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FULL PAPER

Morphological and molecular characterization of two ITS groups of *Erysiphe* (Erysiphales) occurring on *Syringa* and *Ligustrum* (Oleaceae)

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Abstract ITS sequences determined for 53 *Erysiphe* specimens on *Syringa* and *Ligustrum* collected in Europe, East Asia, and North and South America were divided into two ITS groups, S and K types. Phylogenetic analysis showed that these two ITS types do not share a common ancestor and form separate clades. The K type on *Ligustrum* was identified as *Erysiphe ligustri* based on the three-dimensional branching pattern of appendages. Morphological observations showed that there are some morphological differences—pigmentation of appendages and number of ascospores per ascus—between the S and K types on *Syringa*. Based on these morphological observations, the S and K types on *Syringa* were identified as *E. syringae* and *E. syringae-japonicae*, respectively. The recent abundant production of chasmothecia by lilac powdery mildew in Europe was caused by *E. syringae-japonicae* introduced from East Asia. DNA sequence analyses of the rDNA ITS region and the 28S rDNA, *tub2*, *CYP51*, and *Chs1* genes did not support an interspecific hybrid origin for *E. syringae-japonicae*. Haplotype analysis suggested that *E. syringae* originated in North America and independently migrated to East Asia and Europe/South America.

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Introduction

The lilacs (*Syringa*) are well known for their beautiful and fragrant flowers, and many species of this genus are widely cultivated as ornamentals. Two *Erysiphe* species (*E. syringae* and *E. syringae-japonicae*) are known to occur on *Syringa*. *Erysiphe syringae* was described in 1834 based on a specimen collected in North America and is considered a species of North American origin. *Erysiphe syringae-japonicae* was described by Braun (1982) as *Microsphaera syringae-japonicae* based on a specimen collected in Japan. According to Braun (1987), the most conspicuous morphological differences between *E. syringae* and *E. syringae-japonicae* are pigmentation of chasmothecial appendages and number of ascospores per ascus. In *E. syringae*, appendages are not pigmented or are only slightly pigmented at the base. On the other hand, appendages are pigmented up to the middle of the stalk in *E. syringae-japonicae*. The number of ascospores is mostly 4–5 in *E. syringae* and 6–8 in *E. syringae-japonicae*. *Erysiphe syringae* was introduced in Europe from North America in the nineteenth century and became widely distributed in Europe by the middle of the twentieth century (Blumer 1951). Chasmothecia formation is common in North America, while it has been very rare in Europe (Blumer 1951; Braun 1987). However, since 1998, a striking increase has been observed in the number of chasmothecia produced on lilacs in Germany and Switzerland. The causal fungus was identified as *E. syringae-japonicae* (Braun 1998; Bolay 2005), which suggests that *E. syringae-japonicae* entered Europe from East Asia and produced chasmothecia on lilacs. Bolay (2005) examined

two North American *E. syringae* specimens, collected in 1900 and 1955, and further specimens collected more recently in North America. All of these American specimens differed from *E. syringae* in their morphological patterns, and they were clearly identifiable as *E. syringae-japonicae*. Based on this result, Bolay (2005) concluded that *E. syringae-japonicae* is conspecific to *E. syringae*. If this is accepted, the earlier proposal that the recent increase in chasmothecia on lilac powdery mildew in Europe is the result of the introduction and spread of *E. syringae-japonicae* in these countries needs to be reconsidered.

To resolve this problem, Seko et al. (2008) conducted a molecular analysis of the rDNA ITS region for a number of isolates of lilac powdery mildew collected in wide areas of the world. Their analysis revealed that the lilac powdery mildew isolates can be divided into two ITS groups (S and K types) with only 94% sequence homology, and that the two ITS groups do not share a common ancestor. Furthermore, they revealed that the recent abundant chasmothecia formation in Europe was caused by the K-type fungus, which was recently introduced to Europe. However, morphological differences between the S and K types were not clear in their observations, so they could not assign any species names to the two ITS types.

This study was conducted to identify the two ITS types based on morphological and molecular characteristics using many specimens. During this study, we noticed that the K type is highly similar to *E. ligustri* on *Ligustrum* spp. in ITS sequence but clearly different in morphology. On the other hand, the K type is more similar to the S type in morphology than to *E. ligustri*. To explain the apparent conflict between molecular and morphological characteristics, we made a working hypothesis that the K type could be an interspecific hybrid between *E. ligustri* and the S type, and investigated this possibility using sequence analyses of five DNA regions.

Materials and methods

Sample collection

A total of 205 *Erysiphe* specimens on *Syringa* spp. and *Ligustrum* spp. were collected in East Asia, Europe, and North and South America, or obtained from existing collections. Whole-cell DNA was extracted from chasmothecia (when available) or mycelia by the chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996).

