SOYBEANS:
Improvement, Production, and Uses

Third Edition

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4   Speciation and Cytogenetics

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Since 1984, there has been an explosion of research activity in the area of speciation and cytogenetics of the genus *Glycine*. This was due to three factors: an increase in plant exploration and formal taxonomy; an increase in cytogenetic investigations; and lastly, the application of both biochemical and molecular approaches to phylogenetic studies. For a detailed review of past research especially formal taxonomy and cytogenetics, the reader is directed to Chapter 3 of the Soybean monograph (Hadley and Hymowitz, 1973) and Chapters 2 and 5 of the Soybean monograph, 2nd edition (Hymowitz and Singh, 1987; Palmer and Kilen, 1987). This chapter covers the literature from January 1984 through January 2002.

4–1 ORIGIN OF THE SOYBEAN AND ITS DISSEMINATION

Linguistic, geographical, and historical evidence suggest that the soybean [*Glycine max* (L.) Merr.] emerged as a domesticate during the Zhou dynasty in the eastern half of north China. Domestication is a process of trial and error and not a time-datable event. In the case of the soybean, this process probably took place during the Shang dynasty (*ca* 1500–1100 B.C.) or perhaps earlier. By the first century A.D., the soybean probably reached central and south China as well as peninsular Korea. The movement of soybean germplasm within the primary gene center is associated with the development and consolidation of territories and the degeneration of Chinese dynasties (Ho, 1969; Hymowitz, 1970).

According to Chinese legend, Emperor Shen Nong, the Father of Agriculture and Medicine, reported the earliest use of the soybean in the herbal, "Ben Cao Gang Mu". No fewer than six different dates from 2838 B.C. to 2383 B.C. have been acclaimed as the publication date for Shen Nong's book (Hymowitz, 1970). However, the historical analysis of the legitimacy of Emperor Shen Nong by sinologists reveals a completely different story. For example, Hirth (1908) was adamant in his belief that the value of the works of Shen Nong, who was sometimes represented as having the body of a man and the head of an ox, were a fabrication of historians, as perhaps the emperor himself. Statements such as "the soybean is one of the oldest cultivated crops" or "it has been cultivated for over 5000 years" are thus incorrect.

From about the first century A.D. to the Age of Discovery (15th–16th century), soybeans were introduced into several countries with land races eventually developing in Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Myanmar, Nepal, and north India. These regions comprise a secondary gene center. The movement of the soybean throughout this period was due to the establishment of sea and land trade routes, the migrations of certain tribes from China, and the rapid acceptance of the seeds as a stable food by other cultures (Hymowitz, 1990; Hymowitz and Newell, 1980).

For centuries, the soybean has been the cornerstone of East Asian nutrition. Although many different foods were developed from soybean, the four most important were miso, soy sauce, tempeh, and tofu. These traditional foods have little physical or flavor identity with the original bean. Thus, it is not too surprising that the first Europeans who visited China (e.g., Marco Polo) or Japan did not mention the soybean as a crop in their journals (Hymowitz and Newell, 1981).

Starting in the late 16th century and throughout the 17th century, European visitors to China and Japan noted in their diaries the use of a peculiar bean from which various food products were produced (Carletti, 1964; Satow, 1900). In the 17th century, soy sauce was a common item of trade from the East to the West. For example, in 1679, John Locke noted in his journal that "mango and soy are two sauces brought to England from East India" (King, 1858).

By 1705, European pharmacologists were familiar with the soybean from Japan and its culinary value (Dale, 1705). However, it was not until 1712, when Engelbert Kaempfer, who lived in Japan during 1691 and 1692 as a medical officer of the Dutch East India Company, published his book *Amoenitatum Exoticum...* that the western world fully understood the connection between the cultivation of soybean and the utilization of its seed as a food. Kaempfer's drawing of the soybean is accurate and his detailed description of how to make soy sauce and miso are correct (Kaempfer, 1906).

The soybean must have arrived in the Netherlands before 1737, as Linnaeus described the soybean in the *Hortus Cliffortianus* which was based on plants cultivated in the garden at Hartecamp (Linnaeus, 1737). In 1739, soybean seeds sent by missionaries in China were planted in the Jardin des Plantes, Paris (Paillieux, 1880). In 1790, soybean was planted at the Royal Botanic Garden at Kew, England (Aiton, 1812), and in 1804 were planted near Dubrovnik, Yugoslavia (Buconjic, A. n.d. A Monograph of Dubrovnik 1800–1810. The Church of St. Luka Marunic. Unpubl. Ms. [In Serbo-Croatian]). In the Netherlands, France, and England soybean was grown for taxonomic or display purposes. However, soybean grown in Yugoslavia was harvested, cooked, mixed with cereal grain and then fed to chickens (*Gallus gallus*) for increased egg production.

The soybean was introduced into North America by Samuel Bowen. Henry Yonge, the surveyor General of the Colony of Georgia, planted soybean on his farm at the request of Samuel Bowen in 1765. Mr. Bowen, a former seaman employed by the East India Company, brought soybean to Savannah from China via London. From 1766, Mr. Bowen planted soybean on his plantation "Greenwich" located at Thunderbolt, a few miles east of Savannah. Today the property is a city cemetery. The soybean grown by Bowen were used to manufacture soy sauce and vermicelli (soybean noodles). In addition, he manufactured a sago powder substitute from sweet potato (*Ipomoea batatas* L.). The three products were exported to England. Samuel Bowen received a patent (No. 878) for his manufacturing inventions for producing these products. He was awarded a gold medal from the Society of Arts, Manufactures, and Commerce and received a present of 200 guineas from King George III. In addition, Bowen sent soy sauce and soybean to the American Philosophical Society in Philadelphia and was elected to membership of the society. Unfortunately, when Bowen died in London on 30 Dec.1777, his soybean enterprise in Georgia ended (Hymowitz and Harlan, 1983).

Another early introduction of soybean to North America was by Benjamin Franklin. In 1770, he sent seeds from London to the botanist John Bartram who likely planted them in his garden, which was situated on the west bank of the Schuykill River below Philadephia (Hymowitz and Harlan, 1983).

In 1851, the soybean was introduced first to Illinois and subsequently throughout the Corn Belt. The introduction came through a series of very unusual circumstances. In December 1850, the barque *Auckland* left Hong Kong for San Francisco carrying sugar and other general merchandise. About 830 km (500 mi) off the coast of Japan the ship came across a Japanese junk foundering on the sea. The Japanese crew was removed from the junk and placed on board the Auckland which continued on to San Francisco. In San Francisco, the Japanese fishermen were not permitted to go ashore because of the possibility of spreading diseases. By coincidence, waiting for a passenger ship, to take him back to Alton, IL via the Panama overland route, was Dr. Benjamin Franklin Edwards. Dr. Edwards examined the Japanese fishermen, declared them free of any contagious diseases and received as a gift a packet of soybean that was carried back to Alton. Mr. John H. Lea, an Alton horticulturist, planted the soybean in his garden in the summer of 1851. In 1852, the multiplied soybean were grown in Davenport, IA by Mr. J. R. Jackson and also in Cincinnati, Ohio by Mr. A. H. Ernst. In 1853, Mr. Ernst distributed soybean seeds to the New York State Agricultural Society, the Massachusetts Horticultural Society, and the Commissioner of Patents. The two societies and the Commissioner of Patents sent soybean seeds to dozens of farmers throughout the USA (Hymowitz, 1986).

In mid-1854, Dr. James Morrow, the agriculturist in Commodore Matthew Perry's Expedition to Japan, obtained soybean seed and sent them to the Commissioner of Patents. Subsequently, the seeds were distributed to farmers (Hymowitz, 1986). Thus from 1855 onward, it is difficult to distinguish between soybean seed sources in farmers' reports.

4–2 *GLYCINE* SPECIES

The genus *Glycine* is divided into two subgenera *Glycine* (perennials) and *Soja* (Moench) F.J. Herm. (annuals). The list of species recognized as of 1981, their $2n$ chromosome number and distribution is shown on Table 4–1. The reader is directed to Chapter 2 of the Soybean monograph, Second Edition (Hymowitz and Singh, 1987) for further discussion about each of the seven perennial species described as of 1981. Only new information about each species will be presented in this chapter.

Table 4–1. The genus *Glycine* Willd., subgenera, chromosome number, and distribution (Hymowitz and Newell, 1981).

Species	2n	Distribution
Subgenus *Glycine*		
Glycine clandestina Wendl.	40	Australia
Glycine clandestina var. sericea Benth.	-	Australia
Glycine falcata Benth.	40	Australia
Glycine latifolia (Benth.) Newell and Hymowitz	40	Australia
Glycine latrobeana (Meissn.) Benth.	40	Australia
Glycine canescens F. J. Herm.	40	Australia
Glycine tabacina (Labill.) Benth.	40, 80	Australia, south China, Taiwan, Mariana Island, Ryukyu Island, South Pacific Islands
Glycine tomentella Hayata	38, 40, 78, 80	Australia, south China, Taiwan, Philippines, Papua New Guinea
Subgenus *Soja* (Moench) F. J. Herm.		
Glycine soja Sieb. & Zucc.	40	China, Taiwan, Japan, Korea, Russia
Glycine max (L.) Merr.	40	Cultigen

Since 1981, plant taxonomists have described 15 additional perennial *Glycine* species. This was due primarily to extensive plant exploration activities undertaken by U.S. and Australian scientists (e.g., Anonymous, 1988; Brown et al., 1985; Hymowitz, 1982, 1989, 1998; Newell, 1981). A list of the species in the genus *Glycine* as currently delimited, three-letter code, genomic designation, and distribution is shown on Table 4–2.

The taxonomic description of four of the perennial species occurred while this chapter was being written. These species include *G. aphyonota*, *peratosa*, *pullenii*, and *rubiginosa*. Little is known about these species other than what was presented in the initial publications (Brown et al., 2002; Pfeil et al., 2001).

4–2.1 Subgenus *Glycine*

4–2.1.1 *Glycine albicans* Tind. and Craven

Glycine albicans is a nonstoloniferous subshrub that grows up to 0.6 m high. Leaves are digitately three-foliolate. The central leaflets are obovate to obovate-elliptic. Rhizomes are present bearing underground shoots from which new plants develop and produce cleistogamous flowers and fruit. Leaves have white, soft hairs. The corolla is white with a purplish keel. Chasmogamous inflorescences are borne singly in the axils of the leaves. Pods contain two to four black seeds. The species is endemic to the Mitchell Plateau, Western Australia, Australia (Tindale and Craven, 1988). The few accessions studied cytologically are diploid (2n = 40). At the University of Illinois, under greenhouse conditions, we have been unsuccessful in the multiplication of seed.

4–2.1.2 *Glycine arenaria* Tind.

Glycine arenaria is a nonstoloniferous herb having a deep woody taproot. The leaves are pinnately three-foliolate. The leaflets are thick, very narrowly lanceolate

Table 4–2. List of species in the genus *Glycine* Willd., three letter code, 2n, genome symbol and distribution as of January 2002.

	Code	2n	Genome†	Distribution
Subgenus *Glycine*				
1. *G. albicans* Tind. & Craven	ALB	40	II	Australia
2. *G. aphyonota* B. Pfeil	APH	40	?	Australia
3. *G. arenaria* Tind.	ARE	40	HH	Australia
4. *G. argyrea* Tind.	ARG	40	A2A2	Australia
5. *G. canescens* F. J. Herm.	CAN	40	AA	Australia
6. *G. clandestina* Wendl.	CLA	40	A1A1	Australia
7. *G. curvata* Tind.	CUR	40	C1C1	Australia
8. *G. cyrtoloba* Tind.	CYR	40	CC	Australia
9. *G. dolichocarpa* Tateishi and Ohashi	DOL	80	?	(Taiwan)
10. *G. falcata* Benth.	FAL	40	FF	Australia
11. *G. hirticaulis* Tind. & Craven	HIR	40	H1H1	Australia
		80	?	Australia
12. *G. lactovirens* Tind. & Craven	LAC	40	I1I1	Australia
13. *G. latifolia* (Benth.) Newell & Hymowitz	LAT	40	B1B1	Australia
14. *G. latrobeana* (Meissn.) Benth.	LTR	40	A3A3	Australia
15. *G. microphylla* (Benth.) Tind.	MIC	40	BB	Australia
16. *G. peratosa* B. Pfeil & Tind.	PER	40	?	Australia
17. *G. pindanica* Tind. & Craven	PIN	40	H2H2	Australia
18. *G. pullenii* B. Pfeil, Tind. & Craven	PUL	40	?	Australia
19. *G. rubiginosa* Tind. & B. Pfeil	RUB	40	?	Australia
20. *G. stenophita* B. Pfeil & Tind.	STE	40	B3B3	Australia
21. *G. tabacina* (Labill.) Benth.	TAB	40	B2B2	Australia
		80	Complex‡	Australia, West Central and South Pacific Islands
22. *G. tomentella* Hayata	TOM	38	EE	Australia
	TOM	40	DD	Australia, Papua New Guinea
		78	Complex§	Australia, Papua New Guinea
		80	Complex¶	Australia, Papua New Guinea, Indonesia, Philippines, Taiwan
Subgenus *Soja* (Moench) F. J. Herm.				
23. *G. soja* Sieb. & Zucc.	SOJ	40	GG	China, Russia, Taiwan, Japan, Korea (Wild Soybean)
24. *G. max* (L.) Merr.	MAX	40	GG	Cultigen (Soybean)

† Genomically similar species carry the same letter symbols.
‡ Allopolyploids (A and B genomes) and segmental allopolyploids (B genomes).
§ Allopolyploids (D and E, A and E, or any other unknown combination).
¶ Allopolyploids (A and D genomes, or any other unknown combination).

with the terminal leaflet often larger than the lateral leaflets. Chasmogamous inflorescences are borne in the axils of the upper leaves. The corolla is mauve. The style is slightly curved, glabrous having a small capitate stigma. The pods contain two or three black seeds. The species occurs in Western Australia and the Northern Territory, Australia (Tindale, 1986b). The few accessions studied cytologically are diploid (2n = 40).

4–2.1.3 *Glycine argyrea* Tind.

Glycine argyrea is a twining herb having slender stems with a woody taproot. The stems are densely covered with downward pointing hairs. The leaves are pin-

nately trifoliolate and the leaflets are narrowly lanceolate. The inflorescences are axillary. The corolla is light purple. The style is slightly incurved having a terminal capitate stigma. Pods when ripe contain up to nine or more black seeds. The species is endemic to Queensland and New South Wales, Australia (Tindale, 1984). Accessions studied cytologically are diploid ($2n = 40$). This species has both cleistogamous and chasmogamous flowers on the same plant (Schoen and Brown, 1991). Using allozyme polymorphism as measured by starch gel electrophoresis, Brown et al.(1986) determined that natural outcrossing via insect pollination is common in the species.

4–2.1.4 *Glycine canescens* F.J. Herm.

Glycine canescens is restricted to Australia. It is a twining herb with pinnately trifoliolate leaves. The leaflets are elliptic-linear to oblong-lanceolate and pinnately veined. The whole plant processes a hoary, silky-strigose pubescence which produces a silvery appearance in extreme cases. Flowers are pink and pods are linear. Plants are diploid ($2n = 40$) (Hymowitz and Singh, 1987).

4–2.1.5 *Glycine clandestina* Wendl.

Glycine clandestina is a slender twiner restricted to Austrialia. Leaves of *G. clandestina* are digitate; the leaflets range from ovate-lanceolate or obong to linear, and exhibit reticulate venation. Flowers vary in color from pale pink to rose-purple. The accessions studied cytologically are diploid ($2n = 40$) (Hymowitz and Singh, 1987).

4–2.1.6 *Glycine curvata* Tind.

Glycine curvata is a trailing herb having a woody taproot. The leaves are weakly pinnately trifoliolate, and the terminal leaflet is usually slightly larger than the lateral leaflets. The leaflets are very narrowly elliptical to very narrowly lanceolate. The standard is white to cream. First seedling leaves are unifoliolate. The pods are narrowly oblanceolate, curved, and contain five to seven seeds. The seeds are black. The species is endemic to Queensland, Australia (Tindale, 1986a). The plants are diploid ($2n = 40$).

4–2.1.7 *Glycine cyrtoloba* Tind.

Glycine cyrtoloba is a twining herb having a woody taproot. The leaves are pinnately trifoliolate. The leaflets are narrowly lanceolate, ovate, or elliptical. The terminal leaflet is slightly larger than the two lateral leaflets. The standard is pink to purple. The first seedling leaves are trifoliolate. The pods are oblong to oblong-linear, curved, and contain three to nine seeds. The seeds are dark brown to almost black. The plants are diploid ($2n = 40$) (Newell and Hymowitz, 1978). The species is endemic to Queensland and New South Wales, Australia (Tindale, 1984).

4–2.1.8 *Glycine dolichocarpa* Tateishi et Ohashi

Glycine dolichocarpa is a twining or prostrate herb. The hairs on the stems and petioles are deflexed. The leaves are pinnately trifoliate. The leaflets are lanceolate to ovate. The stems and leaves have numerous long tawny hairs. The species has both chasmogamous and cleistoganous flowers. The flowers are pinkish. The pods are linear with constriction between seeds. The five to nine seeds are dark brown or black. The few accessions studied cytologically are polyploid ($2n = 80$).

The species described was collected in Taiwan by Ohashi et al. (1991) and Tateishi and Ohashi (1992). However, it is very likely that the species is endemic to Australia (Hymowitz, 1990) and has been naturalized on Taiwan (Yeh et al., 1997).

4–2.1.9 *Glycine falcata* Benth.

Glycine falcata is restricted to Australia. It has a decumbent or erect growth habit and strigose pubescence. Flower recemes are long and stock with white to pale lilac flowers. Pods are broadly falcate and hirsute-strigose with oblong or ovoid seeds. In addition, the plant contains pods on underground rhizomes. The few accessions studied cytologically are diploid ($2n = 40$) Hymowitz and Singh (1987).

