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The genomic relationships among six wild perennial species of the genus *Glycine* subgenus *Glycine* Willd. *

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Summary. Based on meiotic chromosome behavior in intra- and interspecific hybrids, genome symbols were assigned to the following diploid ($2n=40$) *Glycine* species: *G. canescens* = *AA*; *G. clandestina*-Intermediate pod (Ip) = A_1A_1; *G. clandestina*-Short pod (Sp) = BB; *G. latifolia* = B_1B_1; *G. tabacina* = B_2B_2; *G. cyrtoloba* = *CC*; and *G. tomentella* = *DD*. Genome symbol *GG* was reserved for the soybean, *G. max*. At metaphaseI, loose chromosome associations were observed in completely sterile interspecific hybrids whose parents differed in their genomes, suggesting some chromosome homologies among species. Although *G. clandestina*-Sp, *G. latifolia* and *G. tabacina* are morphologically distinct species, they differ only by a paracentric inversion. Similar observations were recorded for *G. canescens* and *G. clandestina*-Ip. Evidence is presented that demonstrates that *G. tabacina* ($2n=80$) and *G. tomentella* ($2n=78$, 80) are allotetraploid species complexes. Hybrid weakness, sterility, seedling lethality and seed inviability were found in intra- and interspecific hybrids.

Key words: *Glycine canescens* – *Glycine clandestina* – *Glycine cyrtoloba* – *Glycine latifolia* – *Glycine tabacina* – *Glycine tomentella* – Intraspecific hybridization – Interspecific hybridization

Introduction

The genus *Glycine* Willd. has been divided into two subgenera, *Glycine* and *Soja* (Moench) F.J. Herm. The subgenus *Soja* consists of the cultivated soybean, *G. max* (L.) Merr. and its annual wild progenitor, *G. soja* Sieb. & Zucc. The subgenus *Glycine* is composed of nine wild perennial species. Prior to 1983 the following seven species were recognized: *G. canescens* F.J. Herm., *G. clandestina* Wendl., *G. falcata* Benth., *G. latifolia* (Benth.) Newell & Hymowitz, *G. latrobeana* (Meissn.) Benth., *G. tabacina* (Labill.) Benth., and *G. tomentella* Hayata (Hymowitz and Newell 1981). *G. clandestina* was found to be highly variable (Hermann 1962; Newell and Hymowitz 1983). Recently, Tindale (1984) removed the curved pod forms from *G. clandestina* and established a new species, *G. cyrtoloba* Tind. In addition, based on recent *Glycine* collections in Australia she described a new species, *G. argyrea* Tind. Among the currently recognized nine wild perennial species, *G. tabacina* ($2n=40$, 80) and *G. tomentella* ($2n=38$, 40, 78, 80) have a wide geographical distribution. They are found in Australia as well as in certain South Pacific Islands and/or in the West-Central Pacific Basin. The other seven species are diploid ($2n=40$) and are indigenous to Australia (Hymowitz and Newell 1981; Tindale 1984).

The classification of the wild perennials of the subgenus *Glycine* has been based on classical taxonomy. However, during the past six years, extensive cytogenetic studies have been conducted with the aim of establishing the phylogenetic relationships among the wild perennial *Glycine* (Putievsky and Broué 1979; Newell and Hymowitz 1983; Grant et al. 1984a, b; Singh and Hymowitz 1985a). These studies demonstrated that the crossability rate, hybrid seed viability and seed fertility of F_1 plants in intra- and interspecific hybrids depends upon the closeness of the parental genomes. Singh and Hymowitz (1985a) presented evidence that chromosomal structural changes, such as paracentric inversions and to a lesser extent reciprocal translocations, together with geographic isolation played a major role in the speciation process of the perennial *Glycine*.

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The objectives of the present study were to establish the genomic relationships among six wild perennial *Glycine* species (*G. canescens, G. clandestina, G. cyrtoloba, G. latifolia, G. tabacina, G. tomentella*) of the subgenus *Glycine* based upon the cytogenetic results published earlier (Newell and Hymowitz 1983; Singh and Hymowitz 1985a) and from results obtained in the present investigation. The intergration of these data facilitated the designation of genome symbols to the diploid wild perennial *Glycine* species.

Materials and methods

The wild perennial *Glycine* species selected for this study are listed in Table 1. Of the 28 accessions, five were *G. canescens,* two *G. clandestina,* two *G. cyrtoloba,* four *G. latifolia,* eleven *G. tabacina* and four *G. tomentella.* Within *G. clandestina* there are at least three morphological groups easily separated by pod length: short pod = Sp (< 25 mm), intermediate pod = Ip (25–35 mm), and long pod-Lp (> 35 mm). Plant Introduction (P.I.) 339664 belongs to the Sp group and P.I. 440948 belongs to the Ip group. Newell and Hymowitz (1983) designated P.I. 440948 as a long pod type *G. clandestina.* All accessions were grown in the greenhouse. Crossing procedures and cytological techniques described by Singh and Hymowitz (1985a) were used. In certain hybrid combinations pods started to abort 2–3 weeks after pollination. In such cases, attempts were made to germinate the immature seeds utilizing in vitro culture technique (Newell and Hymowitz 1982).