PCR and DNA sequencing

PCR primers to amplify the β-tubulin gene (*tub2*), the cytochrome P450 14α-demethylase gene (*CYP51*), and the chitin synthase gene (*Chs1*) were newly designed in this study based on sequence data retrieved from DDBJ and our unpublished sequence data (Table 1). These three DNA regions, the rDNA ITS region, and the D1/D2 domains of the 28S rDNA were amplified by two sequential PCRs using nested primer sets. PCR reactions were conducted with TaKaRa Taq DNA polymerase (TaKaRa, Tokyo, Japan) in a thermal cycler TP-400 (TaKaRa) under the following conditions: an initial denaturing step for 2 min at 95°C for 30 cycles, where each cycle consisted of 30 s at 95°C, followed by 30 s at 52°C for annealing and 30 s at 72°C for extension, and a final extension cycle of 7 min at 72°C. A negative control that lacked template DNA was included for each set of reactions. The PCR amplicons were separated by electrophoresis on 1.5% agarose gels in TAE buffer. The desired band was visualized under long-wavelength ultraviolet light and cut out of the gel. Purification of the DNA fragment was performed utilizing the JETSORB Kit (GENOMED, Germany), as described by the manufacturer's protocol. Both strands of the amplicons were sequenced using the primers shown in Table 1. The sequence reactions were conducted using the CEQ Dye Terminator Cycle Sequencing Kit (Beckman Coulter, Fullerton, CA, USA) according to the manufacturer's instructions and run on a DNA sequencer CEQ2000XL (Beckman Coulter).

Representative sequences determined in this study were deposited in DNA databases (DDBJ, EMBL, GenBank) under the accession numbers of AB295456–AB295462 and AB571048–AB571065.

Phylogenetic analysis

The sequences were initially aligned using the Clustal X package (Thompson et al. 1997). Alignment was then visually refined with a word processing program, using color-coded nucleotides. The alignments were deposited in TreeBASE (http://www.treebase.org/) under the accession number of 10667 (for accession: http://purl.org/phylo/treebase/phylows/study/TB2:S10677). Phylogenetic trees were obtained from the data using the maximum parsimony (MP) method and Bayesian analysis. MP analyses were performed with the parsimony ratchet (Nixon 1999) in PAUP* 4.0 (Swofford 2001) and PAUPRat ver. 1 (Sikes and Lewis 2001) with the heuristic search option using the tree bisection-reconstruction (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting trees was tested with bootstrap (BS) analyses (Felsenstein 1985), using 1000 replications with the stepwise addition option set to simple and a maximum tree number of 100. BS values of 70% or higher are provided.

Table 1 PCR primers used in this study

Target DNA	Primer name	Direction	Primer sequence (5′–3′)	Reference
ITS	ITS1	Forward	TCCGTAGGTGAACCTGCGG	White et al. (1990)
	ITS5	Forward	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
	PM7	Forward	RYYGACCCTCCACCCGTGY	Seko et al. (2008)
	T4	Forward	TCAACAACGGATCTCTTGGC	Hirata and Takamatsu (1996)
	ITS4	Reverse	TCCTCCGCTTATTGATATGC	White et al. (1990)
	P3	Reverse	GCCGCTTCACTCGCCGTTAC	Kusaba and Tsuge (1995)
	ITS2	Reverse	GCTGCGTTCTTCATCGATGC	White et al. (1990)
28S rDNA	PM3	Forward	GKGCTYTMCGCGTAGT	Takamatsu and Kano (2001)
	NL1	Forward	AGTAACGGCGAGTGAAGCGG	Mori et al. (2000)
	NL3	Forward	AGACCGATAGCGAACAAGTA	Mori et al. (2000)
	NL2	Reverse	TACTTGTTCGCTATCGGTCT	Mori et al. (2000)
	TW14	Reverse	GCTATCCTGAGGGAAACTTC	G. Saenz (pers. commun.)
	NLP2	Reverse	GGTCCCAACAGCTATGCTCT	Mori et al. (2000)
tub2	TU-E1	Forward	TTCAYCATGCGCGAGATC	This study
	TU5	Reverse	ATAYGTTCCTCGWGCYGT	This study
	TU6	Reverse	TGTWCGWGCYGGYCCWT	This study
CYP51	CYP51-5f	Forward	CATGAYCTWGATATGGGMTTYAC	This study
	CYP51-E1f	Forward	ATCAACTTTACATTYYACTGGG	This study
	CYP51-6r	Reverse	TGGRCAACAYTCTTCRTCATC	This study
CHS1	CHS1-E1f	Forward	TACACAYGAYATYACTTGGGG	This study
	CHS1-M1f	Forward	AGGCGAYGATAARGCTGAAC	This study
	CHS1-M2r	Reverse	ACAAGAAGATTAYTATAGRGGRT	This study

