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SEROTAXONOMY OF *SIMMONDSIA CHINENSIS* (SIMMONDSIACEAE)

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Introduction

Simmondsia Nutt. is a monotypic genus consisting of the single species *Simmondsia chinensis* (Link) C. K. Schneid. A variety of systematic affiliations has been suggested for the genus *Simmondsia*. It has been placed in the order Euphorbiales as a member of the Euphorbiaceae (Bentham and Hooker 1883), as a member of a monotypic family, the Simmondsiaceae (Takhtajan 1969), or as a member of the Buxaceae (Cronquist 1968), although other workers are dubious of a close alliance between *Simmondsia* and the Buxaceae (Wiger 1935; Wunderlich 1967). Additional workers have suggested membership in the orders Hamamelidales (Hutchinson 1959), Celastrales (Baillon 1877), or the Chenopodiales (van Tieghem 1897). Still others have suggested a close relationship with the Garryaceae (Nuttall 1844) or Simaroubaceae (R. F. Thorne, pers. comm.). Thorne (1976) has listed *Simmondsia* as a *taxon incerta sedis*.

Earlier phytochemical work by Scogin and Brown (1979) showed the leaf flavonoids of *Simmondsia* to be of little utility as a systematic discriminant because of their uniqueness. The present study was undertaken to determine whether comparative serology would favor a particular systematic relationship for *Simmondsia* among the plethora of suggested possibilities.

Materials and Methods

Antigen preparation.—Meal was prepared from *Simmondsia chinensis* seed collected from plants in cultivation at the Rancho Santa Ana Botanic Garden by grinding in a micromill and was delipified by extraction twice with n-heptane. Fifteen grams of delipified seed meal were extracted overnight at 4 C with 250 ml of 2.5% NaCl solution at pH 7.0. This extract was clarified by centrifugation (10 min @ 5,000 × g) and the protein content was determined by the method of Murphy and Kies (1960).

Preparation of antiserum.—*Simmondsia* seed meal extract containing 1.2 mg/ml of protein was thoroughly emulsified with an equal volume of Freund's complete adjuvant (Calbiochem) and was injected into a single New Zealand white rabbit according to the following schedule. Day 1: ½ ml injected subcutaneously (one site) and 1 ml injected intramuscularly at each of two sites. Day 20: 1 ml injected intramuscularly (one site) and ½ ml injected subcutaneously at each of two sites. The rabbit was bled from the

Table 1. Taxa examined for serological cross-reactivity with *Simmondsia* antiserum. An asterisk indicates the presence of cross-reactivity.

Taxon (Seed source)
Celastrales
Celastraceae
Celastrus scandens L. (G)
Euonymus europaeus L. (R)
E. japonicus L. (R)
E. latifolius Mill. (B)
Mortonia utahensis (Cov.) A. Nels. (RSA)
Chenopodiales
Amaranthaceae
Amaranthus albus L. (MSU)
A. caudatus L. (MSU)
Cactaceae
Opuntia erinacea Engelm. & Bigel. (RSA)
O. phaeacantha Engelm. (RSA)
Caryophyllaceae
Silene lemmonii Wats. (RSA)
Chenopodiaceae
Chenopodium ambrosioides L. (MSU)
Phytolaccaceae
Rivina humilis L. (D)
Portulacaceae
Calyptridium monandrum Nutt. in T. & G. (RSA)
Lewisia nevadensis (Gray) Rob. in Gray (CR)
Cornales
Garryaceae
Garrya elliptica Dougl. (RSA)
G. veatchii Kell (RSA)
Euphorbiales
Euphorbiaceae
Acalyphoideae
Acalypha californica Benth. (RSA)
**Mallotus japonica* Muell. Arg. (R)
* *Ricinus communis* L. (RSA)
Crotonoideae
Jatropha curcas L. (D)
Ricinocarpos bowmanii F. Muell. (GW)
R. pinifolius Desf. (GW)
Euphorbioideae
Euphorbia corollata L. (MSU)
E. marginata Pursh. (R)
E. peplus L. (N)
**Sapium sebiferum* Roxb. (GW)
Phyllanthoideae
Antidesma venosum E. Mey. ex Tul. (GW)
Hamamelidales
Hamamelidaceae
Hamamelis japonica Sieb. & Zucc. (B)
H. virginica L. (B)
Liquidambar styraciflua L. (G)
Pittosporales
Buxaceae
Buxus microphylla Lieb. & Zucc. (MSU)
B. sempervirens L. (MSU)
Sarcocca hookeriana Baill. (D)
S. humilis Stapf. (OX)
Rutales
Simaroubaceae
Ailanthus giraldii Dode (N)
Holocantha emoryi Gray (RSA)

Seed sources: B, Arboretum des Barres (France); CR, Clyde Robbins (private seed purveyor); D, Technisch Hogeschool Delft; G, University of Guelf Arboretum; GW, Dr. G. L. Webster, University of California, Davis; MSU, Beal Botanic Garden, Michigan State University; N, Jardin Botanique, Nantes; OX, Botanic Gardens, University of Oxford (England); R, Istituto ed Orto Botanico, Rome; RSA, Rancho Santa Ana Botanic Garden.

ear vein on days 36 and 62 and serum was prepared by standard methods (Campbell et al. 1964).

Preparation of test extracts.—Seed of numerous test species were received from various botanic gardens as indicated in Table 1. Seed material was ground in a micromill to yield a fine meal and sufficient 0.85% NaCl was added to form a slurry which remained overnight at 4 C. Text extracts were prepared by centrifugation (10 min @ 5,000 × g) of the slurry and the opalescent supernatants were used immediately for analysis.

