Mitochondrial DNA variation in North American melanopline grasshoppers

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Restriction fragment analysis of mitochondrial DNA (mtDNA) was used to examine genetic variation within and among 11 species of grasshoppers in the subfamily Melanoplinae. Total DNA of 236 individuals was digested with 10 restriction enzymes and probed with three cloned *EcoRI* fragments representing the entire mitochondrial genome of *Melanoplus sanguinipes*. The average size of mtDNA for these species was about 16.1 kilobases. Average nucleotide diversities within species ranged from 0.00 to 0.31 per cent. Values are considered low and possibly reflect chronically small population sizes in pre-agricultural times as well as bottlenecks engendered by various glacial episodes. Species regarded as major pests appear to have larger diversities. For each species, common genotypes occur throughout the sampled region suggesting very little spatial differentiation although in a couple of melanoplins, one or two haplotypes are geographically restricted. Comparisons among species led to a hypothesis concerning their phylogenetic relationships. Not all affinities accorded with expectations based on morphological comparisons. The estimated time of divergence for the genus *Melanoplus* is at least 4 million years.

Keywords: grasshoppers, *Melanoplus*, mitochondrial DNA, population structure, phylogeny.

Introduction

Contemporary orthopterists recognize about five subfamilies of North American Acrididae (Otte, 1981; Vickery & Kevan, 1986). The largest one, Melanoplinae, is dominated by the genus *Melanoplus*, comprising over 80 species (Helfer, 1987). These include species of economic importance, such as the migratory grasshopper *M. sanguinipes* and, up to the turn of the century, the notorious Rocky Mountain grasshopper *M. spretus* (Lockwood, 1989), now extinct. In spite of their obvious agricultural impact and dominance as grazing herbivores in prairie communities (Otte, 1981), very little is known about the systematic relationships among the various species in this subfamily. Availability of phylogenetic information would not only satiate the curiosity of acridologists but could, for example, provide an evolutionary perspective for comprehending factors which underlie pest status.

Melanoplins differ in their distribution ranges, habitats, food preferences and bionomics (Brooks, 1958; Vickery & Kevan, 1986), egg and egg pod characteristics (Onsager & Mulkern, 1963), nymphal

external features (Handford, 1946), and isozyme patterns (Chapco, 1989). Species are generally identified by genital characteristics of the male. By contrast, females of some species are difficult to distinguish, at least those which, according to Helfer (1987), are deemed closely related. Attempts have been made, based on morphology, to erect several 'species-groups' or 'series' (Scudder, 1898; Gurney, 1977) but no indication is given as to how the species-groups are interconnected among themselves. It would seem that Brooks' (1958, p. 19) statement regarding *Melanoplus* that 'at present the exact relationship of the many species (of *Melanoplus*) is unknown' is still true today. Splitting the genus has been proposed on the basis of its heterogeneity (Vickery, 1987) and some unpublished cytological results (Vickery & Scudder, 1987) but the exact nature of this division should await a more complete genetic analysis, preferably one based on a variety of independent traits.

One trait that has been exploited extensively for addressing both population genetics and systematics issues is based on mitochondrial DNA (mtDNA) investigations (Wilson *et al.*, 1985; Avise *et al.*, 1987). Advantages and properties of the macromolecule rendering it suitable for such research are well known and have been reviewed elsewhere (Harrison, 1989). In this paper, an analysis of fragment patterns generated

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by restriction enzyme digestion of mtDNA obtained from several melanopline species is presented. The objective is twofold. The first is to compare and contrast levels of variation and population structures among species, some of which differ in their status as pests, and the second aim is to establish a phylogeny of these species whereby connections among members can be compared with those suggested by the taxonomic literature.

Materials and methods

Relevant data for *M. sanguinipes* reported previously (Chapco *et al.*, 1992) have been extracted and are summarized and included here for comparative purposes. Eight other species of the genus *Melanoplus* were assayed for their mtDNA fragment patterns: *M. angustipennis*, *M. bivittatus*, *M. confusus*, *M. dawsoni*, *M. femurrubrum*, *M. gladstoni*, *M. infantilis* and *M. packardii*. Two other melanoplins, *Hesperotettix viridis* and *Phoetaliotes nebrascensis* were included and their status as potential outgroup species was explored. A total of 236 individuals was collected during 1989 and 1990 from 19 locations in Saskatchewan and the following places outside the province: Mauston, Wisconsin; Alexandria, Minnesota; Turtle Lake, North Dakota; Santa Fe, New Mexico and Tobermory, Ontario (Appendix A).

