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Genetic structure of *Glycine canescens*, a perennial relative of soybean

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Summary. Allozyme variation as detected by starch gel electrophoresis was used to assess the extent and spatial organization of genetic variation across the entire range of *Glycine canescens* sensu lato. Eleven enzyme systems were assayed in 116 accessions of this taxon and 102 alleles were detected at a total of 31 loci. Eighty-one percent of loci were polymorphic. Most of this variation occurred between and very little within accessions. Three major groupings were detected. These groupings (groups 1, 2, and 3) also differed with respect to mean seed size and their geographic distribution. A further ten accessions stood out from these distinct groups. These accessions were most closely related to group 3 but were variable among themselves. In general, they were collected from highly dissected terrain, often in the remote interior of the continent. A final group of 18 problematic accessions (group X), originally tentatively identified as *G. canescens* on morphological grounds, was shown to be isozymically distinct from this species and was reclassified as one form of the polytypic species *G. clandestina*.

Key words: *Glycine canescens* – Allozyme variation – Geographic distribution – Collection strategies – Core collection

Introduction

Wild species related to the major world crops are receiving increased attention as genetic resources (Hawkes 1977; Harlan 1984; Brown et al. 1989). Wild relatives serve as a source both of new genes for crop improvement and of model systems for studying modes of adaptation. Yet the collection, conservation, and use of such variation poses problems different from those encountered when considering cultivated stocks. To exploit these resources and to supplement them with further collections will require knowledge of the structure of intraspecific genetic variation and its geographic patterns. Further, to rationalize the size of collections and the difficult task of mobilizing their variation into breeders' populations, a streamlined, "core" collection of a limited number of accessions needs to be designated (Brown 1989a, b). This too requires information on the genetic structure of the species. Can these activities be guided by the environment from which the samples were obtained (Rick 1973) and, if so, on what scale?

In order to address this question, the diploid ($2n = 40$), perennial species *Glycine canescens* F. J. Herm., a wild relative of soybean [*Glycine max* (L.) Merr.], was chosen for study. The species is indigenous to Australia and has continent-wide distribution. It is adapted to the dry inland, offering a contrasted adaptive norm to that of soybean. It provides a useful model system for in vitro culture (Newell and Luu 1985; Hammatt et al. 1987; Rech et al. 1988) and it has been hybridized with soybean (Newell et al. 1987). The species has been studied intensively as a source of resistance to soybean leaf rust caused by *Phakopsora pachyrhizi* Syd. (Burdon 1986, 1987, 1988), and is a source of a set of lines to identify different races of the pathogen (Burdon and Speer 1984). In the present study, we examined allozyme variation in 11 isozyme systems in 116 accessions to obtain an overview of the genetic structure of the species as a whole (Brown 1978; Hamrick and Godt 1989). The aims were: (1) to determine the amount and patterns of genetic variation; (2) to test whether genetic variation was distributed geographically and, hence, whether a core collection could be selected efficiently; and (3) to clarify the taxonomic status of certain problematic accessions.

Materials and methods

Accessions

The 98 accessions of *G. canescens* tested for allozyme variation comprise the current collection of this taxon held in the CSIRO collection of Australian perennial *Glycine*. The collection essentially covers the known geographic range of the species and the morphological heterogeneity present. In addition, another 18 accessions, whose classification as either *G. canescens* or *G. clandestina* Wendl. was unresolved, were included. About 75% of the 116 accessions are original field collections made by the authors or their colleagues; the remainder are donations from other collectors. The original locations are known for all but five of the accessions. Most accessions consist of very few plants (<5), as the species is often locally rare. All accessions were multiplied in the glasshouse at Canberra, and 100-seed weight was measured on the dried sample prepared for long-term storage. The appendix lists the locality of origin of all accessions by state of origin and isozyme group as determined below.