4–2.1.10 *Glycine hirticaulis* Tind. and Craven

Glycine hirticaulis (Tindale and Craven, 1988) is a nonstoloniferous and non-rhizomatous herb. Opportunistic amphicarpy occurs in the species. The leaves are digitately trifoliolate. The leaflets are linear with the middle leaflet longer than the two laterals. Plants have both chasmogamous and cleistogamous seed. The chasmogamous flowers are mauve. The pods contained one to three seeds, not curved. The seeds are purplish black. The few accessions examined are diploid or polyploid ($2n = 40, 80$). The species is endemic to the Northern Territory, Australia.

4–2.1.11 *Glycine lactovirens* Tind. and Craven

Glycine lactovirens is a prostrate nonstoloniferous herb (Tindale and Craven, 1988). The leaves are digitately trifoliolate. The leaflets are light green, narrowly obovate or elliptical. The middle leaflet is larger than the two lateral leaflets. The flower color is milky green. The pods contain two to four black seeds. The few accessions examined cytologically are diploid ($2n = 40$). The species is endemic to Western Australia, Australia. Under greenhouse conditions at the University of Illinois we have not succeeded in the multiplication of seed.

4–2.1.12 *Glycine latifolia* (Benth.) Newell and Hymowitz

Glycine latifolia is a trailing or twining species having long robust stems. It roots freely form stolons. The leaves are pinnately trifoliolate with large rhombic leaflets. The long inflorescence contains flowers from lavender to purple. The species sets seeds from chasmogamous and cleistogamous flowers. The straight pods contain two to four seeds. Accessions studied cytologically are diploid ($2n = 40$). The species is restricted to eastern Australia and shows promise as a pasture legume on clay soils in tropical and subtropical Australia (Newell and Hymowitz, 1980a; Jones et al., 1996).

4–2.13 *Glycine latrobeana* (Meissn.) Benth.

Glycine latrobeana is an uncommon species found in the Australian States of South Australia, Tasmania, and Victoria. The species is considered vulnerable

and has been placed on the List of Threatened Australian Flora (ANZECC, 1993). It is a small herb with a compact, decumbent or somewhat twining growth habit and a thickened tap root. The leaves are digitately trifoliolate with obovate or suborbicular leaflets. The flowers are pink to purple. The pods contain three to five cylindrical shaped seeds. Accessions studied cytologically are diploid ($2n = 40$). Under greenhouse conditions at the University of Illinois, seed multiplication has been accomplished, but only rarely and with difficulty (Lynch, 1994).

4–2.1.14 *Glycine microphylla* (Benth.) Tind.

Glycine microphylla (Tindale, 1986b) has prostate stems that can root at the nodes. The stems are almost glabrous. The upper leaves are weakly pinnately trifoliolate. The leaflets vary from obovate, narrowly elliptical to broadly elliptical. The middle leaflet is often larger than the lateral leaflets and has both chasmogamous and cleistogamous inflorescences. The flowers are usually purple. The straight pods have four to six black seeds. Accessions studied cytologically are diploid ($2n = 40$). The species is native to Australia (primarily the eastern states), but also has been collected on Norfolk Island.

4–2.1.15 *Glycine pindanica* Tind. and Craven

Glycine pindanica (Tindale and Craven, 1993) is a climbing or creeping nonrhizomatous and nonstoloniferous species. Opportunist amphicarpy (Tindale and Craven, 1988) occurs in the species. The leaves are trifoliolate. The central leaflet is larger than the lateral leaflets. Chasmogamous and cleistogamous inflorescences are present. The flowers are light purple to dark purple. Pods have two to five seeds. The few accessions studied cytologically are diploid ($2n = 40$). The species appears to be restricted to the Pindan region of Western Australia, Australia.

4–2.1.16 *Glycine stenophita* B. Pfeil and Tind.

Glycine stenophita (Doyle et al., 2000) is a nonstoloniferous scrambling or climbing herb. The leaves are pinnately trifoliolate. The terminal leaflets are usually slightly larger than the lateral leaflets. The stem hairs are white. The flowers are pink to purple. The pods are straight and contain from three to seven seeds. The few accessions studied cytologically are diploid ($2n = 40$). The species is found in southern Queensland and northern New South Wales, Australia.

4–2.1.17 *Glycine tabacina* (Labill.) Benth.

Glycine tabacina is found in Australia, China, West Central and South Pacific Islands (Hymowitz and Singh, 1987; Li et al., 1983). Recently, the species was collected on the Islet of Kinmen (Quemoy) (Yeh et al., 1997; Hymowitz, 1998). The stems trail or twine and bear pinnately trifoliolate leaves. The leaflets have reticulate venation. The deep rose-purple flowers often are fragrant. Pods are stout and linear with oblong or ovoid black, sometimes brown seeds. Diploids ($2n = 40$) and tetraploids ($2n = 80$) have been reported (Hymowitz and Singh, 1987).

4–2.1.18 *Glycine tomentella* Hayata

Glycine tomentella is an extremely variable species distributed in Australia, China (Li et al., 1983), Papua New Guinea, Philippines, and Taiwan. Recently, the species was collected on the Islet of Kinmen (Quemoy)(Hymowitz 1998; Yeh et al., 1997). Four cytotypes have been reported for *G. tomentella* ($2n = 38, 40, 78, 80$). The polyploids form a species complex (Singh et al., 1987b). Hill (1998) conducted an extensive morphological and biochemical analysis of the diploid *G. tomentella*. He concluded that the currently delimited diploid *G. tomentella* ($2n = 38, 40$) is not monophyletic and suggested that the species be divided into four separate species: (i) A central and south Queensland group containing 38 chromosomes; (ii) A northern Queensland-Papua New Guinea group; (iii) A central Queensland big pod group; and (iv) A group of accessions from Western Australia -Northern Territory.

4–2.2 Subgenus *Soja*

The subgenus *Soja* includes the cultivated soybean, *G. max* and *G. soja*, the wild annual soybean (Table 4–2).

4–2.2.1 *Glycine soja* Sieb. and Zucc.

Glycine soja grows wild in the China, Japan, Korea, Russia, and Taiwan. It grows in fields, hedgerows, along roadsides, and riverbanks. The plant is an annual procumbent, or slender twiner having pinnately trifoliolate leaves and often tawny, strigose, or hirsute pubescence. The leaflets may be narrowly lanceolate, ovate, or oblong-elliptic. The purple or very rare white flowers are inserted on short, slender racemes. The pods are short with a strigose to hirsute pubescence and oval-oblong seeds (Hermann, 1962). Plants are diploid ($2n = 40$).

Ohashi (1982) and Ohashi et al. (1984) proposed that the scientific name of the annual wild soybean be changed to *G. max* (L.) Merr. subsp. *soja* (Sieb. and Zucc.) Ohashi. Although the International Legume Database (ILDIS) in the United Kingdom has accepted this proposal in nomenclature (http://www.ildis.org), the U.S. National Plant Germplasm System (USDA-GRIN) has not (http://www.ars.grin. gov).

Glycine max (Merr.) L. subsp. *formosana* (Hosokawa) Tateishi and Ohashi appears to be a form of *G. soja* having abnormally narrow leaflets, and smaller pods (Tateishi and Ohashi, 1992; Thseng et al., 2000).

In this chapter, we will continue to use *G. soja* as the scientific name of the annual wild soybean. The primary reason for conserving the name is that *G. soja* is so well known and hundreds of manuscripts have been published under that name. Secondly, unless there are compelling reasons to make the change, no benefit can be gained for the adaptation of a new name for the wild annual soybean.

4–2.2.2 *Glycine max* (L.) Merr.

Glycine max (L.) Merr., the cultivated soybean, is a true domesticate. In the absence of human intervention, the species would not exist. It is an annual that generally exhibits an erect, sparsely branched, bush-type growth habit with pinnately

trifoliolate leaves. The leaflets are broadly ovate, but can be oval to elliptic-lance-olate. The purple, pink, or white flowers are borne on short axillary racemes or re-duced peduncles. The pods are either straight or slightly curved, usually hirsute. The one to three seeds per pod are usually ovoid to subspherical. Seed coats of com-mercial cultivars are yellow. However, the germplasm collections contain soybean with seed coats having olive green, buff, brown, and black to reddish black color-ing. A 100-seed sample of commercial cultivars has a mass of about 10 to 20 g. How-ever, some accessions in the soybean germplasm collection have seeds with a mass of up to 50 g.

The soybean is morphologically extremely variable. This is due primarily to the development of soybean land races in East Asia. Individual farm families have grown soybean containing specific traits generation after generation for use as food, feed, medicinal, religious or ceremonial value. Today, these land races provide the major sources of genetic diversity within soybean germplasm collections. However, these land races or other unusual strains should not to be considered exotic. The term exotic applies only to the wild perennial and annual species (Singh and Hy-mowitz, 1999).

The subgenus *Soja* contains, in addition to *G. max* and *G. soja*, a form known as *G. gracilis*. This semi-cultivated or weedy form known only from Northeast China is somewhat intermediate in morphology between *G. max* and *G. soja* and was pro-posed as a new species of *Glycine* by Skvortzow (1927). However, Hermann (1962) considered *G. gracilis* a variant of *G. max*, a thesis supported by Wang (1976) and Shoemaker et al. (1986). Crossability barriers were not observed in hybrids among *G. soja*, *G. max*, and *G. gracilis* because putative crossed pods matured in the plants, hybrid seed germinated normally, and F_1 plants were totally pollen- and seed-fer-tile (Singh and Hymowitz, 1989). Recently, the taxonomic position of *G. gracilis* was again questioned by Wu et al. (2001). They used SSR markers to evaluate ge-netic diversity among *Glycine* species. They concluded that *G. gracilis* should be separated from *G. max*. However, neither ILDIS nor USDA-GRIN recognizes *G. gracilis* as a species. Thus, the continued use of the gracilis ephithet is not warranted.

4–3 GENERIC RELATIONSHIPS

The genus *Glycine* Willd. is a member of the family Leguminosae, subfam-ily Papilionoideae, and tribe Phaseoleae. The Phaseoleae is the most economically important tribe of the Leguminosae. It contains members that have considerable im-portance as sources of food and feed, such as soybean [*Glycine max* (L.) Merr.]; pigeon pea [*Cajanus cajan* (L.) Millsp.]; sweet-hyacinth bean [*Lablab purpureus* (L.)]; common bean, lima bean, and tepary bean (*Phaseolus* spp.); winged bean [*Psophocarpus tetragonolobus* (L.) DC.]; and cowpea, mung bean, black gram, adzuki bean, and Bambarra groundnut (*Vigna* spp.).

Within the tribe Phaseoleae, Lackey (1981) recognized 16 genera as be-longing to the subtribe Glycininae. These include *Glycine* as well as *Amphicarpaea* Nutt., *Cologania* Kunth., *Dumasia* DC., *Diphyllarium* Gagnep., *Eminia* Taub., *Mastersia* Benth., *Neonotonia* Lackey, *Nogra* Merr., *Pseudeminia* Verdc., *Pseudovi-gna* Verdc., *Pueraria* DC., *Shuteria* W. and A., *Sinodolichos* Verdc., *Teramnus* P.Br., and *Teyleria* Backer.

Table 4–3. The genera within Glycinae, approximate number of species, chromosome numbers, and geographic distribution. (Adapted from Kumar and Hymowitz, 1981; Lackey, 1981; Lee and Hy-mowitz, 2001.)

General	No. of species	2n	Geographic distribution
Amphicarpaea	3	20, 22, 40	Asia, Africa, and North America
Calopogonium	8	36	South and Central America
Cologania	10	44	Mexico, Central and South America
Dumasia	8	--	Asia and Africa
Diphyllarium	1	20	Indochina
Eminia	5	22	Tropical Africa
Glycine	24	38, 40, 78, 80	Asia and Australia
Mastersia	2	22, 44	Indo-Malaya
Neonotonia	2	22	Africa to Asia
Nogra	3	22	Asia
Pachyrhizus	6	22	Neotropics
Pseudeminia	4	22	Tropical Africa
Pseudovigna	1	22	Tropical Africa
Pueraria	20	22	Asia
Shuteria	5	22	Indo-Malaya
Sinodolichos	2	--	Asia
Teramnus	8	28	Pantropical
Teryleria	1	44	Asia

Viviani et al. (1991) conducted a phenetic analysis of the tribe Phaseoleae. They scored 126 morphological characters and included 11 taxa from eight genera of Glycininae sensu Polhill (1994). Their results revealed that Glycininae was not clustered into a single group because several taxa of Clitoriinae, Diocleinae, and Kennediinae were included within the Glycininae clade. They suggested that the most similar genus to *Glycine* was *Pueraria*, a genus that was considered primitive among the Glycininae by Lackey (1977).

Phylogenetic relationships were investigated within the tribe Phaseoleae based on chloroplast DNA (cpDNA) restriction site mapping of the inverted repeat regions (Doyle and Doyle, 1993; Bruneau, et al., 1994). Glycininae was represented by 12 genera from Lackey's (1977) subtribe Glycininae. The cp DNA restriction study suggested that Glycininae sensu Lackey (1981) are not monophyletic with *Calopogonium* and *Pachyrhizus* of the subtribe Doicleinae arising within the Glycininae clade and with *Shuteria* placed outside of Glycininae. However, the sis-ter genus or genera to *Glycine* within the Glycininae was not clearly resolved. On the basis of these restriction site-based phylogenies, Polhill (1994) transferred *Calopogonium* and *Pachyrhizus* from the subtribe Diocleinae sensu Lackey (1977, 1980) to Glycininae and reorganized 18 genera within Glycininae (Table 4–3).

Lee and Hymowitz (2001) conducted a vigorous analysis of phylogenetic re-lationships within the subtribe Glycininae inferred from cpDNA *rps16* intron se-quence variation. Samples from 15 genera were included in the study. Missing were samples from *Diphyllarium*, *Mastersia*, and *Sinodolichos*. Phylogenies estimated using parisomony, neighbor-joining and maximum likelihood methods clearly re-vealed that (i) Glycininae is monophyletic if *Pachyrhizus* and *Calopogonium* are included within Glycininae; (ii) the genus *Teramnus* is closely related to *Glycine*, and *Amphicarpaea* showed sister relationship to the clade comprising *Teramnus* and *Glycine*; and (iii) the genus *Pueraria* regarded as a closely related genus to *Glycine* is not monophyletic and needs to be divided into at least four genera (Fig. 4–1).
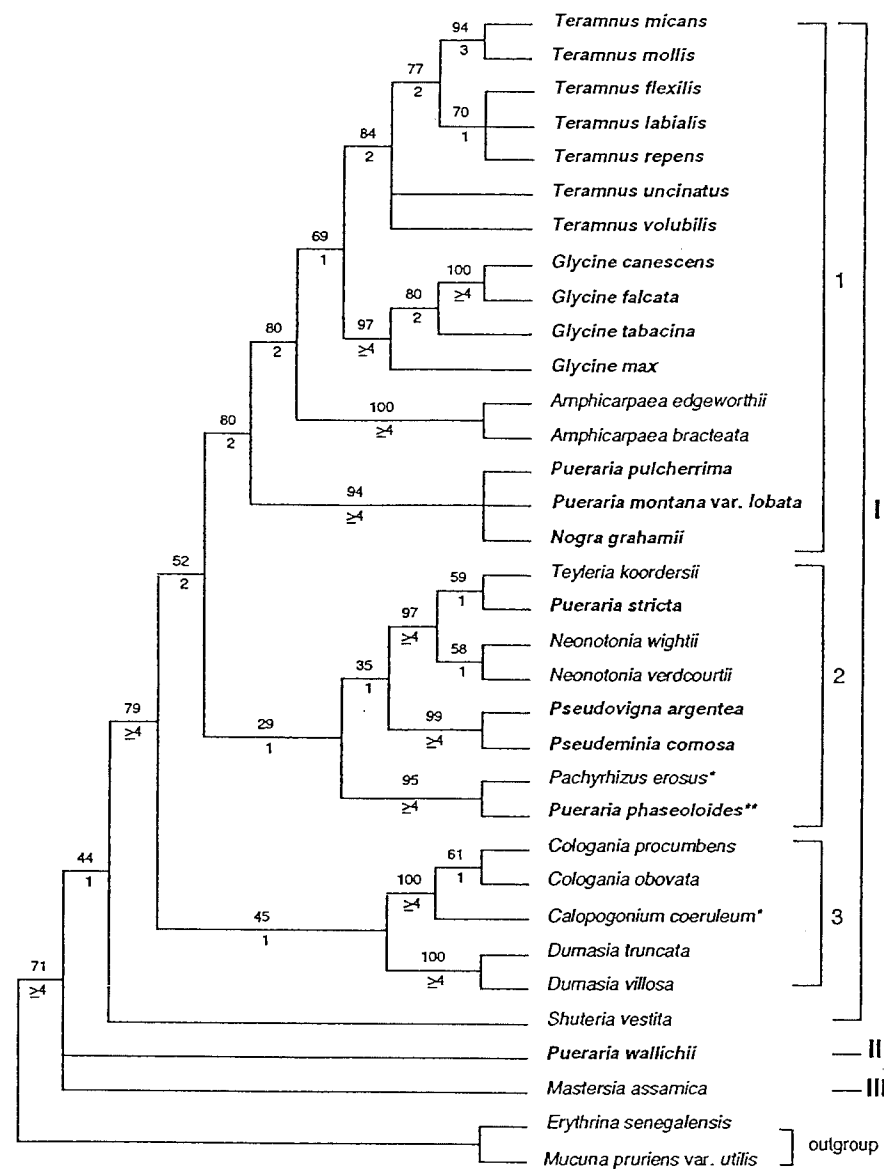
Fig. 4-1 (phylogenetic tree):

94 — Teramnus micans
3
77 — Teramnus mollis
2
Teramnus flexilis
70
84 — Teramnus labialis
2 1
Teramnus repens
Teramnus uncinatus
Teramnus volubilis
69
1
100 — Glycine canescens
80 — Glycine falcata
≥4
97 — Glycine tabacina
2
≥4 — Glycine max
80
2
100 — Amphicarpaea edgeworthii
≥4 — Amphicarpaea bracteata
94 — Pueraria pulcherrima
≥4 — Pueraria montana var. lobata
Nogra grahamii

52
2
59 — Teyleria koordersii
1
97 — Pueraria stricta
≥4
58 — Neonotonia wightii
35 1
1 — Neonotonia verdcourtii
29 99 — Pseudovigna argentea
1 ≥4 — Pseudeminia comosa
95 — Pachyrhizus erosus*
≥4 — Pueraria phaseoloides**

79
≥4
44
1
61 — Cologania procumbens
100 1
≥4 — Cologania obovata
45 — Calopogonium coeruleum*
1
100 — Dumasia truncata
≥4 — Dumasia villosa
Shuteria vestita

71
≥4
Pueraria wallichii — II
Mastersia assamica — III
Erythrina senegalensis
Mucuna pruriens var. utilis — outgroup

Groups: 1, I, 2, 3, II, III

Fig. 4–1. Strict consensus 402 step trees derived from equally weighted parsimony analysis of 34 un-ambiguously aligned chloroplast DNA rps16 intron sequences (consistency indices with and without uninformative characters = 0.769 and 0.627; retention index = 0.751). The numbers above the branches indicate the number of times a monophyletic group occurred in 100 bootstrap replicates. The numbers below the branches indicate the additional number of steps over 402 (the total number of steps in the shortest tree) needed to collapse that branch (decay values). Decay analysis with tree lengths >4 steps longer than the most parsimonious trees could not be performed owing to the computational constraints. Glycine groups of Lackey (1981) are in bold and asterisks indicate species included within Glycininae by Polhill (1994), but not by Lackey (1977a, 1981). Double asterisks indicate Pueraria phaseoloides var. phaseoloides. (Adapted from Lee and Hymowitz, 2001.)

