Microsatellite and ITS sequence variation in wild species and cultivars of pea

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Previous investigations by our laboratory (1, 2) have applied the nucleotide sequence variation found in ribosomal internal transcribed spacer (ITS) regions to the systematic analysis of closely related pea taxa, in part due to the high rate of evolutionary change characterizing these DNA regions (3). No attempt was made to infer evolutionary relationships among the 65 taxa, however, given the relatively few informative sites available to the analysis. Despite a paucity of data, several observations could be made respecting the overall level of genetic variation found across the genus and the topological relationships established among the selected groups of taxa. These include: 1) very close genetic affinities throughout *Pisum*, with *P. fulvum* exhibiting the greatest degree of genetic divergence based upon the data examined; 2) support for *P. fulvum,* northern *P. humile* and southern *P. humile* as distinct taxonomic categories; 3) the independent evolution of a pea chromosomal translocation; 4) the assignment of JI1794 as a "northern" *P. humile*; and 5) inconsistent taxonomic assignments for JI241, JI198, JI1398, JI1096 and JI2055. The data were unable to resolve the very close systematic relationships among *P. elatius, P. abyssinicum* and *P. sativum*.

More recently, our laboratory has developed and/or characterized a series of pea microsatellite loci (4, 5) to serve as informative neutral molecular markers for a number of project applications. In previous studies involving pea, microsatellite-based molecular markers have been used to determine inter-genera transferability of primers (6), to analyze diversity within the genus *Pisum* (7) and to estimate the level of microsatellite polymorphism in *Pisum sativum* L. (8). RAMS (Randomly Amplified MicroSatellites), a novel molecular marker technique (7), applies lower PCR annealing temperatures to microsatellite-specific primers and provides an efficient means to generate greater numbers of less-specific multi-banded DNA profiles.

Unfortunately, in the case of a *P. sativum* ssp. Syriacum accession from the original collection, too few reliable RAMS bands were obtained to include it in the current data set. The goal of the present study, therefore, is to compare and combine RAMS microsatellite variation with nuclear ribosomal DNA variation as a systematic organizing tool for 64 wild and cultivated pea taxa, both to re-examine our previous findings and to explore the results of combining both data sets in a common analysis.

Materials and Methods

Pisum isolates 701-723 are from the Ben Ze'ev and Zohary (1973) collection (courtesy of J.G. Waines), JI accessions are from the John Innes collection (courtesy of M.J. Ambrose), cv. Alaska is from J. Mollema and Son, Inc. (Grand Rapids, MI), cv. (Morse's) Progress #9 is from Ferry-Morse Seeds (Mountain View, CA) and accessions 82-14n, A1078-234 and PI 179449 were kindly provided to this project by G. Marx and N. Weeden.

DNAs from 64 pea accessions representing the range of the genus *Pisum* are amplified with 11 of the 31 primer sets described previously (4, 5) and then separated on polyacrylamide gels to evaluate detectable differences using the RAMS method (see 4). Clearly discernable polymorphic and monomorphic bands between 90-300 bp in size are scored as "present", "absent" or "missing data" for each accession. Sequence data are analyzed using the PAUP computer package (9).

Results and Discussion

Seventy-eight scoreable DNA bands are produced among the 64 representative pea accessions using primer sets 3, 5, 14, 16, 17, 18, 20, 22, 23, 25 and 31 (see 4, 5 and Table 1), resulting in 4992 total data points. Of the 78 RAMS bands generated, 46 are polymorphic and 44 are informative across all of the accessions. A compilation of the 44 informative bands is delineated for all 64 pea taxa in Table 1. As noted previously (1), the table is organized in accordance with the two commonly recognized species of pea (10, 11, 12), the more divergent *P. fulvum* (also see 13) and the typically cultivated *P. sativum*. The former is represented by eight different accessions, while the latter is further differentiated in the table as four subspecies: *humile, elatius, abyssinicum* and *sativum*. Subspecies *humile* is subdivided by northern and southern populations (10). There are six accessions characterized as questionable taxonomic assignments solely based on the RAMS data, as contrasted with five inconsistent assignments (three shared in common) based on the nuclear ribosomal DNA variation reported previously (1).