Isozyme assays

The methods used in the starch gel electrophoresis of imbided seed samples were essentially as previously described (Grant et al. 1984). At least three seeds were assayed from each accession. Table 1 lists the 11 isozyme systems included in the survey. Enzyme assays were as used previously (Grant et al. 1984), except that the TPI assay was that of Pichersky and Gottlieb (1983). (The capital symbols TPI refer to the enzymatic protein, and the italic *Tpi* refers to the putative gene loci.) The GPI, PGM, and TPI assays were made as 1% agar overlays to improve resolution. All zymograms were photographed. Nearly all accessions were apparently homozygous so that their pattern was not complicated by heterozygosity at a locus. When multiple bands appeared on the zymogram, they were allocated to one or more isozyme systems, primarily on the basis of electrophoretic mobility (i.e., zone location on the gel). When two or more zones were identified, they were numbered in decreasing order of anodal mobility, consistent with previous publications (Grant et al. 1984; Brown et al. 1986). For the AAT, GPI, IDH, MDH, MDR, PGM, and TPI zymograms, the proximity of allozyme bands on the gel, the formation of interlocus dimers, or similarity of appearance (intensity, presence of correlated bands) were the basis for allocating the different allozyme bands to isozyme systems. Within each system, allozymes are due to allelic variation at single loci, and/or to gene or genome duplication. In the latter case such apparently related loci were denoted as A or B. Extracts from two seeds of the standard accession G1232 were included on every gel for reference. The gene diversity indices, $\tilde{h}$ (Brown and Weir 1983), were computed as the average per locus probability that two random homozygous accessions within a group would carry different alleles at a locus, and the mean identity was the complementary value.

Results

In general terms the ADH, DIP, ENP, GPI, IDH, MDH, and SDH zymograms resembled those previously described for *Glycine tomentella* (Grant et al. 1984). The features of the additional systems are as given below. Preliminary genetic data for F_2 segregations have been obtained for all systems except IDH.

Asparate aminotransferase (AAT)

Individual seeds displayed the products of at least four loci. Two of these (*Aat1A* and *AatIB*) were responsible for the more anodal zone, and allozyme differences between lines showed that this pair formed an interlocus dimer. In the more cathodal zone, the products of a further two loci (*Aat2A* and *Aat2B*) were evident. These again formed an interlocus dimer. In addition a third system, probably consisting of a pair of loci as anticipated from research on other plant species (Hurka et al. 1989), was evident in some samples but could not be reliably scored. The correlated migration of the interlocus dimers whenever variation was present for the homodimeric position assisted in the scoring. This interpretation of the genetics of the system was confirmed in a series of crosses.

NADH dehydrogenase or menadione reductase (MDR)

Most samples showed five or more bands. The most prominent and reliably resolved was the slowest migrating anodal band, which served as the basis for scoring genotypes. The more anodal and less active bands (presumably heterotetrameric) varied in concert with variation in the slowest zone. Two kinds of homozygous (true breeding) variants at the slow zone were found. In the first variant (e.g., G1069), the slowest homodimer migrated further than the comparable band in the standard G1232. The pattern of the second type of variant (e.g., G1112) had five bands in the slowest anodal zone and a plurality of bands in the more anodal positions. Its slowest isozyme migrated to the same position as the comparable band in the standard. Presumably a two-gene tetrameric system (labelled *Mdr1A* and *Mdr1B*) encodes the slowest band (or bands for G1112).

Phosphoglucomutase (PGM)

The products of two sets of duplicate genes were present, adjacent to each other on the gel. The more anodal, more intense zone was either single- or double-banded, and was assigned to two loci (*Pgm1A* and *Pgm1B*). The less intense set consisted of two doublets, which appeared as either two, three, or four bands, depending on whether the doublets had identical, overlapping, or different migration. This set was assigned to the loci *Pgm2A* and *Pgm2B*.

Triosephosphate isomerase (TPI)

Two well-separated zones of isozymes were resolved on the gel. The allozyme bands in each zone appeared to be coded by a pair of duplicate genes, with heterodimers forming when the products of the gene pair differed in mobility. The more anodal, less intense zone was assigned to the loci *Tpi1A* and *Tpi1B*, and the slower mi-

Table 1. Isozyme variation in *Glycine canescens*

Enzyme assay	E.C. no.	Gene symbol	Buffer system	No. of alleles at locus						
				IA	IB	2A	2B	3A	3B	4
Alcohol dehydrogenase	1.1.1.1	*Adh*	T	3		3		2		
Aspartate aminotransferase	2.6.1.1	*Aat*	L	4	5	3	4			
Dipeptidase	3.4.13.11	*Dip*	L	5						
Endopeptidase	3.4.99	*Enp*	T	3						
Glucosephosphate isomerase	5.3.1.9	*Gpi*	H	5		4	5			
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*Idh*	H	4	1					
Malate dehydrogenase	1.1.1.37	*Mdh*	H	4	1	4		1	3	3
NADH dehydrogenase	1.6.99.2	*Mdr*	L	4	2					
Phosphoglucomutase	2.7.5.1	*Pgm*	H	5	2	2	4			
Shikimate dehydrogenase	1.1.1.25	*Sdh*	H	5						
Triosephosphate isomerase	5.3.1.1	*Tpi*	H	5	1	4	1			

