Analysis of Genetic Variability among *Phalaenopsis* Species and Hybrids Using Amplified Fragment Length Polymorphism

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ABSTRACT. *Phalaenopsis* is the second most valuable potted plant in the United States. Information on the genetic diversity and relationships among species and hybrids is important for breeding purposes and species conservation. In this study, genetic variability of 16 *Phalaenopsis* species and hybrids was analyzed using amplified fragment length polymorphism (AFLP) markers. Ten AFLP primer combinations amplified 1353 DNA fragments ranging in size from 100 to 350 bp and 1285 (95%) of them were polymorphic. The genetic similarity among *Phalaenopsis* species and hybrids ranged from 0.298 to 0.774 based on Dice coefficient. The dendrogram derived by the unweighted pair group method with arithmetic mean analysis clustered the germplasm into two main groups. Bootstrap values for the groups supported 70% of the clustering. A significant linear relationship ($r = 0.724$, $P < 0.0001$) was observed between known pedigrees and AFLP-derived genetic similarity for 136 pairwise comparisons of *Phalaenopsis* species and hybrids. The results of this study demonstrate the usefulness of AFLP analysis in *Phalaenopsis* and its potential application in breeding and species conservation.

Orchids are not only beautiful, but also commercially important and occupy the second place in the potted flowering plant market in the United States (Griesbach, 2002). According to the U.S. Department of Agriculture (USDA) floriculture crops 2007 survey, the potted orchid industry was valued at \$126 million (USDA, 2008). Among potted orchids, *Phalaenopsis* comprises the majority of commercial orchids (50% to 90%) in the United States as a result of their long-lasting flowers, easy care, and low cost (Griesbach, 2002; Laws, 2004).

The orchid family is unique because hybridization is possible not only between species, but also between members of related genera. Moreover, hybrids can interbreed with other species to create novel combinations (Tan and Hew, 1995). As a result of these features, genetic relationships are sometimes difficult to evaluate. However, accurate information on genetic relationships among species and hybrids is needed for the conservation and use of breeding materials.

Through intensive breeding activity, 3500 *Odontoglossum* hybrids and intergenerics, 4300 *Cattleyas* hybrids and intergenerics, and over 5000 *Paphiopedilum* crosses had been made by 1945 (American Orchid Society, 2002). However, only ≈ 140 *Phalaenopsis* hybrids were registered through 1945

(Challis, 1996). An average of 100 new *Phalaenopsis* hybrids was registered in the Royal Horticulture Society every 2 months from 1976 through 2008, underscoring the importance of novel *Phalaenopsis* hybrids for market demands.

Despite the economic importance of *Phalaenopsis*, the genetic potential of *Phalaenopsis* has not been fully exploited. An assessment of genetic variability is important for the use of genetic resources and for determining the uniqueness of genotypes to protect breeders' intellectual property rights and to exploit heterosis (Franco et al., 2001). Genetic diversity can be determined using phenotypic variation and/or molecular markers. Morphological characteristics have limitations because environmental factors and the developmental stage of the plant may influence their expression. In contrast to morphological markers, molecular markers based on DNA polymorphism are more informative, independent of environmental conditions and unlimited in number (Agarwal et al., 2008).

Several types of molecular markers are available; however, amplified fragment length polymorphism (AFLP) markers have notable advantages such as reproducibility, high levels of polymorphism that can be detected in a single reaction, genomewide distribution of markers, and no need for prior DNA sequence information compared with other DNA-based markers (Vos et al., 1995). AFLP analysis uses selective amplification of a subset of restriction enzyme-digested DNA fragments to generate a unique fingerprint of a particular genome (Mueller and Wolfenbarger, 1999). AFLP markers have been used in the assessment of genetic relationships of a wide range of species, including ornamentals such as *Caladium* (Loh et al., 1999), *Calathea* (Chao et al., 2005), *Hemerocallis* (Tomkins et al., 2001), *Cornus* (Smith et al., 2007), and

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Impatiens (Carr et al., 2003). The objectives of this study were to determine the level of genetic variability in *Phalaenopsis* species and hybrids using AFLP fingerprinting and to estimate the correlation of genetic relatedness with the known pedigree in various hybrids.