Table 1. List of parental accessions used in successful intra- and interspecific hybridization in subgenus *Glycine*

P.I.[a]	2*n*	Country of origin
G. canescens		
399478	40	Ooraminna Rockhole, N.T., Australia
440928	40	Broadwater, N.S.W. Australia
440932	40	Goyders Lagoon, Birdville, S.A., Australia
440934	40	Kings Park, Perth, W.A., Australia
440936	40	Condobolin, N.S.W., Australia
G. clandestina		
339664-Sp	40	Grafton, N.S.W., Australia
440948-Ip	40	Mt. Painter, Canberra, A.C.T. Australia
G. cyrtoloba		
440962	40	Brampton Island, Qd., Australia
440963	40	Brampton Island, Qd., Australia
G. latifolia		
253238	40	Capella, Qd, Australia
378709	40	Inverelle, N.S.W., Australia
440980	40	Yallaroi, N.S.W., Australia
446964	40	Inverelle, N.S.W., Australia
G. tabacina		
373985	80	Caramana No. 1, N.S.W., Australia
373992	80	Delungra, N.S.W., Australia
440992	80	Glen Innes, N.S.W., Australia
440994	80	Eidsvold Station, Qd., Australia
440996	80	Grafton, N.S.W., Australia
446970	80	Ouaieme, New Caledonia
446972	80	Iejima, Ryukyu Islands
483202	80	Hufangalupe beach, Tonga
483204	80	Nukú Alofa, Tonga
483208	80	Erromango, Vanuatu
483212	80	Poë beach, New Caledonia
G. tomentella		
441000	40	Mt. Garnet, Qd., Australia
441002	80	Brampton Island, Qd., Australia
441005	80	Lindeman Island, Qd., Australia
446993	40	Nadzab, Popua New Guinea

[a] P.I.; Plant introduction. For additional information about these accessions contact the US Department of Agriculture, Plant Introduction Officer, BARC – West, Beltsville, Maryland 20705, USA
Sp = short pod; Ip = Intermediate pod

Hybrids were identified morphologically and cytologically. Voucher herbarium specimens of accessions and hybrids are deposited in the herbarium of the Crop Evolution Laboratory (CEL), University of Illinois, Urbana.

The following abbreviations for countries or regions will be used throughout the manuscript: Australia = A. Regions within Australia; ACT = Australian Capital Territory; NSW = New South Wales; NT = Northern Territory; Qd = Queensland; SA = South Australia; WA = Western Australia; PNG = Papua New Guinea. South Pacific Islands; F = Fiji; NC = New Caledonia; TO = Tonga; VA = Vanuatu (New Hebrides). West Central Pacific Islands; MI = Mariana Islands; RI = Ryukyu Islands.

Results

Intraspecific hybrids

All intraspecific hybrids of *G. canescens, G. cyrtoloba* and *G. latifolia* showed normal meiosis (Table 2). Both hybrid combinations of *G. cyrtoloba* did not set seed although pollen fertility was 94%. We do not know the cause of sterility in these hybrids.

In tetraploid *G. tabacina,* the intraspecific hybrids were weak or sterile when one of the parental accessions was P.I. 440994, P.I. 440996 or P.I. 446972 (Table 2). Plant introduction 440994 was collected from Eidsvold Station, Qd and P.I. 440996 was collected from Grafton, NSW. The sites are on the east side of the Great Dividing Range separated by about 1,600 km. However, morphologically both accessions are similar in possessing long and narrow trifoliolate leaves which make them distinct from the other tabacinas. On the other hand, P.I. 446972 collected from the Ryukyu Islands can not be distinguished morphologically from the rest of the tabacinas. Sterility in intraspecific hybrids involving these accessions is chromosomal. At metaphase I, the range of bivalents was 16–22. Often, bivalents were arranged at the equatorial plate and univalents were scattered in the cytoplasm. At anaphase I–telophase I, the majority of univalents lagged at the equatorial plates while chromosomes from bivalents had already moved to their respective poles. Chromosome pairing analyses in intraspecific hybrids suggest that P.I. 440994, P.I. 440996, and P.I. 446972 differ from other tabacinas so far studied by one genome. Crosses are being made to establish genomic relationships among these three accessions.

Table 2. Chromosome association at metaphase I and seed-set in intraspecific F_1 *Glycine* hybrids

