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HYBRID ORIGIN OF "*BAUHINIA BLAKEANA*" (LEGUMINOSAE: CAESALPINIOIDEAE), INFERRED USING MORPHOLOGICAL, REPRODUCTIVE, AND MOLECULAR DATA[1]

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Bauhinia blakeana (Leguminosae subfam. Caesalpinioideae tribe Cercideae), or the Hong Kong Orchid Tree, is of great horticultural value. It is completely sterile and is shown here to be the result of hybridization between the largely sympatric species, *B. purpurea* and *B. variegata*. Although the analysis of patterns of morphological variation revealed only a few examples of phenotypic intermediacy, study of intersimple sequence repeat (ISSR) markers enabled unequivocal identification of the parental species due to the presence of additive inheritance of alleles and the absence of any bands that are unique to *B. blakeana*. Investigation of aspects of the reproductive biology of the taxa furthermore revealed that the parental species are largely xenogamous, have flowering periods that overlap seasonally and temporally, and share common pollinators. Evidence is provided to show that *B. blakeana* is not naturally stabilized and is only maintained horticulturally by artificial propagation. It is therefore recommended that the hybrid be regarded as a horticultural cultivar rather than a naturally occurring species; a new cultivar name, *Bauhinia* 'Blakeana', is accordingly validated.

Key words: *Bauhinia blakeana*; *Bauhinia purpurea*; *Bauhinia variegata*; cultivar; Hong Kong; hybridization; ISSR; pollination.

Bauhinia blakeana Dunn (the Hong Kong Orchid Tree) is of considerable horticultural value, and has been extensively planted as a garden, park and roadside ornamental tree in many warm temperate and subtropical regions. It is completely sterile and consequently requires artificial propagation, generally by grafting onto rootstocks of other *Bauhinia* species. This sterility has led to the suggestion that it is probably of hybrid origin, with *B. purpurea* L. and *B. variegata* L. as the most likely candidates as parental species. These latter species are sympatric over much of their distribution ranges, and although they are not indigenous to Hong Kong, they have long been cultivated as ornamentals. The putative parental species share many morphological similarities with *B. blakeana* (Figs. 1–4), including pollen morphology (Larsen, 1975). Chromosome counts of $n = 14$ and $2n = 28$ have furthermore been reported for all three species (Sharma and Raju, 1968; Husaini and Gill, 1985; Yeh et al., 1986; Choudhary and Choudhary, 1988; Kumari and Bir, 1989).

Bauhinia blakeana was first discovered by a French missionary in the 1880s, growing in the grounds of an abandoned house close to the shore near Pokfulam, Hong Kong Island (Dunn, 1904, 1906, 1908). The close proximity of the tree to a former habitation led Dunn (1906) to suggest that it was an introduction. The missionary collector subsequently propagated it in the grounds of the nearby Pokfulam Sanatorium run by the Missions Étrangères de Paris, and from there it was introduced to the Hong Kong Botanic Gardens and the grounds of the Roman Catholic Cathedral in Canton (now Guangzhou). Dunn (1908) subsequently formally named it *B. blakeana* in honor of Sir Henry Blake, Governor of Hong Kong between 1898 and 1903.

By 1903, the tree in the Botanic Gardens was reported to be flowering profusely and persistently (Dunn, 1904). The tree survived being blown over by a typhoon in 1906 (Dunn, 1907) and was subsequently used to propagate new trees vegetatively over the next few years. Beginning in 1914, *B. blakeana* was extensively planted as an ornamental in various regions of Hong Kong (Tutcher, 1915). There is therefore no evidence that *B. blakeana* originated more than once, and there is strong circumstantial evidence suggesting that all trees cultivated today originate from a single ancestor, grown in the Hong Kong Botanic Gardens.

The present study aims to determine the hybrid origin and the parentage of *B. blakeana*. A broad range of approaches are adopted, including: (1) analysis of morphological variation (both macromorphology of floral organs and pollen ultrastructure), (2) determination of flowering phenology at the single flower, individual tree and entire population levels, (3) identification of breeding systems using field-based controlled pollination experiments, (4) assessment of pollen viability in vitro, (5) observations of floral visitors and floral rewards, and (6) analysis of variation in intersimple sequence repeat (ISSR) markers.

MATERIALS AND METHODS

Morphological variation—Differences in floral structure between *B. blakeana*, *B. purpurea*, and *B. variegata* were assessed using the following macromorphological characters (based on 20 flowers from 10 individuals of each species): (1) corolla length (mm), (2) corolla width (mm), (3) hypanthium length (mm), (4) stamen number, (5) staminode number, (6) filament length (mm), (7) anther length (mm), (8) gynophore length (mm), (9) ovary length (mm), (10) style length (mm), (11) stigma length (mm), (12) inferior petal length (mm), (13) inferior petal width (mm), (14) inferior petal length/width ratio, (15) lateral petal length (mm), (16) lateral petal width (mm), (17) lateral

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Figs. 1–4. Floral morphology of *Bauhinia blakeana* and putative parental species. **1.** *B. purpurea*. **2.** *B. blakeana*. **3.** *B. variegata* (variant with darker coloration; stigma held below anthers). **4.** *B. variegata* (variant with paler coloration; stigma held above anthers).

petal length/width ratio, (18) upper petal length (mm), (19) upper petal width (mm), and (20) upper petal length/width ratio. Analysis of variance (ANOVA) was used to compare the means of the continuously variable characters between species, with statistical significance evaluated using Duncan's multiple range test. All statistical analyses were performed using Statistica, version 5.0 software (StatSoft, Inc., 1994).