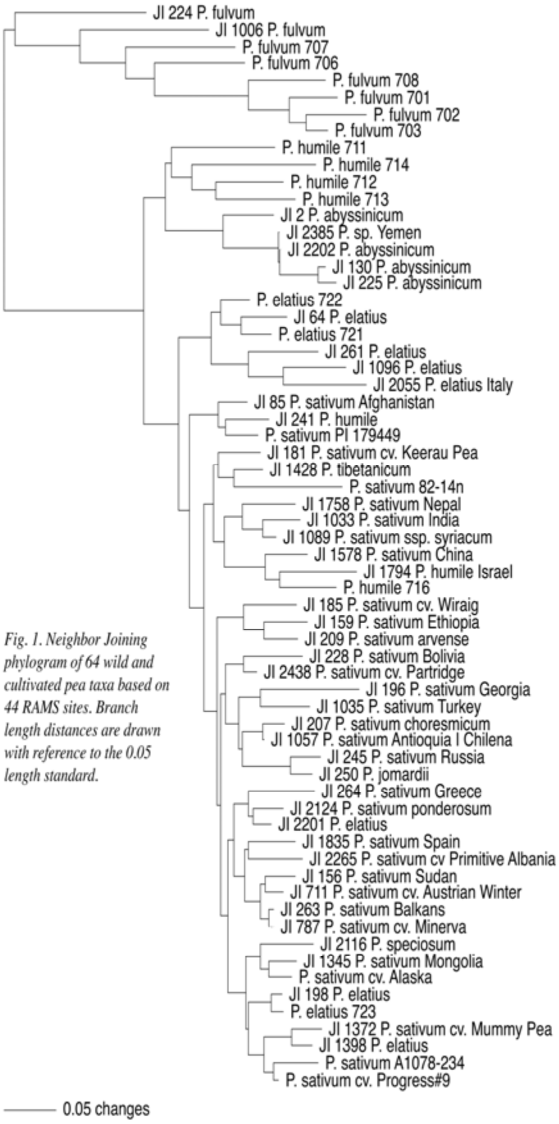

Fig. 1. Neighbor Joining phylogram of 64 wild and cultivated pea taxa based on 44 RAMS sites. Branch length distances are drawn with reference to the 0.05 length standard.

A Neighbor Joining (NJ) distance analysis of the 44 RAMS data points is presented as a phylogram in Figure 1 to provide a basic illustration of the information presented in Table 1. In the figure, *P. fulvum,* northern and southern *P. humile*, *P. abyssinicum* and a half-dozen *P. elatius* accessions maintain distinct group associations; although, the *P. abyssinicum* group includes JI2385, formerly designated as *P. sativum* (1). Two *P. elatius* accessions (JI 1096 and JI 2055) that displayed the largest number of ITS sequence differences with *P. fulvum* in the ribosomal DNA study now group as part of the six *P. elatius* accessions. Four other *P. elatius*, a single putative *P. humile* and the paired northern *P. humile* all intersperse with *P. sativum* in the figure. A second NJ phylogram (Figure 2) combines the 44 RAMS data points with the 21 informative ITS data points from the earlier study (1). The relationships within and among *P. fulvum*, southern *P. humile*, *P. abyssinicum* and the six *P. elatius* remain essentially the same as depicted in Figure 1, and the four other *P. elatius* and one *P. humile* remain dispersed within an otherwise single block of *P. sativum.* With the combination of data sets, however, the pair of northern *P. humile* accessions is associated more closely with the *P. elatius* group and is not interspersed with *P. sativum.*

Both the microsatellite and combined data sets presented in Figures 1 and 2, respectively, support the designation of *P. fulvum* as a distinct taxon; although, the relatively small number of available data points renders any conclusions from this study tentative. Additional support for *P. fulvum* as a distinct species, however, is presented elsewhere (1, 10, 11, 12, 13). With respect to the remaining taxa, southern *P. humile* is least closely associated with *P. sativum* and remains separated from northern *P. humile.* The *P. abyssinicum* group is most closely associated with southern *P. humile* and portions of a dispersed *P. elatius* group. These relationships are not inconsistent with the proposed placement of *P. abyssinicum* between *P. elatius* and *P. sativum* (14) given the dispersed nature of the *P. elatius* subspecies. Northern *P. humile* has been postulated the closest wild progenitor of the cultivated pea based in part on a shared chromosomal translocation (10) and detailed chloroplast studies (15). The current study lends limited support to this assertion, which was not supported by the ITS data alone (1).