Methods of mtDNA isolation, restriction endonuclease digestion of total DNA, gel electrophoresis, Southern transfer, radiolabelling of probes and hybridization are described in Chapco *et al.* (1992). Three *EcoRI* fragments of 8.0, 6.8 and 1.2 kb cloned into pUC18 and representing the entire mitochondrial genome of *M. sanguinipes* were used collectively to probe mtDNA of each species.

Restriction enzyme profiles were designated as a sequence (haplotype) of 10 uppercase letters AAAAAAAAAA, BAAAAAAAAA, etc. where each letter refers to a particular 'morph' or fragment pattern produced by each of the restriction enzymes: *EcoRI*, *BglII*, *PstI*, *AccI*, *PvuII*, *XbaI*, *HinfI*, *HpaI*, *AluI* and *DraI*, respectively. Nucleotide diversities (δ) between pairs of haplotypes were estimated according to equations 5.52 to 5.55 in Nei (1987). In addition, average nucleotide diversities (π) within species and between species (corrected for within-species variation) were calculated; these indices take into account haplotype frequencies (Nei & Li, 1979). To discern patterns of relationship among taxa, the matrix of inter-specific π values was analysed by Saitou and Nei's (1987) neighbour-joining (NJ) method. Haplotypes were also converted into strings of 1s and 0s for

cladistic analysis using the PHYLIP programs, MIX and CONSENSE, provided by J. Felsenstein (1988).

Following Nei (1982), the extent of inter-site variation for each species was estimated for Saskatchewan populations using the statistic γ_{ST} ; its significance was evaluated by a comparison with a distribution of γ_{ST} values generated by 1000 random shufflings of haplotypes among locations (see Palumbi & Wilson, 1990).

Results

Fragment patterns and sizes are set out for each enzyme in Appendix B. Restriction enzymes *AccI*, *AluI*, *DraI* and *HinfI* generated the greatest number of electrophoretic bands. It is likely that this group of enzymes produced an even greater number of (undetected) smaller fragments, given the disparity among fragment size totals. For the remaining endonucleases, average totals of fragment sizes ranged from 15.9 in *H. viridis* to 16.2 kb in *P. nebrascensis*, values in close agreement with the sum of sizes for the cloned *EcoRI* pieces. Two (*EcoRI*, *XbaI* and *HpaI*) to eight (*HinfI*) variants were detected per enzyme. The majority of patterns was shared by two or more species. For example, variant A of *EcoRI* was found in all species except *M. confusus* and *M. gladstoni*. Each of seven enzymes exhibited polymorphism for fragment patterns in at least one of five species. All species except *M. packardii* displayed pattern A of *XbaI*. *H. viridis* possessed the largest number of unique patterns.

Altogether, 30 different composite genotypes were revealed among the 11 species (Appendix A). All haplotypes were species-specific except for haplotype 4, detected in one *M. sanguinipes* and nine *M. dawsoni* individuals.

Table 1 lists two measures of within-species diversity: number of haplotypes and average diversity (π) values. *M. sanguinipes*, *M. femurrubrum*, *M. bivittatus*, *M. packardii* and *M. dawsoni* were polymorphic for 12, 5, 3, 3 and 2 haplotypes, respectively, with certain haplotypes predominating (Appendix A). Over 90 per cent of *M. bivittatus* were haplotype 12; two haplotypes, 15 and 16, were equally represented in *M. packardii*; haplotypes 18 and 19 comprised over 70 per cent of *M. femurrubrum* and *M. dawsoni* was mostly genotype 4. Over 50 per cent of *M. sanguinipes* were either haplotype 1 or 5 (Chapco *et al.*, 1992). Only one haplotype was revealed for each of the remaining species. Divergences (δ — not recorded) between pairs of haplotypes ranged from 0.18 per cent (for several pairs of mtDNA morphs in *M. sanguinipes*) to 1.40 per cent representing the most disparate pair, haplotypes 12 and 14 in *M. bivittatus*. Average percentage nucleotide diversities (π) in polymorphic species

ranged from 0.06 (*M. dawsoni*) to 0.31 (*M. femurrubrum*).

The last column on Table 1 records the extent of intraspecific variation (γ_{ST}) that is attributable to among-site variation. The amount of differentiation for *M. sanguinipes* is significantly greater than that expected by random shuffling. Values for other species are not significant with that of *M. packardii* bordering on significance. Outside the major study area, limited sampling revealed haplotypes which also occur in Saskatchewan. For example, one *M. bivittatus* and two *M. infantilis* individuals collected at Turtle Lake, ND, possessed haplotypes 12 and 22, respectively. One exception is provided by a single *M. femurrubrum* individual collected in Alexandria, MN, which possessed a unique haplotype (30).