4–4 ORIGIN OF THE GENUS GLYCINE

According to Goldblatt (1981), "The base number for Phaseoleae is almost certainly $x = 11$, which is also probably basic in all tribes." Goldblatt also pointed out that aneuploid reduction ($x = 10$) is prevalent throughout the Papilionoideae. Previously, Darlington and Wylie (1955) proposed a $x = 10$ basic chromosome number for the cultivated soybean. Based upon the above views and on recent taxonomic, cytological, and molecular systematics research on the genus Glycine and allied genera, we hypothesize that a putative ancestor of the genus Glycine with $2n = 20$ arose in Southeast Asia (Kumar and Hymowitz, 1989; Singh and Hymowitz, 1999; Lee and Hymowitz, 2001; Singh et al., 2001). However, such a progenitor is either extinct or yet to be collected and identified in Cambodia, Laos, or Vietnam (Fig. 4–2).

Tetraploidization ($2n = 2x = 40$) through auto- or allopolyploidy of the progenitor species occurred either prior to or after dissemination from the ancestral region. The progenitor of the wild perennial species radiated out southward, adapting to ecological niches in the Australian continent. These species were not domesticated (Fig. 4–2).

The path of migration northward (Fig. 4–2) from the ancestral region to China from a commmon progenitor is assumed by Singh et al. (2001) as: wild perennial ($2n = 4x = 40$, unknown or extinct) → wild annual ($2n = 4x = 40$; G. soja) → soybean ($2n = 4x = 40$; G. max, cultigen). All currently described species of the genus

Fig. 4-2 (flowchart):

Cultigen, annual ($2n = 4x = 40$) G. max
Domestication
Wild annual ($2n = 4x = 40$) G. soja
CHINA
Wild perennial (Extinct?) ($2n = 4x = 40$)
Auto- or allopolyploidization?
Putative ancestor of the genus Glycine ($2n = 2x = 20$) (Unknown?)
SOUTHEAST ASIA??
Auto- or allopolyploidization?
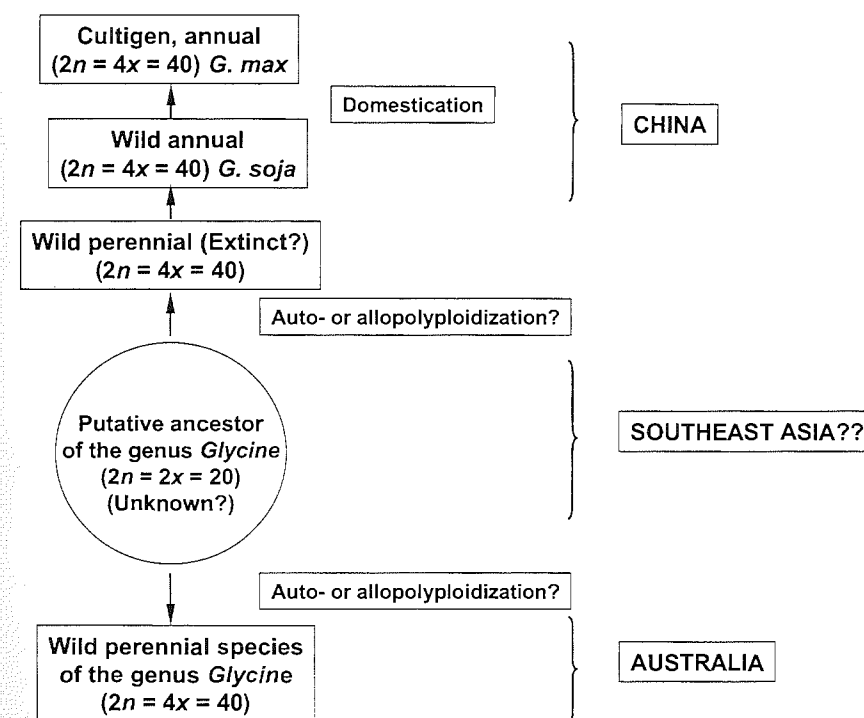Wild perennial species of the genus Glycine ($2n = 4x = 40$)
AUSTRALIA

Fig. 4–2. The origin of the genus Glycine.

Glycine exhibit diploid-like meiosis, are primarily inbreeders and produce cleistogamous seed (Singh and Hymowitz, 1985a).

Allopolyploidization (interspecific hybridization followed by chromosome doubling) via unreduced gametes probably played a major role in the speciation of the genus *Glycine*. This assumption infers that the 40-chromosome *Glycine* species and the 80-chromosome *G. tabacina* and *G. tomentella* are tetraploid and octoploid, respectively. The expression of four rDNA loci in *G. curvata* and *G. cyrtoloba* (Singh et al., 2001) strongly supports a hypothesis of allotetraploid origin that was originally proposed on the basis of cytogenetic evidence (Singh and Hymowitz, 1985a, 1985b; Xu et al., 2000a) and molecular studies (Lee and Verma, 1984; Shoemaker et al., 1996).

Hymowitz et al. (1990), based upon cytogenetic studies, hypothesized that the disjunct allopolyploid distribution of *G. tabacina* and *G. tomentella* between Australia and the islands of the west-central Pacific region was due to long-distance dispersal by migrating shore birds. That hypothesis was verified by Doyle et al. (1990a, 1990b), who examined chloroplast DNA and histone H3-D polymorphism patterns within the *G. tabacina* polyploid complex.

Long-distance dispersal of seeds by birds has been well documented (Carlquist, 1974). Currently, all *G. tabacina* and *G. tomentella* accessions collected on the West-Central Pacific Islands are polyploids. However, all diploids as well as the polyploids are found on the Papua New Guinea-Australian tectonic plate (Table 4–2). Hence, the question must be asked, "Do migrating shore birds selectively ingest seed containing polyploid *Glycine* cytotypes?" (Hymowitz et al., 1990). There is no evidence that diploid *Glycine* seeds are not carried by birds. However, the allopolyploid forms of *G. tabacina* and *G. tomentella*, unlike their diploid counterparts, are aggressive colonizing species (Singh and Hymowitz, 1985b). Thus the inbreeding tetraploids are able to compete successfully and establish themselves in the West-Central Pacific, where the diploids are unsuccessful. The dispersal process is a chance, but continuous annual event that, apparently, has occurred over many thousands of years. Today, the *G. tabacina* populations on the islands off the coast of Taiwan appear to be morphologically similar. However, the populations vary considerably as measured by random amplification of polymorphic DNA (RAPD) analysis (Thseng et al., 1997).

4–5 GENOMIC RELATIONSHIPS AMONG DIPLOID SPECIES

Genomic relationships (Fig. 4–3) among diploid species of the subgenus *Glycine* have been established by (i) cytogenetic analyses (Palmer and Hadley, 1968; Putievsky and Broué, 1979; Newell and Hymowitz, 1983; Grant et al., 1984a, 1986; Singh and Hymowitz, 1985b, 1985d; Singh et al., 1988, 1992b, 1997; Kollipara et al., 1993), (ii) biochemical techniques (Mies and Hymowitz, 1973; Broué et al., 1977; Vaughan and Hymowitz, 1984; Doyle and Brown, 1985; Doyle et al., 1986; Menancio and Hymowitz 1989; Brown, 1990; Domagalski et al., 1992; Kollipara et al., 1995), and (iii) molecular methods (Doyle and Beachy, 1985; Doyle and Brown, 1989; Doyle et al., 1990a, 1990b, 1990c, 1996, 1999a, 1999b; Menancio et al., 1990; Zhu et al., 1995b; Kollipara et al., 1997).

The concept of genome designation of the *Glycine* species was first proposed by Singh and Hymowitz (1985b). Based on hybridization success, hybrid seed viability, fertility of F_1 plants in intra- and interspecific hybrids and degree of meiotic chromosome pairing, the authors proposed the following genome symbols for six diploid ($2n = 40$) wild perennial species (Table 4–2, Fig. 4–3): *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; *G. tomentella* = DD). Due to its small pods and leaflets, *G. clandestina* - Sp was removed from *G. clandestina* and taxonomically renamed *G. microphylla* (Tindale, 1986b). In addition, all B- genome species (*G. microphylla*, *G. latifolia*, and *G. tabacina*) have adventitious roots, while the other species lack adventitious roots (Costanza and Hymowitz, 1987).

Genomically similar *Glycine* species are expected to hybridize, produce viable, vigorous, and fertile F_1 plants, and exhibit normal meiotic chromosome pairing at metaphase I. Still, certain hybrid combinations can show a chromatin bridge and an acentric fragment (paracentric inversion) at anaphase I. By contrast, in genomically dissimilar species...
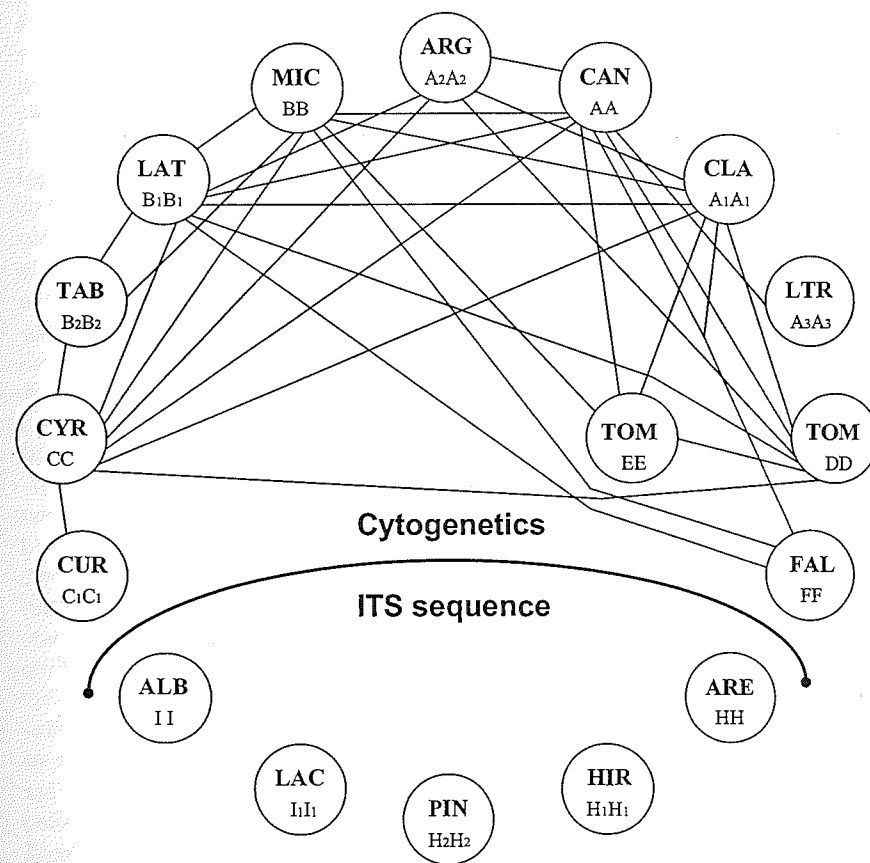

Fig. 4–3. Genomes of the wild perennial *Glycine* species. For the three-letter species abbreviations see Table 4–2.

nomically dissimilar *Glycine* species the interspecific crossability is extremely low, pod abortion is common, and 19- to 21-d old immature seeds must be germinated aseptically in vitro. F_1 hybrids are slow growing, morphologically weak, and completely sterile. The sterility is due to poor chromosome pairing. Furthermore, species distantly related usually produce inviable F_1 seeds, or premature death of germinating seedlings, and seedling and vegetative lethality (Singh et al., 1988, 1992b; Kollipara et al., 1993).

Interspecific crosses within the A-genome species (*G. canescens* = AA; *G. clandestina* = A_1A_1; *G. argyrea* = A_2A_2) or within the B-genome species (*G. microphylla* = BB; *G. latifolia* = B_1B_1; *G. tabacina* = B_2B_2) produced mature pods with normal seed set. F_1 plants were vigorous and completely fertile. Genome A displays partial genome affinities with B- and D-genome species, but a stronger genome homology with D than with B. Genome B shows no genomic affinity with D genome as F_1 hybrids were seedling lethal (Singh et al., 1988). *Glycine cyrtoloba* (C genome) shows slightly stronger (though possibably significant) affinity with B (B × C = 26.2 I + 6.9 II; B_1 × C = 29.9 I + 5.05 II) than with A (A_2 × C = 30.8 I + 4.6 II). Furthermore, A ×C and A_1 × C F_1 plants die prematurely (Singh, 1993). Grant et al. (1984b) reported a higher percentage of meiotic pairing (5.25 I + 17.3 II) in the F_1 hybrid between *G. canescens* and *G. latrobeana*. These results together with seed protein-banding profile similarities within *G. latrobeana, G. canescens, G. clandestina,* and *G. argyrea* prompted the assignment of genome symbol A_3A_3 to *G. latrobeana* (Singh et al., 1992b).

Successful production of hybrid seed is ordinarily a reliable indicator of genomic affinity between species. However, this was not true for *G. cyrtoloba* and *G. curvata*. These two species have curved pods, are morphologically very similar (Tindale, 1984, 1986a), and have similar banding patterns for chloroplast DNA (Doyle et al., 1990a, 1990b) and seed protein (Singh et al., 1992b). Pairwise divergence in the internal transcribed spacer (ITS) sequence of the nuclear ribosomal DNA between *G. cytroloba* and *G. curvata* was 1.3% which was significantly smaller than the mean percent distance between either one and any of other *Glycine* species (Kollipara et al., 1997). Despite close morphological and genetic affinity, the two species did not hybridize even though large number of flowers (748) were pollinated. This suggests that the crossability barrier between *G. curvata* and *G. cyrtoloba* probably are likely physiological than genetic (Singh et al., 1992b). Investigations on cytogenetics (Singh et al., 1988), rDNA loci (Singh et al., 2001), seed protein-banding profiles (Singh et al., 1992b), chloroplast DNA variation (Doyle et al., 1990a, 1990b), and nucleotide sequence variation in the ITS region of nuclear ribosomal DNA (Kollipara et al., 1997) suggest that *G. cyrtoloba* and *G. curvata* contain similar genomes and differ genomically from the other species of the genus *Glycine* (Fig. 4–3).

Intraspecific F_1 hybrids among *Glycine* species usually show normal meiosis and are completely fertile. However, *G. tomentella* ($2n$ = 38, 40) is an exception. Morphologically, the 38- and 40-chromosome accessions of *G. tomentella* are indistinguishable. Based on isozyme differences, Doyle and Brown (1985) proposed separating 38-chromosome *G. tomentella* into two groups D1 and D2 (D = diploid), and that 40-chromosome *G. tomentella* be divided into six isozyme groups D3A, D3B, D3C, D4, D5, and D6. Cytogenetic and biochemical results have shown that

isozyme groups D1 and D2, and D3A, D3B, and D3C are genomically similar (Kollipara et al., 1993). Isozyme D4 group is much closer genomically to A-genome species than to D1, D2, D3, and D5 groups of *G. tomentella* (Singh et al., 1988; Kollipara et al., 1993). Tindale (1986b) split off the D6 group of *G. tomentella* from the Eastern Kimberley District of Western Australia and described it as a new species, *G. arenaria* (Fig. 4–3).

Based on cytogenetic information, Singh et al. (1988) assigned genome symbol EE to *G. tomentella* accessions with $2n$ = 38 chromosomes because in the D3 × E cross at metaphase I, the average chromosome association (range) was 26.6 I (19–33) + 6.2 II (3–10). In these crosses, bivalents are usually rod shaped and are loosely associated at metaphase I. Further cytogenetic and molecular studies (Kollipara et al., 1993, 1997) suggested that the 40-chromosome *G. tomentella* is a species complex. Despite morphological similarity among diploid *G. tomentella* accessions, isozyme D3 group was designated as the true *G. tomentella*. The D4 group accessions are variants close to A-genome species, while the D5 group includes highly heterogeneous accessions from the Western Australia (Singh et al., 1998a). These are tentative desgnations, since classical taxonomy does not split the 38- and 40-chromosome *G. tomentella* into two species.

Of the 22 wild perennial species of the subgenus *Glycine, G. falcata* is unique. It differs from the other species in several morphological traits (Hermann, 1962; Hymowitz and Newell, 1975; Newell and Hymowitz, 1978), seed protein composition (Mies and Hymowitz, 1973; Singh et al., 1992b), presence and absence of leaf flavonoids and isoflavonoids (Vaughan and Hymowitz, 1984), seed oil and fatty acid content (Chaven et al., 1982), 5S ribosomal RNA (Doyle and Beachy, 1985), phytoalexin production (Keen et al., 1986), and sequences from the ITS region of nuclear rDNA (Kollipara et al., 1997). Chloroplast DNA data suggests that *G. falcata* should be grouped with species containing the A-chloroplast (plastome) genome (Doyle et al., 1990a, 1990b). However, cytogenetic results do not support this conclusion. *Glycine falcata* showed negligible chromosome homology with the A- and B-genome species and could not be hybridized with the other species (Putievsky and Broué, 1979; Singh et al., 1988). This information led to the assignment of genome symbol FF to *G. falcata* (Singh et al., 1988) (Fig. 4–3).