Materials and Methods

PLANT MATERIAL. Two commercially available *Phalaenopsis* species and 14 hybrids were used in this study (Table 1). The germplasm represents a range of flower size and colors available in the U.S. market. All the plants were maintained at 20 to 30 °C with 70% humidity in the greenhouse.

DNA EXTRACTION. Genomic DNA was extracted from fresh *Phalaenopsis* leaves according to Doyle and Doyle (1990) with some modifications. Plant tissue (0.5 g) was pulverized in liquid nitrogen and immediately transferred into a 50-mL tube containing 10 mL extraction buffer [2% CTAB, 100 mM Tris (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 0.2% 2-mercaptoethanol, and 4% polyvinyl pyrrolidone]. The samples were incubated at 60 °C for 1 h and 10 mL chloroform:isoamyl alcohol (24:1) was added to each sample and mixed. The samples were centrifuged at 12,000 g for 10 min. The aqueous phase was transferred to a clean tube. DNA was precipitated with 5 mL of ice-cold isopropanol and stored at -20 °C overnight. The samples were centrifuged at 5000 g for 10 min and the supernatant was discarded. The DNA pellet was resuspended in 20 mL wash buffer (10 mM NH₄OAc, 75% ethanol) for 20 min and centrifuged at 12,000 g for 5 min. The DNA was dissolved in TE buffer (100 µL) containing 100 µg of RNase and incubated at 37 °C for 1 h. DNA concentration was measured using a NanoDrop (Thermo Fisher Scientific, Waltham, MA) and quality was checked by electrophoresis on a 0.8% agarose gel in TBE buffer.

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM ANALYSIS. AFLP analysis was carried out according to Vos et al. (1995) with some modifications. Genomic DNA (500 ng) was restriction-digested with *Eco*RI and *Mse*I and *Eco*RI and *Mse*I adapters were ligated in a final volume of 11 µL. The reaction contained 1× T4 ligase buffer [50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM ATP] [New England Biolabs (NEB), Ipswich, MA], 0.05 M NaCl, 0.045 mg·mL⁻¹ BSA, 1 µM *Eco*RI adapter, 5 µM *Mse*I adapter, 5 U *Eco*RI (NEB), 5 U *Mse*I (NEB), and 1 U T4 DNA ligase (NEB). The reactions were gently mixed and incubated at 37 °C for 3 h. After restriction and ligation, the reaction mixture was diluted 10-fold with 0.1× TE buffer [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA].

Preselective amplification was carried out in a final volume of 13 µL consisting of 1× polymerase chain reaction (PCR) buffer [100 mM Tris-HCl (pH 8.3), 500 mM KCl], 2.0 mM MgCl₂, 0.2 mM dNTP, 10 µM *Eco*RI+A primer, 10 µM *Mse*I+C primer, and 3 µL of diluted restriction-ligation product. PCR was carried out in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA) programmed for 72 °C for 2 min followed by 20 cycles of 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 2 min and a final incubation of 72 °C for 2 min and 60 °C for 30 min. The preselective amplification PCR products were diluted 10-fold in 0.1× TE buffer and used as a template for selective amplification.

Selective amplification was carried out in 8 µL reaction volume containing 1× PCR buffer [100 mM Tris-HCl (pH 8.3),

500 mM KCl], 2.0 mM MgCl₂, 0.2 mM dNTPs, 0.625 µM of D4 WellRED dye-labeled *Eco*RI primer (E+3), 0.625 µM *Mse*I primer (M+3), 0.2 U of JumpStart *Taq* DNA polymerase (Sigma-Aldrich, St. Louis, MO), and 2 µL of diluted preselective amplification product. The PCR amplification consisted of an initial denaturation step of 94 °C for 2 min followed by the first cycle of 94 °C for 20 s, 66 °C for 30 s, 72 °C for 2 min, and 1 °C decrease in annealing temperature in each of the next nine cycles. This was followed by 25 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 3 min. The reactions were incubated at 60 °C for 30 min before electrophoresis. PCR products were diluted two-fold with sample loading solution (Beckman-Coulter, Fullerton, CA) and 1.5 µL of diluted reaction products were added to 40 µL of sample loading solution (Beckman-Coulter). DNA size standard 600 (Beckman-Coulter) was also added to each sample. The samples were electrophoresed and detected using a Beckman-Coulter CEQ 8800 Genetic Analysis System. The Frag-4 module of CEQ was used to size all the fragments using internal DNA size standard.