For Bayesian phylogenetic analyses, the best-fit evolution model was determined for each data set by comparing different evolutionary models via the Akaike information criterion (AIC) using PAUP* and MrModeltest 2.2 (Nylander 2004). MRBAYES 3.1.1 (Huelsenbeck and Ronquist 2001) was launched with random starting trees for 5×10^6 generations and the Markov chains were sampled every 100 generations, which resulted in 5×10^4 sampled trees. To ensure that the Markov chain did not become trapped in local optima, we used the MCMCMC algorithm, performing the estimation with four incrementally heated Markov chains. Bayesian posterior probability (PP) values of 0.95 or higher are shown.

Morphological observations

Presence or absence of chasmothecia was observed under a dissecting microscope for 204 specimens on *Syringa* spp. and *Ligustrum* spp. For the specimens on which chasmothecia were available, branching pattern and pigmentation of appendages and number of ascospores per ascus were examined by standard light microscopy (Axio Imager; Carl Zeiss, Göttingen, Germany) and differential interference contrast optical instruments and devices. Five to seven chasmothecia were examined for each specimen.

For scanning electron microscopic (SEM) observations, fresh leaves with chasmothecia were cut into small pieces with a razor blade and then treated by the modified tannic acid fixation method (Kunoh et al. 1977). They were then dried in a critical-point dryer and coated with gold using an ion sputter (model E-1010, Hitachi, Tokyo, Japan). Specimens were observed with a SEM (model S-4000, Hitachi, Tokyo, Japan) at 20 kV accelerating voltage.

Results

Phylogenetic analysis

The 53 ITS sequences of powdery mildew specimens on *Syringa* and *Ligustrum* collected in Europe, East Asia, and North and South America were aligned with 21 sequences of *Erysiphe* spp. retrieved from the DNA database. The alignment data matrix consisted of 74 taxa and 604 characters, of which 185 (30.6%) characters were variable and 113 (18.7%) characters were informative for parsimony analysis. *Erysiphe glycines* was used as an outgroup taxon based on the report of Takamatsu et al. (1999). A total of 154 equally parsimonious trees with 406 steps [consistency

index (CI) = 0.6133, retention index (RI) = 0.8602, rescaled consistency index (RC) = 0.5276] were constructed by the parsimony ratchet analysis. Tree topologies were almost consistent among the 154 trees, except for branching orders of the terminal branches and branch length. One of the 154 trees with the highest log likelihood value is shown in Fig. 1. MrModeltest selected the GTR+I+Γ model as the best for this data set. Using this evolution model, MRBAYES was run for 5×10^6 generations, resulting in approximately 5×10^4 sampling trees. The first 32100 trees were discarded (burn-in) because ASDSF was higher than 0.01. The remaining 17900 trees were summarized in a majority-rule consensus tree, yielding the probability of each clade being monophyletic. The tree topology generated by the Bayesian analysis was almost identical to the MP tree, and thus the tree is not shown.

The 53 sequences of powdery mildew specimens on *Syringa* and *Ligustrum* were divided into two large groups with 93.5–94% sequence similarity between the groups (Fig. 1). One group, corresponding to the S type in Seko et al. (2008), was composed of 25 of the 53 specimens sequenced. The S type was further divided into two haplotypes, S-1 and S-2, based on one base difference in the ITS2 region. Of the 25 specimens, 23 were haplotype S-1 and only two specimens collected in the USA belonged to haplotype S-2. Another group, corresponding to the K type in Seko et al. (2008), was composed of 28 specimens and further divided into four haplotypes based on 1–3 base differences. All 13 specimens on *Syringa* collected in Japan, far-eastern Russia and Europe belonged to haplotype K-1. Four, 9 and 2 specimens on *Ligustrum* belonged to haplotypes K-2, K-3 and K-4, respectively. These specimens on *Ligustrum* were identified as *E. ligustri* based on the three-dimensional branching pattern of the appendage apex (Figs. 2, 3) (Shin 2000; Seko et al. 2008). Two specimens from Argentina belonged to haplotype K-2. The phylogenetic analysis revealed that the two ITS groups do not share a common ancestor and form separate clades. This suggests that the two ITS groups became parasites of *Syringa* on two independent occasions.