Genomic relationships of five described diploid species, *G. albicans, G. arenaria, G. hirticaulis, G. lactovirens,* and *G. pindanica* (Tindale, 1986a; Tindale and Craven, 1988, 1993) were not established by cytogenetics. These species are narrowly distributed in Western Australia and do not grow well under greenhouse conditions at Urbana, IL. Kollipara et al. (1997) sequenced the ITS region of the rDNA of 16 wild perennial species of the subgenus *Glycine* and the two annual species of the subgenus *Soja*. Phylogenetic analysis of the ITS region clearly resolved all the genomic groups and verified the cytogenetic results. They assigned new genome symbols HH to *G. arenaria*, $H_1 H_1$ to *G. hirticaulis*, $H_2 H_2$ to *G. pindanica*, II to *G. albicans*, and I_1I_1 to *G. lactovirens* (Fig. 4–3).

Doyle (1991) eloquently reviewed his collaborative research activities utilizing chloroplast DNA (cpDNA) for phylogenetic studies in the genus *Glycine*. Of the 12 perennial *Glycine* species studied, he identified distinct groups of plastomes. The B and C plastomes were congruent with the BB and CC nuclear genome groups of Hymowitz et al. (1998). For contrast, the A plastome group included the AA, DD,

EE, and FF nuclear groups of Singh and Hymowitz (1985b) resulting in apparent incongruence between the cpDNA phylogenetic studies and classical morphological and cytogenetic based taxonomic groupings (Doyle et al., 1999a, 1990b). However, in all cases, the morphological, cytological, and molecular variation seen in the sequenced ITS region of the rDNA were concordant (Kollipara et al., 1997). In this chapter, the genome designations for all the species in the genus *Glycine* primarily are those assigned by the authors of this chapter, and are based upon classical taxonomy and cytogenetics (Table 4–2, Fig. 4–3).

The genome symbol assigned to the recently name of species, *G. stenophita* is B3B3 (A.J. Lee, personal communication, 2001). This was determined similarly to the procedure that Kollipara et al. (1997) reported. The genomic relationship of 80-chromosome *G. dolichocarpa* is unknown; however, plant morphology suggests this species is related to 80-chromosome *G. tomentella*.

4–6 POLYPLOID COMPLEXES

Based on meiotic chromosome pairing in intra- and interspecific F_1 hybrids, Singh and Hymowitz (1985b) proposed that the tetraploid *G. tabacina* and *G. tomentella* are polyploid species complexes and have probably originated through allopolyploidization. These species behave like diploids, that is, chromosomes pair as bivalents (Singh and Hymowitz, 1985a). In contrast to their diploid ($2n = 38$ or 40) counterparts, tetraploid tabacinas and tomentellas are morphologically diverse, have wide geographical distributions, possess aggressive growth habits, and carry immense heterogeneity. These are characteristic features of allopolyploid species complexes (Singh et al., 1987b).

4–6.1 Glycine tabacina (2n = 80)

Accessions of tetraploid *G. tabacina* in the *Glycine* collection include at least two distinct morphological groups: one with adventitious roots and one without (Costanza and Hymowitz, 1987). Accessions without adventitious roots have longer and narrower leaves than those accessions with adventitious roots. Intraspecific F_1 hybrids within a group were highly fertile. All F_1 plants between groups showed no adventitious roots, suggesting that adventitious rooting is a recessive trait. F_1 hybrids were weak, slow growing, and sterile. This sterility was attributed to disturbed meiotic chromosome association. The mean number of bivalents in the eight hybrid combinations ranged from 14.5 to 19.6 (Singh et al., 1992a). Numerous univalents lagged at the equatorial plate during anaphase I, resulting in an unbalanced chromosome number in male and female spores, which caused sterility.

Singh et al. (1987b) studied meiotic pairing in F_1 hybrids between synthesized allopolyploids (BBB$_2$B$_2$, AABB) and *G. tabacina* ($2n = 80$) accessions with and without adventitious roots. Meiotic pairing of F_1s between the synthesized allopolyploid (BBB$_2$B$_2$) and *G. tabacina* accessions without adventitious roots revealed 40 bivalents at metaphase I and normal seed fertility. Thus, accessions with the adventitious roots are segmental allopolyploids. The genomes may be in any possible combinations (BBB$_1$B$_1$, BBB$_2$B$_2$, B$_1$B$_1$B$_2$B$_2$,) involving only B-genome

diploid species. On the other hand, *G. tabacina*, lacking adventitious roots, are true allotetraploids and may constitute any combination of A- and B-genome species (Singh et al., 1992b). The 38-chromosome *G. tomentella* has no genomic affinity with *G. tabacina*, and likewise, *G. latifolia* did not donate its genome to 80-chromosome *G. tomentella* (Singh et al., 1987b).

Menancio and Hymowitz (1989) used isozyme variation to differentiate diploid and tetraploid tabacinas. The number of bands in tetraploids was always greater than in the diploids. Similarly, by using the soybean Bowman-Birk inhibitor as a genome marker, Kollipara et al. (1995) separated tetraploid tabacinas with and without adventitious roots (Costanza and Hymowitz, 1987). Blackhall et al. (1991) and Hammatt et al. (1991) used flow cytometry to measure DNA content in *G. tabacina*. They were able to differentiate diploid from tetraploid tabacinas. With this technique, chromosome counts may not be needed to separate the cytotypes of *G. tabacina*.

4–6.2 Glycine tomentella (2n = 78, 80)

Based on cytogenetics, Singh and Hymowitz (1985b, 1985d) proposed that the aneutetraploid ($2n = 78$) and tetraploid ($2n = 80$) *G. tomentella* are polyploid species complexes, a proposal supported by additional cytogenetic (Singh et al.,1987b, 1989; Kollipara et al., 1994) and molecular data (Grant et al., 1984a; Doyle and Brown, 1989; Doyle et al., 1990c, 1990d; Kollipara et al., 1994). Isozyme banding patterns indicate three groups (T1, T5, and T6) in aneutetraploid, and three groups (T2, T3, and T4) in tetraploid, *G. tomentella* (Doyle and Brown, 1985; Doyle et al., 1986). Kollipara et al. (1994) tentatively assigned Indonesian accessions to the T7 group based on cytogenetic, biochemical, and molecular studies.

Cytogenetic results, total seed protein profiles, trypsin and chymotrypsin inhibitor migration patterns, anti-KTI, anti-BBI, and anti-SBL immunocrossreactive protein profiles, and RFLP analyses strongly supported three distinct groups in aneutetraploid and four groups in tetraploid *G. tomentella* (Kollipara et al., 1994, 1995). Meiotic chromosome pairing between the synthesized amphidiploid and the aneutetraploid or the tetraploid *tomentella* accessions revealed the genomic constitutions of T1(D3D3EE), T5(AAEE), and T2 (AAD3D3).

The origin of allopolyploidy in 78- and 80-chromosome *G. tomentella* was described by Singh et al. (1987b, 1989). Phylogenetic analyses clearly demonstrated that both 78- and 80-chromosome *G. tomentella* had a common genome. Hybrids among various diploid species were synthesized to determine which diploid ancestors gave rise to polyploid *G. tomentella*. The synthesized amphidiploids (Singh et al., 1987b) were crossed to the T1 through T7 group *G. tomentella* accessions. Morphology, cytology, and seed protein profiles of F_1s showed that the T1 group ($2n = 78$) originated through allopolyploidization of D3 and E genomes of *G. tomentella,* and the T5 group ($2n = 78$) was derived from A- and E-genome species. The parents of 80-chromosome *G. tomentella* from Queensland, Australia (T2 group) were determined to be *G. canescens* (AA) and *G. tomentella* (D3 D3). These results suggested that the isozyme groups in both aneutetraploid (T1, T5, and T6) and tetraploid accessions (T2, T3, T4, and T7) probably originated by multiple independent events. Reconstruction of hypothetical ancestors through production of

synthetic hybrids provide a technique to analyze this complex (Singh et al., 1989; Kollipara et al., 1994).

Blackhall et al. (1991) and Hammatt et al. (1991) used flow cytometry to measure DNA content in four *G. tomentella* cytotypes. They were able to distinguish diploids from tetraploids. However, they were not able to distinguish between the diploid ($2n = 38, 40$) nor the polyploid ($2n = 78, 80$) cytotypes.

Several investigators (Wang and Zhuang, 1994; Hui et al., 1997; Taylor-Grant and Soliman, 1999; Hsieh et al., 2001; Wu et al., 2001) have conducted phylogenetic or species variation experiments within the genus *Glycine*. The results of those studies were difficult to interpret because diploids (having one genome) were compared to polyploids (having two genomes) or because the experiment lacked standards for comparison.

4–7 GENE POOLS

Harlan and deWet (1971) proposed that the concept of three gene pools, primary (GP-1), secondary (GP-2), and tertiary (GP-3), be based on the success rate of hybridization among species. This system, as applied to *Glycine* (Fig. 4–4) is described as follows:

1. Primary Gene Pool (GP-1)
   GP-1 consists of biological species, and crossing within the gene pool is easy. The hybrids are vigorous, exhibit normal meiotic chromosome pairing, and possess total seed fertility; gene segregation is normal and gene
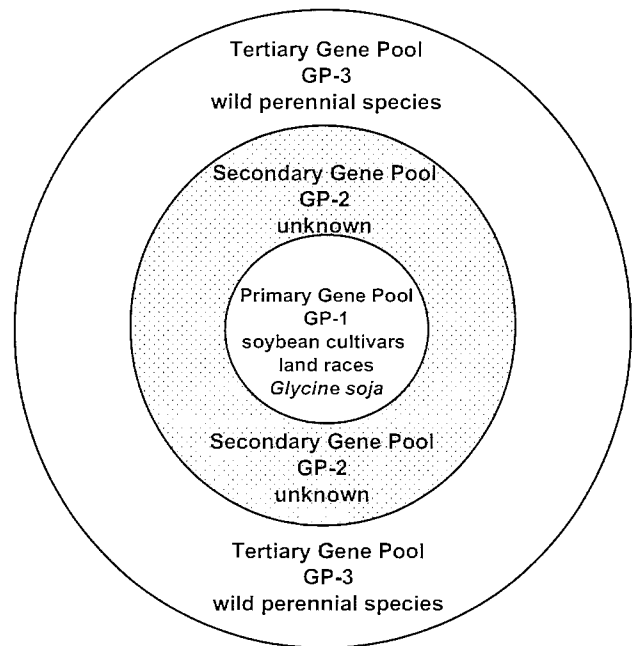
2. Secondary Gene Pool (GP-2)
   As defined by Harlan and deWet (1971), GP-2 consists of species that can be crossed with GP-1 and having some fertility in the F1. By this definition, *Glycine* does not have a GP-2.

3. Tertiary Gene Pool (GP-3)
   The GP-3 is the extreme outer limit of potential genetic resources. Hybrids between GP-1 and GP-3 are anomalous, lethal or completely sterile, and gene transfer is not possible or requires rescue techniques (Harlan and deWet, 1971). Based upon this definition, GP-3 includes the 22 wild perennial *Glycine* species of the subgenus *Glycine*. These species are indigenous to Australia and are geographically isolated from *G. max* and *G. soja*. Intersubgeneric hybrids and fertile-derived lines have been produced (Singh et al., 1990, 1993; Singh and Hymowitz, 1999).

4–8 INTERSUBGENERIC HYBRIDS

Ideally, soybean breeders would like to use the genetic diversity found in the wild perennial *Glycine* species to improve soybean yields. Before this can be accomplished, a method must be developed whereby the soybean can be hybridized with a wild perennial species and fertile lines can be derived from the cross. Several research teams have attempted to overcome species hybridization problems and genomic and fertility barriers (Table 4–4).

All of the intersubgeneric hybrids generated to date were obtained via embryo rescue techniques. The wild perennial *Glycine* parent(s) of subgenus *Glycine*, when used in successful hybrids with the soybean subgenus *Soja*, carried the AA, DD, and/or EE genomes (Hymowitz et al., 1998). It is unknown whether the intersubgeneric hybrid failures with AA, DD, and/or EE genomes might be due to lack of crossing effort rather than due to genomic incompatibility (Table 4–4). Chung and Kim (1991) reported a hybrid in a cross between *G. max* and *G. latifolia*. The F_1 carrying the genomes G and B_1 was sterile.

In general, all the F_1 intersubgeneric hybrid plants were vegetatively vigorous and exihibits a growth habit resembling that of the perennial parent. Meiotic analysis of sporophytic cells revealed that univalents predominated. The cells contained a few rod-shaped bivalents each with a terminalized chiasma. The occurrence of several loosely paired rod bivalents suggested the possibility of allosyndetic pairing (pairing between chromosomes of different genomes). All reported F_1 intersubgeneric hybrids were sterile.

When F_1 intersubgeneric hybrids were treated with colchicine (Cheng and Hadley, 1983; Kollipara et al., 1998), fertility was partially restored in the synthetic amphiploids (Newell et al., 1987; Singh et al., 1990). Bodanse-Zanettini et al. (1996) did not mention restored fertility in their $2n = 118$ synthetic amphiploid plant.

Thus far, only Singh et al. (1990, 1993) have reported successfully backcross-derived fertile progeny from the soybean and a wild perennial relative, *G. tomentella*. The soybean cv. Clark 63 was used as the recurrent parent in the backcrossing scheme. Procedures for embryo-rescue were used to obtain F_1, amphiploid,

Fig. 4–4. The gene pools of the soybean.

Table 4–4. Published intersubgeneric hybrids in the genus *Glycine* (Hymowitz et al. 1998).

Intersubgeneric Hybrids	Authors
1. [*G. tomentella* ($2n = 38$, EE) × *G. canescens* ($2n = 40$, AA)] × *G. max* ($2n = 40$, GG) = F_1 ($2n = 59$, EAG), sterile	Broué et al. (1982)
2. *G. max* ($2n = 40$, GG) × *G. tomentella* ($2n = 78$, DDEE) = F_1 ($2n = 59$, GDE), sterile *G. max* ($2n = 40$, GG) × *G. tomentella* ($2n = 80$, AADD) = F_1 ($2n = 60$, GAD) sterile	Newell and Hymowitz (1982)
3. *G. tomentella* ($2n = 78$, DDEE) × *G. max* ($2n = 40$, GG) = F_1 ($2n = 59$, DEG), sterile	Singh and Hymowitz (1985c)
4. *G. max* ($2n = 40$, GG) × *G. tomentella* ($2n = 80$, AADD) = Hybrid embryo cell, $2n = 64$, no plant	Sakai and Kaizuma (1985)
5. *G. argyrea* ($2n = 40$, A_3A_3) × *G. canescens* ($2n = 40$, AA) × *G. max* ($2n = 40$, GG) $F_1 \rightarrow$ CT $\rightarrow$ amphiploid ($2n = 80$), sterile	Grant et al. (1986)
6. *G. max* ($2n = 40$, GG) × *G. clandestina* ($2n = 40$, A_1A_1) = F_1 ($2n = 40$, GA_1) sterile	Singh et al. (1987a)
7. *G. max* ($2n = 40$, GG) × *G. tomentella* ($2n = 78$, DDEE) = F_1 ($2n = 59$, GDE) $\rightarrow$ CT $\rightarrow$ amphiploid ($2n = 118$, GG DDEE), partially fertile *G. canescens* ($2n = 40$, AA) × *G. max* ($2n = 40$, GG) = F_1 ($2n = 40$, AG) $\rightarrow$ CT $\rightarrow$ amphiploid ($2n = 80$, AAGG), sterile *G. tomentella* ($2n = 78$, DDEE) × *G. max* ($2n = 40$, GG) = F_1 ($2n = 59$, DEG) $\rightarrow$ CT $\rightarrow$ amphiploid ($2n = 118$, DDEEGG), sterile	Newell et al. (1987)
8. *G. max* ($2n = 40$, GG) × *G. tomentella* ($2n = 78$, DDEE) = F_1 ($2n = 59$, GDE), sterile	Coble and Schapaugh Jr. (1990) Shen and Davis (1992)
9. *G. max* ($2n = 40$, GG) × *G. tomentella* ($2n = $ AADD) = F_1, ($2n = 60$, GAD), sterile	Chung and Kim (1990) Kwon and Chang (1991)
10. *G. max* ($2n = 40$, GG) × *G. latifolia* ($2n = 40$, B_1B_1) = F_1, cytology not studied, sterile	Chung and Kim (1991)
11. *G. max* ($2n = 40$, GG) × *G. tomentella* ($2n = 78$, DDEE) = F_1 ($2n = 59$, GDE) $\rightarrow$ CT $\rightarrow$ amphiploid ($2n = 118$, GGDDEE), no further information	Bodanse-Zanettini et al. (1996)
12. *G. max* ($2n = 40$, GG) × *G. tomentella* ($2n = 78$, DDEE) = F_1 ($2n = 59$, GDE) $\rightarrow$ CT $\rightarrow$ amphiploid ($2n = 118$, GGDDEE) × *G. max* $\rightarrow$ BC_1 ($2n = 76$, expected = 79) $\rightarrow$ × *G. max* BC_2 ($2n = 58, 56, 55$; GG+D, E) × *G. max* $\rightarrow$ BC_3 -BC_6 ($2n = 40 + 1, 2, 3$; GG + 1D or 1E or 2E...), fertile lines (monosomic alien addition lines)	Singh et al. (1990, 1993)

BC_1, and BC_2 plants (Singh et al., 1987). The BC_1 plant obtained by backcrossing *G. max* to the synthetic amphiploid was male sterile and carried $2n = 76$ (expected $2n = 79$). Continued backcrossing resulted in three sterile BC_2 plants ($2n = 58, 56, 55$). The range of chromosome numbers among backcrossed BC_3 plants was $2n = 41$ to $2n = 52$ and among backcrossed BC_4 plants was $2n = 40$ to 64 (Fig. 4–5).