DATA ANALYSIS. All AFLP fragments were scored as binary data (1, peak present; 0, peak absent) along with their sizes. The binary scores were manually compared with the electropherograms to reconfirm presence or absence of peaks. NTSYS-pc software version 2.20 (Rohlf, 2005) was used to calculate the genetic distance or similarity between the samples. A cluster analysis was performed using unweighted pair group method with arithmetic mean (UPGMA) based on the Dice index (Nei and Li, 1979). This analysis was also compared using the FreeTree software package (Hampl et al., 2001). Bootstrap values (based on 1000 resamplings) were used to estimate the reliability of the clustering pattern.

Coefficients of coancestry for all possible pairs of *Phalaenopsis* species and hybrids were calculated according to Weir et al. (2006) based on pedigree information in the Royal Horticulture Society (RHS) orchid hybrid registration database information system (RHS, 2002). Simple linear correlation analysis was conducted to determine the association between coancestry coefficient and genetic similarity values using JMP software (version 5.1; SAS Institute, Cary, NC).

Results

A total of 1353 fragments ranging in size from 100 to 350 bp was scored using 10 primer combinations. The number of polymorphic fragments for each primer varied from 94 (E-CAG/M-CCT) to 162 (E-CAG/M-CCA). The average number of polymorphic loci (DNA band/fragment) detected was 121 per primer combination. The average percentage of polymorphic loci (polymorphic loci/total loci) was 95% and the range among primer combinations was from 92% (E-CAG/M-CAG) to 97% (E-CAG/M-CCC, E-CAG/M-CAC, E-CAC/M-CCG) (Table 2). The primer combination with the lowest number of fragments (E-CAG/M-CGA) had too many ambiguous fragments for counting and was not used in the analysis.

Genetic similarities among the 16 individual *Phalaenopsis* species and hybrids were estimated based on the number of common fragments (Table 3). Similarity values among individual samples ranged from 0.298 to 0.774 on the Dice index. The 'Picotee' clone of *Doritaenopsis* Newberry Parfait (13) and *Doritaenopsis* (Luchia Lady × City Girl) (14) were the most closely related, whereas *Phalaenopsis* Sogo Little Angel (4) and *Doritaenopsis* Abed-Nego 'Chadwick' (15) were the most

Table 1. Pedigree information, flower features, and sources of 16 *Phalaenopsis* species and hybrids.