Analysis of activity.—Double diffusion analysis of the precipitin reaction between test extracts and antiserum was performed as described by Brown and Bold (1964). Double diffusion plates were developed for 24–48 hr at 37 C in a humidified atmosphere. For immunoelectrophoretic analysis the test extract protein mixture was fractionated by standard procedures on a 7½% acrylamide tube gel (Ornstein and Davis 1962). This gel was then embedded in Ionagar (as used for double diffusion) supported on a microscope slide. Serum troughs were cut to receive antiserum (Campbell et al. 1964) and the precipitin reaction was developed as described earlier.

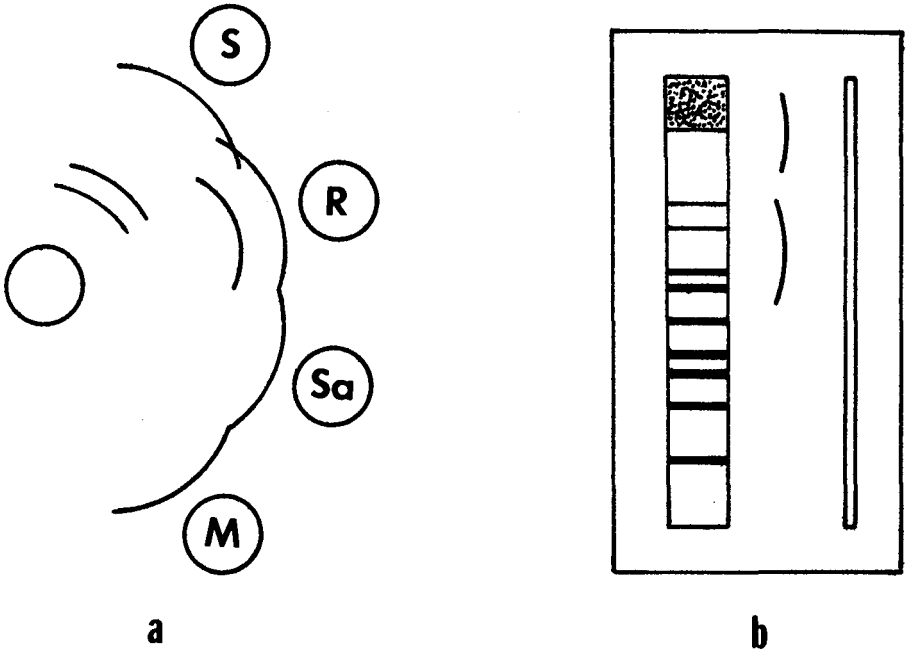

Fig. 1. A. Double diffusion patterns of test extracts against *Simmondsia* antiserum: S, *Simmondsia chinensis*; R, *Ricinus communis*; Sa, *Sapium sebiferum*; M, *Mallotus japonicus*.— B. AGE and IE patterns of the *Simmondsia* vs. *Simmondsia* homologous reaction.

Results

Homologous reaction.—When antiserum to *Simmondsia* seed protein was challenged with *Simmondsia* seed extract in the homologous reaction, three strong immunoprecipitating systems (IPS, "bands") were noted in double diffusion plates (see Fig. 1a). Immunoelectrophoresis (IE) of *Simmondsia* seed protein yielded two IPS arcs, neither of which corresponded to major seed-protein bands as revealed by staining following acrylamide gel electrophoresis (AGE). The IE and AGE patterns of *Simmondsia* seed proteins are shown in Fig. 1b.

Heterologous reaction.—Seed extracts from numerous putatively related taxa gave no cross-reaction when challenged by double diffusion with *Simmondsia* antiserum. Taxa examined are listed in Table 1. Cross-reaction was detected only with three species of the Euphorbiaceae: *Mallotus japonicus, Sapium sebiferum* and *Ricinus communis*. Heterologous precipitin bands were weaker than those of the homologous reaction and exhibited a nonidentity interaction with *Simmondsia* extract bands. Two IPS are detectable between *Simmondsia* and *Ricinus*, but only one each between *Sim-*

mondsia paired with *Sapium* and with *Mallotus*. The one IPS common among the three euphorbiaceous genera exhibits identity interactions (see Fig. 1a).

Discussion

The presence of serological cross-reactivity between *Simmondsia* and three genera of the Euphorbiaceae and the absence of reactivity with all other putatively related taxa which were tested, strongly support a relationship between the Euphorbiaceae and *Simmondsia*. The possibility of the reactivity with the Euphorbiaceae being artifactual and "antisystematic" (Fairbrothers 1977) is made much less likely by its absence in any of a wide taxonomic spectrum of other tested species.

Within the Euphorbiaceae the three reacting genera occur in two subfamilies, the Acalyphoideae (*Ricinus, Mallotus*) and the Euphorbioideae (*Sapium*) (Webster 1975). Uniform reactivity within these subfamilies was, however, not detected. No serological reactivity was noted with *Acalypha californica* or several *Euphorbia* species. This is attributed to the fact that reactivity was detected only in those taxa possessing large seed and for which substantial experimental material was available for extraction. By contrast, the seed of available *Acalypha* and *Euphorbia* species are very small and very limited quantities were available. Cross-reactivity in these genera might be noted if more material were available for extraction.

The serological cross-reactivity data are most consistent with a systematic treatment of *Simmondsia* as a separate family within the Euphorbiales as treated by Takhtajan (1969). These data also indicate a close affinity with the family Euphorbiaceae, especially with the subfamilies Acalyphoideae and Euphorbioideae.

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