Because of extremely low crossability and early pod abortion, wild perennial *Glycine* species have not been exploited in soybean-breeding programs (Ladizinsky et al. 1979; Singh and Hymowitz, 1987). The success of Singh et al. (1990, 1993) vs. other scientists was simply due to two factors: (i) persistence in making crosses and (ii) solving the early pod abortion problem. A hormonal mixture was sprayed on pollinated gynoecia 24 h after pollination. The solution consisted of 100 mg GA, 25 mg NAA, and 5 mg kinetin per liter of distilled water. The solution was stored at 4°C (Singh et al., 1990).

Singh et al. (1998b) reported that they isolated 22 individual monosomic alien addition lines (MAALs, $2n = 41$) from fertile lines derived from the cross of the soybean × *G. tomentella* (Fig 4–5). These alien addition lines are excellent sources of economic traits, for example, resistance to pests and pathogens, for broadening the extremely narrow genetic base of the soybean.

Two papers regarding intersubgeneric hybridization have been omitted from Table 4–4. The first is an abstract and a paper by Hood and Allen (1980, 1987) who attempted to cross *G. max* and a wild perennial relative, *G. falcata*. The abstract has been cited by others as an example of successful hybridization, but no hybrid plants were obtained. The other paper is a report by Shoemaker et al. (1990) that vegetative cuttings from a synthetic amphiploid ($2n = 118$) obtained from *G. max* ($2n = 40$) × *G. tomentella* ($2n = 78$) (Newell et al., 1987), were transferred from the Monsanto Company to Iowa State University during October 1987. The origin of this reputed hybrid was examined in a M.S. thesis by Heath (1989). The plants were maintained in the greenhouse for 1 yr. One plant was reported to have 41 pods with more than 100 F_2 seeds. After further cytogenetic evaluation, it was concluded, "that the *G. tomentella* chromosome complement had been eliminated after genetic exchange and/or genetic modification has taken place between the two genomes." (Heath, 1989, p. 2). No other evidence for hybridity of this plant was presented. The purported hybridization could not be reproduced by the authors or other researchers, and thus may have been artefactual.

4-9 CYTOGENETIC TECHNIQUES

Since the 1980s cytological studies on the genus *Glycine* have drastically increased. The literature cited in the next section is extensive and thereby offers the opportunity for the reader to examine publications of interest. Palmer and Kilen (1987) reviewed the cytological literature up to 1984.

4-9.1 Mitotic Chromosomes

Singh (1993) provided the details of past protocols developed to count soybean mitotic chromosomes, for example, Palmer and Heer (1973). Xu et al. (1998a)

detailed a procedure for mitotic chromosome counts currently used in our laboratory. The procedure yields numerous cells with metaphase chromosomes that are well spread for precise counts.
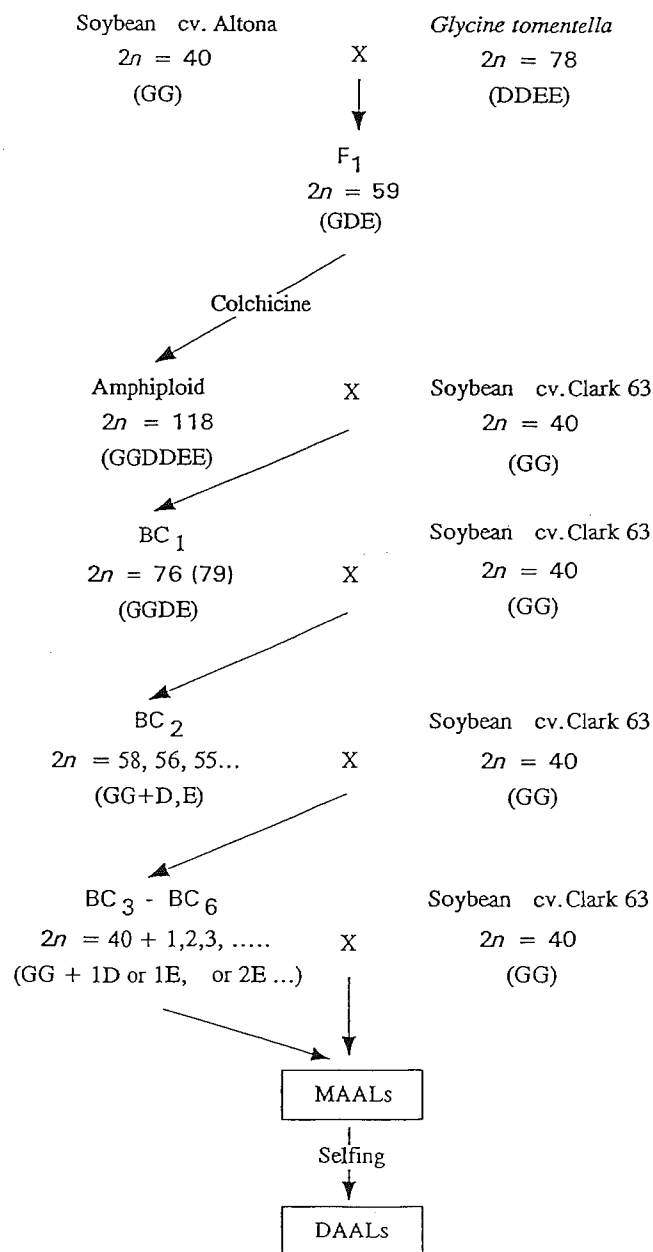
Soybean cv. Altona $2n = 40$ (GG) X Glycine tomentella $2n = 78$ (DDEE)

F_1 $2n = 59$ (GDE)

Colchicine

Amphiploid $2n = 118$ (GGDDEE) X Soybean cv. Clark 63 $2n = 40$ (GG)

BC_1 $2n = 76 (79)$ (GGDE) X Soybean cv. Clark 63 $2n = 40$ (GG)

BC_2 $2n = 58, 56, 55...$ (GG+D,E) X Soybean cv. Clark 63 $2n = 40$ (GG)

$BC_3 - BC_6$ $2n = 40 + 1,2,3, ...$ (GG + 1D or 1E, or 2E ...) X Soybean cv. Clark 63 $2n = 40$ (GG)

MAALs

Selfing

DAALs

Fig. 4–5. The route to successful backcrossed-derived fertile progeny from the soybean and a wild perennial relative, G. tomentella.

The soybean seeds were germinated in a sand bench in the greenhouse. Root tips from actively growing 7- to 10 d-old seedlings were collected in 1.5 mL microcentrifuge tubes containing double-distilled water. Root tips were pretreated with 0.05% 8-hydroxyquinoline for 4 to 5 h at 16°C in a micro-cooler. Pretreated root tips were fixed in a 3:1 (v/v) mixture of 95% ethanol and propionic acid for 24 h. The fixative was removed from the microcentrifuge tubes, and the root-tips were washed once with double-distilled water. Root tips were hydrolyzed in 1 M HCL for 11 to 15 min at 60°C. After hydrolysis, the root tips were rinsed in double distilled water and stained in Schiff's reagent (Fuchsin-sulfate reagent) for 2 to 4 h at room temperature in the dark. The Feulgen stain was removed and the root tops were rinsed with cold double-distilled water and stained with Carbol fuchsin stain overnight at 0 to 4°C in a refrigerator. After Carbol fuchsin staining, the root tips were washed three to four times with cold double-distilled water to remove phenol and stored in cold double-distilled water in a refrigerator. Root tips were squashed in 45% acetic acid on a clean glass slide.

4–9.2 Meiotic Chromosomes

Except for minor changes (Bione et al., 2000) the procedure reported by Singh and Hymowitz (1985a) appears to be generally used. Flower buds undergoing meiosis were fixed in a freshly prepared mixture of 3:1 absolute ethanol: propionic acid. Ferric chloride (1 g 100 mL^{-1} fixative) was added to the fixative to intensify staining of chromosomes. Buds were transferred to 70% ethanol after 48 h of fixation and stored under refrigeration. Anthers with meiotic stages were stained in 0.7% aceto-carmine for 7 d under refrigeration and squashes were made in 45% acetic acid.

4–9.3 Flow Cytometry

The DNA content of nuclei in Glycine species has been correlated to genome size and chromosome number as measured by a flow cytometer. Baranyi and Greilhuber (1996) have published the protocols for ethidium bromide flow cytometry.

Hammatt et al. (1991) isolated nuclei from cotyledons of accessions from 14 species of Glycine. The 4 C amounts for diploid Glycine ranged from 3.80 to 6.59 pg. The accessions were divided into two groups. The first group contained amounts ranging from 3.80 to 5.16 pg and included species from the A, B, D, E, G, and H genomes. The second group had DNA contents ranging from 5.27 to 6.59 pg and consisted of species from the C and F genomes. The polyploid species, G. tabacina ($2n = 80$) and G. tomentella ($2n = 78, 80$) contained amounts approximating to the sums of the respective parental diploid species. Thus flow cytometry easily distinguishes between diploid and tetraploid forms of either G. tabacina or G. tomentella.

Graham et al. (1994) reported a significant correlation between maturity and the genome size of 20 soybean cultivars. In addition, they found a 15% difference in genome size among the cultivars. On the other hand, Greilhuber and Obermayer (1997) observed no reproducible genome size difference among cultivars and a significant correlation with maturity group was not confirmed. Rayburn et al. (1997) reported a 12% range in variation in nuclear DNA content among 90 soybean lines

from China. Obermayer and Greilhuber (1999) observed no significant differences in the high-ranking DNA content vs. the low-ranking DNA content within soybean groups. Furthermore, no evidence was obtained for a difference in DNA content between Chinese and American soybean. Chung et al. (1998) reported a significant correlation between genome size and leaf and seed size in soybean. This intriguing finding has not, however, been independently confirmed.

For comparative measurements in flow cytometry, the growing conditions of experimental materials must be the same and identical conditions must be present during isolation and processing. The internal standards must be carefully controlled.

4–9.4 Fluorescent In-situ Hybridization

Fluorescent in-situ hybridization (FISH) is a physical mapping approach to detect specific DNA sequences in interphase nuclei or on condensed chromosomes of plants. Zhu et al. (1995a) published a detailed protocol for soybean. Thus far, the use of the procedure has been very limited within the soybean research community. Skorupska et al. (1989) located 18 S and 25 S rDNA sites in interphase cells. The in-situ hybridization results revealed that, for ribosomal RNA genes, G. max behaves as a diploid. Griffor et al. (1991) located rDNA sites in metaphase chromosomes of the soybean. In addition, the rDNA probe was detected in plants that were trisomics for the nucleolar-organizing region (NOR) containing chromosomes. Singh et al. (2001) examined the distribution of rDNA loci in 16 Glycine species by FISH using the internal transcribed spacer (ITS) region of nuclear ribosomal DNA as a probe. All species contained one rDNA site except G. curvata, G. cytoloba, G. tabacina (2n = 80) and G. tomentella (2n = 80) which had two rDNA sites. The study demonstrated that the distribution of the rDNA gene in the 16 Glycine species studied is highly conserved within the genus.

Shi et al. (1996) reported on the development of FISH, PCR-primed in-situ labeling (PCR-PRINS) procedures, and molecular probes for the cytological identification and physical mapping of soybean somatic chromosomes. They called the method soybean chromosome painting. The researchers demonstrated the utility of in-situ chromosome analysis for soybean by presenting examples of different DNA probes representative of particular sequence categories.

4–9.5 Sterility Systems

Various sterility systems have been reported in soybean. They have been classified as synaptic, structural, partial-male sterile or partial-female sterile, and completely male-sterile, female-fertile. The genetic control may be either nuclear or nuclear-cytoplasmic. Refer to Chapter 5 (Palmer et al., 2004, this publication) for information about the inheritance and gene symbols for specific male-sterile lines.

4–9.6 Diploid-like Meiosis

Chromosome pairing in wheat (*Triticum aestivum* L.) is controlled by a major gene Ph1 located on the long arm of chromosome 5 and also by minor genes

located on other chromosome of wheat (Sears,1976). When Singh and Hymowitz (1985a) examined the chromosome pairing of six amphiploids (2n = 80, 120, or 160), they observed that all the amphiploids showed diploid-like meiosis in the majority of sporocytes and did not exhibit multivalent associations, a classical example of allopolyploidy. The strong preferential pairing between homologous chromosome is likely to be under genetic control.

4–10 KARYOTYPE ANALYSIS

Halvankar and Patil (1990) summarized the literature concerning the karyotype analysis of soybean mitotic chromosomes, and noted that the information about chromosome morphology of *Glycine* species was meager. Therefore, they attempted to provide new information on karyotypes of six *Glycine* species: *G. canescens*, *G. clandestina*, *G. latifolia*, and *G. max* were 2n = 40 while *G. tabacina* and *G. tomentella* had 80 chromosomes. The camera lucida drawings revealed that the *Glycine* species had either median or submedian centromeres. The results were identical with those of all previous studies, for example, Ahmad et al. (1984). Because of the high chromosome number, similar chromosome sizes, median to submedian centromere position, and the lack of morphological landmarks, the individual somatic chromosomes of the soybean have not been distinguishable (Zhong et al., 1997).

Singh and Hymowitz (1988) conducted a study to determine the genomic relationship between the cultivated soybean and the wild soybean, G. soja. Pachytene analysis of F_1 hybrids aided in the construction of chromosome maps based upon chromosome length, and euchromatic and heterochromatic distribution (Fig. 4–6). The chromosomes were numbered in descending order of size from 1 to 20 (Fig. 4–7). The largest chromosome is about four times the size of the smallest. The NOR is contained on chromosome 13. The smallest arms of six chromosomes—5, 7, 10, 18, 19, and 20—were totally heterochromatic. About 35.8% of the soybean genome was heterochromatic. Except for heteromorphic regions in chromosomes 6 and 11, the pachytene complement of G. max and G. soja were similar. Thus, the genome symbol GG assigned to G. max and G. soja by Singh and Hymowitz (1985b) and Singh et al. (1988) was valid. The paper by Singh and Hymowitz (1988) is an important landmark in soybean cytology, genetics, and molecular biology.

4–11 CHROMOSOME ABERRATIONS—NUMERICAL CHANGES

4–11.1 Polyploidy

In general, natural polyploidization in soybean beyond tetraploidy has not been identified. However, numerous synthesized allotetraploids (2n = 4x = 80) have been created to study the progenitors of 80-chromosome G. tabacina having or lacking adventitious roots, 78-, 80-chromosome G. tomentella, or to elucidate the mechanism of diploid-like meiosis (Singh and Hymowitz, 1985a). See section 4–6

of this chapter for a discussion of the polyploid complexes of *G. tabacina* and *G. tomentella*.

Singh and Hymowitz (1991a) synthesized an allopentaploid ($2n = 5x = 100$). The origin of the pentaploid plant was as follows: *G. clandestina*, $2n = 2x = 40$, A1A1 × *G. canescens*, $2n = 2x = 40$, AA → F$_1$ ($2n = 2x = 40$, AA1) × G. *tomentella*
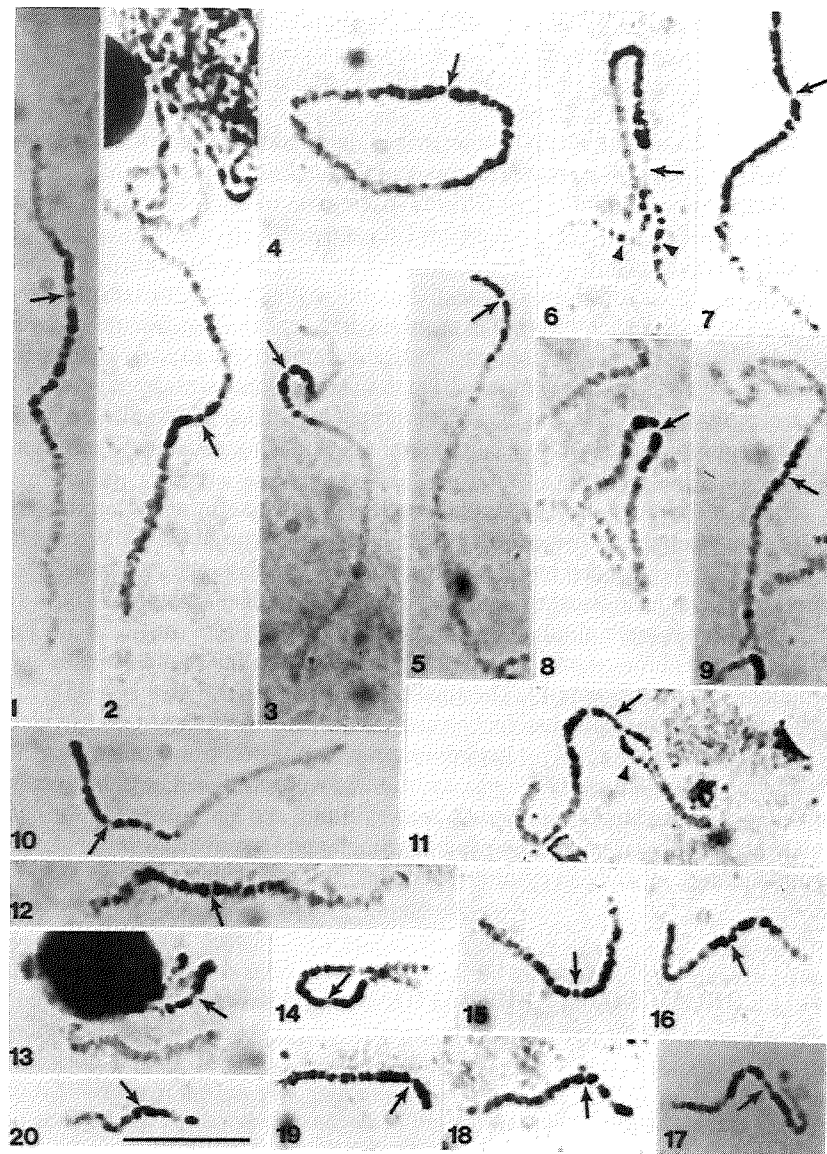
($2n = 4x = 80$, A*A*DD → F$_1$($2n = 3x = 60$, AA*D) → 0.1% colchicine treatment → $2n = 6x = 120$ (AAA*A*DD) × G. *tomentella* ($2n = 4x = 80$, A*A*DD) → BC$_1$, $2n = 5x = 100$ (AA*A*DD).