Sample no.	Sample name	Ancestor species of the hybrids ^z	Generations		Flower characteristics	Source
			(no.) ^x			
1	<i>P. amabilis</i>	<i>P. amabilis</i>	—		White flower; very spread out sepals; very broad petals	Orchid Station, Barboursville, VA
2	<i>P. lindenii</i>	<i>P. lindenii</i>	—		Flowers are in pink tones with paler edges	NA ^y
3	<i>Phalaenopsis</i> Brother Heather Nuclear	<i>P. amabilis</i> , <i>P. amboinensis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. fasciata</i> , <i>P. lueddemanniana</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i>	12		White flower, densely spotted purple and red lip	Carmela Orchids, Hakalau, HI
4	<i>Phalaenopsis</i> Sogo Little Angel	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. sanderiana</i> , <i>P. stuartiana</i>	9		White and faint pink flower with dark red lip	Floradise Orchids, Gordonsville, VA
5	<i>Phalaenopsis</i> Taipei Gold Golden Star	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. venosa</i>	8		Yellow flower	Floradise Orchids
6	<i>Phalaenopsis</i> Tropical Stripes Carmela	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. lueddemanniana</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i>	14		Purple flower with dark purple veining	Carmela Orchids
7	<i>Phalaenopsis</i> Neon Stripes Red Avenger	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. lueddemanniana</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i>	14		NA	Carmela Orchids
8	<i>Phalaenopsis</i> Baldan's Kaleidoscope Golden Treasure	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. fasciata</i> , <i>P. lueddemanniana</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i>	10		Vivid golden yellow with dark red stripes and red blush	Floradise Orchids
9	<i>Phalaenopsis</i> (Taisuco Windian × Sogo Yukidian)	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i>	11		White flower	Bedford Orchids, Montreal, Canada
10	<i>Phalaenopsis</i> (Meller × Taisuco Kochdian)	NA	NA		Yellow flower	Floradise Orchids
11	<i>Phalaenopsis</i> pink-flowered unknown hybrid	NA	NA		Pink flower	Bedford Orchids
12	<i>Phalaenopsis</i> Luchia Pink	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. fasciata</i> , <i>P. lueddemanniana</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i>	14		Faint pink flower with red lip	Orchid Station
13	<i>Doritaenopsis</i> Newberry Parfait Picotee	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i> , <i>P. pulcherrima</i>	11		White flower with pink veining	Orchid Station
14	<i>Doritaenopsis</i> (Luchia Lady × City Girl)	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. lueddemanniana</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i> , <i>P. pulcherrima</i>	9		White flower with dark purple lip	Floradise Orchids
15	<i>Doritaenopsis</i> Abed-Nego Chadwick	<i>P. ambionensis</i> , <i>P. fasciata</i> , <i>P. lueddemanniana</i> , <i>P. violacea</i> , <i>P. pulcherrima</i>	3		Red flower with purple lip	Chadwick Orchids, Powhatan, VA
16	<i>Phalaenopsis</i> Schilleriana Pink Butterfly	<i>P. amabilis</i> , <i>P. aphrodite</i> , <i>P. equestris</i> , <i>P. lueddemanniana</i> , <i>P. sanderiana</i> , <i>P. schilleriana</i> , <i>P. stuartiana</i>	10		Pink and purple flower	NA

^zRoyal Horticulture Society (2002).^yNA = information not available.^xNumber of generation that were used to document contributing ancestor species in a particular hybrid.

Table 2. Total number of amplified fragment length polymorphism loci examined using 10 primer combinations as well as percentage of polymorphic loci per primer combination among 16 *Phalaenopsis* species and hybrids.

Primer combination		Loci detected (no.)	Polymorphic loci (no.)	Polymorphic loci (%)
E-CAG	M-CCG	143	136	95.1
E-CAG	M-CCA	169	162	95.9
E-CAG	M-CCT	100	94	94.0
E-CAG	M-CCC	139	135	97.1
E-CAG	M-CGG	131	123	93.9
E-CAG	M-CGC	162	151	93.2
E-CAG	M-CGT	136	128	94.1
E-CAG	M-CAC	108	105	97.2
E-CAG	M-CAG	129	119	92.2
E-CAC	M-CCG	136	132	97.1
Total		1,353	1,285	
Mean		135.3	128.5	95.0

distantly related. Relationships among 16 *Phalaenopsis* species and hybrids are shown in a dendrogram using UPGMA (Fig. 1). Two groups (Group I and Group II) were obtained. Group I contains the *Phalaenopsis* hybrids Taipei Gold 'Golden Star', Tropical Stripes 'Carmela', Neon Stripes 'Red Avenger', and Baldan's Kaleidoscope 'Golden Treasure'. Group II was further partitioned into two subgroups (Subgroup A and Subgroup B). Subgroup A contains (Taisuco Windian $\times$ Sogo Yukidian), *Phalaenopsis* (Meller $\times$ Taisuco Kochdian), pink-flowered unknown hybrid, and *Phalaenopsis* Luchia Pink. Two *Doritaenopsis* hybrids were closely associated in Subgroup B.

