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Where are the diploid ($2n = 2x = 20$) genome donors of *Glycine* Willd. (Leguminosae, Papilionoideae)?

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Summary

There are about 16 genera in the subtribe Glycininae, tribe Phaseoleae, family Leguminosae. The overwhelming reason for the economic importance of the subtribe Glycininae is due to the cultivated soybean, *Glycine max* (L.) Merrill. The soybean, its wild annual counterpart and most of wild perennial members of the genus *Glycine* carry $2n = 40$ chromosomes. It is reasonable to assume that the base number of the genus is $x = 10$. However *Glycine* plants carrying $2n = 20$ have not been reported. Herein we report on the 2n chromosome situation of the remaining 15 genera in the subtribe Glycininae and two genera (*Galactia, Ophrestia*) once thought to be allied with *Glycine*. Certain species in *Dumasia, Galactia* and *Ophrestia* were found to carry $2n = 20$ chromosomes. All the other genera carry $2n = 22$, 28 or 44 chromosomes. The 2n chromosome number of *Teyleria* (44) and *Pseudeminia* (22) is being reported for the first time. No cytological information is available for *Diphyllarium, Masteria* and *Sinodolichos*.

Introduction

The members of the subtribe Glycininae, tribe Phaseoleae belong to the family Leguminosae, subfamily Papilionoideae. Lackey (1977, 1981) divided the Glycininae into two groups – the *Glycine* and the *Shuteria*. The *Glycine* group is composed of about 8 genera: *Eminia* Taub., *Glycine* Willd., *Nogra* Merr., *Pseudeminia* Verdc., *Pseudovigna* Verdc., *Pueraria* DC., *Sinodolichos* Verdc., and *?Teramnus* P. Br. The *Shuteria* group also contains about 8 genera: *Amphicarpa* Nutt., *Cologania* Kunth., *?Diphyllarium* Gagnep., *Dumasia* DC., *?Masteria* Benth., *Neonotonia* Lackey, *Shuteria* W. & A., and *Teyleria* Backer.

Although *Neonotonia, Pueraria* and *Teramnus* species are used as forage, pasture and/or for soil conservation purposes in tropical and subtropical zones, the overwhelming reason for the economic importance of the subtribe Glycininae is due to the cultivated soybean, *Glycine max* (L.) Merrill.

The genus *Glycine* is divided into two subgenera *Soja* (Moench) F.J. Herm. and *Glycine*. The annual cultivated soybean ($2n = 40$) and its wild annual counterpart *G. soja* Sieb. & Zucc. ($2n = 40$) comprise the subgenus *Soja*. *Glycine soja* is distributed in China, Japan, Korea, Taiwan, and the USSR (Hymowitz & Singh, 1987).

The subgenus *Glycine* contains 12 wild perennial species (Singh and Hymowitz, 1985a; Singh et al., 1988). Ten of the species [*G. arenaria* Tind., *G. argyrea* Tind., *G. canescens* F.J. Herm., *G. clandestina* Wendl., *G. curvata* Tind., *G. cyrtoloba* Tind., *G. falcata* Benth., *G. latifolia* (Benth) Newell and Hymowitz, *G. latrobeana* (Meissn.) Benth., and *G. microphylla* (Benth.) Tind.] are indigenous to Australia and carry $2n = 40$ chromosomes. *Glycine tabacina* (Labill.) Benth. with $2n = 40$, 80 chromosomes, has been found in Australia, Taiwan, South Pacific Islands (New Caledonia, Fiji, Tonga, Vanuatu) and West Central Pacific Islands (Mariana, Ryukyu). All accessions of *G. tabacina* collected outside of Australia are tetraploid (2n = 80) and even within Australia, tetraploids predominate over the diploid forms. *Glycine tomentella* Hayata has been found in Australia (2n = 38, 40, 78, 80) Papua New Guinea (2n = 40, 78, 80), Philippines (2n = 80), and Taiwan (2n = 80). Singh et al. (1987) demonstrated that the complexes of *G. tabacina* and *G. tomentella* evolved through allopolyploidization.

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. As indicated above, all *Glycine* species identified thus far carry two main cytotypes 2n = 40 and 2n = 80. Thus, it is quite reasonable to assume that the 40 and 80 chromosome species are tetraploids (2n = 4 x = 40) and octoploids (2n = 8 x = 80), respectively. Since chromosome pairing is under genetic control (Singh & Hymowitz, 1985b) the *Glycine* species behave cytologically as diploids.