Cross PI[a]	Origin of F_1	No. F_1 plants	2*n*	Chromosome configurations			Total PMC	Anaphase I	Seed-set
				I	II	IV			
G. canescens									
440932 (40)[b] SA[c] × 399478 (40) NT	S[d]	3	40	0.0	20.0[e]	0.0	50		Fertile
G. cyrtoloba									
440963 (40) Qd × 440962 (40) Qd	S	2	40	0.0	20.0	0.0	50		Sterile
440962 (40) Qd × 440963 (40) Qd	S	1	40	0.0	20.0	0.0	25		Sterile
G. latifolia									
446964 (40) NSW × 253238 (40) Qd	S	2	40	0.0	20.0	0.0	50		Fertile
× 378709 (40) NSW	S	2	40	0.0	20.0	0.0	20		Fertile
× 440980 (40) NSW	S	2	40	0.0	20.0	0.0	20		Fertile
G. tabacina									
1. Within Australia									
373992 (80) NSW × 440996 (80) NSW	S	2[g]							
440996 (80) NSW × 373985 (80) NSW	S	2[g]							
2. Australian × South Pacific Islands									
373992 (80) NSW × 483202 (80) TO	S	2	80	4.0 (0–10)[f]	38.0 (33–40)	0.0	25	Bridges, laggards	Fertile
× 483204 (80) TO	S	2	80	3.8 (0–10)	38.1 (35–40)	0.0	27	Laggards	Fertile
× 483212 (80) NC	S	2	80	2.0 (0– 6)	38.8 (37–40)	0.1 (0–1)	8	Laggards	Pt. Fertile
483204 (80) TO × 440992 (80) NSW	S	2	80	4.3 (0–10)	37.9 (35–40)		28	Bridge + frag.	Fertile
× 440994 (80) Qd	S	1	80	42.0 (36–48)	19.0 (16–22)	0.0	15	Laggards	Sterile
3. South Pacific Islands × West Central Pacific Islands									
483208 (80) VA × 446972 (80) RI	SC	1	80	41.6 (38–48)	19.0 (16–21)	0.1 (0–1)	10	Laggards	Sterile

[a] Plant Introduction; [b] Chromosome number; [c] Abbreviations: see "Materials and methods"; [d] S = germination form mature seed, SC = seed culture; [e] Average chromosome association; [f] Range of chromosome configuration; [g] Hybrid plants in both combinations were weak and did not reach the flowering stage

The other four *Glycine tabacina* hybrid combinations derived from the hybridization between Australian and South Pacific Island accessions did not exhibit morphological abnormalities. These hybrids were fertile, although the range of univalents at metaphase I was wide, and sporocytes with laggards and chromatin bridges with acentric fragments were recorded at anaphase I.

Interspecific hybrids

Several new interspecific hybrids were obtained and studied morphologically and cytologically (Figs. 1 and 2, Table 2). Repeated attempts to hybridize *G. latifolia* with *G. canescens* yielded several pods that began to abort 20 days after pollination. Three seeds germinated among the 21 seeds cultured. Meiosis was studied in two plants. The true hybridity of F_1 plants was revealed by leaf size and shape (Fig. 1). Hybrid plants inherited the pubescence density trait from *G. canescens,* and their flowers were larger than those of both parents. The plants were completely sterile. Average chromosome associations and (ranges) were 20.9I (12–32)+ 9.5II (4–14). Figure 2a and b show metaphase I cells with 20I+10II and 32I+4II chromosomal configurations, respectively. Chromosomes lagged during anaphase I (Fig. 2c). Moreover, some cells also showed a chromatin bridge and acentric fragment (Fig. 2d).

The *G. canescens* (P.I. 440934) parent of the *G. canescens* × *G. tomentella* hybrids, was collected from Kings Park, Perth, WA while the diploid *G. tomentella* (P.I. 446993) parent came from PNG. Hybrid seed germination was normal when *G. canescens* was used as the female parent. On the other hand, only five out of 35 hybrid seeds germinated when *G. tomentella* was the female parent, and only two of these produced plants that reached the flowering stage. The plants were sterile. In the latter hybrids, an intraspecific hybrid (P.I. 440936 × P.I. 440928) of *G. canescens* was used as the pollen parent. Chromosome pairing at metaphase I was similar in both hybrids (Table 3).

One plant from the *G. canescens* × *G. clandestina*-Sp cross was produced by immature seed culture. It was weak and slow in growth. The plant flowered profusely but did not set seed. This may be due to disturbed chromosome pairing (Table 3).

The immature seeds of *G. tomentella* ($2n=40$) × *G. clandestina*-Ip turned into calluses in culture and several plantlets were regenerated through organogenesis. A total of 12 plants were transferred to the greenhouse and all carried $2n=40$ chromosomes. Detailed meiotic analysis was carried out in three plants. At metaphase I, bivalents ranged from 9–18 with an average chromosome association of 13.6I + 13.3II per sporocyte.

The range of total bivalents in all of the above interspecific hybrids was 9.5–13.3 per sporocyte. The average frequency of ring bivalents per sporocyte was much lower than the frequency of open bivalents (Table 3). These results suggest that the genomes of the species involved in their respective hybrids are not closely related, yet may have some homologies in their genomes.

In *G. tabacina* ($2n=80$) × *G. canescens* ($2n=40$) hybrids, the same accession (P.I. 440932) of *G. canescens* was hybridized with four different tetraploid accessions of *G. tabacina.* All F_1 plants carried the expected $2n=60$ chromosomes, except plant number 1 in hybrid P.I. 373992 × P.I.440932, which had $2n=59$ chromosomes (Table 3). The plant with $2n=59$ chromosomes was slower in growth than those with $2n=60$ chromosomes. In addition, chromosome pairing analysis at