Pollen ultrastructure was studied using scanning electron microscopy (SEM), without acetolysis. Standard SEM preparation techniques were employed, and specimens were examined using a Cambridge Stereoscan 440 SEM.

Floral phenology—The floral phenology of the three species was investigated to determine events and changes at the single flower, individual tree, and entire population levels. The flowering periods of populations were assessed over a 5-yr period (1997–2001). The timing and duration of flowering events in individual trees were measured by tagging 20–50 unopened flower buds on three or more individuals of each species; observations were made daily prior to flower opening, and every 2 h during anthesis.

The onset and duration of stigmatic receptivity was determined in the field by immersing stigmas (of known age from the time of anthesis) in 3% hydrogen peroxide solution. The formation of gas bubbles on the stigmatic surface is due to the presence of the enzyme peroxidase, and indicates receptivity (Galen and Plowright, 1987).

Breeding systems—A total of six different controlled pollination treatments were conducted (Dafni, 1992) to determine the breeding system: (1) control—flowers tagged and left to freely pollinate; (2) induced self-pollination—flowers bagged, emasculated, and artificially pollinated with self-pollen; (3) geitonogamy—flowers bagged, emasculated, and artificially pollinated with pollen from another flower of the same individual; (4) artificial cross-pollination—flowers bagged, emasculated, and artificially pollinated with pollen from flowers of different individuals of the same genet; (5) natural cross-pollination—flowers emasculated, but neither bagged nor artificially pollinated; and (6) agamospermy—flowers bagged and emasculated, but not artificially pollinated.

Pollen viability—Pollen viability was estimated by calculating pollen germination rates in vitro. Fresh pollen grains were collected from recently dehisced anthers, and incubated in 5, 10, 15, 20, and 25% sucrose solutions in 50% (w/v) H_3BO_3 and 50% (w/v) $Ca(NO_3)_2$ for 24 h at ambient temperatures

TABLE 1. Provenance of accessions sampled in Hong Kong, China.

Species/Location	Coordinates	No. of accessions	Sample codes
B. purpurea			
University of Hong Kong, Hong Kong Island	22°17′N, 114°08′E	10	P01–P10
B. blakeana			
Kennedy Road, Central, Hong Kong Island	22°17′N, 114°09′E	2	B01, B02
Wong Nai Chung Road, Hong Kong Island	22°17′N, 114°11′E	1	B03
Wong Nai Chung Gap Road, Hong Kong Island	22°16′N, 114°12′E	1	B04
St. Stephens Road, Stanley, Hong Kong Island	22°12′N, 114°13′E	1	B05
Tai Po Kau Park, Tai Po, New Territories	22°26′N, 114°11′E	2	B06, B07
Bride's Pool Road, Plover Cove, New Territories	22°28′N, 114°12′E	1	B08
Ho Pui Road, Kam Tin, New Territories	22°28′N, 114°00′E	2	B09, B10
B. variegata			
Sha Wan Drive, Hong Kong Island	22°17′N, 114°07′E	8	V01–V08

(Dafni, 1992). A total of 200 pollen grains of each species were assessed, and the germination rates calculated as a percentage.

Floral visitors and floral rewards—Extensive observations of the activities of floral visitors were undertaken during daylight hours (0700–1700 hours), supplemented with occasional night-time observations (1800–2200 hours). More detailed studies of the behavior of the floral visitors were achieved by videotaping 2–5 individuals of each species for 30-min periods over several days. The number of flowers observed on the tree and the number and types of floral visitors were recorded, with samples collected for subsequent identification. Visitation rates were calculated as $N_V/N_F \times N_H$, where N_V = number of visits observed, N_F = number of flowers observed, and N_H = number of hours of observations (Dafni, 1992). The microclimate around the flowers and ambient weather conditions were recorded during the observation periods using a digital psychrometer.

Nectar sugar composition was determined using a Dionex HPLC system (Dionex Corp., Sunnyvale, California, USA) fitted with a CarboPac PA-1 (4 × 250 mm) column, and a 10 μL sample loop, with 10 mM NaOH isocratic elution at 1 mL/min. An ED40 electrochemical detector fitted with a pulsed amperometric cell was used, and peaks were compared with authentic sugar standards.

Intersimple sequence repeat (ISSR) markers—A total of 28 accessions (eight of *B. variegata*, and 10 each of *B. purpurea* and *B. blakeana*) were studied from nine different roadside and park localities in Hong Kong (Table 1). The extent of sampling was constrained by the fact that all three taxa have been artificially planted in Hong Kong, and therefore natural populations do not occur locally. Voucher specimens have been deposited in the HKU herbarium.