Morphologically, the pentaploid plant very closely resembled the tetraploid *G. tomentella* parent. The pentaploid did not breed true, as chromosomes in the 14 examined progeny ranged from $2n = 86$ to 97. Progeny of a plant with $2n = 90$ segregated for plants with $2n = 81$–86 chromosomes. This suggests that the preferential elimination of *G. canescens* (A genome) chromosomes is rapid and that eventually A*A*DD-genome chromosomes will prevail.

In the process of obtaining backcrossed-derived fertile plants from G. *max* and G. *tomentella* hybrids, an amphiploid containing $2n = 118$ chromosomes was synthesized. For the origin of the amphiploid, refer to section 4–8 of this chapter. The amphiploid plant produced four small black seed that bred true (Singh et al., 1990, 1993).

Singh and Hymowitz (1985a) synthesized four allohexaploids ($2n = 6x = 120$). The origin of the allohexaploids was as follows: (i) *G. clandestina* ($2n = 40$) × *G. tabacina* ($2n = 80$) = F$_1$ ($2n = 3x = 60$) → 0.1% colchicine treatment → $2n = 6x = 120$, (ii) *G. tabacina* ($2n = 80$) × *G. canescens* ($2n = 40$) = F$_1$ ($2n = 3x = 60$) → 0.1% colchicine treatment → $2n = 6x = 120$, (iii) *G. tomentella* ($2n = 80$) × *G. canescens* ($2n = 40$) = F1 ($2n = 3x = 60$) → 0.1% colchicine treatment → $2n = 6x = 120$, and (iv) *G. canescens* ($2n = 40$) × *G. tomentella* ($2n = 80$) = F$_1$ ($2n = 3x = 60$) → 0.1% colchicine treatment → $2n = 6x = 120$.

Sterile F$_1$ interspecific triploid *Glycine* hybrids set seed only after colchicine treatment (i.e., chromosome doubling), establishing that the mechanism of unreduced gamete formation is a rare event in *Glycine*. The only publication reporting
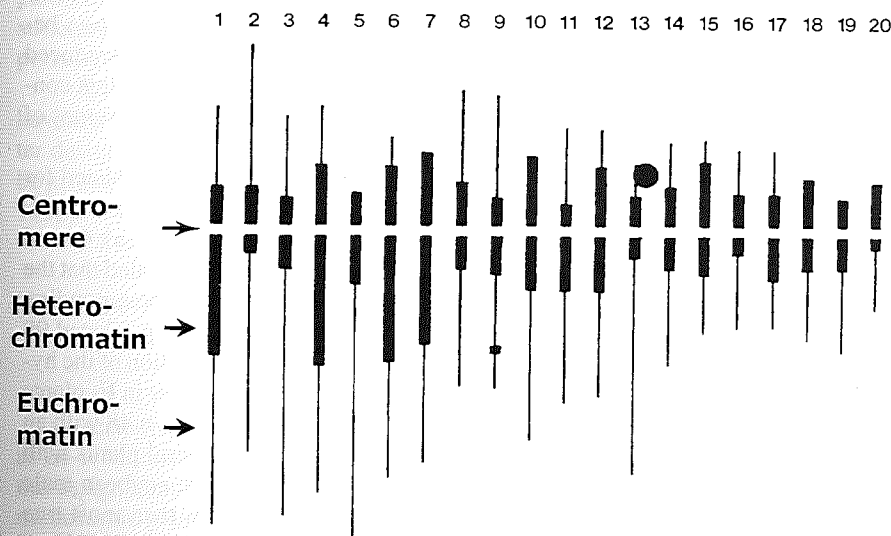

Fig. 4–6. Photomicrographs of the pachytene chromosome complement of *G. max* × *G. soja* F$_1$ hybrids. Each figure shows a different chromosome. Arrows indicate centromere locations. Arrow heads in chromosomes 6 and 11 show heteromorphic regions. Bar represents 10µ. (Adapted from Singh and Hymowitz, 1988.)

Fig. 4–7. Proposed idiogram of the pachytene chromosomes of the soybean. Arrows indicate centromeres, heterochromatin, and euchromatin. Chromosome 13 carries the nucleolus-organizing region (NOR). (Adapted from Singh and Hymowitz, 1988.)

unreduced gamete formation in *Glycine* was by Newell and Hymowitz (1980b). In a natural population of tetraploid *G. tabacina* they isolated a hexaploid *G. tabacina* ($2n = 6x = 120$) plant. It appears that an unreduced female gamete ($2n = 80$) was fertilized by a reduced ($n = 40$) male gamete. The plant was an autohexapoloid because some cells exhibited up to 16 trivalents.

A synthesized allooctoploid ($2n = 8x = 160$) was produced by Singh and Hymowitz (1985a). This is the highest reported chromosome number in the genus *Glycine*. The origin of the allooctoploid plant was as follows: *G. tomentella* ($2n = 4x = 80$) × *G. tabacina* ($2n = 4x = 80$) → F_1 ($2n = 4x = 80$) → 0.1% colchicine treatment → $2n = 8x = 160$. The average chromosome association at diakinesis was 10.4 I (4–18) + 74.8 II (71–78). The high frequency of univalents resulted in laggards at anaphase I. This meiotic irregularity in the allooctoploids is believed to be the cause of complete seed sterility in the C1 generation.

4–11.2 Triploidy

Chen and Palmer (1985) obtained autotriploids ($2n = 3x = 60$) from genetic male-sterile (*ms1ms1*) soybean plants. Meiosis in fertile and sterile autotriploids revealed no distinguishable difference in chromosome associations. Chromosomes of the true autotriploids are expected to associate in a trivalent configuration during diakinesis and metaphase I; however, multivalents, bivalents, and univalents also were observed. Pollen fertility in male-fertile triploid plants (*Ms1ms1ms1*) varied from 57 to 82% with an average of about 71%. Chromosome numbers of progenies obtained from these fertile triploids varied from $2n = 40$ to 71. Zhang and Palmer (1990) could not obtain triploids by crossing diploids *ms1ms1* soybean plants with tetraploid plants.

A hypertriploid plant ($2n = 64$) was isolated in BC_4 plants from a cross between the soybean and *G. tomentella*. Singh et al. (1993) believe that an unreduced egg ($n = 44$) was fertilized by a normal haploid ($n = 20$) male spore. The plant contained three doses of the soybean genome (GGG) plus four extra chromosomes of *G. tomentella*. Although vigorous in growth the plant was sterile.

A spontaneous hypertriploid ($2n = 3x + 1 = 61$) in soybean was isolated for the first time from the progeny of the cross between soybean line T31 and a primary trisomic line T190-47-3 (Xu et al., 2000c). Crosses with cultivar Clark 63 and selfed populations were used to evaluate breeding behavior, which revealed that the hypertriploid expressed more vigorous vegetative growth than the corresponding disomic siblings. The hypertriploid showed 63% pollen fertility and produced 98 seeds from self-pollination. Chromosome numbers in the self-pollination of the hypertriploid ranged from $2n = 50$ to $2n = 69$. The chromosome number in F_1 plants from the cross of hypertriploid × Clark 63 ranged from $2n = 44$ to $2n = 48$ with an exception of one plant that contained $2n = 56$ chromosomes. Several plants with $2n = 44$ to $2n = 47$ had high seed fertility. These data suggest that soybean male and female spores tolerate a higher number of extra chromosmes than most true diploid plant species, corroborating the hypothesis that the soybean is a diploidized polyploid species.

4–12 CHROMOSOME ABERRATIONS—STRUCTURAL CHANGES

4–12.1 Translocations

Translocations are sometimes called interchanges. They are created when there is a reciprocal exchange of terminal segments of nonhomologous chromosomes (Singh, 1993). Translocations have been used very rarely in chromosome mapping studies in the soybean (Sacks and Sadanaga, 1984; Sadanaga and Grindeland, 1984).

A total of 56 *G. soja* accessions from the People's Republic of China and Russia were evaluated for translocations (Palmer et al., 1987). Forty-six accessions had translocations with the same chromosome involved and likely they had the same chromosome structure.

Six different translocation lines were identified genetically and cytologically in soybean. Cytological analysis of F_1 progeny suggested that six of the 20 chromosomes were involved in reciprocal translocations (Mahama et al., 1999).

4–12.2 Inversions

In the genus *Glycine*, paracentric inversions appear to have played a major role in speciation. A dicentric bridge and an acentric fragment at anaphase I is a common configuration for a paracentric inversion. Thus far, inversions have not been used in soybean for mapping studies.

Palmer et al. (2000) conducted a study to determine if the paracentric inversions in two Chinese soybean landraces that were identical to each other and a paracentric inversion identified in *Glycine soja*. The results indicate that the soybean paracentric inversions were the same, but the *G. soja* accession had a chromosome structure differing from that of the two soybean accessions. Thus, the previous reports by Ahmad et al. (1977, 1979) were confirmed.

Extensive hybridization studies among diploid wild perennial *Glycine* species have led to morphologically distinct species being assigned the same genome letter designation but different number subscripts. The latter distinction appeared to be primarily due to paracentric inversions (Hymowitz et al., 1991; Hymowitz et al., 1998).

4–13 CHROMOSOME ABERRATIONS—ANEUPLOIDY

4–13.1 Monosomics

An individual lacking one chromosome is called monosomic and is designated as $2n-1$. Monosomics are useful for locating genes to specific chromosomes and in the assignment of linkage groups. Monosomics are rare in diploid crops. Two monosomic plants were identified among progenies of Triplo 3 (BC3) and Triplo 6 (BC4) trisomic plants, backcrossed to Clark 63 as the recurrent parent. The two monosomics were designated as Mono-3 and Mono-6 (Xu et al., 2000a). Morpho-

logically, Mono-6 was similar to the disomic, while Mono-3 was smaller with reduced vigor. Both monosomics showed 19II +1I chromosome association at Metaphase I. Pollen fertility in Mono-3 was 8.8% and in Mono-6 was 20.0%.

Among the progeny of partially male-sterile plants, a deficiency aneuploid 39-chromosome plant was identified. Of the 130 seeds produced, two plants had 39 chromosomes and the rest were diploids. The low-transmission of n-1 gametes and high abortion rate make these plants unreliable as source of monosomics by sexual reproduction (Skorupska and Palmer, 1987).

4–13.2 Primary Trisomics

An individual with a normal chromosome complement plus an extra complete chromosome ($2n = 2x + 1$) is designated as a simple primary trisomic, and is called a "Triplo". For the soybean, a triplo is $2n = 40 + 1$. Primary trisomics are superb marker stocks for determining the gene-chromosome-linkage group relationships in soybean (Singh, 1993).

Palmer (1974, 1976) made an early attempt to develop primary trisomics of soybean. Five primary trisomics ($2n = 41$) were characterized and arbitrarily designated TRI A, TRI B, TRI C, TRI D, and TRI S (Palmer, 1976; Sadanaga and Grindeland, 1984; Gwyn et al., 1985; Gwyn and Palmer, 1989). The five primary trisomics were not identified karyotypically. Honeycutt et al. (1990) demonstrated that the *v2* locus was located on the extra chromosome of TRI A. Hedges and Palmer (1991) reported that *Dia1* was located on the extra chromosome of TRI D.

All 20 primary trisomics of the soybean were tentatively identified by pachytene analysis (Singh and Hymowitz, 1991b; Ahmad et al., 1992; Ahmad and Hymowitz, 1994; Xu et al., 2000b) and were designated as Triplo 1 through Triplo 20. The previously reported primary trisomics TRI A, C, D, and S were identified as Triplo 5, 1, 4, and 13, respectively (Xu et al., 2000b). The sources of primary trisomics in soybean were aneuploid lines ($2n = 41, 42, 43$) derived from asynaptic and desynaptic mutants (Palmer, 1974; Palmer and Heer, 1976), male sterile (*ms*) lines (Chen and Palmer, 1985; Sadanaga and Grindeland, 1981; Zhang and Palmer, 1990), neutron irradiated plants (Sadanaga and Grindeland, 1981), tissue culture induced sterile mutants (Graybosch et al., 1987; Palmer and Skorupska, 1994), and crosses between 'Funman' sterile plants with Clark 63 (Xu et al., 2000b).

4–13.3 Tetrasomics

An individual with a normal chromosome complement plus two extra similar chromosomes ($2n = 2x + 2$) is designated a tetrasomic and that individual is called "Tetra". For the soybean a tetra is $2n = 40 + 2$. A double trisomic is an individual with a normal chromosome complement plus two extra dissimilar chromosomes ($2n = 2x + 1 + 1$). For the soybean, a double trisomic is $2n = 40 + 1 + 1$.

Gwyn and Palmer (1989) compared 5 tetrasomics, 10 double trisomics and their related disomics for 25 morphological traits. Their analysis suggested that tetrasomics and double trisomics can be accurately distinguished on the basis of phenotype from disomics and from each other. The five tetrasomics were not identified karyotypically.

Tetrasomics have been identified from selfed progenies of primary trisomics by counting somatic chromosomes (Xu et al., 1997, 1998b). A.R.J. Singh (personal communication, 2001) believes that he has isolated and individually identified 15 different tetrasomics.

4–14 CONCLUSIONS

Within the past two decades, due to extensive plant exploration activities and with increased cytological, classical taxonomic and biosytematic investigations, the number of species in the genus *Glycine* has increased from 9 to 24. The genomic relationships among species has been established. As plant exploration activity continues, especially in remote areas of Australia, the number of new species assigned to the genus *Glycine* is expected to increase. The wild perennial species in *Glycine* offers great promise in expanding the narrow germplasm base of cultigen soybean by the introgression of useful traits from the wild species via wide hybridization.

Although soybean is one of the world's major crops, basic cytological and cytogenetic studies have lagged behind crops such as wheat, tomato (*Lycopersicum esculentum* Mill.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.). The soybean chromosomes ($2n = 40$) are smaller than most other crop plants and individual chromosome identification at mitotic metaphase is not possible with current technologies. Within the past two decades, cytological techniques have greatly improved. All 20 chromosome pairs now have been differentiated based upon pachytene chromosome analysis. Cytological and marker stocks have been identified, for example, primary trisomics, tetrasomics, monosomics, translocations, inversions, and monosomic alien addition lines. All of these newly developed stocks have established a firm foundation for conducting genetic studies on the soybean as well as providing information for improving the soybean as a commercial crop

REFERENCES

Ahmad, Q.N., E.J. Britton, and D.E. Byth. 1977. Inversion bridges and meiotic behavior in species hybrids of soybeans. J. Hered. 68:360–364.
Ahmad, Q.N., E.J. Britton, and D.E. Byth. 1979. Inversion heterozygosity in the hybrid soybean × *Glycine soja*. J. Hered. 70:358–364.
Ahmad, Q.N., E.J. Britton, and D.E. Byth. 1984. The karyotype of *Glycine soja* and its relationship to that of the soybean. Cytologia (Tokyo) 49:645–658.
Ahmad, F., and T. Hymowitz. 1994. Identification of five new primary simple trisomics in soybean based upon pachytene chromosome analysis. Genome 37:133–136.
Ahmad, F., R.J. Singh, and T. Hymowitz. 1992. Cytological evidence for four new primary trisomics in soybean. J. Hered. 83:221–224.
Aiton, W.T. 1812. Hortus Kewensis. Vol. 4. p. 295. Longman, Hurst, Rees, Orme, and Brown, London.
Anonymous. 1988. Final report on the Australian *Glycine* and *Gossypium* exploration mission in 1987. Plant Genetic Resources Newsl. 73/74: Rep. 88/9. p.55.

Australian and New Zealand Environment and Conservation Council. 1993. Proposed list of the threatened Australian flora. Report for the ANZECC, ANZECC Endangered Flora Network, Canberra.

Baranyi, M., and J. Greilhuber. 1996. Flow cytometric and Feulgen densitometric analysis of genome size variation in *Pisum*. Theor. Appl. Genet. 92:297–307.

Bione, N.C.P., M.S. Pagliarini, and J.F.F. deToledo. 2000. Meiotic behavior of several Brazilian soybean varieties. Genet. Mol. Biol. 23:623–631.

Blackhall, N.N., N. Hammatt, and M.R. Davey. 1991. Analysis of variation in the DNA content of *Glycine* species: A flow cytometric study. Soybean Genet. Newsl. 18:194–200.

Bodanese-Zanettini, M.H., M.S. Lauxen, S.N.C. Richter, S. Cavalli-Molina, C.E. Lange, P.J. Wang, and C.Y. Hu. 1996. Wide hybridization between Brazilian soybean cultivars and wild perennial relatives. Theor. Appl. Genet. 93:703–709.

Broué, P., J. Douglass, J.P. Grace, and D.R. Marshall. 1982. Interspecific hybridization of soybeans and perennial *Glycine* species indigenous to Australia via embryo culture. Euphytica 31:715–724.

Broué, P., D.R. Marshall, and W.J. Müller. 1977. Biosystematics of subgenus *Glycine* (Verdc.): Isoenzymatic data. Aust. J. Bot. 25:555–566.

Brown, A.H.D. 1990. The role of isozyme studies in molecular systematics. Aust. Syst. Bot. 3:39–46.

Brown, A.H.D., J.L. Doyle, J.P. Grace, and J.J. Doyle. 2002. Molecular phylogenetic relationships within and among diploid races of *Glycine tomentella* (Leguminosae). Aust. Syst. Bot. 15:37–47.

Brown, A.H.D., J.E. Grant, J.J. Burdon, J.P. Grace, and R. Pullen. 1985. Collection and utilization of wild perennial *Glycine*. p. 345–352 *In* R. Shibles (ed.) World Soybean Res. Conf., Iowa State Univ., Ames. 12–17 Aug. 1984. Westview Press, Boulder, CO.

Brown, A.H.D., J.E. Grant, and R. Pullen. 1986. Outcrossing and paternity in *Glycine argyrea* by paired fruit analysis. Biol. J. Linn. Soc. 29:283–294.

Bruneau, A., J.J. Doyle, and J.L. Doyle. 1994. Phylogenetic relationships in Phaseoleae: Evidence from choloroplast DNA restriction site characters. p. 309–330. *In* M. Crisp and J.J. Doyle (ed.) Advances in legume systematics. Royal Botanic Gardens, Kew, England.