Morphological observations of the S and K types

Formation of chasmothecia was observed for 138 specimens on *Syringa* and 25 specimens on *Ligustrum*. When chasmothecia were found, the pigmentations and branching patterns of appendages and the number of ascospores per ascus were checked. Of the 138 specimens on *Syringa*, 32 specimens were of the S type and 106 specimens were of the K type. Of the 25 specimens on *Ligustrum*, 5 specimens were of the S type and 20 specimens were of the K type. The 20 K-type specimens on *Ligustrum* were identified as *E. ligustri*, and these specimens were excluded from a morphological comparison of the S and K types in Figs. 4 and 5. Pigmentation of appendages in the K type on *Syringa* varied from pigmented only at the base to pigmented up to two-thirds of the stalk, and many were pigmented up to the middle of the stalk (Fig. 4). On the other hand, appendages were not pigmented or were pigmented only at the base in the S type, except for one specimen (KW 30901) collected in Ukraine in 1990, in which appendages were pigmented up to the middle of the stalk. In the K type on *Syringa*, the number of ascospores per ascus was 5–8, and mostly 6–7 (Fig. 5). In the S type, it was 4–7, excepting for one specimen (KW 30901) with 6–7(–8) ascospores per ascus. In both the S and K types, the appendage apex was dichotomously branched 4–6 times (Fig. 3). The branching pattern was two-dimensional in both types, and was clearly different from the three-dimensional pattern of *E. ligustri*.

Is the K type on *Syringa* an interspecific hybrid of *E. ligustri* and the S type?

The above results indicate that the K type on *Syringa* is closely related to *E. ligustri* in ITS sequences, but only distantly related to the S type. The two ITS types evolved independently from different ancestors. On the other hand, the branching pattern of appendages is three-dimensional in *E. ligustri*, while it is two-dimensional in the S and K types on *Syringa*. We made an interspecific hybrid hypothesis as a working assumption to explain this apparent conflict between molecular characteristics and morphology. The apparent conflict might be explained if the ITS sequence of the K type on *Syringa* is derived from *E. ligustri* and the DNA region concerning appendage morphology was derived from the S type. If this hypothesis were to be true, the K type could have chimeric DNA that came partly from *E. ligustri* and partly from the S type. To check this possibility, we performed a multigene sequence analysis.

Five DNA regions—rDNA ITS (553–558 bp), 28S rDNA (797 bp), β-tubulin gene (*tub2*) (ca. 240 bp), *CYP51* gene (216 bp), and *Chs1* gene (168 bp)—were sequenced for 18 S-type (one on *Ligustrum* and 17 on *Syringa*), 9 K-type (on *Syringa*) and 6 *E. ligustri* specimens. As shown in Table 2, the 18 S-type specimens were divided into three haplotypes (A, B, C), and the 6 *E. ligustri* specimens were divided into four haplotypes (E, F, G, H). All nine K-type specimens on *Syringa* had identical DNA sequences for all five DNA regions. DNA sequences of the K type on *Syringa* were similar to *E. ligustri* in all five DNA regions sequenced, but there is no haplotype shared by the K type on *Syringa* and *E. ligustri*. KW 30901 on