Total DNA was extracted from young leaves in liquid nitrogen using a CTAB protocol (modified from Doyle, 1991), and samples purified using a Wizard Plus SV Minipreps DNA purification system (Promega Corp., Madison, Wisconsin, USA). Eight ISSR primers (UBC SSR Primer, Oligonucleotide Set 100/9, Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada) were used in single-primer PCR amplifications, viz.: 807 [$(AG)_8$-T], 810 [$(GA)_8$-G], 834 [$(AG)_8$-YT], 835 [$(AG)_8$-YC], 842 [$(CA)_8$-YG], 844 [$(CT)_8$-RC], 866 [$(CTC)_6$], and 889 [DBD-$(AC)_7$].

After optimization, standard reaction conditions were: reaction volumes of 25 μL, consisting of 0.25 μM primer, 1 × *Taq* polymerase buffer, 0.1 mM dNTPs, 1.25 U *Taq* DNA polymerase (Gibco BRL), 1.5 mM $MgCl_2$, and 4 μL of DNA. Reaction tubes were transferred to a PTC-100 Programmable Thermal Cycler (MJ Research, Inc., Waltham, Massachusetts, USA) with the thermocycler program set for 5 min at 94°C; followed by 35 cycles of 30 s at 94°C, 45 s at 48°C, 2 min at 72°C, 7 min at 72°C, and 4°C indefinitely. PCR reactions were characterized on 1.5% agarose gels in 0.1 × TAE buffer. The gels were stained in ethidium bromide and banding patterns were captured for each gel using an image system (UVP Gel Documentation System; UVP Inc., Upland, California, USA) and interpreted manually. Fragment sizes were estimated based on a 1-kb ladder size standard (3000–100 bp) (Gibco-BRL; now Invitrogen, Carlsbad, California, USA) and their sizes were used to assign loci for each primer; bands were scored as diallelic for each assigned locus (1 = present; 0 = absent).

The presence–absence data matrix was analyzed with POPGENE (Yeh et al., 1997). Nei's (1972) genetic identity (*I*) between populations was computed at the species level, and dendrograms of Nei's genetic distances were constructed using the unweighted pair group method with arithmetic averages (UPGMA).

RESULTS

Morphological variation—Analysis of patterns of variation in the 20 macromorphological floral characters examined revealed considerable taxonomic differences between the three species (Table 2). *Bauhinia purpurea* differed from *B. variegata* in 17 of the characters, as assessed using Duncan's multiple range test: only corolla width, staminode number and gynophore length were not statistically different. The putative hybrid, *B. blakeana*, differed from *B. purpurea* in all 20 characters and differed from *B. variegata* in 15 characters (all except hypanthium length, stamen number, and the widths of the three petal types). In most cases, however, there was little evidence that *B. blakeana* was intermediate between the putative parental species: of the nine continuously variable size characters that were significantly different between all three species, *B. blakeana* was only intermediate in one (stigma length). The three petal length/width ratio characters, however, all showed that *B. blakeana* is intermediate between *B. purpurea* and *B. variegata*; this suggests that the shape of petals rather than the size of floral organs is a better indicator of intermediacy in *B. blakeana*.

Pollen of all three species was tricolporate, oblate-spheroidal to prolate-spheroidal and rounded-triangular (see also Larsen, 1975). *Bauhinia purpurea* pollen had a reticulate exine (Fig. 5), whereas *B. variegata* pollen had a supra-striate exine (Fig. 7); significantly, the pollen of *B. blakeana* had an intermediate exine structure (Fig. 6). The pollen of *B. blakeana* was noticeably irregular in size, with a high proportion (ca. 30–40%) of ''micropollen'' that was developmentally arrested and hence smaller than normal pollen (40–43 × 29–32 μm vs. 48–52 × 35–38 μm; Fig. 6). The micropollen was furthermore fully sterile.

Floral phenology—The flowering periods of the three *Bauhinia* tree species overlapped significantly: *B. purpurea* flow-

TABLE 2. Means (± standard deviations, SD) of morphological characters for *B. purpurea*, *B. blakeana*, and *B. variegata*. The continuously variable characters have superscript letters that summarize the results of the Duncan's multiple range test. Species with the same letters do not differ significantly for that character ($P < 0.05$).