The key question to be answered in elucidating the evolutionary pathways which produced the 40 chromosome *Glycine* species is the following. Where are the diploid progenitor species or genome donors which carry 2n = 2 x = 20 or 22 chromosomes? Several possibilities exist.

1. The diploid progenitor species are extinct, perhaps swamped out by the more aggressive polyploids.
2. The diploid progenitor species exist but have not been collected.
3. The diploid progenitor species have been collected but misclassified by systematists.
4. The diploid *Glycine* forms never existed. One or more genera allied to *Glycine* are the genomic donors to forms that evolved into *Glycine.*

Herein we report on the 2n chromosome number of 8 genera in the subtribe Glycininae. In addition, we also report on the 2n chromosome number of *Galactia* P.Br. of the subtribe Diocleinae and *Ophrestia* H.M.L. Forbes of the subtribe Ophrestiinae. Both genera have in the past been reported as having an affiliation with *Glycine* (Lackey, 1977).

Materials and methods

All species were screened for their somatic (2n) chromosome number from root tip squashes (Table 1). The seed were scarified to facilitate germination and the technique of Palmer & Heer (1973) suggested for the soybean was used for the preparation of root tips with some modifications with regard to the pre-treatment. The following pretreatments were tried:

1. Ice cold water – 24 hours.
2. Saturated solution of paradichlorobenzene (PDB) – 4 hours.
3. 0.002 M solution of 8 – hydroxyquinoline – 3 hours.

The roots pre-treated in 8 – hydroxyquinoline gave much better chromosome spreads for the species used in the study than those pretreated with ice cold water or PDB both of which work well for soybeans. A minimum of 10 well spread cells were counted to determine the somatic chromosome numbers.

Meiotic cytological techniques described by Singh & Hymowitz (1985c) were used. Herbarium specimens of accessions are deposited in the herbarium of the Crop Evolution Laboratory (CEL), University of Illinois at Urbana-Champaign.

Results and discussion

The accessions utilized in this study, their somatic chromosome number, origin, and seed source are presented in Table 1. Following is a discussion of results.

Amphicarpa – Two species, *A. bracteata* and *A. edgeworthii* had somatic chromosome numbers of 22 (Fig. 2). Lackey (1977) cited previously reported somatic chromosome counts of 20, 22, and 40. Most probably misidentification of specimens and/or the lack of a good pretreatment for chromosome spreading resulted in erroneous counts. Our results show that the basic chromosome number of this

Table 1. Species, somatic chromosome number, origin, and seed sources of genera, in the subtribe Glycininae, *Galactia* of the subtribe Diocleinae, and *Ophrestia* of the subtribe Ophrestiinae.