Interspecific nucleotide diversities (π), corrected for within species variation, are recorded in Table 2. Within the genus *Melanoplus*, values ranged from 0.29 per cent (between *M. dawsoni* and *M. sanguinipes*) to 4.38 per cent (between *M. packardii* and *M. confusus*). On average, the most distant species was *H. viridis* with a maximum value of 7.96 per cent separating it from *P. nebrascensis*. Interestingly, the latter species, although outside the *Melanoplus* genus, was more similar to some members (e.g. *M. bivittatus*) than some *Melanoplus* species were to each other (e.g. *M. packardii* and *M. confusus*).

An overall interpretation of these distances is provided by the NJ network depicted in Fig. 1. Connections among taxa agree reasonably well with those suggested by a majority-rule consensus tree (Fig.

Table 1 Mitochondrial DNA variation and interpopulational diversities for 11 species of melanoplines

Species	Sample size ^a	Number of populations ^a	Number of haplotypes ^a	Percentage diversity (π)	Percentage interpopulational diversity (γ_{ST})
<i>M. sanguinipes</i>	75 (89)	14 (19)	12	0.26	27.4 (0.003) ^b
<i>M. bivittatus</i>	53 (54)	12	3	0.12	<0.0 (0.75)
<i>M. packardii</i>	30	9	3	0.20	27.5 (0.05)
<i>M. femurrubrum</i>	11 (12)	4 (5)	4 (5)	0.31	53.1 (0.08)
<i>M. dawsoni</i>	10	4	2	0.06	—
<i>M. angustipennis</i>	4	1	1	0.00	—
<i>M. confusus</i>	4 (12)	2	1	0.00	—
<i>M. gladstoni</i>	5	1	1	0.00	—
<i>M. infantilis</i>	10 (12)	3 (4)	1	0.00	—
<i>P. nebrascensis</i>	4	1	1	0.00	—
<i>H. viridis</i>	4	1	1	0.00	—

^aValues are for Saskatchewan populations. Values in parentheses in columns 2–4 are for all populations. ^bProportion of 1000 simulated values which exceed the observed value.

Table 2 Percentage nucleotide diversity (π) within species (diagonal) and between species (corrected for within species variation)

Species	1	2	3	4	5	6	7	8	9	10	11
1. <i>M. sanguinipes</i>	0.256										
2. <i>M. bivittatus</i>	1.163	0.123									
3. <i>M. packardii</i>	3.260	3.275	0.196								
4. <i>M. femurrubrum</i>	0.910	1.895	2.472	0.312							
5. <i>M. dawsoni</i>	0.289	1.286	3.281	1.087	0.058						
6. <i>M. confusus</i>	2.814	2.105	4.380	3.702	2.849	0.000					
7. <i>M. infantilis</i>	3.061	1.755	3.426	1.664	3.185	3.926	0.000				
8. <i>M. gladstoni</i>	1.892	1.466	2.438	2.985	1.512	1.390	3.822	0.000			
9. <i>M. angustipennis</i>	0.847	1.377	3.207	1.836	0.510	2.633	3.519	0.948	0.000		
10. <i>P. nebrascensis</i>	2.888	2.264	6.294	4.708	2.979	3.854	4.751	2.880	2.608	0.000	
11. <i>H. viridis</i>	4.598	4.647	7.171	5.071	5.303	6.262	5.826	6.471	6.032	7.761	0.000

2) based on over 100 equally parsimonious trees (115 steps). The latter was constructed using the most disparate taxon, *H. viridis*, as an outgroup. Either topology illustrates: (1) a close association among *M. sanguinipes*, *M. angustipennis* and *M. dawsoni*, (2) a connection between *M. bivittatus* and *P. nebrascensis*, and (3) linkages among *M. packardii*, *M. femurrubrum* and *M. infantilis*. The latter is apart from a larger group consisting of (1), (2), *M. confusus* and *M. gladstoni*.

Discussion

All surveyed species possess mtDNA of approximately the same length, about 16.1 kb on average, well within the range of most metazoans, including insects (Chapco *et al.*, 1992). None of the banding patterns displayed by single individuals suggests that these species might be heteroplasmic for two or more size classes of mtDNA, as is the case for a number of invertebrates (Boyce *et al.*, 1989).