Floral characters	*B. purpurea* (mean ± SD)	*B. blakeana* (mean ± SD)	*B. variegata* (mean ± SD)
Corolla length (mm)	41.2 ± 5.5[a]	65.2 ± 7.5[b]	46.8 ± 8.6[c]
Corolla width (mm)	93.6 ± 8.7[a]	117.3 ± 10.5[b]	90.4 ± 14.0[a]
Hypanthium length (mm)	9.8 ± 1.0[a]	17.9 ± 1.5[b]	18.4 ± 2.4[b]
Stamen number	3 (–4)	5	5
Staminode number	5–6	2–5	5
Filament length (mm)	44.3 ± 2.2[a]	58.4 ± 3.3[b]	33.8 ± 3.8[c]
Anther length (mm)	7.4 ± 0.8[a]	9.8 ± 0.9[b]	5.8 ± 0.7[c]
Gynophore length (mm)	14.6 ± 1.6[a]	23.8 ± 2.0[b]	15.6 ± 1.7[a]
Ovary length (mm)	13.7 ± 1.0[a]	18.6 ± 1.2[b]	15.0 ± 1.0[c]
Style length (mm)	14.8 ± 1.8[a]	19.7 ± 2.3[b]	10.3 ± 1.8[c]
Stigma length (mm)	2.1 ± 0.2[a]	1.9 ± 0.2[b]	1.3 ± 0.2[c]
Inferior petal length (mm)	65.0 ± 4.7[a]	79.8 ± 7.2[b]	57.1 ± 5.0[c]
Inferior petal width (mm)	22.4 ± 3.4[a]	30.2 ± 3.5[b]	29.0 ± 4.0[b]
Inferior petal length/width ratio	2.96 ± 0.47[a]	2.66 ± 0.22[b]	1.99 ± 0.18[c]
Lateral petal length (mm)	63.5 ± 5.3[a]	75.8 ± 7.0[b]	54.5 ± 3.3[c]
Lateral petal width (mm)	21.7 ± 2.5[a]	31.1 ± 2.7[b]	31.6 ± 3.0[b]
Lateral petal length/width ratio	2.95 ± 0.32[a]	2.45 ± 0.18[b]	1.73 ± 0.12[c]
Upper petal length (mm)	61.9 ± 4.8[a]	72.9 ± 6.4[b]	51.7 ± 3.5[c]
Upper petal width (mm)	20.3 ± 2.4[a]	32.3 ± 3.1[b]	33.9 ± 3.6[b]
Upper petal length/width ratio	3.08 ± 0.34[a]	2.27 ± 0.20[b]	1.53 ± 0.10[c]

ered from September to January (5 mo); *B. blakeana* had two flowering periods, from September to April and from May to June (flowering over a total of 10 mo); and *B. variegata* flowered between late December and April (5 mo).

Anthesis occurred between 1300 and 1500 hours in *B. purpurea*, 1600 and 1700 hours in *B. blakeana*, and 1500 and 1600 hours in *B. variegata*, with floral receptivity extending for 24–72 h after opening in all three species. The anthers dehisced either just before the flowers opened (*B. purpurea* and *B. variegata*) or immediately afterwards (*B. blakeana*). The stigma was not receptive for the first ca. 24 h, during which phase the style was initially horizontal but subsequently descended slightly (Fig. 3), and the staminal filaments were curved upwards. As the stigma became receptive after ca. 24 h, the style gradually curved upwards and was elevated above the anthers (Fig. 4); during this phase both the stamens and the carpel were functional. Nectar production was initiated immediately after floral opening, with average accumulated volumes (± standard deviation, after 10 h) of 239 ± 44 μL in *B. blakeana*, 178 ± 28 μL in *B. purpurea*, and 187 ± 3 μL in *B. variegata*.

Breeding systems—*Bauhinia blakeana* failed to produce fruits under any of the treatments and was therefore totally sterile. All treatments except the test for agamospermy, however, resulted in fruit set in both *B. purpurea* and *B. variegata* (Table 3).

Pollen viability—The optimal sucrose concentration for pollen germination was 15%. Pollen germination percentages for *B. purpurea*, *B. blakeana*, and *B. variegata* were variable, ranging between 8.6–27.3%, 7.0–21.9%, and 9.7–39.6%, respectively, with mean values (± standard errors) of 16.7 ± 6.3%, 13.5 ± 5.6%, and 21.8 ± 9.2% respectively. The pollen germination values calculated for *B. blakeana* were therefore the lowest of the three (inclusive of ''micropollen''). This variation was shown to be statistically significant, using Duncan's multiple range test ($P < 0.05$).

Floral visitors and floral rewards—*Bauhinia purpurea* and *B. variegata* had several floral visitors in common (Table 4). Field observations revealed that *B. purpurea* and *B. variegata* were both primarily visited by honeybees (*Apis cerana* and *A.*

Figs. 5–7. Pollen exine ultrastructure of *Bauhinia blakeana* and putative parental species (scanning electron micrographs). **5.** *B. purpurea*. **6.** *B. blakeana* (normal pollen on left, ''micropollen'' on right). **7.** *B. variegata*. Scale bars = 20 μm.

TABLE 3. Percentage fruit-set (mean ± standard deviation, SD) resulting from controlled pollination experiments in *Bauhinia*. N = number of trees; n = number of flowers. Superscript letters summarize the results of the Duncan's multiple range test. Treatments with the same letters do not differ significantly ($P < 0.05$).