CU[1]	Species	2n	Origin	Seed source
			Subtribe Glycininae	
200	*Amphicarpa bracteata* (L.) Fernald	22	Belle Plaine, IA	C.A. Laible, Bloomington, IL
208	*Amphicarpa bracteata* (L.) Fernald	22	Tompkins Co., NY	J.J. Doyle, Cornell Univ, Ithaca, NY
209	*Amphicarpa bracteata* (L.) Fernald	22	East Peoria, IL	#63448[2]
210	*Amphicarpa edgeworthii* Benth.	22	Korea	#47549[2]
293	*Dumasia villosa* DC	20	Pakistan	#45614[2]
147	*Neonotonia wightii* (Arnott) Lackey	22	Australia (?)	P.I.258381[8]
150	*Neonotonia wightii* (Arnott) Lackey	22	India	J.M.-2297[10]
153	*Neonotonia wightii* (Arnott) Lackey	44	Malawi	CPI 52607[3]
159	*Neonotonia wightii* (Arnott) Lackey	22	Tanzania	CPI 52614[3]
11	*Pseudeminia comosa* (Bak.) Verdc.	22	Mtoko, Zimbabwe	H.D.L. Corby, Salisbury, Zimbabwe
28	*Pseudovigna argentea* (Willd.) Verdc.	22	?	CPI 33429[2]
33	*Pueraria lobata* (Willd.) Ohwi	22	?	#17321[2]
35	*Pueraria lobata* (Willd.) Ohwi	22	?	#48977[2]
7	*Pueraria phaseoloides* (Rox.) Benth.	22	Singapore	Pretoria, South Africa[4]
8	*Pueraria phaseoloides* (Rox.) Benth.	22	Singapore	Pretoria, South Africa[4]
10	*Pueraria phaseoloides* (Rox.) Benth.	22	Taipei	Pretoria, South Africa[4]
29	*Pueraria phaseoloides* (Rox.) Benth.	22	Philippines	Los Banos, Philippines[5]
81	*Pueraria phaseoloides* (Rox.) Benth.	22	?	#17298[6]
85	*Pueraria phaseoloides* (Rox.) Benth.	22	?	#17305[6]
114	*Pueraria phaseoloides* (Rox.) Benth.	22	Indonesia	Bogor, Indonesia[7]
211	*Teramnus labialis* (L.f.) Spreng	28	India	#62315[2]
164	*Teramnus micans* (Bak.) Bak.f.	28	India	?
163	*Teramnus unicinatus* (L.) SW.	28	India	P.I. 213514[8]
42	*Teyleria koordersii* (Backer) Backer	44	Hainan, PRC	Guangzhou, PRC
294	*Teyleria koordersii* (Backer) Backer	44	Hainan, PRC	#18064[6]
			Subtribe Diocleinae	
216	*Galactia acapulcensis* Rose	20	Honduras	P.I. 188883[8]
218	*Galactia jussiaeana* Kunth.	20	Brazil	P.I. 367914[8]
284	*Galactia longifolia* (Jacq.) Benth	20	Australia	100764[9]
285	*Galactia longifolia* (Jacq.) Benth	20	Australia	100812[9]
286	*Galactia longifolia* (Jacq.) Benth	20	Australia	TPI70[9]
219	*Galactia* species	20	El Salvador	P.I. 200744
231	*Galactia* species	20	Honduras	P.I. 200745[8]
			Subtribe Ophestiinae	
250	*Ophrestia hedysaroides* (Willd.) Verdc.	20	Tanzania	P.I. 274229[8]
245	*Ophrestia radicosa* (A. Rich.) Verdc.	22	Zaire	P.I. 231465

[1] University of Illinois internal code number.
[2] Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois.
[3] Department of Primary Industries, Brisbane, Queensland, Australia.
[4] Division of Plant and Seed Control, Pretoria, South Africa.
[5] Institute of Plant Breeding, College of Agriculture, University of the Philippines, Los Banos, Philippines.
[6] CIAT, Cali, Columbia.
[7] Botanic Gardens of Indonesia, Bogor, Indonesia.
[8] U.S. Department of Agriculture, Beltsville, Maryland.
[9] Division of Tropical Crops and Pastures, CSIRO, St. Lucia, Queensland, Australia.
[10] L.J.G. van der Maesen, ICRISAT, India.

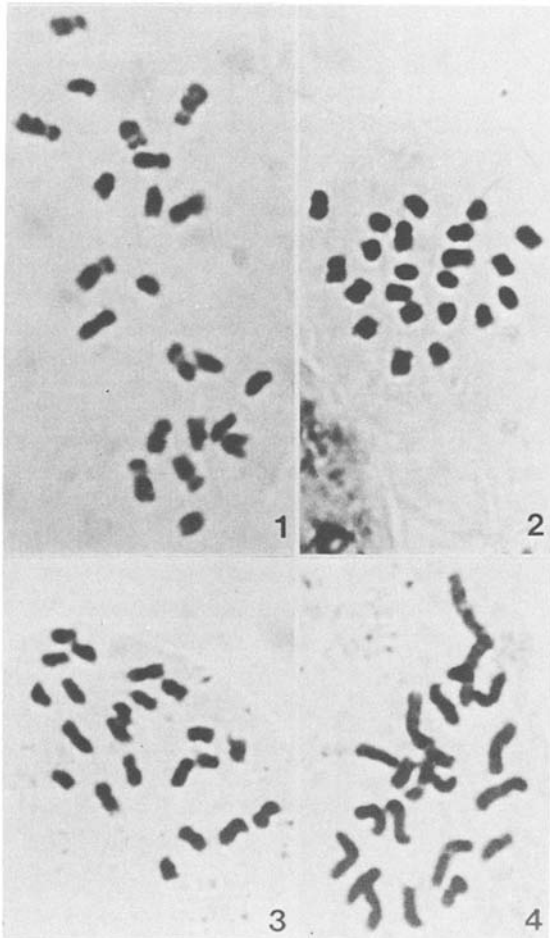
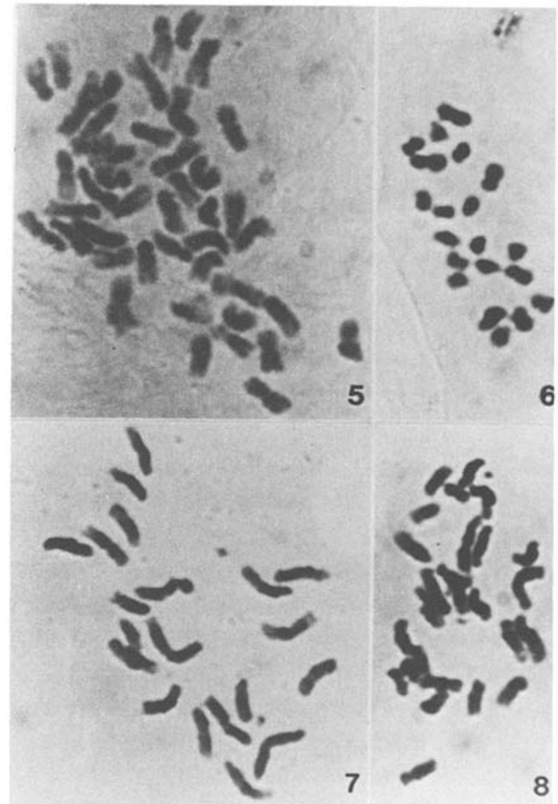