Intraspecific diversities ($\pi = 0.00$ to 0.31 per cent) are about the same or less than that reported previously for *M. sanguinipes* and are in the lower spectrum of values for most insects (Chapco *et al.*, 1992). Small sample sizes ($n = 4, 5$) could reasonably account for a failure to detect more than one haplotype in studies involving *M. angustipennis*, *M. gladstoni*, *H. viridis* and *P. nebrascensis*. This could also be true for *M. dawsoni* and *M. infantilis* for which numbers of assayed insects are somewhat larger ($n = 10-12$) although *M. femurrubrum* with a comparable sample size is much more diverse. Low mutation rates and purifying selection, among other factors, have been discussed in relation to low diversity (Martin & Simon, 1990). A more probable and appealing explanation, however, rests in the sensitivity of genetic variation, especially mtDNA variation, to events associated with reductions in population size (Wilson *et al.*, 1985). Such was probably the case during various glacial

episodes and prior to the transition to an agroecosystem (see Chapco *et al.*, 1992 for further discussion). Irregular fluctuations in abundance have also occurred in this century that have been attributed to climatic variation, disease, changes in the agricultural landscape and herbicide use (Riegert, 1968, 1986). Of course the relative impact of these historical events can only be speculated upon. Indeed, it is only now that theory is being developed for inferring connections between previous demographic occurrences and sequence or restriction site data (Slatkin & Hudson, 1991; Rogers & Harpending, 1992).

Fig. 1 Neighbour-joining tree based on average nucleotide divergence ($\pi \times 100$) between pairs of melanoplina species. Letters represent the first letter of the species name (Appendix A). Dotted line connecting species *v* = 4.4 per cent.

Fig. 2 Majority-rule consensus of 100 trees of equal length (115 steps), each constructed by the maximum parsimony method. Letters refer to the first letter of the species name. Subscripted letters indicate specific haplotypes. *Hesperotettix viridis* (*v*) was used to root the tree.

The first four species listed in Table 1 are frequently listed among the top six grasshopper pests in North America (Beirne, 1972; Vickery & Kevan, 1986); the greatest amount of mtDNA variation is also exhibited by these species, including *M. femurrubrum* in spite of the small sample taken. Whether there is a connection between variability and fluctuation in abundance (see Lorimer (1979) and Carson (1990) for theoretical arguments) is a hypothesis certainly worth pursuing in the case of grasshoppers. Present data, although indicative of a relationship, are unfortunately insufficient to be definitive. Larger sample sizes and more extensive samplings of the genome are required.

In the previous study involving *M. sanguinipes*, it was concluded on the basis of the numerical value of Nei's interpopulational nucleotide diversity index γ_{ST} that there was very little differentiation among sites for this species. While the statistical test indicates otherwise, an examination of Table 1 in Chapco *et al.* (1992) reveals that this results mainly from the presence of a few geographically restricted haplotypes (10 and 20 in Hudson Bay and 7 and 8 in Assiniboia and Horizon). If these data are omitted, γ_{ST} reduces to 6.49, a non-significant value ($P=0.22$). The near-significant γ_{ST} for *M. packardii* stems largely from the presence of a single haplotype in one pocket of the study area (Chamberlain); γ_{ST} drops to 9.09 ($P=0.34$) if this datum is omitted. No biological meaning should be ascribed to the negative value for *M. bivittatus*. Given the particular array of haplotype frequencies and the hypothesis of no differentiation, about 25 per cent of γ_{ST} values are expected to be 0 or less. In general, common genotypes occur throughout the sampled region, in North Dakota, MN, and in the case of *M. sanguinipes* as far as Ontario and New Mexico. The apparent geographical restriction of certain genotypes is curious, however, given the relative openness of the prairies and an absence of any obvious barriers. A more comprehensive survey of these regions is clearly required.