Carletti, F. 1964. My voyage around the world. Trans. by H. Weinstock. Pantheon Books, NY.

Carlquist, S. 1974. Island biology. Columbia Univ. Press, New York.

Chaven, C., T. Hymowitz, and C.A. Newell. 1982. Chromosome number, oil and fatty acid content of species in genus *Glycine* subgenus *Glycine*. J. Am. Oil Chem. Soc. 59:23–25.

Chen, L.F., and R.G. Palmer. 1985. Cytological studies of triploids and their progeny from male-sterile (*ms1*) soybean (*Glycine max*). Theor. Appl. Genet. 71:400–407.

Cheng, S.H., and H.H. Hadley. 1983. Studies in polyploidy in soybeans: A simple and effective colchicine technique of chromosome doubling for soybean (*Glycine max* (L.) Merr.) and its wild relatives. Soybean Genet. Newsl. 10:23–24.

Chung, G.H., and J.H. Kim. 1990. Production of interspecific hybrids between *Glycine max* and *G. tomentella* through embryo culture. Euphytica 48:97–101.

Chung, G.H., and K.S. Kim. 1991. Obtaining intersubgeneric hybridization between *Glycine max* and *Glycine latifolia* through embryo culture. Korean J. Plant Tissue Cult. 18:39–45.

Chung, J., J.H. Lee, K. Arumuganathan, G.L. Graef, and J.E. Specht. 1998. Relationships between nuclear DNA content and seed and leaf size in soybean. Theor. Appl. Genet. 96:1064–1068.

Coble, C.J., and W.T. Schapaugh, Jr. 1990. Nutrient culture medium components affecting plant recovery from immature embryos of three *Glycine* genotypes and an interspecific hybrid grown *in vitro*. Euphytica 50:127–133.

Costanza, S.H., and T. Hymowitz. 1987. Adventitious roots in *Glycine* subg. *Glycine* (Leguminosae): Morphological and taxonomic indicators of the B genome. Plant Syst. Evol. 158:37–46.

Dale, S. 1705. Pharmacologiae. p. 184. S. Smith and B. Walford, Londoni (Latin).

Darlington, C.D., and A.P. Wylie. 1955. Chromosome atlas of flowering plants. George Allen & Unwin Ltd., London.

Domagalski, J.M., K.P. Kollipara, A.H. Bates, D.L. Brandon, M. Friedman, and T. Hymowitz. 1992. Nulls for the major soybean Bowman-Birk protease inhibitor in the genus *Glycine*. Crop Sci. 32:1502–1505.

Doyle, J.J. 1991. The pros and cons of DNA systematic data: Studies of the wild perennial relatives of soybean. Evol. Trends Plants 5:99–104.

Doyle, J.J., and R.N. Beachy. 1985. Ribosomal gene variation in soybean (*Glycine*) and its wild relatives. Theor. Appl. Genet. 70:369–376.

Doyle, J.J., and A.H.D. Brown. 1989. 5S nuclear ribosomal gene variation in the *Glycine tomentella* polyploid complex (Leguminosae). Syst. Bot. 14:398–407.

Doyle, J.J., and J.L. Doyle. 1993. Chloroplast DNA phylogy of the Papilionoid legume tribe Phaseoleae. Syst. Bot. 18:309–327.

Doyle, J.J., J.L. Doyle, and A.H.D. Brown. 1990a. Analysis of polyploid complex in *Glycine* with chloroplast and nuclear DNA. Aust. Syst. Bot. 3:125–136.

Doyle, J.J., J.L. Doyle, and A.H.D. Brown. 1990b. A chloroplast-DNA phylogeny of the wild perennial relatives of soybean (*Glycine* subgenus *Glycine*); Congruence with morphological and crossing groups. Evolution 44:371–389.

Doyle, J.J., J.L. Doyle, and A.H.D. Brown. 1990c. Chloroplast DNA polymorphism and phylogeny in the B genome of *Glycine* subgenus *Glycine* (Leguminosae). Am. J. Bot. 77:772–782.

Doyle, J.J., J.L. Doyle, and A.H.D. Brown. 1999a. Incongruence in the diploid B-genome species complex of *Glycine* (Leguminosae) revisited: Histone H3-D alleles versus chloroplast haplotypes. Mol. Biol. Evol. 16:354–362.

Doyle, J.J., J.L. Doyle, and A.H.D. Brown. 1999b. Origins, colonization, and lineage recombination in a widespread perennial soybean polyploid complex. Proc. Natl. Acad. Sci. USA 96:10741–10745.

Doyle, J.J., J.L. Doyle, A.H.D. Brown, and J.P. Grace. 1990d. Multiple origins of polyploids in the *Glycine tabacina* complex conferred from chloroplast DNA polymorphism. Proc. Natl. Acad. Sci. USA 87:714–717.

Doyle, J.J., J.L. Doyle, A.H.D. Brown, and B.E. Pfeil. 2000. Confirmation of shared and divergent genomes in the *Glycine tabacina* polyploid complex (Leguminosae) using histone H3-D sequences. Syst. Bot. 25:437–448.

Doyle, J.J., V. Kanazin, and R.C. Shoemaker. 1996. Phylogenetic utility of histone H3 intron sequences in the perennial relatives of soybean (*Glycine*: Leguminosae). Mol. Phylog. Evol. 6:438–447.

Doyle, M.J., and A.H.D. Brown. 1985. Numerical analysis of isozyme variation in *Glycine tomentella*. Biochem. Syst. Ecol. 13:413–419.

Doyle, M.J., J.E. Grant, and A.H.D. Brown. 1986. Reproductive isolation between isozyme groups of *Glycine tomentella* (Leguminosae), and spontaneous doubling in their hybrids. Aust. J. Bot. 34:523–535.

Goldblatt, P. 1981. Cytology and the Phylogeny of Leguminosae. p.427–463 *In* R.M. Polhill and P.H. Raven (ed.) Advances in legume systematics. Part 2. Royal Botanic Garden, Kew, England.

Graham, M.J., D.C. Nickell, and A.L. Rayburn. 1994. Relationship between genome size and maturity group in soybean. Theor. Appl. Genet. 88:429–437.

Grant, J.E., A.H.D. Brown, and J.P. Grace. 1984a. Cytological and isozyme diversity in *Glycine tomentella*. Hayata (Leguminosae). Aust. J. Bot. 32:665–677.

Grant, J.E., J.P. Grace, A.H.D. Brown, and E. Putievsky. 1984b. Interspecific hybridization in *Glycine* Willd. subgenus *Glycine* (Leguminosae). Aust. J. Bot. 32:655–663.

Grant, J.E., R. Pullen, A.H.D. Brown, J.P. Grace, and P.M. Gresshoff. 1986. Cytogenetic affinity between the new species *Glycine argyrea* and its congeners. J. Hered. 77:423–426.

Graybosch, R.A., M.E. Edge, and X. Delannay. 1987. Somaclonal variation in soybean plants regenerated from the cotyledonary node tissue culture system. Crop Sci. 27:803–806.

Greilhuber, J., and R. Obermayer. 1997. Genome size and maturity group in *Glycine max* (soybean). Heredity 78:547–551.

Griffor, M.C., L.O. Vodkin, R.J. Singh, and T. Hymowitz. 1991. Fluorescent *in situ* hybridization to soybean metaphase chromosomes. Plant Mol. Biol. 17:101–109.

Gwyn, J.J., and R.G. Palmer. 1989. Morphological discrimination among some aneuploids of soybean (*Glycine max* [L.] Merr.): 2. Double trisomics, tetrasomics. Can. J. Genet. Cytol. 27:608–613.

Gwyn, J.J., R.G. Palmer, and K. Sadanaga. 1985. Morphological discrimination among some aneuploids in soybean [*Glycine max* [L.] Merr.]: 1. Trisomics. Can. J. Genet. Cytol. 27:608–613.

Hadley, H.H., and T. Hymowitz. 1973. Speciation and cytogenetics. p. 97–116. *In* B.E. Caldwell (ed.) Soybeans: Improvement, production and uses. Agron. Monogr. 16. ASA, Madison, WI.

Halvankar, G.B., and V.P. Patil. 1990. Inter-specific variation in the karyotypes of the genus *Glycine* Willd. Cytologia (Tokyo) 55:273–279.

Hammatt, N., N.W. Blackhall, and M.R. Davey. 1991. Variation in the DNA content of *Glycine* species. J. Exp. Bot. 42:659–665.

Harlan, J.R., and J.M.J. deWet. 1971. Toward a rational classification of cultivated plants. Taxon 20:509–517.

Heath, M.S. 1989. Analysis of hybrid plants and progeny of a cross between *Glycine max* (L.) Merr. and *Glycine tomentella* Hayata. M.S. thesis. Iowa State Univ., Ames.

Hedges, B.R., and R.G. Palmer. 1991. Tests of linkage of isozyme loci with five primary trisomics in soybean. *Glycine max* (L.) Merr. J. Hered. 82:494–496.

Hermann, F.J. 1962. A revision of the genus *Glycine* and its immediate allies. USDA Tech. Bull. 1268. USDA, Washington, DC.

Hill, J.L. 1998. Morphological and biochemical analysis of variation in diploid *Glycine tomentella* Hayata (2n = 38, 40). Ph.D. diss. Univ. of Illinois, Urbana (Diss. Abstr. ADG9921694).

Hirth, F. 1908. The ancient history of China to the end of the Chou Dynasty. Columbia Univ. Press, NY.

Ho, P.T. 1969. The loess and the origin of Chinese agriculture. Am. Hist. Rev. 75:1–36.

Honeycutt, R.J., K.E. Newhouse, and R.G. Palmer. 1990. Inheritance and linkage studies of a variegated leaf mutant in soybean. J. Hered. 81:123–126.

Hood, M.J., and F.L. Allen. 1980. Interspecific hybridization studies between cultivated soybean, *Glycine max* and a perennial wild relative, *G. falcata*. p. 58 *In* Agronomy abstracts. ASA Madison, WI.

Hood, M.J., and F.L. Allen. 1987. Crossing soybeans with a wild perennial relative. Tenn. Farm Home Sci. Issue 144:25–30.

Hsieh, J.S., K.L. Hsieh, Y.C. Tsai, and Y.I. Hsing. 2001. Each species of *Glycine* collected in Taiwan has a unique seed protein pattern. Euphytica 118:67–73.

Hui, D., S.Y. Chen, and B.C. Zhuang. 1997. Phylogeny of 12 species of genus *Glycine* Wild. reconstructed with internal transcribed region in nuclear ribosomal DNA. Sci. China (Ser. C) 40:137–144.

Hymowitz, T. 1970. On the domestication of the soybean. Econ. Bot. 24:408–421.

Hymowitz, T. 1982. Exploration for a wild relative of the soybean on Vanuatu. Naika 7:1–4.

Hymowitz, T. 1986. Introduction of the soybean to Illinois. Econ. Bot. 41:28–32.

Hymowitz, T. 1989. Exploration for wild perennial *Glycine* species on Taiwan. Soybean Genet. Newsl. 16:92–93.

Hymowitz, T. 1990. Soybean: The success story. p.159–163. *In* J. Janick and J.E. Simon (ed). Advances in new crops. Timber Press, Portland, OR.

Hymowitz, H. 1998. Plant exploration trip to Taiwan and islet of Kinmen (Quemoy). Soybean Genet. Newsl. 25:127.

Hymowitz, T., and J.R. Harlan. 1983. The introduction of the soybean to North America by Samuel Bowen in 1765. Econ. Bot. 37:371–379.

Hymowitz, T., and C.A. Newell. 1975. A wild relative of the soybean. Illinois Res. 17(4):18–19.

Hymowitz, T., and C.A. Newell. 1980. Taxonomy, speciation, domestication, dissemination, germplasm resources, and variation in the genus *Glycine* p. 251–264. *In* R.J. Summerfield and A.H. Bunting (ed.) Advances in legume science. Royal Botanic Garden, Kew, England.

Hymowitz, T., and C.A. Newell. 1981. Taxonomy of the genus *Glycine*, domestication and uses of soybean. Econ. Bot. 35:272–288.

Hymowitz, T., R.G. Palmer, and R.J.Singh.1991.Cytogenetics of the genus *Glycine*. p. 53–63. *In* T. Tsuchiya and P.K.Gupta (ed.) Chromosome engineering in plants: Genetics, breeding, evolution. Part B. Elsevier, NY.

Hymowitz, T., and R.J. Singh. 1987. Taxonomy and speciation. p. 23–48. *In* J.R. Wilcox (ed.) Soybeans: Improvement, production, and uses. 2nd ed. Agron. Monogr. 16. ASA, CSSA, and SSSA, Madison, WI.

Hymowitz, T., R.J. Singh, and K.P. Kollipara. 1998. The genomes of the *Glycine*. Plant Breed. Rev. 16:289–317.

Hymowitz, T., R.J. Singh, and R.P. Larkin. 1990. Long distance dispersal. The case for the allopolyploids *Glycine tabacina* Benth. and *G. tomentella* Hayata in the west-central pacific. Micronesica 23:5–13.

Jones, R.M., A.H.D. Brown, and J.N. Coote 1996. Variation in growth and forage quality of *Glycine latifolia* (Benth.) Newell and Hymowitz. CSIRO Trop. Agric., Genet. Resources Com. 26:1–11.

Kaempfer, E. 1906. The history of Japan together with a description of the Kingdom of Siam 1690–1692. Translated by J. G. Scheuchzer from the original edition of 1727, Vol I. p. 188. James Maclehose, Glasgow.

Keen, N.L., R.L. Lyne, and T. Hymowitz. 1986. Phytoalexin production as a chemosystematic parameter with *Glycine* spp. Biochem. Syst. Ecol. 14:481–486.

King, P. 1858. The life and letters of John Locke. p.134. Henry G. Bohn, London.

Kollipara, K.P., R.J. Singh, and T. Hymowitz. 1993. Genomic diversity in aneudiploid (2n = 38) and diploid (2n = 40) *Glycine tomentella* revealed by cytogenetic and biochemical methods. Genome 36:391–396.

Kollipara, K.P., R.J. Singh, and T. Hymowitz. 1994. Genomic diversity and multiple origins of the tetraploid (2n = 78, 80) *Glycine tomentella*. Genome 37:448–459.

Kollipara, K.P., R.J. Singh, and T. Hymowitz. 1995. Genomic relationships in the genus *Glycine* (Fabaceae:Phaseoleae): Use of a monoclonal antibody to the soybean Bowman-Birk inhibitor as a genome marker. Am. J. Bot. 82:1104–1111.

Kollipara, K.P., R.J.Singh, and T.Hymowitz.1997. Phylogenetic and genomic relationships in genus *Glycine* Willd. based on sequences from the ITS region of nuclear rDNA. Genome 40:57–68.

Kollipara, K.P., R.J. Singh, and T. Hymowitz. 1998. Induction of amphidiploids through colchicine treatment in the genus *Glycine*. Soybean Genet. Newsl. 16:94–96.

Kumar, P.S., and T. Hymowitz. 1989. Where are the diploid (2n = 2x = 20) genome donors of *Glycine* Willd. (Leguminosae, Papilionoideae)? Euphytica 40:221–226.

Kwon, C.S., and K.Y. Chang. 1991. *In vitro* germination of young hybrid embryos between *Glycine max* and *G. tomentella*. Korean J. Breed. 22:379–383.

Lackey, J.A. 1977. A revised classification of the tribe Phaseoleae (Leguminosae, Papilionoideae) and its relation to canavanine distribution. J. Linn. Soc. Bot. 74:163–178.

Lackey, J.A. 1980. Chromosome numbers in the Phaseoleae (Fabaceae; Faboideae) and their relation to taxonomy. Am. J. Bot. 67:595–602.

Lackey, J.A. 1981. Tribe 10 Phaseoleae DC. (1825) p.301–307. *In* R.M. Polhill and P.H. Raven (ed.) Advances in legume systematics. Royal Botanic Gardens, Kew, England.

Ladizinsky, G., C.A. Newell, and T. Hymowitz. 1979. Wide crosses in soybeans: Prospects and limitations. Euphytica 28:421–423.

Lee, J., and T. Hymowitz. 2001. A molecular phylogenetic study of the subtribe Glycininae (Leguminosae) derived from the chloroplast DNA *rps*16 intron sequences. Am. J. Bot. 88:2064–2073.

Lee, J.S., and D.P.S. Verma. 1984. Structure and chromosomal arrangement of leghemoglobin genes in kidney bean suggest divergence in soybean leghemoglobin gene loci following tetraploidization. EMBO J. 3:2745–2752.

Li, F., R. Chang, and S. Shu. 1983. The plants of the genus *Glycine* in China. (In Chinese.) Soybean Sci. 2:109–116.

Linnaeus, C. 1737. Hortus Cliffortianus, Historiae Naturalis Classica. p. 499. *In* J. Cramer and H.K. Swann (ed.) Vol. 63. Reprint 1968. Stechert-Hafner Service Agency, NY.

Lynch, A.J.J. 1994. The identification and distribution of *Glycine latrobeana* (Meissn.) Benth. in Tasmania. Papers and Proc. R. Soc. Tasmania 128:17–20.

Mahama, A.A., L.M. Deaderick, K. Sadanaga, K.E. Newhouse, and R.G. Palmer. 1999. Cytogenetic analysis of translocations in soybean. J. Hered. 90:648–653.

Menancio, D.I., and T. Hymowitz. 1989. Isozyme variation between diploid and tetraploid cytotypes of *Glycine tabacina* (Labill) Benth. Euphytica 42:79–87.

Menancio, D.I., A.G. Hepburn, and T. Hymowitz. 1990. Restriction fragment length polymorphism (RFLP) of wild perennial relatives of soybean. Theor. Appl. Genet. 79:235–240.

Mies, D.W., and T. Hymowitz. 1973. Comparative electrophoretic studies of trypsin inhibitors in seed of the genus *Glycine*. Bot. Gaz. 34:121–125.

Newell, C.A. 1981. Distribution of *Glycine tabacina* (Labill.) Benth. in the West-Central Pacific. Micronesica 17:59–65.