Buffer systems: T – Tray buffer, 0.3 *M* borate, 0.1 *M* sodium hydroxide; gel buffer, 4 m*M* citrate, 13 m*M* TRIS; H – Tray buffer, 0.4 *M* sodium citrate, pH 8.0; gel buffer, 5 m*M* histidine pH 8.0; L – Tray buffer, 75 m*M* lithium hydroxide, boric acid to pH 8.5; gel buffer, 6% tray buffer, 95% 9.1 m*M* citrate, 65 m*M* TRIS

Table 2. Summary of allozyme variation within groups

Isozyme group	Typical line	*N*	Alleles per locus	Proportion of polymorphic loci	Gene diversity ($\tilde{h}$)	Mean identity
1	G1232	21	1.45	0.39	0.077	0.92
2	G1340	39	1.87	0.61	0.157	0.84
3	G1120	28	1.77	0.52	0.132	0.87
Others		10	2.16	0.71	0.257	0.74
Total *G. canescens*		98	3.10	0.81	0.31	0.69
X	G1123	18	1.52	0.45	0.121	0.88

grating strongly staining zone was assigned to the loci *Tpi2A* and *Tpi2B*.

Overall variation

Table 1 gives the number of distinct alleles observed at each locus in the whole collection of 116. A total of 101 alleles at the 31 loci was found, with an average of 3.26 alleles per locus. The proportion of polymorphic loci was 0.81. The collection is very diverse genetically, with the estimate of gene diversity being 0.336. As progeny from the same plant were usually uniform, most of the variation occurred in the species as variation between different homozygous lines. The level of true heterozygosity per locus per plant (as opposed to polymorphism due to different homozygous lines within an accession) was 0.008. In common with all species of *Glycine, G. canescens* produces both showy chasmogamous flowers on racemes and cleistogamous flowers in the leaf axils. The former are cross-pollinated in natural populations to an unknown extent. Heterozygosity in *G. canescens* for a rust resistance gene has also been recorded (Burdon and Speer 1984).

Organization of allozyme variation

Despite the very high level of variation at the loci screened, it was clear from inspection of the multilocus genotypes that the variation was itself highly structured. Of the total 116 accessions, 88 accessions of *G. canescens* fell into one of three distinct isozyme groups. The groups are as follows.

Group 1, characterized by line G1232, corresponds to a typical *G. canescens*. It included the line G1850 (=USDA PI 440928), selected by Singh et al. (1988) to typify the *G. canescens* chromosome complement.

Group 2 has G1340 as a typical line and is the most common in the collection.

Group 3 is typified by line G1120. Plants of this group when grown in the glasshouse have narrower, more linear leaves, and smaller seeds.

The 10 remaining lines of *G. canescens* did not group closely with these three major groups, but some close grouping among them was clear-cut. Finally, the 18 problematic lines formed a coherent isozyme group X.

Overall this classification, based only on isozymes, was strongly developed, as is seen when the estimates of

Table 3. Allele frequencies for ten of the more polymorphic loci surveyed that differentiated among the major groups of *Glycine canescens*