Apart from some species-group units erected by Scudder (1898), there have been very few published accounts suggesting evolutionary relationships among melanopline grasshoppers. It is even admitted that '...in most of these (series) ... the collocation is rather forced' (Scudder, 1898, p. 122). In an attempt to identify common factors underlying population fluctuations, Mulkern (1980) clustered various acridids according to a number of bionomic parameters, including the extent of overlap with respect to resource utilization. It is, however, uncertain whether proximity of species on the basis of these parameters stems from convergent evolutionary events or common inheritance. A phylogenetic interpretation of these clusters must, for now, be viewed as premature; indeed, the resolution of the

very question of convergence versus common descent can best be addressed when an independently derived evolutionary tree can be obtained, preferably one that is free from environmental vicissitudes. The trees emerging from the current analysis are to be viewed as first approximations of the group's phylogeny owing to the limitations and resolving power of the fragment method (Wilson *et al.*, 1985). Still, given the (apparent) absence of heteroplasmy and generally small δ values, the approach is a valid one (Nei, 1987; Dowling *et al.*, 1990) and does yield a number of interesting findings. For instance, females of *M. sanguinipes*, *M. femurrubrum*, *M. gladstoni* and *M. confusus* are to varying extents similar morphologically and difficult to distinguish (Guenther & Chapco, 1990); yet, based on mtDNA profiles, *M. sanguinipes* is most similar to *M. dawsoni*, a small, usually brachypterous insect and quite unlike the other *Melanoplus* species. The rather distinctly different mtDNA profiles of *M. packardii* and *M. bivittatus* represent another apparent conflict with morphology; these two species bear a superficial resemblance to one another (Scudder, 1898); members are among the largest organisms in the genus. Analysis of species outside the genus yielded strikingly different outcomes. mtDNA profiles of the green striped grasshopper, *H. viridis*, are unquestionably distinct from those of the other species whereas the large headed grasshopper, *P. nebrascensis*, has an affinity with certain *Melanoplus* species (e.g. *M. bivittatus*), closer than that among some *Melanoplus* species. These results suggest that caution should be exercised in inferring phylogenetic relationships solely on the basis of one type of trait. Indeed, Moritz & Hillis (1990) argue that both morphological and molecular approaches should be utilized in reconstructing phylogenies since both types of characters are subject to different evolutionary forces.

Assuming a conventionally used evolutionary rate of 1.0 per cent substitutions/base pair/lineage/million years (Wilson *et al.*, 1985), the estimated time of common ancestry of the most distant haplotypes (those of *P. nebrascensis* and *H. viridis*) is about 4 million years. The comparable figure for the genus *Melanoplus* is 2.2 million years. Although these estimates should be regarded as extremely crude, owing to errors of ascertainment associated with evolutionary rates and δ values, it seems reasonable to suppose that these taxa, or to be more precise — these haplotypes — are fairly recently evolved relative to times that have been speculated for Acrididae and the subfamily Melanoplineae (Gangwere, 1965, 1967; Hewitt, 1979; Jago, 1983).

Portions of the mitochondrial genome of these and other species are currently being sequenced to examine more precisely the populational and phylogenetic

hypotheses suggested by these restriction fragment data.

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Appendix A

Sampling locations and mtDNA haplotypes of 11 melanopline species

Species	Location	Haplotypes	N
<i>M. angustipennis</i>	Springside	(51.4, 102.8)†	31‡(4)§
<i>M. bivittatus</i>	Assiniboia	(49.6, 106.0)	12(1)
	Avonlea	(50.0, 105.0)	12(2)
	Balcarres	(50.5, 103.2)	12(1)
	Chamberlain	(50.5, 105.3)	12(5), 13(1)
	Crestwynd	(50.1, 105.1)	12(1), 13(2)
	Hanley	(51.4, 106.3)	12(1), 14(1)
	Horizon	(49.3, 105.1)	12(4)
	Kipling	(49.6, 102.4)	12(10)
	Lumsden	(50.3, 104.5)	12(6)
	Odessa	(50.2, 103.5)	12(4)
	Parry	(49.5, 104.5)	12(4)
	Rowatt	(50.2, 104.4)	12(10)
	Turtle L. (ND)	(47.3, 100.8)	12(1)
			54
<i>M. confusus</i>	Jameson	(50.2, 104.2)	21(9)
	Mauston (WI)	(43.6, 89.5)	21(3)
			12
<i>M. dawsoni</i>	Balcarres	4(2)	2
	Chamberlain	4(2), 24(1)	3
	Cypress Hills	(49.4, 109.3)	4(3)
	Hanley	4(2)	2
			10
<i>M. femurrubrum</i>	Balcarres	19(2)	2
	Crestwynd	18(1)	1
	Kipling	18(2), 19(1), 29(1)	4
	Rowatt	18(2), 23(2)	4
	Mauston (WI)	30(1)	1
			12
<i>M. gladstoni</i>	Last Mountain	25(5)	5
<i>M. infantilis</i>	Balcarres	22(3)	3
	Chamberlain	22(4)	4
	Last Mountain	22(3)	3
	Turtle L. (ND)	22(2)	2
			12