The same analysis was carried out using the FreeTree program with the Dice index and UPGMA for the comparison. Figure 2 shows the dendrogram obtained from the FreeTree program. The result was similar for Groups I and II, which contained the same two subgroups as clustering using NTSYSpc software. Bootstrap values for Group I and Group II supported 70% of the clustering. Group I containing four *Phalaenopsis* hybrids had bootstrap values ranging from 84% to 100%. Two *Doritaenopsis* hybrids in Subgroup II exhibited a bootstrap value of 100%. All other nodes had values greater than 64. The plants that did not fall into obvious groups were the distinct species (*Phalaenopsis amabilis* and *Phalaenopsis lindenii*) and some of the complex hybrids with unusual parents (*Phalaenopsis violacea* for *Doritaenopsis* Abed-Nego 'Chadwick' and *Phalaenopsis amboinensis* for *Phalaenopsis* Brother Heather 'Nuclear'). The genetic contribution of these parents, unique to single entries in our study of 16 clones, may have been sufficient to differentiate them from the groups.

The range of available ancestry information of *Phalaenopsis* hybrids was three to 14 generations (Table 1). Therefore, coefficients of coancestry were calculated on the basis of three generations. Coefficients of coancestry based on pedigree data ranged from 0 to 0.320 with a mean of 0.100 (Table 4). From a total of 136 pairs of *Phalaenopsis* species and hybrids, 73 pairs did not share a common parent until the third generation. The correlation between coefficient of coancestry and AFLP-based genetic similarity was 0.724 ($P < 0.0001$), indicating a strong linear association between these two

Table 3. Similarity matrix for 16 *Phalaenopsis* species and hybrids based on the Dice coefficient.^a

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 ^b	1.000															
2	0.417	1.000														
3	0.504	0.458	1.000													
4	0.371	0.379	0.424*	1.000												
5	0.427	0.390	0.505	0.343*	1.000											
6	0.497	0.432*	0.533	0.369*	0.593	1.000										
7	0.492	0.418*	0.512	0.352*	0.562	0.752	1.000									
8	0.478	0.408*	0.571	0.376*	0.586	0.670	0.643	1.000								
9	0.488	0.404*	0.522	0.389*	0.484	0.573	0.575	0.577	1.000							
10	0.464	0.383*	0.568	0.349*	0.549	0.573	0.567	0.582	0.654	1.000						
11	0.460	0.387*	0.499	0.367*	0.468	0.572	0.563	0.521	0.629	0.622	1.000					
12	0.493	0.420*	0.533	0.389*	0.490	0.597	0.579	0.570	0.694	0.656	0.732	1.000				
13	0.480	0.387*	0.499	0.365*	0.492	0.573	0.557	0.545	0.567	0.539	0.588	0.623	1.000			
14	0.488	0.384*	0.490	0.384*	0.492	0.549	0.547	0.545	0.591	0.546	0.585	0.616	0.774	1.000		
15	0.336	0.298	0.398*	0.292	0.474	0.382*	0.376*	0.402*	0.350*	0.405*	0.367*	0.374*	0.419*	0.421*	1.000	
16	0.444	0.375	0.479	0.345	0.452	0.510	0.496	0.470	0.468	0.469	0.548	0.511	0.573	0.554	0.413	1.000

^aNei and Li, 1979.

^bThe numbers in the first column and first row represent the *Phalaenopsis* genotypes as numbered in Table 1.

*Coefficients followed by the asterisk are significantly different at the $\alpha = 0.05$ level using *t* test.

Fig. 1. Dendrogram for 16 *Phalaenopsis* species and hybrids generated by unweighted pair group method with arithmetic mean analysis based on amplified fragment length polymorphism data using the Dice coefficient of genetic similarity estimates (Nei and Li, 1979).

measures of genetic relationships within our *Phalaenopsis* germplasm (Fig. 3).