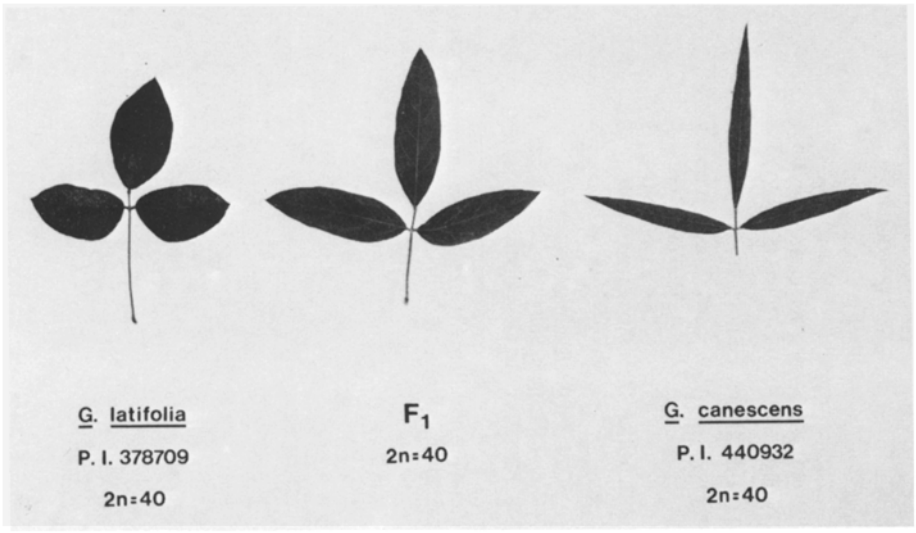

Fig. 1. Herbarium leaf specimens of *G. latifolia* (female-*left*), *G. canescens* (male-*right*) and their hybrid (*middle*). Note the differences in leaf shape and size between the F_1 hybrid and its parents

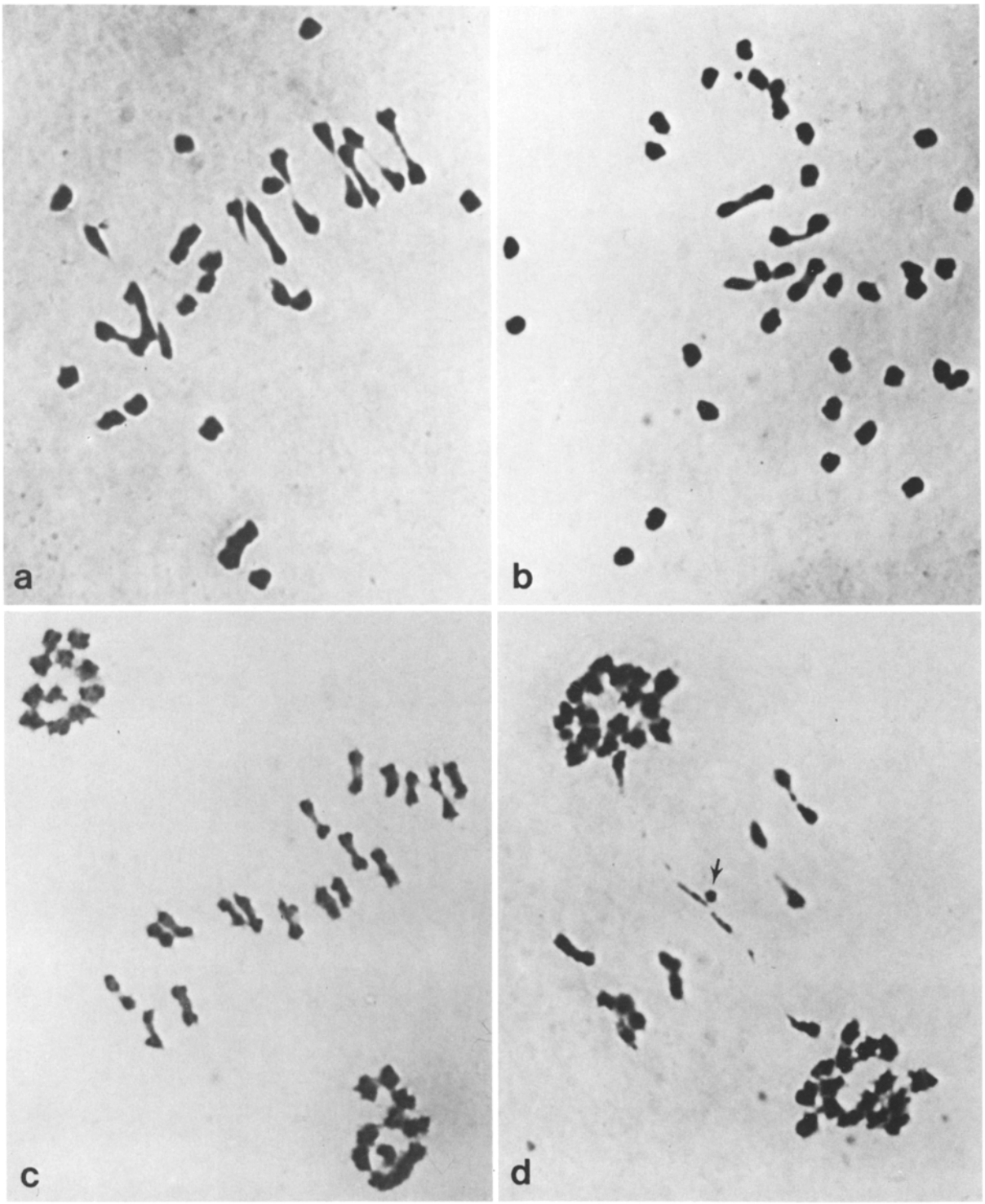

Fig. 2a–d. Meiosis in *G. latifolia* P.I. 378709 ($2n=40$)×*G. canescens* P.I. 440932 ($2n=40$) hybrid. **a** Metaphase I showing 10II+20I; **b** Metaphase I showing 4II+32I; **c** Telophase I showing 11-18-11 chromosome separation; **d** Telophase I showing six lagging chromosomes and a chromatin bridge with an acentric fragment (*arrow*). All figures × 1,600