Controlled pollination treatment	*B. purpurea* (N = 13, n = 282–637)	*B. blakeana* (N = 5, n = 49–115)	*B. variegata* (N = 11, n = 121–301)
Control	13.9 ± 8.0[a]	0	6.2 ± 8.2[a]
Induced self-pollination	18.7 ± 7.2[a,b]	0	15.6 ± 6.5[a]
Geitonogamy	27.2 ± 8.3[b]	0	18.9 ± 7.6[a]
Artificial cross-pollination	23.6 ± 7.5[a,b]	0	17.1 ± 11.6[a]
Natural cross-pollination	11.7 ± 6.9[a]	0	9.8 ± 11.9[a]
Agamospermy	0	0	0

mellifera) and to a lesser extent by bamboo carpenter bees (*Xylocopa iridipennis*) and the common mormon butterfly (*Papilio polytes*). Pollen was observed adhering to the bodies of these insects, indicating that they were likely to be effective pollinators. The nectar produced by all three species was sucrose dominated (66, 63, and 80% sucrose, respectively, for *B. purpurea*, *B. blakeana*, and *B. variegata*); this is typical of flowers visited by long-tongued bees, wasps and lepidopterans (Martınez del Rio et al., 1992; Perret et al., 2001).

Intersimple sequence repeat (ISSR) markers—A total of 105 ISSR bands were scored for the eight primers across the three species, with *B. blakeana*, *B. purpurea*, and *B. variegata* having 72, 69, and 70 bands, respectively (Table 5). Of the total 105 bands, 49 (46.7%) of the bands were polymorphic within individual species, and 30 (28.6%) were common to all three species. The putative hybrid, *B. blakeana*, had no unique (species-specific) bands (Table 5; Figs. 8, 9): 18 of its bands were shared with *B. purpurea*, and 25 were shared with *B. variegata*. In contrast, *B. purpurea* had 17 unique bands, and *B. variegata* had 11. Restricting discussion to the 90 polymorphic fragments that were polymorphic across all accessions, it is noteworthy that 28 (31.1%) were species-specific, and 43 (47.8%) were shared either by *B. purpurea* and *B. blakeana* or by *B. variegata* and *B. blakeana*: only six (6.7%) of the fragments were common to *B. purpurea* and *B. variegata* (Table 5).

Among the three species, *B. purpurea* and *B. variegata* had the highest genetic distance (1.020), and *B. blakeana* and *B. variegata* had the lowest (0.394) (Table 6). Genetic identities (I) among the three species averaged 0.530; *B. blakeana* was closer to *B. variegata* (I = 0.674) than to *B. purpurea* (I = 0.556), whilst the genetic identity between the latter two species was 0.361 (Table 6).

An unrooted UPGMA dendrogram was constructed to reveal the genetic relatedness of the populations sampled for all three species (Fig. 10). The dendrogram showed that *B. blakeana* was approximately equidistant between the two clusters of *B. purpurea* and *B. variegata* (although slightly closer to

TABLE 4. Floral visitors and visitation rates for *Bauhinia*. Floral visitors with visitation rates <1 for all three *Bauhinia* species are excluded. N_H = total number of hours of observation; N_F = total number of flowers under observation.

Floral visitor	*B. purpurea* (N_H = 14, N_F = 132)	*B. blakeana* (N_H = 10, N_F = 91)	*B. variegata* (N_H = 15, N_F = 150)
Hymenoptera: Apidae			
Apis cerana	7.3	4.1	7.2
Apis mellifera	12.9	5.6	8.8
Apis sp.	—	1.2	—
Xylocopa collaris	—	—	2.4
Xylocopa iridipennis	17.2	—	1.6
Hymenoptera: Vespidae			
Vespa affinis	8.5	8.2	—
Vespa bicolor	4.3	—	—
Polistes olivaceous	4.3	—	—
Eumenes pyriformis	4.3	—	—
Lepidoptera: Hesperiidae			
Notocrypta currifascia	3.8	—	—
Lepidoptera: Papilionidae			
Papilio polytes	4.3	4.1	1.6
Papilio paris	3.8	—	—
Lepidoptera: Pieridae			
Eurema blanda	12.9	—	—
Eurema hecabe	3.8	—	—
Rodentia: Sciuridae			
Callosciurus erythraeus	—	—	1.6
Total visitation rate (all visitors)	87.1	23.2	24.6

TABLE 5. Summary of the results of intersimple sequence repeat (ISSR) fragments from *Bauhinia purpurea*, *B. blakeana*, and *B. variegata* genomic DNA with eight primers.

Number of fragments	*B. purpurea*	*B. blakeana*	*B. variegata*	Total
Total scored	69	73	71	105
Polymorphic	23	0	26	90
Unique	17	0	11	28

TABLE 6. Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) (Nei, 1972) among the three *Bauhinia* taxa.