Appendix A *Continued*

Species	Location	Haplotypes	<i>N</i>
<i>M. packardii</i>	Avonlea	15(1), 16(2)	3
	Chamberlain	16(1), 17(4)	5
	Crestwynd	16(2)	2
	Hanley	15(3)	3
	Lumsden	15(4), 16(1)	5
	Odessa	15(1), 16(2)	3
	Parry	15(2), 16(1)	3
	Rowatt	15(2), 16(1)	3
	Springside	15(1), 16(2)	3
			30
<i>M. sanguinipes</i>	19 Locations	Haplotypes 1–11, 20	89
<i>Hesperotettix viridis</i>	Last Mountain	32(4)	4
<i>Phoetaliotes nebrascensis</i>	Jameson	33(4)	4

†Latitude and longitude.

‡Haplotypes: 1–11 and 20 defined in Chapco *et al.* (1992). Letters refer to pattern variants for restriction enzymes listed in the same order as in Appendix B: 4 = ABAAAADBAA, 12 = ACABBAAABAA, 13 = ACBBBABBA, 14 = ACBCBABBA, 15 = ACACBBCAAB, 16 = ACACBBDAAB, 17 = ACCCBBCAAB, 18 = ABAAAAABAB, 19 = ABAAAAAAAB, 21 = BCACBAEBBA, 22 = ACABCAFBCB, 23 = ABABAAABAB, 24 = ABAAAAGBAA, 25 = BCACBAGBAA, 29 = AAAAAAABAB, 30 = ABAABAABAB, 31 = ACAAAAAGBAA, 32 = ADBDEAAADC, 33 = ACAEDAHBAA.

§Number of individuals. *N* = sample size.

Appendix B**Restriction fragment patterns and sizes**

Enzyme	Pattern	Fragment sizes (bp)	Species†
<i>EcoRI</i>	A	8000, 6800, 1200	a, b, d, f, i, n, p, s, v
	B	8000, 3800, 3000, 1200	c, g, s
<i>BglII</i>	A	6800, 5600, 2400, 1200	f, s
	B	7800, 6800, 1200	d, f, s
	C	16300	a, b, c, g, i, n, p
	D	10500, 5000	v
<i>PstI</i>	A	16300	a, b, c, d, f, g, i, n, p, s
	B	12000, 4300	b, v
	C	8000, 7800	p
<i>AccI</i>	A	8800, 4800, 2600	a, d, f, s
	B	12000, 4200	b, f, i
	C	7500, 4300, 2600, 1300	b, c, g, p
	D	8600, 4000, 1600, 1400	v
	E	6500, 5800, 4000	n
<i>PvuII</i>	A	13400, 2600	a, d, f, s
	B	16300	b, c, f, g, p, s
	C	11000, 5000	i
	D	9000, 7500	n
	E	uncut	v
<i>XbaI</i>	A	7300, 4400, 2100, 1600, 600	a, b, c, d, f, g, i, n, s, v
	B	8500, 2600, 1900, 1800	p
<i>HinfI</i>	A	3700, 2600, 1200, 700, 600, 500, 450	b, f, s, v
	B	4000, 2300, 1200, 700, 600, 500, 450	b
	C	3700, 2600, 1400, 900, 700, 600, 450, 400	p
	D	3700, 2600, 1200, 900, 700, 600, 500, 450, 400	d, s, p
	E	2600, 1700, 1500, 1200, 700, 600, 500, 450	c
	F	3700, 2600, 1700, 1200, 600, 500, 450	i
	G	2600, 1200, 900, 700, 600, 550, 450, 400	a, d, g, s
	H	2800, 1200, 950, 750	n
<i>MpaI</i>	A	13300, 1700, 1000	f, p, s, v
	B	15000, 1000	a, b, c, d, f, g, i, n, s
<i>DraI</i>	A	1750, 1600, 1250, 1000, 850, 700, 575, 400	a, b, d, f, g, n, p, s
	B	3900, 2300, 1600, 1250, 1150, 600, 550, 400	c
	C	3700, 1750, 1000, 850, 575, 400	i
	D	2100, 1100, 850, 700, 650, 550, 450	v
<i>AluI</i>	A	2100, 1300, 900, 800, 700, 600, 450, 400, 375	a, b, c, d, g, n, s
	B	2000, 1600, 1300, 900, 800, 700, 565, 375	f, i, p
	C	1400, 950, 800, 600, 500	v

†Letters represent the first letter of the species name.