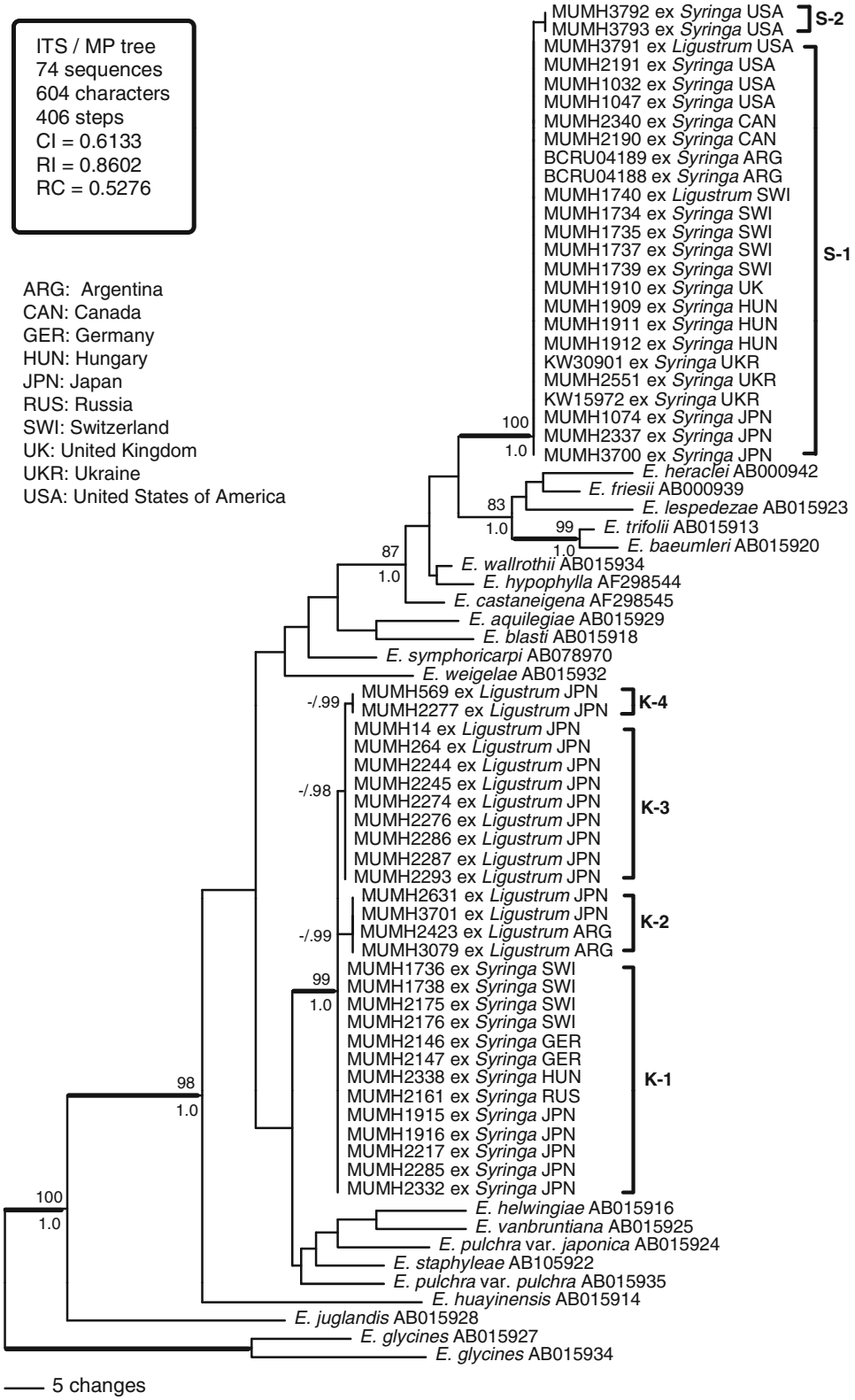
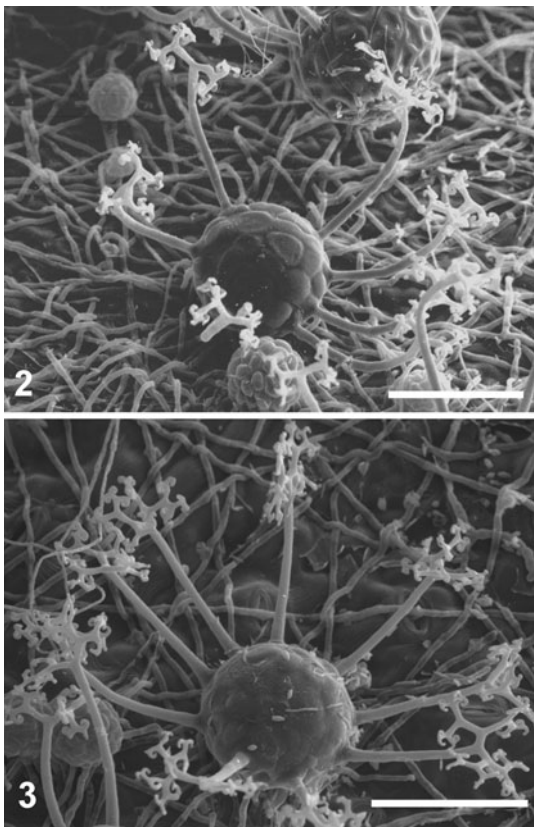


Fig. 1 One of the 154 MP trees based on ITS sequences from 74 sequences of *Erysiphe* spp., including 53 isolates from *Syringa* and *Ligustrum*. Percentage bootstrap support (1000 replications; ≥70%) and Bayesian posterior probability (≥0.95) are shown on and under branches, respectively. *S-1–S-2* and *K-1–K-4* indicate ITS haplotypes found in *Erysiphe* spp. on *Syringa* and *Ligustrum*. *Bold lines* denote branches supported by ≥95% bootstrap support

Syringa is of S type in its ITS, 28S rDNA and *tub2* gene, while it is of K type in its *Chs1* gene. In addition, peaks on electropherograms obtained for the *CYP51* gene were overlapping in the S K types. We repeated the sequencing of DNA extracted from this specimen and got the same result.