Locus	Allele	Accession group			
		Gp1	Gp2	Gp3	GpX
Enp	a	0.03	–	–	–
	b	0.97	–	–	–
	c	–	0.95	1.0	1.0
Mdr1A	a	–	0.05	0.96	–
	b	1.0	0.81	0.04	1.0
	c	–	0.14	–	–
Aat1A	b	1.0	0.79	1.0	–
	c	–	0.21	–	0.83
	d	–	–	–	0.17
Aat1B	a	–	–	0.14	–
	b	0.03	–	0.86	–
	c	0.97	0.74	–	1.0
	d	–	0.26	–	–
	e	–	–	–	–
Aat2A	a	–	–	1.0	1.0
	b	1.0	1.0	–	–
	c	–	–	–	–
Aat2B	a	–	–	–	0.06
	b	–	–	–	0.75
	c	–	0.97	1.0	–
	d	1.0	0.03	–	0.19
Dip	a	–	–	–	–
	b	–	–	–	–
	c	1.0	0.97	0.97	–
	d	–	0.03	0.03	1.0
	e	–	–	–	–
Sdh	a	0.31	–	–	–
	b	–	–	–	0.17
	c	0.69	0.92	0.11	–
	d	–	–	0.07	0.83
	e	–	0.08	0.82	–
Tpi2A	a	0.11	–	–	–
	b	–	0.03	–	–
	c	0.89	0.94	0.04	0.94
	d	–	0.03	0.96	–
	e	–	–	–	0.06
Mdh3	a	0.17	–	0.93	1.0
	b	0.83	–	0.07	–
	c	–	1.0	–	–

mean genetic identities within each group (Table 2) are compared with those between groups. Thus, the genetic identity between groups 1 and 2 is 0.705, between 1 and 3 it is 0.455, and between 2 and 3 it is 0.543. These values are markedly less than the mean genetic identities within the three groups (0.92, 0.84, and 0.87). The identities shown by group X with groups 1, 2, and 3 were 0.436, 0.496, and 0.530, respectively, marginally smaller than the values between the three groups of *G. canescens*.

Table 4. Seed weight and geographic distribution of the major isozyme groups

	1	2	3	X
Seed wt (mg)				
Mean	11.1	7.1	4.6	5.4
Range	8.2–16.2	4.8–9.9	2.8–6.6	3.8–6.5
Latitude (°S)				
Mean	28°38′	24°23′	31°34′	32°37′
Range	19°–35°	20°–31°	26°–35°	30°–34°
Longitude (°E)				
Mean	140°22′	134°31′	145°24′	148°27′
Range	133°–146°	120°–146°	136°–149°	146°–151°
Altitude (m)				
Mean	171	426	218	351
Range	10–600	100–700	90–400	130–560

Table 3 lists the allele frequencies for ten of the more polymorphic loci, which were well differentiated among the main groups. Of course, similarity of electrophoretic mobility at the protein level may not mean a lack of differences at the DNA level. For example, the MDR homodimer coded by the allele *Mdr1Ab* for group X differs slightly but consistently from that bearing the same label in the groups. However, this difference would not be resolved in a mixed sample or in a heterozygous condition, and was therefore not included as an isozyme difference. Hence, the level of divergence is underestimated. Yet some loci were highly diagnostic, e.g., the alleles *Enpb* for group 1, *Mdh3c* for group 2, *Mdr1Aa* for group 3, and *Dipd* for group X.

The mean weight of individual seed for the accessions as classified into the isozyme groups is given in Table 4. The seed weight of group 1 accessions ranged between 8.2 and 16.2 mg per seed, with a mean of 11.3 mg. This value was more than twice the means of groups 3 and X, showing a marked difference between the groups for this character. The seed weights for group 2 were intermediate. These results show a striking morphological divergence between the groups.

Geographic distribution of the groups

Table 4 summarizes the data on the original locality of the accessions in groups as the average and the range for the latitude (°S), longitude (°E) and altitude (m). The centers of occurrence for the groups (the average of the geographic coordinates for the accessions in the collection) formed a southeast transect from group 2, group 1, group 3, to group X. The distribution of altitudes differed between group 1 with a lower average and the peak of values below the midpoint of the range (positively skewed), and group 2 having the opposite pattern (negatively skewed).