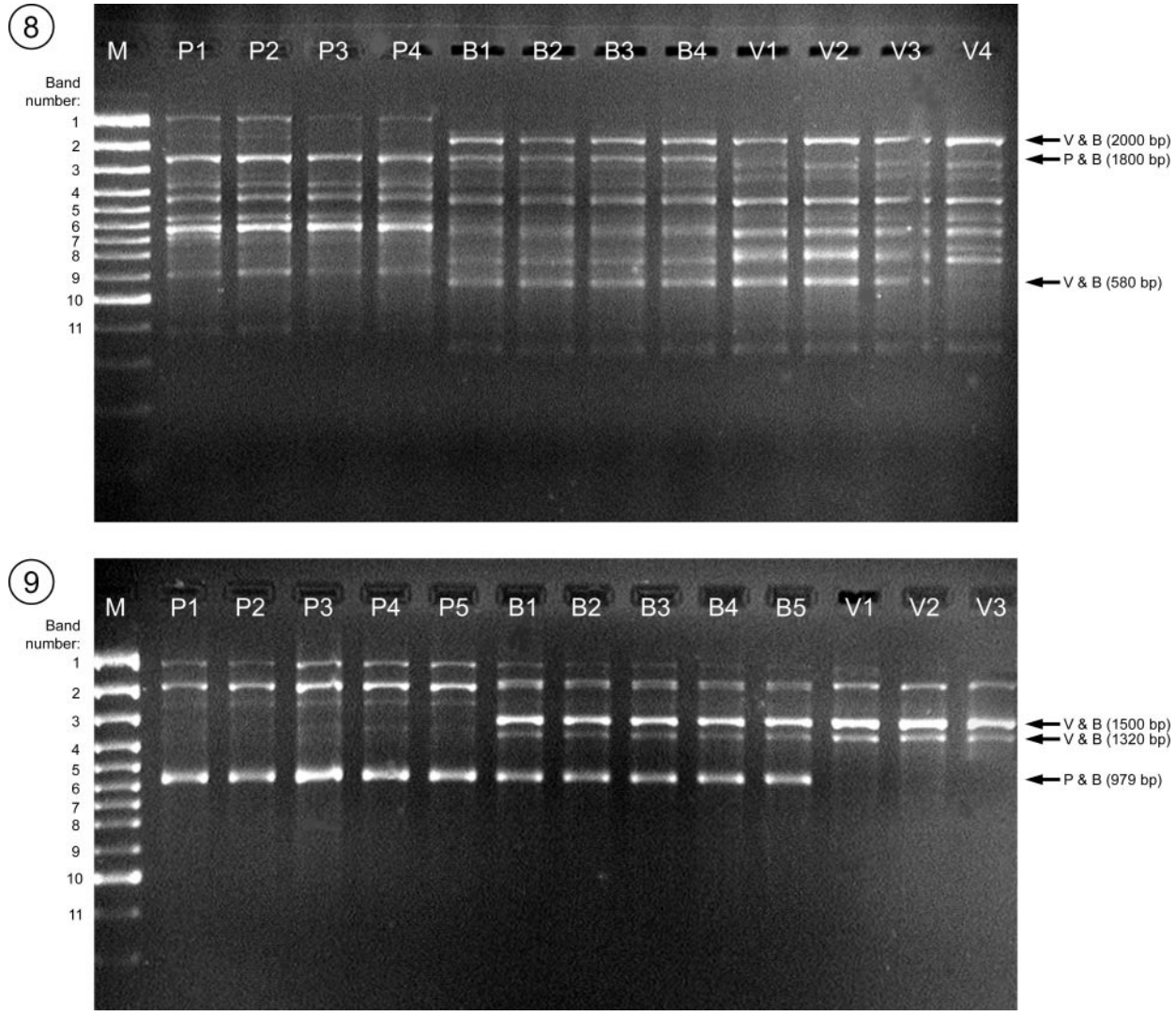
Taxon	*B. purpurea*	*B. blakeana*	*B. variegata*
B. purpurea	—	0.556	0.361
B. blakeana	0.588	—	0.674
B. variegata	1.020	0.394	—

B. variegata) and that all 10 DNA accessions of *B. blakeana* were genetically identical.

DISCUSSION

Is Bauhinia blakeana a hybrid?—It has previously been suggested that *B. blakeana* is of hybrid origin, and that *B. purpurea* and *B. variegata* are the most likely candidates as parental species. Larsen (1975) made this proposal on the basis of pollen morphology, citing the intermediate structure of the exine and the high proportion of ''micropollen'' that shows arrested development and is accordingly sterile. The ultrastructural intermediacy of the pollen and the occurrence of micropollen is corroborated here (Figs. 5–7).

Phenotypic intermediacy is not evident, however, in many of the macromorphological floral characters assessed for *B. blakeana*: only four of the 20 characters studied are shown here to be statistically intermediate between the putative parents (Table 2). Most of the characters showing intermediacy are derived length/width ratios, and therefore reflect differenc-



Figs. 8–9. Typical intersimple sequence repeat (ISSR) marker banding patterns for *Bauhinia purpurea* (P), *B. blakeana* (B) and *B. variegata* (V). **8.** Primer 807. **9.** Primer 866. M = 100 bp DNA ladder; 'P & B' = bands shared between *B. purpurea* and *B. blakeana*; 'V & B' = bands shared between *B. variegata* and *B. blakeana*.