Figs. 2, 3 Scanning electron microscopy of chasmothecia of *Erysiphe* spp. on *Ligustrum* and *Syringa*. **2** *E. ligustri* on *Ligustrum tschonoskii*. **3** Haplotype K-1 on *Syringa vulgaris*. These two fungi were closely related in terms of rDNA sequences (99.8% similarity), but were distinguished by the apical branching pattern of appendages: three-dimensional type in *E. ligustri* and two-dimensional in haplotype K-1. *Bars* 100 μm

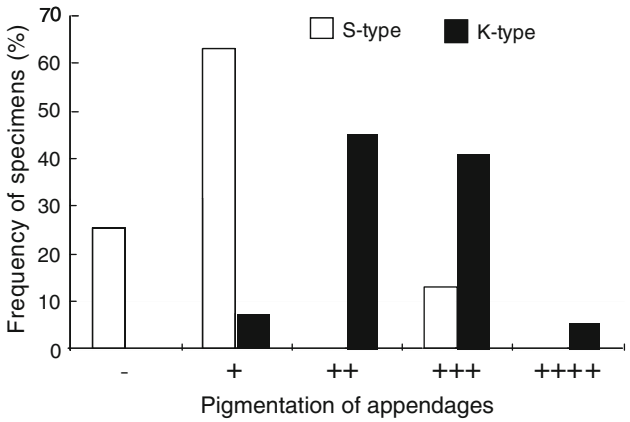


Fig. 4 Pigmentation of chasmothecial appendages in S and K types of powdery mildew (*Erysiphe* spp.) isolates from *Syringa* spp. − not pigmented, + pigmented only at the base, ++ up to one-third of the appendage length is pigmented, +++ up to two-thirds of the appendage length is pigmented, ++++ more than two-thirds of the appendage length is pigmented

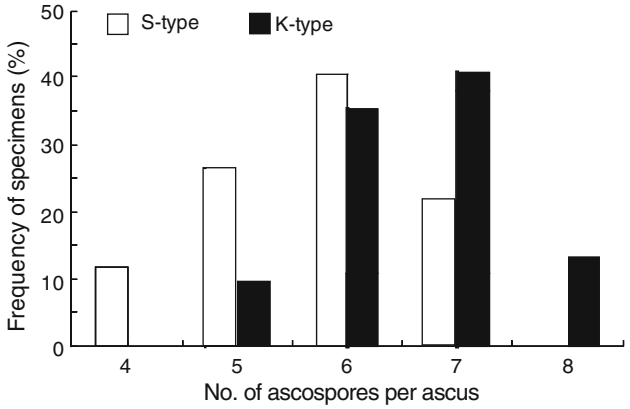


Fig. 5 Number of ascospores per ascus in S and K types of powdery mildew (*Erysiphe* spp.) isolates from *Syringa* spp.

Discussion

Species identification of S and K types

In this study, we performed morphological observations of the two ITS types found on *Syringa* spp., especially with regard to the number of ascospores per ascus and the pigmentation of appendages, which are the most conspicuous morphological differences between *E. syringae* and *E. syringae-japonicae* (Braun 1987). As a result, we detected morphological differences between these two ITS types, although the characteristics often overlapped. The number of ascospores per ascus was somewhat smaller in *E. syringae* (4–7) than in *E. syringae-japonicae* (5–8). The most conspicuous difference between the two ITS types was observed in the pigmentation of appendages. In the S type, they are hyaline or only pigmented at the base, while they are pigmented at the base or for up to two-thirds of the appendage length in the K type. Based on these results, the S type can be assigned to *E. syringae*, although the number of ascospores does not completely agree with the description of Braun (1987). The K type can be assigned to *E. syringae-japonicae*.

Erysiphe syringae-japonicae is closely related to *E. ligustri* based on DNA sequences. *Syringa* and *Ligustrum* belong to the family Oleaceae and are closely related to each other in molecular phylogeny (Li et al. 2002). *Syringa* is paraphyletic to *Ligustrum*, and the two genera form a monophyletic group. The close genetic relatedness of *E. syringae-japonicae* and *E. ligustri* would appear to be an intuitive result considering the nature of the host relationship. However, these two species can be clearly distinguished by their branching patterns: the two-dimensional pattern in *E. syringae-japonicae* and the three-dimensional pattern in *E. ligustri*. Sequence analyses of the five DNA regions showed that there is no haplotype shared by *E. syringae-japonicae* and *E. ligustri*. Thus, there is a

Table 2 Sequence analyses of five DNA regions for *Erysiphe* spp. on *Syringa* and *Ligustrum*