Discussion

In this study, 95% of the AFLP-generated loci were polymorphic among 16 *Phalaenopsis* species and hybrids. This is not surprising considering the complex interspecific hybridization used to derive most modern *Phalaenopsis* and *Doritaenopsis* hybrids (Table 1). In another orchid genus, *Dendrobium*, 83% of the AFLP-generated loci were polymorphic among 43 hybrids (Xiang et al., 2003). In our dendrogram, *P. amabilis* and *P. lindenii* were distantly clustered (0.417); this is consistent with known pedigrees of *Phalaenopsis* based on random amplified polymorphic DNA (RAPD) markers. *P. amabilis* can be found within the pedigrees of nearly all *Phalaenopsis* cultivars, whereas *P. lindenii* has not been used frequently in *Phalaenopsis* hybrid breeding and the dendrograms reflect these relationships. Traditionally, *P. amabilis* and *P. lindenii* were classified in separate sections, *Phalaenopsis* and *Staur-oglottis*, respectively, within the subgenus *Phalaenopsis* (Christenson, 2001). Fu et al. (1997) reported that *P. amabilis*, section *Phalaenopsis*, was taxonomically close to *Phalaenopsis*

equestris, section *Staur-oglottis* using RAPD markers. *P. amabilis* and *P. equestris* exhibited a genetic similarity of 0.339 in their study. Thus, the genetic similarity of 0.417 between *P. amabilis* and *P. lindenii* in our study is close to the previous RAPD analysis result (Fu et al., 1997). Furthermore, based on other molecular data analyses, clustering between *P. amabilis* and *P. lindenii* was supported as well. Goh et al. (2005) assembled 46 species of *Phalaenopsis* into seven main groups based on RAPD analysis and reported that *P. amabilis* and *P. lindenii* occurred in the same group but belonged to two different sections, *Phalaenopsis* and *Staur-oglottis*, respectively. In other examples, *P. amabilis* and *P. lindenii* were treated as different members using the internal transcribed Spacers 1 and 2 of nuclear ribosomal DNA (Tsai et al., 2003, 2006) and DNA sequences of the plastid genome (*matK* and *trnK* introns) and the nuclear genome (Yukawa et al., 2005).

The relatively high bootstrap values (70%) support two major clades in *Phalaenopsis* hybrids used in this study (Fig. 2). *Phalaenopsis* hybrids Taipei Gold 'Golden Star', Tropical Stripes 'Carmela', Neon Stripes 'Red Avenger', and Baldan's Kaleidoscope 'Golden Treasure' were placed in Group I with 84% bootstrap value. In this study, we had five hybrids (1, 3, 4,

Fig. 2. Genetic relationships of 16 *Phalaenopsis* species and hybrids based on amplified fragment length polymorphism data. Dendrogram was generated using FreeTree program (Hampl et al., 2001), the Dice index and unweighted pair group method with arithmetic mean analysis. Numbers on the branches are bootstrap values and range from 64% to 100%.

13, and 14) with white flowers, four (2, 11, 12, and 16) with pink flowers, and three (5, 8, and 10) with yellow flowers. The dendrograms revealed that a simple classification based on only flower color would not have accurately reflected genetic relatedness.

Phalaenopsis hybrids classified by AFLP clustering were found to be consistent with known pedigrees. Group I and Group II on the dendrogram cluster several complex *Phalaenopsis* hybrids together. Genetic relationship derived from our AFLP analysis was verified in the RHS orchid hybrid registration database information system (RHS, 2002). The RHS parentage records reveal that clustered hybrids shared common ancestry. For example, Taipei Gold 'Golden Star', Tropical Stripes 'Carmela', Neon Stripes 'Red Avenger', and Baldan's Kaleidoscope 'Golden Treasure' are placed in Group I. These hybrids shared *P. amabilis* and *Phalaenopsis aphrodite* as common ancestors (Table 1). Tropical Stripes 'Carmela' clustered closely to Neon Stripes 'Red Avenger' at genetic distance 0.752 in Group I. These hybrids shared seven common parents: *P. amabilis*, *P. aphrodite*, *P. equestris*, *Phalaenopsis lueddemanniana*, *Phalaenopsis sanderiana*, *Phalaenopsis schilleriana*, and *Phalaenopsis stuartiana*. Thus, Tropical Stripes 'Carmela' is genetically close to Neon Stripes 'Red Avenger' and this fact was confirmed by our AFLP analysis.