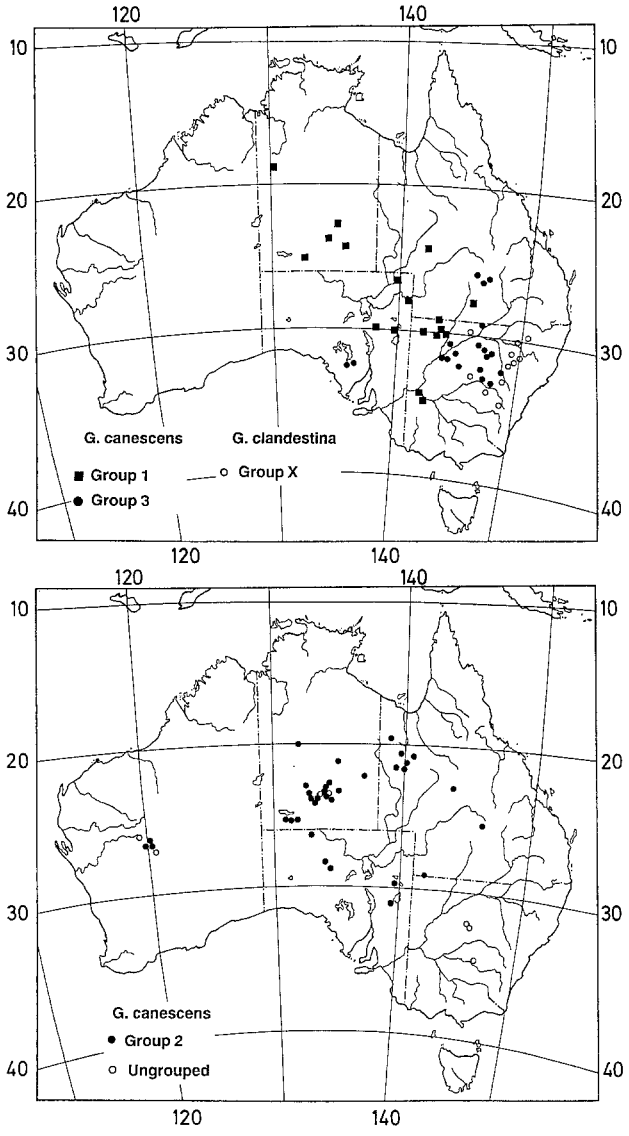

Fig. 1. Origin of accessions of *Glycine canescens* isozyme groups 1, 2, and 3, and of *G. clandestina* group X. When more than one accession traces to a single locality, only one symbol is shown

Figure 1 maps the origin of all accessions where definitely known. Accessions in group 1 occurred from far western New South Wales across into central Australia. The distribution broadly followed the Great Inland Basin. Those in group 2 were in all but the southeastern portion of group 1's range and extended further westwards and northwards across the Northern Territory and Western Australia. In contrast, group 3 accessions were found on the eastern and southern margins of the central *G. canescens* range and on the lower western slopes of the Great Dividing Range.

Problematic accessions – (a) group X

The accessions belonging to group X were collected mostly on the upper western slopes of the Great Dividing Range, in New South Wales. This forms the western margin of the distribution of *Glycine clandestina* and overlaps the eastern margin of *canescens* group 3. *G. clandestina* is mainly a species of the moist, temperate coastal belt and the uplands of the Great Dividing Range of southeastern Australia, whereas *G. canescens* is predominantly found in the arid inland of the continent.

The two species *G. canescens* and *G. clandestina* are closely related (Doyle et al. 1990) and can be crossed readily in the greenhouse (Putievsky and Broué 1979; Singh et al. 1988). The hybrids are usually partially or completely fertile. Generally the species are distinguished (Hermann 1962) by the following *G. canescens* characters: (1) pinnately as opposed to digitately trifoliate leaves, (2) legumes curved abaxially with fewer (4–8) seeds, (3) rectangular as opposed to rounded, oval seeds, and (4) hoary plants, whereas most accessions of *G. clandestina* are sparsely hirsute to glabrous plants and only a few are densely pubescent.

The accessions placed in group X resemble *G. clandestina* more than they resemble *G. canescens*, particularly in fruit and seed morphology. Ambiguity in their classification arose from their leaflet morphology, as these plants show a partially pinnate condition with petiolule length for the mid leaflet of usually 1 mm, and rarely up to 1 cm. The occasional presence of a pinnately trifoliate condition in which the petiolule of the central leaflets is noticeably longer than those of the two outer leaflets indicates that group X is more related to typical *G. canescens* than are typical forms of *G. clandestina* related to *G. canescens*. *G. clandestina* is itself a morphologically variable species.

Extensive crosses have been made between the three main groups of *G. canescens*, and *G. canescens* with group X, in order to study the genetics of resistance to soybean leaf rust (Burdon 1987, 1988). In a few crosses, F_1 fertility was incomplete and other crosses showed some segregation distortion. This occurred as frequently in crosses between *G. canescens* groups 1, 2, and 3, as it did in crosses between these *G. canescens* groups and group X. In general, however, hybrids appeared to be fertile, and the segregation ratios of resistance genes conformed to Mendelian expectations.