ITS type	Host plant	Specimen no.	Country and year collected	ITS haplotype	28S rDNA haplotype	*tub2* haplotype	*CYP51* haplotype	*CHS1* haplotype	Genetic group
S	*Ligustrum japonicum*	MUMH 3791	USA/2002	S-1	S-a	BTS-1	CPS-2	CSS-1	A
	Syringa vulgaris	BCRU 04188	Argentina/2000	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		BCRU 04189	Argentina/1999	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		MUMH 1032	USA/1999	S-1	S-a	BTS-1	CPS-1	CSS-1	B
		MUMH 1074	Japan/2000	S-1	S-a	BTS-1	CPS-1	CSS-1	B
		MUMH 1910	UK/2000	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		MUMH 1911	Hungary/2001	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		MUMH 1912	Hungary/2000	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		KW 15972	Ukraine/1981	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		KW 30901	Ukraine/1990	S-1	S-a	BTS-1	CPS-2/CPK-1	CSK-2	–
		MUMH 2337	Japan/1998	S-1	S-a	BTS-1	CPS-1	CSS-1	B
		MUMH 2340	Canada/2003	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		MUMH 2551	Ukraine/2003	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		MUMH 3700	Japan/2005	S-1	S-a	BTS-1	CPS-1	–	B
		MUMH 3793	USA/2004	S-2	S-a	BTS-1	CPS-2	CSS-1	C
	Syringa sp.	MUMH 2190	Canada/2003	S-1	S-a	BTS-1	CPS-1	CSS-1	B
		MUMH 2191	USA/2003	S-1	S-a	BTS-1	CPS-2	CSS-1	A
		MUMH 3792	USA/2004	S-2	S-a	BTS-1	CPS-2	CSS-1	C
K	*Syringa* × *persica*	MUMH 2175	Switzerland/2002	K-1	K-a	BTK-3	CPK-1	CSK-2	D
	Syringa reflexa	MUMH 2176	Switzerland/2002	K-1	K-a	BTK-3	CPK-1	CSK-2	D
	Syringa vulgaris	MUMH 1916	Japan/1993	K-1	K-a	BTK-3	CPK-1	CSK-2	D
		MUMH 2146	Germany/2001	K-1	K-a	BTK-3	CPK-1	CSK-2	D
		MUMH 2147	Germany/2002	K-1	K-a	BTK-3	CPK-1	CSK-2	D
		MUMH 2161	Russia/1989	K-1	K-a	BTK-3	CPK-1	CSK-2	D
		MUMH 2217	Japan/2003	K-1	K-a	BTK-3	CPK-1	CSK-2	D
		MUMH 2285	Japan/2003	K-1	K-a	BTK-3	CPK-1	CSK-2	D
		MUMH 2338	Hungary/2003	K-1	K-a	BTK-3	CPK-1	CSK-2	D
	Ligustrum obtusifolium	MUMH 569	Japan/1998	K-4	K-d	BTK-2	CPK-1	CSK-2	E
		MUMH 2244	Japan/2003	K-3	K-c	BTK-4	CPK-1	CSK-2	F
		MUMH 2245	Japan/2003	K-3	K-c	BTK-4	CPK-1	CSK-2	F
		MUMH 3701	Japan/2005	K-2	K-b	BTK-1	CPK-1	CSK-1	G
	Ligustrum tschonoskii	MUMH 2287	Japan/2003	K-3	K-d	BTK-4	CPK-1	CSK-2	H
		MUMH 2293	Japan/2003	K-3	K-d	BTK-4	CPK-1	CSK-2	H

BCRU Instiutional Herbarium of Centro Regional Universitario Bariloche, San Carlos de Bariloche, Argentina; *KW* National Herbarium of the M.G. Kholodny Institute of Botany, Kiev, Ukraine; *MUMH* Mie University, Mycological Herbarium, Japan

genetic isolation between *E. syringae-japonicae* and *E. ligustri* that supports the present species delimitation of the two species. *Erysiphe ligustri* is endemic to East Asia and restricted to Japan and Korea based on its geographical distribution. However, the present study showed that specimens with DNA sequences of *E. ligustri* were collected in Argentina and Ukraine (data not shown). *E. ligustri* may be distributed in these countries too.

Chasmothecium formation by *E. syringae* is common in North America, but it is rare in Europe. In contrast, *E. syringae-japonicae* formed abundant chasmothecia in Europe as well as East Asia (Seko et al. 2008). Recent abundant chasmothecium production of lilac powdery mildew in Europe may have been due to *E. syringae-japonicae* introduced from East Asia.