The 'Picotee' clone of *Doritaenopsis* Newberry Parfait clustered close to *Doritaenopsis* (Luchia Lady x City Girl) in Group II; and both had *P. amabilis*, *P. aphrodite*, *P. equestris*, *P. sanderiana*, *P. schilleriana*, *P. stuartiana*, and *Phalaenopsis pulcherrima* as common parents. Our pink-flowered unknown hybrid clustered with *Phalaenopsis* Luchia Pink at genetic distance 0.732. Although the information for the pink-flowered unknown hybrid was unavailable as a result of a lost tag during shipping, the close relationship between it and *Phalaenopsis* Luchia Pink indicates their common parentage. The complex origin of *Phalaenopsis* hybrids ensures considerable polymorphism even when individuals share several common parents, revealing AFLP as a powerful tool for DNA fingerprinting in this valuable crop.

From the dendrogram, AFLP data placed Sogo Little Angel (4) and Schilleriana 'Pink Butterfly' (16) as outliers of Groups I and II despite their shared common ancestry and more distant from *P. amabilis* than either group. *P. amabilis* was found in the ancestry of 11 of the entries, the only exclusions being *P. lindenii* (2) and *Doritaenopsis* Abed-Nego 'Chadwick' (15) which logically occurred as outliers. The greater genetic distance of Sogo Little Angel (4) and Schilleriana 'Pink Butterfly' (16) from *P. amabilis* and the other hybrids may be the result of uneven gene flow and nuclear distribution of the

Table 4. Coefficient of coancestry for all possible pairs of the 16 *Phalaenopsis* from pedigree data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 ^z	0.5000															
2	0.0000	0.5000														
3	0.0000	0.0000	0.5000													
4	0.0000	0.0000	0.0000	0.5000												
5	0.0000	0.0000	0.0000	0.0938	0.5000											
6	0.0000	0.0000	0.0625	0.0000	0.0000	0.5000										
7	0.0000	0.0000	0.0000	0.0000	0.0000	0.3200	0.5000									
8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0313	0.5000								
9	0.0000	0.0000	0.0000	0.0313	0.0938	0.0000	0.0000	0.0000	0.5000							
10	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.5000						
11	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.1250	0.5000					
12	0.0000	0.0000	0.0313	0.1250	0.0000	0.0000	0.0000	0.0000	0.0000	0.1250	0.1250	0.5000				
13	0.0000	0.0000	0.0625	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1250	0.1250	0.1875	0.5000			
14	0.0000	0.0000	0.0000	0.1250	0.0000	0.0000	0.0000	0.0313	0.0000	0.1250	0.1250	0.5000	0.0000	0.5000		
15	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0625	0.0000	0.1250	0.1250	0.0000	0.0000	0.0000	0.5000	
16	0.0000	0.0000	0.0000	0.0000	0.0938	0.0000	0.0000	0.0313	0.0000	0.1250	0.1250	0.0000	0.0625	0.0000	0.0313	0.5000

^zThe numbers in the first column and first row represent the *Phalaenopsis* genotypes as numbered in Table 1.

Fig. 3. Plot of amplified fragment length polymorphism-based genetic similarity coefficient (Nei and Li, 1979) and coefficient of coancestry for 136 pairs of *Phalaenopsis* species and hybrids.