Table 3. Chromosome association at metaphase I and seed-set in interspecific F_1 *Glycine* hybrids

Cross PI[a]	Origin of F_1	No. F_1 plants	$2n$	Mean chromosome configurations					Total PMC	Seed-set
				I	IIr	IIo	Total II	III		
G. latifolia × *G. canescens*										
378709 (40)[b] NSW[c] × 440932 (40) SA	SC[d]	2	40	20.9[e] (12–32)[f]	2.0 (0– 5)	7.5 (3–13)	9.5 (4 –14)	0.0	24	Sterile
G. canescens × *G. tomentella*										
440934 (40) WA × 446993 (40) PNG	S	1	40	19.2 (12–24)	1.1 (0– 4)	10.4 (5–13)	11.5 (8 –14)	0.0	20	Sterile
G. tomentella × *G. canescens*										
446993 (40) PNG × F_1[440936 (40) × 440928 (40)]	S	2	40	14.0 (8–22)	1.5 (0– 4)	11.5 (7–15)	12.0 (9 –16)	0.0	20	Sterile
G. canescens × *G. clandestina*-Sp										
440932 (40) SA × 339664 (40) NSW	SC	1	40	22.2 (14–26)	2.1 (0– 6)	6.8 (5– 8)	8.9 (7 –13)	0.0	10	Sterile
G. tomentella × *G. clandestina*-Ip										
441000 (40) Qd × 440948 (40) ACT	SC	3	40	13.6 (4–22)	2.3 (0– 7)	11.0 (6–15)	13.3 (9 –18)	0.0	25	Sterile
G. tabacina × *G. canescens*										
373985 (80) NSW × 440932 (40) SA	S	2	60	34.4 (22–50)	4.6 (1– 9)	8.1 (2–14)	12.7 (5 –19)	0.05 (0–1)	22	Sterile
373992 (80) NSW × 440932 (40) SA	S	2–1[g]	59	43.2 (27–55)	1.8 (0– 5)	6.1 (1–12)	7.9 (2 –16)	0.0	13	Sterile
		2–2[g]	60	33.0 (24–46)	2.7 (0– 7)	10.8 (6–15)	13.5 (7 –18)	0.0	13	Sterile
440992 (80) NSW × 440932 (40) SA	S	1	60	33.2 (26–40)	3.3 (0– 7)	10.2 (7–13)	13.5 (10–17)	0.0	15	Sterile
446970 (80) RI 440932 (40) SA	S	2	60	32.4 (28–42)	3.8 (0–10)	9.8 (5–16)	13.6 (9 –16)	0.13 (0–1)	15	Sterile
G. tomentella × *G. tabacina*										
446993 (40) PNG × 483204 (80) TO	S	2	60	32.4 (24–42)	2.5 (0– 7)	11.3 (4–17)	13.8 (9–18)	0.0	30	Sterile
G. canescens × *G. tomentella*										
440928 (40) NSW × 441005 (80) Qd	SC	3	60	24.9 (18–34)	5.6 (1–10)	11.3 (7–18)	16.9 (13–21)	0.44 (0–1)	25	Sterile
G. latifolia × *G. tomentella*										
378709 (40) NSW × 441000 (40) Qd	S	Seedling lethality								
446964 (40) NSW × 441000 (40) Qd	S	Seedling lethality								
G. tomentella × *G. cyrtoloba*										
446993 (40) PNG × 440962 (40) Qd	S	Seed inviability								

[a] Plant introduction; [b] Chromosome number; [c] Abbreviations: See "Materials and methods"; [d] S: germination from mature seed; SC: seed culture; [e] Average chromosome association; [f] Range of chromosome configuration; r = ring bivalents; o = open bivalents; Ip = Intermediate pod; Sp: short pod; [g] Two plants were grown; plant number 1 had $2n = 59$ chromosomes while plant number 2 carried $2n = 60$ chromosomes

