively to produce healthy crops ? In: Hebblethwaite PD, Heath MC, Dawkins TCK (eds) The pea crop, a basis for crop improvement. Butterworths, London, pp 215–229

Liu F, Sun GL, Salomon B, von Bothmer R (2001) Distribution of allozymic alleles and genetic diversity in the American Barley Core Collection. Theor Appl Genet 102:606–615

Lu H, Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor Appl Genet 103:613–617

Lu J, Knox MR, Ambrose MJ, Brown JKM, Ellis THN (1996) Comparative analysis of genetic diversity in pea assessed by RFLP and PCR based methods. Theor Appl Genet 93:1103–1111

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:311–335

Métais I, Hamon B, Jalouzot R, Peltier D (2002) Structure and level of genetic diversity in various bean types evidenced with microsatellite markers isolated from a genomic enriched library. Theor Appl Genet 104:1346–1352

Narvel JM, Walter RF, Chu WC, Grant D, Shoemaker RC (2000) Simple sequence repeat diversity among soybean plant introductions and elite genotypes. Crop Sci 40:1452–1458

Pilet-Nayel ML, Muehlbauer FJ, McGee RJ, Kraft JM, Baranger A, Coyne CJ (2002) Quantitative trait loci for partial resistance to *Aphanomyces* root rot in pea. Theor Appl Genet 106:28–39

Posvec Z, Griga M (2000) Utilisation of isozyme polymorphism for cultivar identification of 45 commercial peas (*Pisum sativum* L.). Euphytica 113:251–258

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK (2000) The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet 100:584–592

Prioul S, Onfroy C, Tivoli B, Baranger A (2002) Controlled environment assessment of partial resistance to *Mycosphaerella pinodes* in pea (*Pisum sativum* L.) seedlings. Euphytica 131:121–130

Rogers JS (1972) Measures of similarities and genetic distances. In: Studies in genetics VII. University of Texas Publication 7213:145–153

Rongwen J, Akkaya MS, Bhagwat AA, Lavi U, Cregan PB (1995) The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90:43–48

Russell JR, Fuller JD, Young G, Thomas B, Taramino G, Macaulay M, Waugh R, Powell W (1997) Discriminating between barley genotypes using microsatellite markers. Genome 40:442–450

Russell JR, Ellis RP, Thomas WTB, Waugh R, Provan J, Booth A, Fuller J, Lawrence P, Young G, Powell W (2000) A retrospective analysis of spring barley germplasm development from 'foundation genotypes' to currently successful cultivars. Mol Breed 6:553–568

Samec P, Nasinec V (1995) Detection of DNA polymorphism among pea cultivars using RAPD technique. Biol Planta 37:321–327

Santalla M, Power JB, Davey MR (1998) Genetic diversity in mung bean revealed by RAPD markers. Plant Breed 117:473-478

SAS Institute (1988) SAS/STAT User's Guide: release 603. SAS Institute, Cary, N.C.

Simioniuc D, Uptmoor R, Friedt W, Ordon F (2002) Genetic diversity and relationships among pea cultivars revealed by RAPDs and AFLPs. Plant Breed 121:429–435

Slinkard AE, Murray GA (1972) Registration of Fenn field pea. Crop Sci 12:127

Smartt J (1984) Evolution of grain legumes. I. Mediterranean pulses. Exp Agric 20:275–296

Smartt J (1990) Pulses of the classical world. In: Grain legumes: evolution and genetic resources. Cambridge University Press, Cambridge, p 176

Soleimani VD, Baum BR, Johnson DA (2002) AFLP and pedigree-based genetic diversity estimates in modern cultivars of durum wheat [*Triticum turgidum* L. subsp. d*urum* (Desf.) Husn.]. Theor Appl Genet 104:350–357

Steiger DL, Nagai C, Moore PH, Morden CW, Osgood RV, Ming R (2002) AFLP analysis of genetic diversity within and among *Coffea arabica* cultivars. Theor Appl Genet 105:209–215

Ward JH (1963) Hierarchical grouping to optimize an objective function. Am Stat Assoc J 56:236–244

Weeden NF, Ellis THN, Timmerman-Vaughan GM, Swiecicki WK, Rozov SM, Berdnikov VA (1998) A consensus linkage map for *Pisum sativum*. Pisum Genet 30:1–4

White S, Doebley J (1998) Of genes and genomes and the origin of maize. Trends Genet 14:327–332