Fig. 1–4. Somatic chromosomes – 1. *Pueraria phaseoloides* (2n = 22); 2. *Amphicarpa bracteata* (2n = 22); 3. *Pseudovigna argentea* (2n = 22), and 4. *Pseudeminia comosa* (2n = 22).



Figs. 5–8. Somatic chromosomes – 5. *Teyleria koordersii* (2n = 44); 6. *Galactia striata* (2n = 20); 7. *Ophrestia hedysaroides* (2n = 20) and 8. *Teramnus micans* (2n = 28).

genus is 11, although it is possible, but highly unlikely that *Amphicarpa* is a dibasic genus carrying x = 10 or 11.

Dumasia – The somatic chromosome number of *D. villosa* was found to be 20 and it confirms the only available report for this genus (Bir & Sidhu, 1966).

Neonotonia – Somatic chromosome numbers of 22 and 44 were found in *N. wightii* which is consistent with previously published reports for the genus (Isely et al., 1980; Pritchard & Wutch, 1964).

Pseudeminia – The somatic chromosome number of *P. comosa* was 22 (Fig. 4). This is the first chromosome number report for the genus.

Pseudovigna – The somatic chromosome number of *Pseudovigna argentea* was found to be 22 (Fig. 3) confirming an earlier report of Maréchal (1970).

Pueraria – Both *Pueraria lobata* and *P. phaseoloides* exhibited 22 chromosomes in all accessions studied (Fig. 1). Somatic chromosome counts of 20, 22, and 24 have been reported previously for members of this genus (Goldblatt, 1981). In his revision of *Pueraria,* van der Maesen (1985) suggested that additional studies were required in order to establish the chromosome number(s) of the genus. A range of accessions used in this study (Table 1) consistently showed 22 chromosomes, so we conclude that the somatic chromosome number of this genus is 22.

Teramnus – The three species of *Teramnus* studied, *T. labialis, T. micans* and *T. uncinatus* had 28

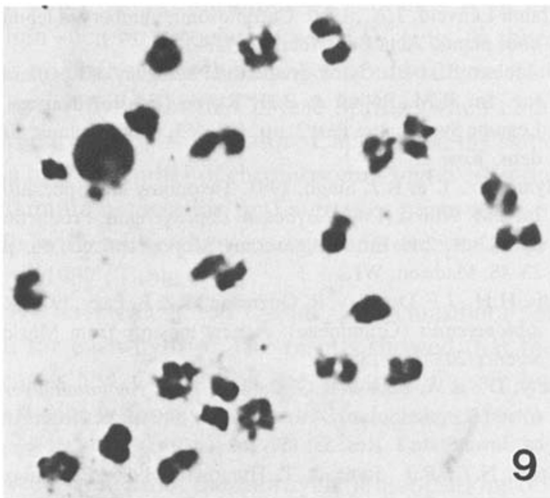


Fig. 9. Teyleria koordersii showing 22 bivalents in meiosis.

somatic chromosomes (Fig. 8). The earlier report of n = 10 for *T. labialis* (Bir & Kumari, 1973) appears to be either a miscount or more likely a count for a misidentified plant. The chromosome number of *T. micans* is being reported for the first time. Our results confirm the earlier report of Frahm-Leliveld (1960) for *T. uncinatus.*

Teyleria – The somatic chromosome number of *T. koordersii* was found to be 44 (Fig. 5). Meiotic analysis of this species revealed 22 bivalants (Fig. 9). This is the first chromosome report for the genus.