Problematic accessions – (b) ungrouped accessions of G. canescens

The ten ungrouped accessions are listed in Table 5 together with the alleles they possess for the ten differentiated loci in Table 3. Overall, these accessions shared more alleles with group 3 (e.g., *Mdr1Aa*, *Aat2Aa*, and *Sdhe*) than with the other main groups. Of the five lines from Western Australia, the locality of collection is known for only two (G1481, G1485, the southeastern slopes of the Robinson Ranges). Accessions G1301 and

Table 5. Genotype of ungrouped accessions of *G. canescens* at allozyme loci in Table 3

Locus	Accessions and origin					
	1104 1485	1299 1904	1301	1481	1672	1998 1999 2711
	W.A.	W.A.	N.T.	W.A.	N.T.	N.S.W.
Enp	ac	a	c	ab	c	ab
Mdr1A	a	a	b	a	a	a
Aat1A	b	c	b	c	d	c
Aat1B	cd	c	c	c	de	ce
Aat2A	a	a	a	a	a	a
Aat2B	c	c	c	c	cd	c
Dip	a	c	b	e	c	d
Sdh	e	e	e	d	e	e
Tpi2A	b	b	b	b	b	b
Mdh3	a	a	a	a	a	b

W.A. – Western Australia; N.T. – Northern Territory; N.S.W. – New South Wales

G1672 from the MacDonnell Ranges, Northern Territory, were also found in remote, dissected terrain. The form G1998/G1999/G2711 is from the eastern margins of group 2 in the isolated hilltops and western foothills of the Great Divide. Each of these six sets of accessions (Table 5) has a distinctive morphology, and subsequent study may remove one or more of them from the species *G. canescens*.

Discussion

This survey of the genetic variation present in *Glycine canescens* has revealed several features of organization. The species is highly polymorphic as a whole. It is predominantly homozygous, indicating that the main mode of reproduction is self-fertilization. Associations between allozymes at different loci provided the basis for dividing into groups or types. Three of these groups are widespread, whereas others are rare and highly localized. Isozyme analysis of the related species *Glycine tomentella* also split the collection into several distinct tetraploid and diploid groups (Doyle and Brown 1985). In this latter case, hybrids between the groups are sterile, whereas the isozyme groups of *G. canescens* are not reproductively isolated.

Perhaps the most striking feature is the distinctive distribution of the three major groups of *G. canescens* on a macrogeographic scale (Fig. 1). The distribution of type 1 through the Great Inland Basin suggests that seed may be transported widely during extensive flooding.

The broad pattern of partial differentiation into types that can occur in the same general area has been found in other autogamous species [e.g., *Avena barbata* in California; Clegg and Allard (1972); Allard et al. (1978)]. This pattern might be anticipated after long-distance colonization, but is not expected to be a feature of the native range of a species.

A corollary to this pattern of variation is that estimates of correlation between many genetic (allozyme) variables and any environmental or geographic variable are almost bound to reach some level of statistical significance. Presumably, the general underlying basis of this result would be the overlapping occurrence of the three common races. Thus, the highly structured nature of genetic variation in predominantly autogamous species indicates that due caution must be taken to avoid overinterpretation of statistically significant correlation coefficients.

A second main aspect of geographic structure in *G. canescens* is the occurrence of zones of high genetic diversity, mostly associated with valleys in the lower slopes of mountain ranges. Classically it has been anticipated that such terrain would harbor more genetic diversity because of increased isolation and local habitat diversity. The dual mating system affording opportunity for outcrossing in chasmogamous flowers and self-fertilizing cleistogamous flowers may give increased flexibility to generate new genotypes in such pockets. If this is true, the level of genetic diversity in such species will vary markedly through its distributional range.

This picture of the genetic structure of *G. canescens* has major implications for sampling strategies aimed at capturing the maximum of variation. It argues against an even distribution of effort on a spatial scale, in favor of more intense sampling in areas of pronounced physiographic variation.

Further, the data can be used to guide the choice of accessions for inclusion in a core collection. While a random sample of 10% of lines can be reasonably efficient, a better strategy is to choose from each of the groups in proportion to the logarithm of their size (Brown 1989b), and take six of the nine unusual types. On the basis of this study, the core collection of *G. canescens* (Brown et al. 1987) was modified as denoted in the Appendix.