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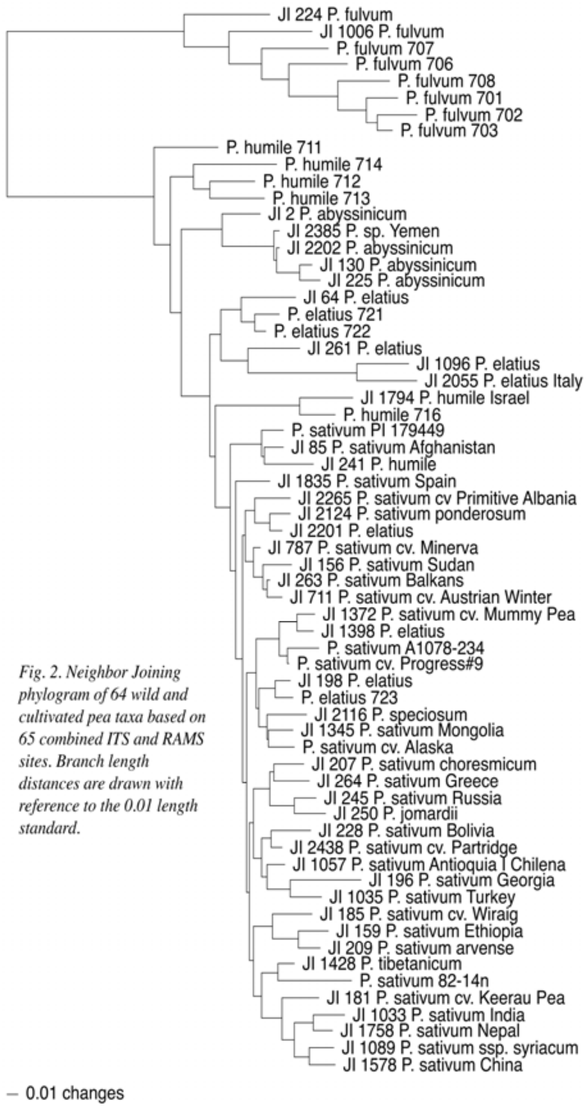

Fig. 2. Neighbor Joining phylogram of 64 wild and cultivated pea taxa based on 65 combined ITS and RAMS sites. Branch length distances are drawn with reference to the 0.01 length standard.

1. Polans, N.O. and Saar, D.E. 2002. *Pisum* Genetics 34:9-14.
2. Saar, D.E. and Polans, N.O. 2000. *Pisum* Genetics 32:42-45.
3. Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S. and Donoghue, M.J. 1995. Annals Missouri Bot. Gard. 82:247-277.
4. Moreno, R.R. and Polans, N.O. 2006. *Pisum* Genetics 38:10-14.
5. Moreno, R.R. and Polans, N.O. 2007. *Pisum* Genetics 39:10-12.
6. Pandian, A., Ford, R. and Taylor, P.W.J. 2000. Plant Mol Bio. Rep. 18:395a-395h.
7. Ford, R., Le Roux, K., Itman, C., Brouwer J.B. and Taylor, P.W.J. 2002. Euphytica 124: 397-405.
8. Burstin, J., Deniot, G., Potier, J.,Weinachter, C., Aubert, G. and Baranger, A. 2001. Plant Breeding 120**:** 311-317.
9. Swofford, D.L. 1998. PAUP, Version 4.0b4a. Sinauer Associates, Sunderland, Massachusetts.
10. Ben Ze'ev, N. and Zohary, D. 1973. Israel J. Bot. 22:73-91.
11. Hoey, B.K., Crowe, K.R., Jones, V.M. and Polans, N.O. 1996. Theor. Appl. Genet. 92:92-100.
12. Marx, G.A. 1977. *In:* Physiology of the Garden Pea. Eds. Sutcliffe, J.F. and Pate, J.S., Academic Press, New York, pp. 21-43.
13. Jing, R., Johnson, R., Seres, A., Kiss, G., Ambrose, M.J., Ellis, T.H.N. and Flavell, A.J. 2007. Genetics 177:2263-2275.
14. Weeden, N.F. and Wolko, B. 2001. *Pisum* Genetics 33:21-25.
15. Palmer, J.D., Jorgensen, R.A. and Thompson, W.F. 1985. Genetics 109:195-213.

Table 1. Variable RAMS sites for wild and cultivated taxa of pea.