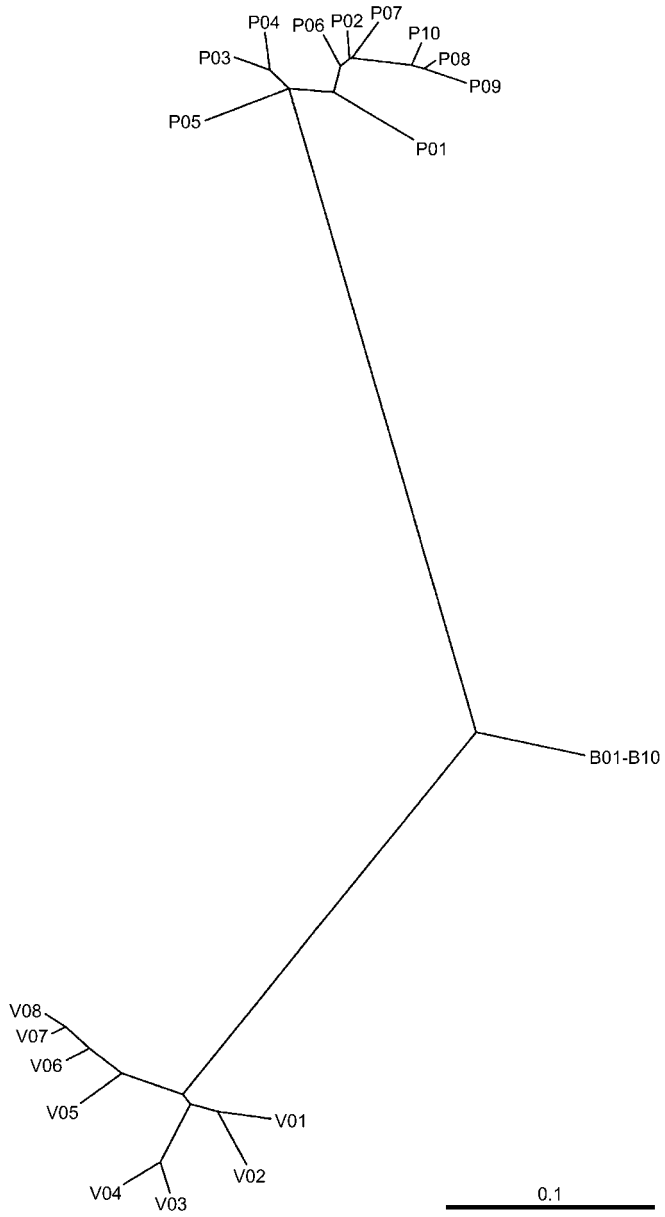


Fig. 10. Unrooted UPGMA dendrogram, based on variation in ISSR markers for 28 accessions of *Bauhinia purpurea* (P01–P10), *B. blakeana* (B01–B10), and *B. variegata* (V01–V08). Scale bar represents genetic distance.

es in shape rather than size. Inevitably, however, the demonstration of the presence of phenotypic intermediacy provides rather equivocal evidence for hybridization as there are many circumstances where hybridization occurs but intermediacy is not evident, including Mendelian inheritance of qualitative characters, and the occurrence of matrocliny, in which maternal traits are more likely to be exhibited in the offspring (Rieseberg and Ellstrand, 1993; Rieseberg, 1995).

The controlled pollination experiments conducted during the present research provide clear evidence that *B. blakeana* is completely sterile: no seeds were set after any of the six experimental treatments (Table 3). This is in marked contrast to both *B. purpurea* and *B. variegata*, which are shown to be capable of both autogamy and xenogamy. Similar evidence of reduced fertility is seen in the data presented for pollen viability. Reduced fertility is common in hybrids, although it is again rather equivocal evidence of hybridization.

The phenological data presented here indicates that the flowering periods of *B. purpurea* and *B. variegata* partially overlap both seasonally and temporally. The results of the controlled pollination experiments (Table 3) furthermore indicate that both putative parental species are xenogamous, although also capable of autogamy. The flower-level phenological data suggests that xenogamy is promoted by two factors: partial temporal separation of the staminate and pistillate functions within individual flowers (because the receptivity of the stigmas lags ca. 24 h behind anther dehiscence); and spatial separation between the stigmas and anthers resulting from the upward curving of the style. Similar results have been reported previously for *B. purpurea* by Reddi and Rao (1993). Analysis

of floral visitors indicate that both *B. purpurea* and *B. variegata* are largely pollinated by the same range of bee and butterfly species (Table 4). The phenological, breeding system and floral visitation data therefore indicate that it is clearly feasible for *B. purpurea* and *B. variegata* to interbreed.