Galactia – The four species analysed had 20 somatic chromosomes (Fig. 6). Our counts are consistent with the previous reports in the genus (Goldblatt, 1981).

Ophrestia – The somatic chromosome number of *O. hedysaroides* was 20 (Fig. 7) and confirms an earlier report for this species (Lackey, 1977). However, the somatic chromosome number for *O. radicosa* was founds to be 22. This is the first chromosome report for the species. Lackey (1977) has placed *Ophrestia* in the subtribe Ophrestiinae together with *Cruddasia* Prain and *Pseudoeriosema* Hauman. The somatic chromosome number of *Pseudoeriosema borianii* (Schweinf.) Hauman has been reported as 22 (Cheng 1963; Pritchard, 1972).

Teyleria with a somatic chromosome number of 44 and 22 meiotic bivalents leads us to the conclusion that it has evolved as an amphiploid involving two 22 chromosome species. The *Teyleria* example gives further credence to the role of allopolyploidy in the evolution of Glycininae, a phenomenon well established in the genus *Glycine* (Singh et al., 1987). Thus the search for at least one or perhaps two genome donors of *G. max* would seem logical. Intensive wild perennial *Glycine* germplasm collecting expeditions have been conducted only within the past decade. It is quite possible that relic diploid populations still exist in Australia and may be discovered by additional *Glycine* germplasm collecting expeditions. The situation may be analogous to the recent discovery of *Zea diploperennis* Iltis, Doebley and Guzma near Jalisco, Mexico (Iltis et al. 1979).

Based on chromosome numbers it would be logical to conclude that the 20 chromosome species viz. *Dumasia villosa, Ophrestia hedysaroides* and the species of *Galactia* should be the primary targets for further investigations aimed at the speciation of *G. max,* but it would not be prudent to over look the role of 22 chromosome species since that would amount to overlooking the role of aneuploid reduction in the evolution of *G. max*. The 38 and 78 chromosome species of *Glycine* are examples of the role of aneuploid reduction in the evolution of the genus *Glycine.*

The seed collections of genera allied to *Glycine* are sparse to nonexistent. Realistically, chromosome counts of one or two accessions per species per genus is not very satisfactory in so far as establishing genomic affinities or even possible genome donors to *Glycine.* In the absence of extensive germplasm collections of these genera, it would seem logical to pursue a complementary course to determine the affinities of genera to *Glycine.* For example, investigations on the qualitative variation in the phytoalexins, (glyceollins) produced by members of the Glycininae appears to be a useful screening procedure for systematic utility (Keen et al., 1986).

It is evident that considerable confusion and contradictions existed with regard to the chromosome numbers of genera in subtribe Glycininae. The present study has helped in establishing and/or con-

Table 2. Summary of chromosome numbers in genera of the subtribe Glycininae.

Genus	Chromosome numbers (2n)	
Glycine Group	Previous reports	This report
Eminia	22[c]	–
Glycine	38, 40, 78, 80[b]	–
Nogra	22[c]	–
Pseudeminia	–	22
Pseudovigna	22[a]	22
Pueraria	20, 22, 24[a]	22
Sinodolichos	–	–
?*Teramnus*	20, 28[a]	28
Shuteria group		
Amphicarpa	20, 22, 40[a]	22
Cologania	44[a]	–
?*Diphyllarium*	–	–
Dumasia	20, 22[a]	20
?*Masteria*	–	–
Neonotonia	22, 44[a]	22, 44
Shuteria	11[c]	–
Teyleria	–	44

[a] Lackey, 1977.
[b] Hymowitz and Singh, 1987.
[c] Goldblatt, 1981.

firming the chromosome numbers of these genera. *Diphyllarium, Masteria,* and *Sinodolichos* are three genera for which no chromosome counts are available (Table 2).

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