Newell, C.A., X. Delannay, and M.E. Edge. 1987. Interspecific hybrids between the soybean and wild perennial relatives. J. Hered. 78:301–306.

Newell, C.A., and T. Hymowitz. 1978. A reappraisal of the subgenus *Glycine*. Am. J. Bot. 65:168–179.

Newell, C.A., and T. Hymowitz. 1980a. A taxonomic revision in the genus *Glycine* subgenus *Glycine* (Leguminosae). Brittonia 32:63–69.

Newell, C.A., and T. Hymowitz. 1980b. Cytology of *Glycine tabacina* a wild hexaploid relative of the soybean. J. Hered. 17:175–178.

Newell, C.A., and T. Hymowitz. 1982. Successful wide hybridization between the soybean and a wild perennial relative, G. *tomentella* Hayata. Crop Sci. 22:1062–1065.

Newell, C.A., and T. Hymowitz. 1983. Hybridization in the genus *Glycine* subgenus *Glycine* Willd. (Leguminosae, Papilionoideae). Am. J. Bot. 70:334–348.

Obermayer, R., and J. Greilhuber. 1999. Genome size in Chinese soybean accessions-stable or variable? Ann. Bot. (London) 84:259–262.

Ohashi, H. 1982. *Glycine max* (L.) Merr. subsp. *soja* (Sieb and Zucc.) Ohashi, *comb. nov.* (In Japanese.) J. Jpn. Bot. 57:30

Ohashi, H., Y. Tateishi, T.C. Huang, and T.T. Chen. 1984. Leguminosae of Taiwan. Sci. Rep. Tohoku Univ. 4th Ser. (Biology) 38:315.

Ohashi, H., Y. Tateishi, T. Nemoto, and H. Hoshi. 1991. Taxonomic studies on the Leguminosae of Taiwan IV. Sci. Rep. Tohoku Univ., 4th Ser. (Biology) 40:23–27.

Paillieux, A. 1880. (In French.) Bull. Soc. Natl. Acclim. Fr. Ser. 2. Ann. 71:414.

Palmer, R.G. 1974. Aneuploids in the soybean, *Glycine max*. Can. J. Genet. Cytol. 16:441–447.

Palmer, R.G. 1976. Chromosome transmission and morphology of three primary trisomics in soybeans (*Glycine max*). Can. J. Genet. Cytol. 18:131–140.

Palmer, R.G., and H.H. Hadley. 1968. Interspecific hybridization in *Glycine*, Subgenus *Leptocyamus*. Crop Sci. 8:557–563.

Palmer, R.G., and H. Heer. 1973. A root tip squash technique for soybean chromosome. Crop Sci. 13:389–391.

Palmer, R.G., and T.C. Kilen. 1987. Qualitative genetics and cytogenetics. p. 135–209. *In* J.R. Wilcox (ed.) Soybeans: Improvement, production, and uses. 2nd ed. Agron. Monogr. 16. ASA, CSSA, and SSSA, Madison, WI.

Palmer, R.G., K.E. Newhouse, R.A. Graybosch, and X. Delannay. 1987. Chromosome structure of wild soybean accessions from China and the Soviet Union of *Glycine soja* Sieb. & Zucc. [sic], Chromosome structure of wild soybean (*Glycine soja* Sieb. and Zucc.). Accessions from China and the Soviet Union. J. Hered. 78:243–247.

Palmer, R.G., T.W. Pfeiffer, G.R. Buss, and T.C. Kilen. 2004. Qualitative genetics. p. 137–234. *In* Soybeans: Improvement, production, and uses. 3rd ed. ASA, CSSA, and SSSA, Madison, WI.

Palmer, R.G., and H.T. Skorupska. 1994. Aneuploids from a male sterile mutant from tissue culture. Soybean Genet. Newsl. 21:228–229.

Palmer, R.G., H. Sun, and L.M. Zhao. 2000. Genetics and cytology of chromosome inversions in soybean germplasm. Crop Sci. 40:683–687.

Pfeil, B.E., M.D. Tindale, and L.A. Craven. 2001. A review of the *Glycine clandestina* species complex (Fabaceae, Phaseolae) reveals two new species. Aust. Syst. Bot. 14:891–900.

Polhill, R.M. 1994. Classification of the Leguminosae. p. XXXV–XLVII. *In* F.A. Bisby et al. (ed.) Phytochemical dictionary of the Leguminosae. Chapman and Hall, New York.

Putievsky, E., and P. Broué. 1979. Cytogenetics of hybrids among perennial species of *Glycine* subgenus *Glycine*. Aust. J. Bot. 27:213–223.

Rayburn, A.L., D.P. Birdar, D.G. Bullock, R.L. Nelson, C. Gourmet, and J.B. Wetzel. 1997. Nuclear DNA content diversity in Chinese soybean introductions. Ann. Bot. (London) 80:321–325.

Sacks, J.M., and K. Sadanaga. 1984. Linkage between the male-sterility gene (*ms1*) and a translocation breakpoint in soybean *Glycine max*. Can. J. Genet. Cytol. 26:401–404.

Sadanaga, K., and R.L. Grindeland. 1984. Locating the *w1* locus on the satellite chromosome in soybean. Crop Sci. 24:147–151.

Sakai, T., and N. Kaizuma. 1985. Hybrid embryo formation in an intersubgeneric cross of soybean (*Glycine max* Merrill) with a wild relative (*G. tomentella* Hayata). Jpn. J. Breed. 35:363–374.

Satow, E.M. 1900. The voyage of Captain John Saris to Japan, 1613. Vol. 5. Ser. 2. p.126. Hakluyt Soc., London.

Schoen, D.J., and A.H.D. Brown. 1991. Whole and part flower self-pollination in *Glycine clandestina* and *G. argyrea* and the evolution of autogamy. Evolution 45:1651–1664.

Shen, B., and L.C. Davis. 1992. Nodulation and nodulin gene expression in an interspecific hybrid between *Glycine max* and *Glycine tomentella*. Austr. J. Plant Physiol. 19:693–707.

Shi, L., T. Zhu, M. Morgante, J.A. Rafalski, and P. Keim. 1996. Soybean chromosome painting: A strategy for somatic cytogenetics. J. Hered. 87:308–313.

Shoemaker, R.C., P.M. Hatfield, R.G. Palmer, and A.G. Atherly. 1986. Chloroplast DNA variation in the genus *Glycine* subgenus *Soja*. J. Hered. 77:26–30.

Shoemaker, R.C., M.S. Heath, H. Skorupska, X. Delannay, M. Edge, and C.A. Newell. 1990. Fertile progeny of a hybridization between soybean [*Glycine max* (L.) Merr.] and *G. tomentella* Hayata. Theor. Appl. Genet. 80:17–23.

Shoemaker, R.C., K. Polzin, J. Labate, J. Specht, E.C. Brummer, T. Olsen, N. Young, V. Concibido, J. Wilcox, J.P. Tamulonis et al. 1996.Genome duplication in soybean (*Glycine* subgenus *soja*). Genetics 144:329–338.

Singh, R.J. 1993. Plant cytogenetics. CRC Press, Boca Raton, FL.

Singh, R.J., and T. Hymowitz. 1985a. Diploid-like meiotic behavior in synthesized amphiploids of the genus *Glycine* Willd. subgenus *Glycine*. Can. J. Genet. Cytol. 27:655–660.

Singh, R.J., and T. Hymowitz. 1985b. The genomic relationships among six wild perennial species of the genus *Glycine* subgenus *Glycine* Willd. Theor. Appl. Genet. 71:221–230.

Singh, R.J., and T. Hymowitz. 1985c. An intersubgeneric hybrid between *Glycine tomentella* Hayata and the soybean, *G. max* (L.) Merr. Euphytica 34:187–192.

Singh, R.J., and T. Hymowitz. 1985d. Intra-and interspecific hybridization in the genus *Glycine*, Subgenus *Glycine* Willd.: Chromosome pairing and genome relationships. Z. Pflanzenzuecht. 95:289–310.

Singh, R.J., and T. Hymowitz. 1987. Intersubgeneric crossability in the genus *Glycine* Willd. Plant Breed. 98:171–173.

Singh, R.J., and T. Hymowitz. 1988. The genomic relationships between *Glycine max* (L.) Merr. and *G. soja* Sieb. and Zucc. as revealed by pachytene chromosome analysis. Theor. Appl. Genet. 76:705–711.

Singh, R.J., and T. Hymowitz. 1989. The genomic relationships among *Glycine soja* Sieb. and Zucc., *G. max* (L.) Merr. and '*G. gracilis*' Skvortz. Plant Breed. 103:171–173.

Singh, R.J., and T. Hymowitz. 1991a. Cytogenetics of a synthesized allopentaploid (*2n = 5x = 100*) in the genus *Glycine* subgenus *Glycine*. Genome 34:751–756.

Singh, R.J., and T. Hymowitz. 1991b. Identification of five primary trisomics of soybean by pachytene chromosome analysis. J. Hered. 82:75–77.

Singh, R.J., and T. Hymowitz. 1999. Soybean genetic resources and crop improvement. Genome 42:605–616.

Singh, R.J., H.H. Kim, and T. Hymowitz. 2001. Distribution of rDNA loci in the genus *Glycine* Willd. Theor. Appl. Genet. 103:212–218.

Singh, R.J., K.P. Kollipara, F. Ahmad, and T. Hymowitz. 1992. Putative diploid ancestors of 80-chromosome *Glycine tabacina*. Genome 35:140–146.

Singh, R.J., K.P. Kollipara, and T. Hymowitz.1987a. Intersubgeneric hybridization of soybeans with a wild perennial species, *Glycine clandestina* Wendl. Theor. Appl. Genet. 74:391–396.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1987b. Polyploid complexes of *Glycine tabacina* (Labill.) Benth. and *G. tomentella* Hayata revealed by cytogenetic analysis. Genome 29:490–497.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1988. Further data on the genomic relationships among wild perennial species (*2n = 40*) of the genus *Glycine* Willd. Genome 30:166–176.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1989. Ancestors of 80- and 78-chromosome *Glycine tomentella* Hayata (Leguminosae). Genome 32:796–801.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1990. Backcross-derived progeny from soybean and *Glycine tomentella* Hayata intersubgeneric hybrids. Crop Sci. 30:871–874.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1992a. Genomic relationships among diploid wild perennial species of the genus *Glycine* Willd. subgenus *Glycine* revealed by crossability, meiotic chromosome pairing and seed protein electrophoresis. Theor. Appl. Genet. 85:276–282.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1993. Backcross (BC$_2$ - BC$_4$) -derived fertile plants from *Glycine max* and *G. tomentella* intersubgeneric hybrids. Crop Sci. 33:1002–1007.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1997. Intergenomic relationships among wild perennial *Glycine* species. p. 40–43. *In* B. Napompeth.(ed.) Soybean feeds the world. World Soybean Res. Conf. V Proc., Chiang Mai, Thailand. 21–27 Feb. 1994. Kasetsart Univ. Press, Bangkok, Thailand.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1998a. The genomes of *Glycine canescens* F. J. Herm. and *G. tomentella* Hayata of Western Australia and their phylogenetic relationships in the genus *Glycine*. Genome 41:669–679.

Singh, R.J., K.P. Kollipara, and T. Hymowitz. 1998b. Monosomic alien addition lines derived from *Glycine max* (L.) Merr. and *G. tomentella* Hayata.: Production, characterization and breeding behavior. Crop Sci. 38:1483–1489.

Skorupska, H., M.C. Albersten, K.D. Langholz, and R.G. Palmer. 1989. Detection of ribosomal RNA genes in soybean. *Glycine max* (L.) Merr., by in situ hybridization. Genome 32:1091–1095.

Skorupska, H., and R.G. Palmer. 1987. Monosomics from synaptic KS mutant. Soybean Genet. Newsl. 14:174–178.

Skvortzow, B.V. 1927. The soybean-wild and cultivated in Eastern Asia. Proc. Manchurian Res. Soc. Pub. Ser. A. Nat. History Sec. 22:1–8.

Tateishi, Y., and H. Ohashi. 1992. Taxonomic studies on *Glycine* of Taiwan. J. Jpn. Bot. 67:127–147.

Taylor-Grant, N., and K.M. Soliman. 1999. Detection of polymorphic DNA and taxonomic relationships among 10 wild perennial soybean species using specific and arbitary nucleotide primers. Biol. Plant. 42:25–37.

Thseng, F.S., T.K. Lin, and W.S. Tu. 2000. The relations of genus *Glycine* subgenus *soja* and *Glycine formosana* Hosok. collected from Taiwan revealed by RAPD analysis. J. Jpn. Bot. 75:270–279.

Thseng, F.S., H.T. Tsui, and S.T. Wu. 1997. Population variation of wild soybean in Taiwan IV. Intraspecific variation of *Glycine tabacina* (labill.) Benth. in Ponghu Islets off Taiwan: Leaflet and pod morphology and DNA polymorphism. (In Chinese.) J. Agric. Assoc. China No. 178:52–62.

Tindale, M.1984. Two new eastern Australian species of *Glycine* Willd. (Fabaceae). Brunonia 7:207–213.

Tindale, M.D. 1986a. A new north Queensland species of *Glycine* Willd. (Fabaceae). Brunonia 9:99–103.

Tindale, M.1986b. Taxonomic notes on three Australian and Norfolk Island species of *Glycine* Willd. (Fabaceae: Phaseolae) including the choice of a neotype for *G. clandestina* Wendl. Brunonia 9:179–191.

Tindale, M.D., and L.A. Craven. 1988. Three new species of *Glycine* (Fabaceae: Phaseolae) from North-Western Australia, with notes on amphicarpy in the genus. Aust. Syst. Bot. 1:399–410.

Tindale, M.D., and L.A. Craven. 1993. *Glycine pindanica* (Fabaceae, Phaseolae), a new species from West Kimberley, Western Australia. Aust. Syst. Bot. 6:371–376.

Vaughan, D.A., and T. Hymowitz. 1984. Leaf flavonoids of *Glycine* subgenus *Glycine* in relation to systematics. Biochem. Syst. Ecol. 12:189–192.

Viviani, T., L. Conte, G. Christofolini, and M. Speranza. 1991. Sero-systematic and taximetric studies on the Phaseoleae (Fabaceae) and related tribes. Bot. J. Linn. Soc. 105:113–136.

Wang, C.L. 1976. Review on the classification of soybeans. (In Chinese.) Acta Phytotaxon. Sin. 14:22–30.

Wang, Y.M., and B.C. Zhuang. 1994. Study on the biology of the genus *Glycine* III. Analysis of SOD zymogram pattern of different subgenera and species. (In Chinese.) Soybean Sci. 13:340–344.

Wu, X.L., C.Y. He, S.Y. Chen, B.C. Zhuang, K.J. Wang, and X.C. Wang. 2001. Phylogenetic analysis of interspecies in the genus *Glycine* through SSR markers. (In Chinese.) Acta Genet. Sin. 28:359–366.

Xu, S.J., R.J. Singh, and T. Hymowitz. 1997. Establishment of a cytogenetic map of soybean: Progress and prospective. Soybean Genet. Newsl. 24:121–122.

Xu, S.J., R.J. Singh, and T. Hymowitz. 1998a. A modified procedure for mitotic chromosome counts in soybean. Soybean Genet. Newsl. 25:107.

Xu, S.J., R.J. Singh, and T. Hymowitz. 1998b. Establishment of a cytogenetic map of soybean: Current status. Soybean Genet. Newsl. 25:120–122.

Xu, S. J., R. J. Singh, and T. Hymowitz. 2000a. Monosomics in soybean: Origin, identification, cytology and breeding behavior. Crop Sci. 40:985–989.

Xu, S.J., R.J. Singh, K.P. Kollipara, and T. Hymowitz. 2000b. Primary trisomics in soybean: Origin, identification, breeding behavior, and use in linkage mapping. Crop Sci. 40:1543–1551.

Xu, S.J., R.J. Singh, K.P. Kollipara, and T. Hymowitz. 2000c. Hypertriploid in soybean: Origin, identification, cytology, and breeding behavior. Crop Sci. 40:72–77.

Yeh, M.S., C.D. Liu, and S.H. Cheng. 1997. Collection and variation of wild soybean germplasm in Taiwan and Kinmen areas. (In Chinese.) J. Agric. Assoc. China No 177:11–27.

Zhang, F., and R.G. Palmer. 1990. The *ms1* mutation in soybean: involvement of gametes in crosses with tetraploid soybean. Theor. Appl. Genet. 80:172–176.

Zhong, Z.P., D.J. Liu, and Q.F. Chen. 1997. Study on the karyotypes of soybean species. p.12–14. *In* B. Napompeth (ed.) World Soybean Res. Conf. V, Chiang Mai, Thailand. 21–27 Feb. 1994. Kaset-sart Univ. Press, Bangkok, Thailand.

Zhu, T., L. Shi, J.J. Doyle, and P. Keim. 1995b. A single nuclear locus phylogeny of soybean based on DNA sequence. Theor. Appl. Genet. 90:991–999.

Zhu, T., L. Shi, and P. Keim. 1995a. Detection by in-situ fluorescence of short, single copy sequence of chromosomal DNA. Plant Mol. Biol. Rep.13:270–277.

5   Qualitative Genetics

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In the first edition of *Soybeans: Improvement, Production, and Uses*, a separate chapter was devoted to qualitative genetics (Bernard and Weiss, 1973). The second edition combined qualitative genetics and cytogenetics (Palmer and Kilen, 1987). Recent advances in qualitative genetics and cytogenetics necessitated separate chapters for the third edition. This chapter summarizes existing information and highlights new information on soybean qualitative genetics with an emphasis on publications and data reported since 1986. The tables are inclusive extending from the data provided in the first edition of the monograph.

5–1 SOYBEAN GENETICS COMMITTEE

The Soybean Genetics Committee, established in 1955, carries out the following functions: (i) establishes guidelines and rules for assigning gene symbols and (ii) acts as a review committee for manuscripts concerning qualitative genetic interpretation and gene symbols in the genus *Glycine*. Soybean scientists are encouraged to submit manuscripts to the committee for a gene symbol assignment and to add seeds of the genetic line to the Soybean Genetic Type Collection. This procedure helps to ensure that an orderly nomenclature is followed to symbolize genes