The ISSR data shows that the 10 *B. blakeana* accessions investigated have a fixed heterozygous genotype and are therefore genetic clones (Fig. 10). Significantly, the data furthermore indicates that the accessions are composed exclusively of alleles that are also found in either one or both of the putative parental species, *B. purpurea* and *B. variegata* (Table 5; Figs. 8, 9). Additive inheritance of alleles that are otherwise unique in the putative parental species is convincing evidence of hybridization. The absence of unique loci as observed in many studies of angiosperms (e.g., Gallez and Gottlieb, 1982; Wang et al., 1994; Barker et al., 1996; Wolfe et al., 1998) indicates that few mutations have occurred since the initial hybridization event, and that *B. blakeana* is therefore of relatively recent hybrid origin. This suggestion is supported by the published historical data: the naturally formed F_1 hybrid of *B. blakeana* was first discovered in the 1880s, and because it is unlikely that *Bauhinia* trees are particularly long-lived (ca. 50 yr), it can be tentatively postulated that hybridization occurred 120–170 years ago. It is also possible, however, that the discovery of species-specific markers is an artefact of the necessarily limited sampling, resulting from the absence of natural populations in Hong Kong.

The values of the genetic identities (Table 6) between *B. blakeana* and the parental species are high (0.556 with *B. purpurea*, and 0.674 with *B. variegata*). The slightly higher value with *B. variegata* is clearly due to the fact that *B. blakeana* shares 25 loci in the eight primers with this species, whereas only 18 loci are shared with *B. purpurea*. No definite conclusion can be drawn from this data, however, due to the limited number of ISSR primers used. The mean genetic identities previously reported for congeneric species vary between 0.67 (Gottlieb, 1981) and ca. 0.75 (Crawford, 1983; Trapnell et al., 2004).

The causes of sterility in *B. blakeana* are rather obscure. Hybrid sterility may result from abnormal segregation at meiosis of either whole chromosomes or of blocks of genes contained in chromosomal segments. The chromosomes of the parental species cannot pair at all during meiosis if they are strongly differentiated, whereas pairing can occur if the parents of a hybrid are more closely related to each other. In the latter situation, pairing is imperfect, resulting in the segregation of the gametes to give abnormal combinations of genes due to differences in the arrangement and structure of genes on the chromosomes. Although *B. blakeana* shares the same diploid chromosome number as its parental species, the karyotypes have been shown to differ slightly (Sharma and Raju, 1968): *B. blakeana* and *B. purpurea* both consist of six type ''A'' chromosomes (long, with a satellite at the distal end of the shorter arm) and 22 type ''B'' chromosomes (short, without any satellite), whereas *B. variegata* consists of four type ''A'' chromosomes and 24 type ''B'' chromosomes. The karyotype differences between the parental species may therefore explain the sterility of *B. blakeana*. It is also significant that *B. blakeana* is reported to possess a variable number of carmine-stainable structures (indicative of nuclei acids) in addition to the 14 bivalent chromosomes (Sharma and Raju, 1968). These may be the result of an imbalance in the settled ratio of DNA and RNA in each cell.

Is Bauhinia blakeana a species?—Grant (1971) enumerated seven different ways in which plant hybrids can become ''stabilized'' to form new entities that can be recognized as distinct species: (1) vegetative propagation, (2) agamospermy, (3) translocation heterozygosity, (4) unbalanced polyploidy, (5) amphidiploidy, (6) recombinational speciation, and (7) hybrid speciation.

Vegetative propagation occurs in *B. blakeana*, but only artificially, as a result of active horticultural practices such as grafting and rooting of cuttings: there is no evidence that *B. blakeana* is capable of self-propagating. The breeding system experiments reported here (Table 3) furthermore indicate that agamospermy does not occur; this is further corroborated by the absence of fruit formation and seed set in mature individuals of *B. blakeana*. Translocation heterozygosity and unbalanced polyploidy (collectively referred to as the heterogamic complex by Grant, 1971) are rare mechanisms, involving, for example, the formation of multivalent rings of chromosomes (e.g., *Oenothera*). Amphidiploidy furthermore does not occur in *B. blakeana*, because all published chromosome number reports indicate that it shares the same diploid chromosome number ($2n = 28$) with the parental species (Sharma and Raju, 1968; Husaini and Gill, 1985; Yeh et al., 1986; Choudhary and Choudhary, 1988; Kumari and Bir, 1989). Recombinational speciation and hybrid speciation (collectively referred to as the homoploid complex by Grant, 1971) are also not applicable to *B. blakeana*, because they are characterized by sustained levels of sexuality, without reduced fertility.

It is therefore evident that although *B. blakeana* is a hybrid that has resulted from a cross (probably natural) between *B. purpurea* and *B. variegata*, it has only been perpetuated genetically by artificial horticultural practices: it is not capable of reproducing itself independently. It is therefore inappropriate to regard it as a distinct species and is better referred to as an artificially maintained cultivar. A new cultivar name is accordingly formally published here, replacing the previous specific binomial published by Dunn (1908):

Bauhinia purpurea × *variegata* 'Blakeana', cv. nov.= *Bauhinia blakeana* Dunn, *Journal of Botany* 46: 325 (1908). Type: Cult. Hong Kong Botanic Garden, 24 June 1905, *anonymous 1722* (holotype: K; isotype: HK!).

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