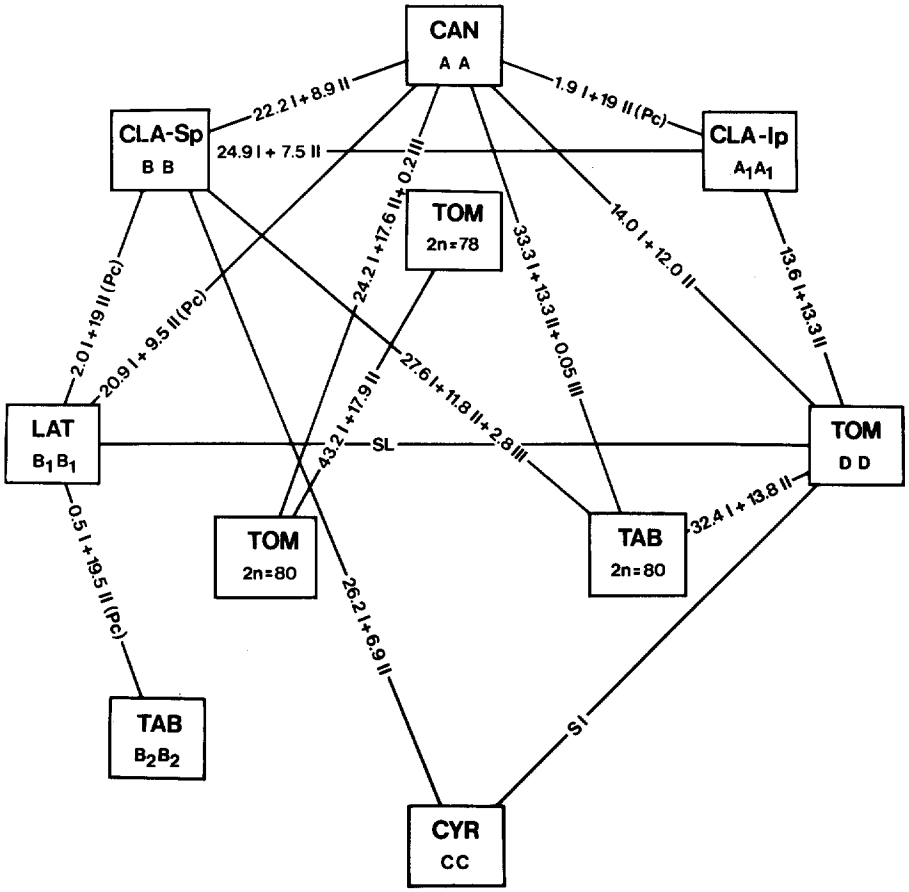

Fig. 3. Summary of genomic relationships, based on an average of data reported by Newell and Hymowitz (1983), Singh and Hymowitz (1985 a), and Table 3, demonstrated by meiotic chromosome behavior among wild perennials of subgenus *Glycine*. *Abbreviations:* CAN = *Glycine* canescens, CLA-Lp = *Glycine clandestina*-Long pod, CLA-Sp = *Glycine clandestina*-Short pod, CYR = *G. cyrtoloba*, LAT = *Glycine latifolia*, TAB = *Glycine tabacina*, TOM = *G. tomentella*, PC = Paracentric inversion, SI = seed inviability, SL = seedling lethality. All the species in the periphery carry $2n = 40$ chromosomes

metaphase I in the plant with $2n = 59$ chromosomes indicated a lower bivalent chromosome association (43.2I + 7.9II) than those plants which had $2n = 60$ chromosome. It is difficult for us to generalize, based on the analysis of one plant, that a missing chromosome influenced both plant growth and chromosome pairing. Chromosome associations in triploid plants with $2n = 60$ chromosomes ranged from 12.7–13.6 for bivalents (Table 3). Furthermore, the frequency of ring bivalents was appreciably less than that of open bivalents (Table 3).

One mature pod was harvested from the cross of diploid *G. tomentella* (P.I. 446993) and tetraploid *G. tabacina* (P.I. 483204). All five seeds were germinated, however, morphological and cytological analyses were conducted on only two plants. Morphologically, the hybrid plants showed longer rachises, internodes, peduncles, and racemes and larger flowers than both parents. The sterile, hybrid plants carried $2n = 60$ chromosomes that associated at metaphase I on an average as of 32.4I + 13.8II (Table 3) with the bivalents ranging from 9–18.

Three hybrid plants of the cross, *G. canescens* × *G. tomentella* ($2n = 80$), obtained by the immature seed culture technique, were studied. The reciprocal cross of this particular hybrid did not require immature seed culture (Singh and Hymowitz 1985 a). At metaphase I, cells with 20I + 20II chromosome configuration were recorded suggesting that one genome of tetraploid *G. tomentella* is similar to the genome of *G. canescens*.

Hybrid seedling lethality was observed in both combinations of *G. latifolia* × *G. tomentella* ($2n = 40$). Seed germination was normal but the cotyledons turned yellow after seedling emergence and further growth ceased (Table 3). Seed inviability was recorded in a *G. tomentella* ($2n = 40$) × *G. cyrtoloba* hybrid (Table 3).

Genomic relationships and genome designations

Combining the results of the present paper with the results from our previously published papers (Newell and Hymowitz 1983; Singh and Hymowitz 1985 a), genomic relationships among six diploid ($2n = 40$) wild perennial species of the subgenus *Glycine* can be established (Fig. 3). An attempt was made to use one or two representative accessions of a species in producing interspecific hybrids. Sometimes another accession of a particular species was used when crosses were not

successful, flowering of parental plants did not coincide or F_1 plants were dead. In all cases, intraspecific hybrids were made to ascertain the intragenomic relationship of accessions within species. In designating genomes, we have considered $2n=40$ chromosomes as the diploid chromosomes number, although there have been controversies on this issue in the literature (Hadley and Hymowitz 1973).