	RAMS BANDS*
	1111111111111111111122222222222233333
	3333555554466667888888888888800222233333511111
	ABCDABCDEBEABCDBBCDFGHIJKLMNAEACDFBDIJAABDEF
Pisum fulvum	
701	11001?110100?1101000001100000000011000110110
702	110?1101000001101000001100000000011000?10101
703	10011?110000111?100000110000000001100001 01??
706	????11?1?0011111100011111000000001????01110?
707	1100111100001110111001110001000111 0??0011101
708	1?0011110000111010100011001000000000?0010100
JI224	11101111?011010?1000001100100001110110011111
JI1006	10101??10001101?1010001100110000001101011100
Pisum sativum	
ssp. humile (northern)	
716	000?0?10101110?1110100100111001111001111 10??
JI1794	000001101111101111110110001100111101111101?
ssp. humile (southern)	
711	01011110101110 1?1100001101110000011011011010
712	0101111010110001110000101110010111 1?11111010
713	0101111010100001110100000111010111101101 1010
714	01011100111010111000010011110?01111011011010
ssp. elatius	
721	010?111010111011101101000111000111 1?11111010
722	010?1110101110111011011011110001111111111010
JI64	0100111010111011101100000111100101111111110?0
JI261	000111101011101110 1?0100111100011111100110??
JI1096	00011?101111101100110 1?001110011110111 0?????
JI2055	00111110111110111011010011110010010?11011010
ssp. abyssinicum	
JI2	01001110101110011?001010011111001011110110?0
JI130	01011110101110111?0010000110010010111101 1000
JI225	01011110101110011000 10?0011001001011110 11000
JI2202	01011110101110111?00101001100101101111 0110?0
ssp. sativum	
82-14n	010?1110111110?01101001011110011110111?01111
A1078-234	0010111011111001110101100111100111011111 1011
PI179449	01101110101110?1100100101111001111011111 1010
Alaska	01101110101110111111011001111001 11?111101011
Progress#9	0?10111011111011 1?01011001111001111111111011
JI85 Afghanistan	0100111010111011111010010111100111111111110?0
JI156 Sudan	010011101011101111010010111110001 1?111111011
JI159 Ethiopia	00101010101110?1100101100111000011?111111011
JI181 Keerau Pea	000011101011101111010010111100011101110 11011
JI185 Wiraig	????11101011101110010010011100001101 1?101011
JI196 Georgia	00101110101110110001011001111000111110001010
JI207 choresmicum	01101110101110111101001001111101111111001011
JI209 arvense	01101010101110 1?10010110011110011101111 1011
JI228 Bolivia	00101110101110011001001001110001111111011011
JI245 Russia	01101110101110111 1?10110111111011 1?111001001
JI250 (P. jomardii)	01001110101110111101011011110?0111111100100?
JI263 Balkans	011?111010111011110100101111100111111111 11011
JI264 Greece	00101110101110101101001011111101111111101011
JI711 Austrian Winter	01101110101110111101001011111001110111111011
JI787 Minerva	01101110101110111101001011111001111111 1?1011
JI1033 India	001?1110111110111101001011110000110111011 0?0
JI1035 Turkey	00101110101110111101001001110 10?1111110010?0
JI1057 Antioquia I Chilena	01101110101110111101001001111001111111001011
JI1089 Syriacum	00101110101110111101001001110000110111011010
JI1345 Mongolia	01101110101110111101111001111001110111 1?1011
JI1372 Mummy Pea	01001110111110111101111001111001111111111011
JI1428(P. tibetanicum)	0000111011111 01?1101001001111 00?11?111001011
JI1578 China	0000??10111110111101011001110000110111?11010
JI1758 Nepal	00101110111110111101001011111010111111011010
JI1835 Spain	010011101011101 1?1010010011110111111111111011
JI2116(P. speciosum)	01101010101110111101011001111011111111101011
JI2124 ponderosum	00101110101110111101011011110001111111101011
JI2265 Primitive Albanian	0010111010111011 1?11001011111011111 1?1111011
JI2438 Partridge	0010111010111011 11?100100111100111111 1011011
Inconsistent assignments:	
JI241 (1)	01?01110101110111011001011110011111111011010
723 (2)	00101110101110111101011001111001111111111010
JI198 (2)	01101110101110111101011001111001111111111010
JI1398 (2)	01?0111011111010110 1?110011110?1111111011011
JI2201 (2)	00101110101110111101001011110001111111111011
JI2385(P. sp. Yemen) (3)	01011110101110111?00101001100101101111 0110?0

*1=present, 0=absent, ?=missing data.

(1) JI241 is listed as ssp. humile, but it displays ssp. Sativum characteristics.

(2) 723, JI198, JI1398 and JI2201 are listed as ssp. elatius, but they display ssp. sativum characteristics.

(3) JI2385 is listed as ssp. sativum, but it displays ssp. Abyssinicum characteristics.