Possibility of an interspecific hybrid origin of *E. syringae-japonicae*

To investigate the possibility of an interspecific hybrid origin of *E. syringae-japonicae*, we sequenced five DNA regions: the ITS, 28S rDNA, *tub2*, *CYP51*, and *Chs1* genes.

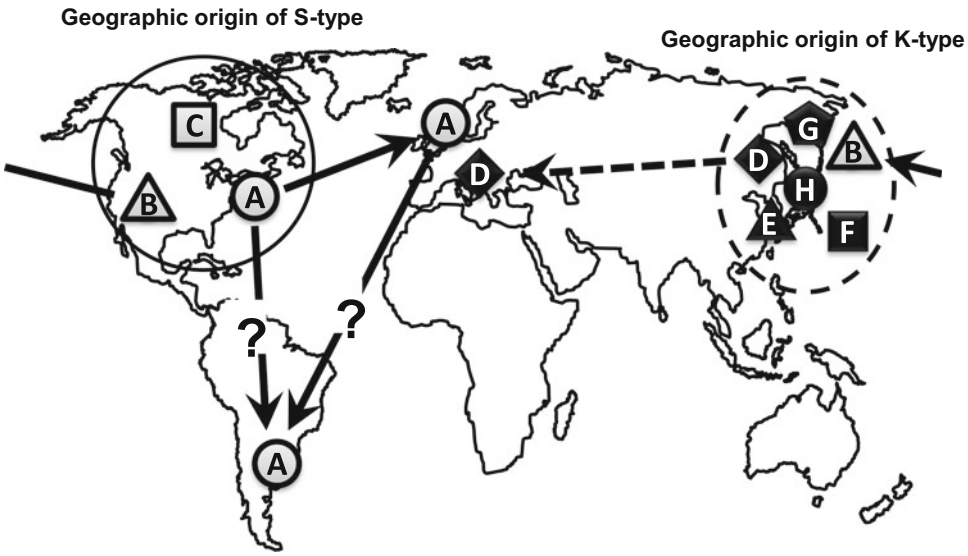


Fig. 6 Geographic distributions of the haplotypes of *Erysiphe* spp. occurring on *Syringa* and *Ligustrum*, and their putative migration routes

All five DNA regions sequenced for *E. syringae-japonicae* were similar to those of *E. ligustri*, but not to *E. syringae*, which does not support an interspecific hybrid origin of *E. syringae-japonicae*.

KW 30901, collected in Ukraine in 1990, was of S type in its ITS, 28S rDNA and *tub2* genes, but of K type in the *Chs1* gene. Peaks from the S and K types overlapped in the *CYP51* gene. A possible explanation for this result may be that both the K and S types simultaneously existed on the same specimen. However, if this was the case, why was only the S-type sequence detected in ITS, 28S rDNA and *tub2* genes, and why did only the K-type sequence appear in the *Chs1* gene? To answer this question, we made the working hypothesis that either sequence was selectively amplified by PCR even when the two different template sequences were mixed in the DNA extraction. To address this possibility, we extracted DNA from a mixture of the same number of chasmothecia of the S and K types, amplified the mixed DNA by PCR, and sequenced the ITS region. Peaks of S and K types were found to overlap in this sequence, which forced us to reject our working hypothesis. Thus, it is possible that this specimen has chimeric DNA of both S and K (or *E. ligustri*) type at the *Chs1* locus. However, further investigations are required to verify that this specimen is a possible hybrid between the S type and the K type, because we had only a single specimen of this kind.

Geographic origin and migration of the S and K types

The S and K types were divided into three and five haplotypes, respectively, based on the five DNA sequences (Table 2). All three haplotypes of the S type were distributed in North America (Fig. 6). Of these, only haplotype B was found in East Asia and only haplotype A was found in Europe and South America. If we accept that the area with the highest genetic diversity is the center of origin of a species, the S type originates from North America. This is consistent with previous reports (Blumer 1951; Braun 1987) suggesting that the geographic origin of *E. syringae* is North America. The present result, that different haplotypes were found in East Asia and Europe/South America, suggests that the S type may have migrated to East Asia and to Europe/South America on separate occasions (Fig. 6).

Among the five haplotypes of the K type, only haplotype D was *E. syringae-japonicae*; the other four haplotypes were *E. ligustri*. Assuming that the most divergent group is ancestral, *E. ligustri* appears to be ancestral to *E. syringae-japonicae*. However, the phylogenetic tree of the ITS region indicates that haplotype K-1 (*E. syringae-japonicae*) has the most ancestral characters in the K-type clade, while the other three haplotypes (*E. ligustri*) have one or two derived characters (Fig. 1). Further analyses are needed to clarify the evolutionary process of this powdery mildew species on *Syringa* and *Ligustrum*.

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