The genome of *G. canescens* is designated as *AA* (Fig. 3). The hybrids between *G. canescens* and *G. clandestina*-Ip showed an average chromosome association of 1.9I + 19.0II per sporocyte (Newell and Hymowitz 1983). Subsequently, Singh and Hymowitz (1985a) observed that these two species differ by a paracentric inversion. Thus, the genome of *G. clandestina*-Ip is designated as A_1A_1. *Glycine clandestina*-Sp is given the genome designation *BB* because F_1 hybrids of *G. clandestina*-Sp × *G. canescens* (Table 3), *G. clandestina*-Sp × *G. clandestina*-Ip (Newell and Hymowitz 1983), and *G. clandestina*-Sp × *G. cyrtoloba* (Singh and Hymowitz 1985a) were sterile and showed low chromosome pairing (Fig. 3). Hybrid plants involving *G. clandestina*-Sp and *G. latifolia* showed 20II in a majority of the sporocytes, but a chromatin bridge and an acentric fragment was noticed at anaphase I (Singh and Hymowitz 1985a). This indicates that only a paracentric inversion differentiate these two species, suggesting the genome designation B_1B_1 for *G. latifolia*. Similarly, the genome of diploid *G. tabacina* is designated as B_2B_2 because it differs from *G. latifolia* by a paracentric inversion (Singh and Hymowitz 1985a). The genomes of *G. cyrtoloba* and *G. tomentella* ($2n=40$) are designated as *CC* and *DD*, respectively based on chromosome pairing and hybrid seed inviability (Fig. 3, Table 3).

Neither the genomes of diploid *G. argyrea*, *G. clandestina*-Lp, *G. falcata* and *G. latrobeana*, nor both genomes of tetraploid *G. tabacina* and *G. tomentella* have been elucidated. However, based on crossability and chromosome pairing of triploid hybrids, we believe that *G. canescens*, *G. clandestina*-Sp, *G. tomentella* ($2n=40$) or perhaps some other diploid species have contributed to the genomes of tetraploid tabacinas. Similarly, *G. canescens* appears to be a one of the genome donors for tetraploid *G. tomentella*. It is evident from Fig. 3 that *G. tomentella* with $2n=80$ and $2n=78$ chromosomes have only one common genome. In addition, we are suggesting that the genome designation *GG* be reserved for the soybean, *G. max*.

Discussion

Grant et al. (1984a) used the basic chromosome number of $x=10$, and a genome affinity index (GAI) to demonstrate the evolutionary developments in the subgenus *Glycine*. This suggests that $2n=2x=20$ is the diploid chromosome number. Thus, the species in the genus *Glycine* with $2n=40$ and $2n=80$ should be regarded as tetraploids and octoploids, respectively. However, there is no concrete evidence to support $x=10$ as the basic chromosome number except the molecular approach by analyzing the DNA contents of soybean genomes (Gurley et al. 1979). However, based on (1) disomic inheritance (Bernard and Weiss 1973), (2) chromosome pairing, (3) all the species in the genus *Glycine* carry $2n=40$ chromosomes (Hymowitz and Newell 1981), and (4) meiotic chromosome pairing (0–4II) of twenty chromosomes in the haploid, *G. max* (Crane et al. 1982), it is evident that the species in the genus *Glycine* with $2n=40$ behave like diploids. One may argue that in the past, plants (*Glycine*?) with $2n=20$ chromosomes existed but now are extinct and the present day forms are tetraploid plants with diploidized genomes. Pending the success of isolating a complete set of primary trisomics in soybeans (Palmer 1974; Palmer and Heer 1976), we are left with no other alternative but to regard $2n=40$ as the diploid chromosome number.

Intra- and interspecific hybrids analyzed in the present study and those reported by Newell and Hymowitz (1983) and Singh and Hymowitz (1985a) have facilitated the establishment of the genomic relationships among six wild perennials of the subgenus *Glycine* (Fig. 3). The intraspecific hybrids studied, thus far, reveal that *G. canescens*, *G. latifolia*, *G. cyrtoloba* and the diploid form of *G. tabacina* are rather homogeneous. In contrast, *G. clandestina* has been observed to be highly variable morphologically (Hermann 1962; Newell and Hymowitz 1983). Recently, the curved pod type has been removed from *G. clandestina* and recognized as a new species, *G. cyrtoloba* (Tindale 1984). The separation of the curved pod form from clandestinas is logical because it also differs from the other species cytogenetically (Fig. 3). However, we still have three distinct forms of *G. clandestina* in our collection: (1) short pod (< 25 mm); (2) intermediate pod (25–35 mm); and (3) long pod (> 35 mm). Short pod accessions have pinnate leaflets, while with one or two exceptions, long and intermediate pod accessions have digitate leaflets. Chromosome pairing results indicate that intermediate pod clandestinas are genomically different from short pod clandestinas and are closer to *G. canescens*. On the other hand, short pod clandestinas are closely related to *G. latifolia* and diploid *G. tabacina*, although they are three distinct species (Hymowitz and Newell 1981). The relationship of long pod clandestinas with other clandestinas and also with other species has not been established yet. However, the long pod clandestinas hybridize easily with *G. clandestina*-Ip and *G. tomentella* ($2n=38$, 40, 80), *G. canescens* (Singh and Hymowitz, unpublished data).