interspecific hybrids (Lexer et al., 2006). Although *P. amabilis* has shared alleles with other species such as *P. aphrodite*, *P. equestris*, *P. lueddemanniana*, *P. sanderiana*, *P. schilleriana*, and *P. stuartiana*, it is possible that the genomes of Sogo Little Angel and Schilleriana ‘Pink Butterfly’ comprise a different subset of the genomes of other species found in their parentage. Another possibility for Little Angel (4) and Schilleriana ‘Pink Butterfly’ (16) occurring as outliers is that these hybrids may represent somaclones derived from tissue culture during cultivar development. Tissue culture has been widely used for mass propagation in *Phalaenopsis* (Tokuhara and Mii, 1993). Somaclonal variants with altered phenotypes have been reported to occur in a range of 0% to 14% (Tokuhara and Mii, 1998, 2001). Somaclonal variation at the molecular level has been documented in *Phalaenopsis* using RAPD (Chen et al., 1998), cDNA-RAPD (Chen et al., 2005), and cDNA-AFLP (Hsu et al., 2008). Genetic polymorphism associated with the somaclones, some of which demonstrated remarkable phenotypic variation as well as differential gene expression have been identified. Hsu et al. (2008) found slightly more than 1% distinct sequences in somaclones among 2269 transcript-derived fragments.

The correlation tests revealed that the AFLP-derived genetic similarity values showed a statistically significant correlation with the coefficient of coancestry values based on known pedigree data (Fig. 3). In Austrian wheat, close associations between coefficient of parentage and RFLP-based genetic similarity ($r = 0.535$) and between coefficient of parentage and simple sequence repeat-based genetic similarity ($r = 0.633$) were reported (Parker et al., 2002). In European maize inbred lines, a highly significant relationship ($r = 0.71$ and 0.86) was reported between coefficient of parentage and RFLP-based genetic similarity (Messmer et al., 1993). Therefore, both the coefficient of coancestry based on pedigree data and DNA marker-based estimates of genetic similarity provided similar information about the genetic relationships among germplasm.

The taxonomy of *Phalaenopsis* is complex. Intensive breeding over many years has generated numerous commercial hybrids. Two of the most important hybrids, *Phalaenopsis* Gilles Gratiot (*P. aphrodite* × *P. rimestadiana*) and *Phalaenopsis* Elisabethae (*P. amabilis* × *P. rimestadiana*), were

developed in 1920 and led to a major advance in *Phalaenopsis* breeding (Griesbach, 2002). Most of our 14 *Phalaenopsis* hybrids were also derived from *Phalaenopsis* Gilles Gratiot and *Phalaenopsis* Elisabethae. Commercial *Phalaenopsis* hybrids were developed through both inbreeding and outcrossing, leading to high rates (95%) of polymorphism detected in our AFLP analysis.

Previous *Phalaenopsis* genome studies (Kao et al., 2001; Lin et al., 2005) also provide information about genetic variation through species relationship. Arends (1970) separated *Phalaenopsis* into large and small chromosome groups. *Phalaenopsis* species with large chromosomes include *P. amboinensis* and *P. pulcherrima*. *Phalaenopsis* species with small chromosomes include *P. amabilis*, *P. aphrodite*, *P. equestris*, *P. sanderiana*, *P. lueddemanniana*, and *P. stuartiana*. High frequencies of bivalents were reported in hybrids between species with large chromosomes (e.g., *P. amboinensis* × *P. pulcherrima*) and between species both with small chromosomes (e.g., *P. amabilis* × *P. lueddemanniana*). Kao et al. (2001) suggested that chromosome size was associated with repetitive genomic DNA content and mixed genome size through inbreeding and outcrossing may have led to the diversity found in *Phalaenopsis* (Flavell, 1982).

This is the first study of genetic variability among *Phalaenopsis* species and hybrids based on AFLP analysis. Analyzing AFLP among *Phalaenopsis* species and hybrids demonstrated significant genetic diversity. Assessments of genetic variation and relationships using both the known pedigree data and genetic fingerprinting will be useful in orchid breeding. This will allow breeders to select appropriate parents based on their genetic distance in addition to the morphological traits for future breeding. Because of all of the other factors to be considered in parent selection, e.g., chromosome number and size, phenotypic traits, species composition, and ploidy, the influence of genetic distance on performance of hybrids (hybrid vigor) may not be obvious. The genetic variability at the DNA level identified by markers such as AFLP will also be useful in species and variety conservation by identifying core collections for preservation in orchid germplasm banks and breeders' proprietary rights.

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