Finally, the accessions that belong to group X and that were originally classified as *G. canescens* should more properly be regarded as a form of *Glycine clandestina*. They are more distant genetically from group 1 than any other group or unusual line. This conclusion is supported by analysis of restriction site variation in the spacer region of the *18S–25S* rDNA genes (A. H. D. Brown, M. T. Clegg, J. J. Doyle, unpubished data).

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Appendix

Origin and isozyme group assignment of G. canescens and related accessions

State[a]	Isozyme group	Accessions
N.S.W.	1	1232, 1958, 2446 Cobham Lake; 1238 Yancannia; 1249 Delalah; 1351 Urella Downs; 2636 Tero Creek, Yancannia
	2	1432 Tibooburra
	3	1112–1113, 1699 Condobolin; 1114–1118 Lake Cargelligo; 1119–1120 Hillston; 1237 White Cliffs; 1239 Wilcannia; 1240 Neckarboo; 1302 Nymagee; 1959 MacCulloch's Range; 1960 Cobar; 1962–1963 Nyngan; 1964 Coolabah; 1965 Bourke; 1966 Engonia; 1968 Warren
	X	1122–1128 Young; 1250 Gilgunnia Range; 1295 Wellington; 1344 Cowra; 1486 Narrabri; 1500 Warialda; 1501 Narrabri; 1511 Coonabarabran; 1514 Mendooran Rd.; 1668 Binnaway; 1967 Engonia; 2637 Parkes
	U	1998 Yathong HS; 1999 Mount Hope; 2711 Wagga
N.T.	1	1235 Simpson Desert; 1245 Harts Range; 1341 Alice Springs; 1473 Ayers Rock; 2402 Mt Winnecke
	2	1339 Ooraminnia; 1340 Murray Downs; 1342, 1701, 2403 Alice Springs; 1470–1472 Standley Chasm; 1474, 1673, 2532 Ayers Rock; 1563 Arltunga Ruins; 2048 Ormiston Gorge; 2518, 2520; Palm Valley; 2519 Undandita; 2521 Tarlton Downs; 2533 Mt Olga; 2534 Palm Valley; 2535 Yuendumu
	U	1301 Alice Springs; 1672 Standley Chasm
Qld	1	2417 Cunnamulla; 2447 Bedourie
	2	1069 Charleville; 2443 Longreach; 2523 Malbon, Duck Ck.; 2524 Dronfield; 2525 Duchess, Wills Ck.; 2526 Dajarra, Pigeon Ck.; 2527 Mt Isa, Mica Ck.; 2528 Thorntonia; 2661 Toona Gate, Grey Range
	3	2191 Sommariva; 2192 Angellala Ck; 2444 Charleville
S.A.	1	1851 Innamincka; 1852 Leigh Ck.; 1853 Goyder's Lagoon; 2092 North Mulga
	2	1895 Moolawatana; 2352 Weetootla Gorge; 2529 Mabel Ck.; 2530 Pootnoura; 2531 Agnes Ck.
	3	2353 Thuralga; 2354 Lake Gairdner
Vic.	1	1270 Colignan; 2351 Lake Hattah
W.A.	2	1482–1484 Wiluna;
	U	1481 Agnew; 1485 Peak Hill; 1104, 1299, 1904 unknown

[a] N.S.W. – New South Wales; N.T. – Northern Territory; Qld – Queensland; S.A. – South Australia; Vic. – Victoria; W.A. – Western Australia

Isozyme groups are *G. canescens* groups 1, 2, 3 and U = unknown, *G. clandestina* group X

Accessions of unknown origin are G1850 (=USDA PI 440928), G2404 (=USDA PI 440927) both of isozyme group 1 and G2445 of group 3. The published records of origin for the former two are invalid, because the records refer to a *G. clandestina* and a *G. microphylla* specimen from the east coast

Revised core collection

	Retained	New
G. canescens–group 1	1232, 1270, 1853	1245, 2402
G. canescens–group 2	1340, 1482	2352, 2528
G. canescens–group 3	1120	2192, 2354
G. canescens–ungrouped	1301, 1485, 1672	1481, 1999
G. clandestina–group X	1500	1123

Accessions deleted from previous core: 1104, 1113, 1249, 1852, 1966

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