Hybrid seedling lethality and seed inviability is a common occurrence in interspecific hybrids (Stebbins 1958). Hybrid seedling lethality in *G. latifolia* × *G. tomentella* ($2n=40$) and hybrid seed inviability in *G. tomentella* ($2n=40$) × *G. cyrtoloba* suggests that the genome of *G. tomentella* ($2n=40$) is not closely related with the genomes of *G. latifolia* and *G. cyrtoloba*. Alternatives for studying such hybrids are to germinate immature hybrid seeds in vitro or to use different accessions. Both of these approaches are currently being tried.

The genome designations of six *Glycine* species (Fig. 3) has been done arbitrarily assuming the diploid chromosome number as $2n=40$. Beginning with *G. canescens* (*AA*), the genomes of the other species were designated based on chromosome pairing, hybrid seedling lethality and hybrid seed inviability in interspecific hybrids. Different genomes were assigned to *G. clandestina*-Sp (*BB*), *G. cyrtoloba* (*CC*), and *G. tomentella* (*DD*), although interspecific hybrids showed higher chromosome associations than expected (Figs. 2 and 3). An examination of the chromosome pairing data (Table 3) reveals that the majority of bivalents at metaphase I were open type (Figs. 2 a, b). These results suggest some chromosome homology among the genomes of the different species. We can not answer the question as to how much chromosome homology is present because, thus far, pachytene chromosome analysis has not been feasible in *Glycine*.

The above discussion concentrated on the diploid species of the subgenus *Glycine*. Among the nine wild perennial species, tetraploid ($2n=80$) forms have been found in *G. tabacina* and *G. tomentella*. *Glycine tomentella* with $2n=78$ chromosomes has been collected from NSW and the adjoining region of Qd, Australia. Compared to the diploid species, the tetraploids have a wide distribution (Hymowitz and Newell 1981). It is note worthy that only tetraploid tabacinas have been found on various islands in the South Pacific (F, NC, TO, VA) and West Central Pacific (MI, RI). Perhaps diploids, having a weak and slow growth habit, could not compete with the tetraploids and thus became extinct, or they still might be present on the islands but as yet not collected. Similarly, the tetraploid tomentellas are more vigorous than the diploid and aneuploid ($2n=38$, 40, 78) forms.

How did the tetraploid tabacinas and tomentellas originate? On the basis of crossability and chromosome pairing results, we proposed that tetraploid tabacinas and tomentellas are allopolyploid species complexes (Singh and Hymowitz 1985 a). Two tetraploid tabacinas (P.I. 440994, P.I. 440996) with long narrow leaves from Australia differ from the rest of the Australian accessions by one complete genome (Table 2), suggesting that the origin of these two accessions differs from other Australian tabacinas. Singh and Hymowitz (1985 a) demonstrated that tabacinas from South Pacific Islands differ from Australian tabacinas by paracentric inversions, but essentially carry the same genome. The range of univalents was wide in intraspecific hybrids from the South Pacific Islands and Australia. The third *G. tabacina* complex is from the West Central Pacific Islands. One *G. tabacina* (P.I. 446972) from the RI differs from other tabacinas by one complete genome. The above results suggest that several diploid species may have participated in the formation of the *G. tabacina* complex.

Like tetraploid *G. tabacina*, tetraploid tomentellas have a wide geographical distribution (Hymowitz and Newell 1981). The intra- and interspecific hybrids revealed that *G. tomentella* with $2n=78$ and 80 chromosomes are allopolyploid species complexes. All intraspecific hybrids between $2n=78$ and $2n=80$ chromosome plants have been found to be sterile and chromosome pairing at metaphase I (Fig. 3) suggests one genome common to both forms while the second genomes different (Newell and Hymowitz 1983; Singh and Hymowitz 1985 a). A contradictory result has been reported by Grant et al. (1984 b). They observed almost normal chromosome pairing and complete pollen fertility (98, 99%) in two reciprocal hybrid combinations (1136 × 1133, 1133 × 1136) between $2n=78$ and $2n=80$ chromosomes. However, according to our germplasm collection records their accession, designated as 1133, is our P.I. 441001, which possesses $2n=78$ chromosomes. Thus Grant et al. (1984 b) studied chromosome pairing in hybrids where both parents carry $2n=78$ chromosomes.

The results presented in the present paper and published elsewhere (Newell and Hymowitz 1983; Singh and Hymowitz 1985 a) have demonstrated that there is both maintained and hidden genetic diversity in the wild perennial species of the subgenus *Glycine*. Genomic analysis provides information about the relationships among wild species and the feasibility of gene transfer to cultivated soybeans, *G. max*. Intersubgeneric hybrids of *G. max* × *G. tomentella* ($2n=78$, 80) have been obtained (Broué et al. 1982; Newell and Hymowitz 1982; Singh and Hymowitz 1985 b). Moreover, *G. tomentella* has been hybridized with *G. canescens* (Putievsky and Broué 1979; Singh and Hymowitz 1985 a) and *G. canescens* is easily hybridized with other species directly or by bridge species are possible candidates for gene transfer to soybeans.

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