

**Table S1. Nonhybrid *Epichloë* species used in this study**

<b>Endophyte</b>		<b>ATCC or CBS</b>		
<b>species</b>	<b>Isolate ID</b>	<b>number</b>	<b>Host species</b>	<b>Reference or source</b>
<i>E. amarillans</i>	E52	ATCC 200743	<i>Sphenopholis obtusata</i>	(1-4)
<i>E. amarillans</i>	E57	ATCC 200744	<i>Agrostis hyemalis</i>	(1-5)
<i>E. amarillans</i>	E4668			(6)
<i>E. baconii</i>	As6	ATCC 90167	<i>Agrostis capillaris</i>	(7)
<i>E. baconii</i>	E424	ATCC 200746	<i>Agrostis tenuis</i>	(8)
	E357 =			
<i>E. baconii</i>	E1031	ATCC 200745	<i>Calamagrostis villosa</i>	(2-4, 6, 8)
			<i>Brachyelytrum</i>	
<i>E. brachyelytri</i>	E1040	ATCC 200752	<i>erectum</i>	(2-4)
			<i>Brachyelytrum</i>	
<i>E. brachyelytri</i>	E1045	ATCC 200753	<i>erectum</i>	(3)

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			<i>Brachyelytrum</i>	
<i>E. brachyelytri</i>	E1124	ATCC 201560	<i>erectum</i>	(3)
			<i>Brachyelytrum</i>	
<i>E. brachyelytri</i>	E4804		<i>erectum</i>	(5)
<i>E. bromicola</i>	E501	ATCC 200749	<i>Bromus erectus</i>	(2, 4, 8)
<i>E. bromicola</i>	E502	ATCC 200750	<i>Bromus erectus</i>	(5, 8)
<i>E. bromicola</i>	E799	ATCC 201559	<i>Bromus benekenii</i>	(8)
<i>E. elymi</i>	E56	ATCC 201551	<i>Elymus canadensis</i>	(1-5, 9)
<i>E. elymi</i>	E184	ATCC 200850	<i>Elymus virginicus</i>	(2-4, 9)
				S. Marek, Oklahoma
<i>E. elymi</i>	EC1		<i>Elymus canadensis</i>	State University
				S. Marek, Oklahoma
<i>E. elymi</i>	EC4		<i>Elymus canadensis</i>	State University
				C.A. Young, The Samuel
<i>E. elymi</i>	WWG2		<i>Pascopyrum smithii</i>	Roberts Noble

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<i>E. festucae</i>	E189	ATCC 90661	<i>Festuca rubra</i>	(1, 4, 8, 9)
		ATCC MYA-		
<i>E. festucae</i>	E434	434	<i>Festuca gigantea</i>	(2, 8)
		ATCC MYA-		
<i>E. festucae</i>	E1035.33	446	NA	(10)
<i>E. festucae</i>	E2368		NA	(5, 10)
<i>E. festucae</i>	Fg1		<i>Festuca glauca</i>	(9)
		ATCC MYA-		
<i>E. festucae</i>	Fl1	3407	<i>Festuca longifolia</i>	(9, 11)
			<i>Festuca rubra</i> subsp.	
<i>E. festucae</i>	Frc5		<i>commutata</i>	(9)
			<i>Festuca rubra</i> subsp.	
<i>E. festucae</i>	Frc7		<i>commutata</i>	(9)
<i>E. festucae</i>	Fr1		<i>Festuca rubra</i>	(9, 11)

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			<i>Festuca rubra</i> subsp.	
<i>E. festucae</i>	Frr1		<i>rubra</i>	(9)
<i>E. glyceriae</i>	E2772	ATCC 200755	<i>Glyceria striata</i>	(2-4)
	E3601 =			
<i>E. mollis</i>	AL9924		<i>Holcus mollis</i>	(6)
			<i>Brachypodium</i>	
<i>E. sylvatica</i>	E354	ATCC 200748	<i>sylvaticum</i>	(2-4, 8)
			<i>Brachypodium</i>	
<i>E. sylvatica</i>	E503	ATCC 200751	<i>sylvaticum</i>	(2-4, 8)
<i>E. typhina</i>	E8	ATCC 200736	<i>Lolium perenne</i>	(3-5, 8, 9)
			<i>Brachypodium</i>	
<i>E. typhina</i>	E505	ATCC 200739	<i>pinnatum</i>	(2-4, 8)
				C.A. Young, The Samuel
				Roberts Noble
<i>E. typhina</i>	ORE04		<i>Dactylis glomerata</i>	Foundation, Oklahoma

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				C.A. Young, The Samuel Roberts Noble Foundation, Oklahoma
<i>E. typhina</i>	ORE06		<i>Dactylis glomerata</i>	
<i>E. typhina</i> subsp. <i>clarkii</i>	Holcus 3	ATCC 90168	<i>Holcus lanatus</i>	(7)
<i>E. typhina</i> subsp. <i>poae</i>	E1022	ATCC 201668	<i>Poa nemoralis</i>	(4, 8)
<i>E. typhina</i> subsp. <i>poae</i>	E5819		<i>Poa nemoralis</i>	(5)

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**Table S2. *Epichloë* species from endophyte-infected plant material used in this study**

<b>Species</b>	<b>Isolate ID</b>	<b>Plant ID</b>	<b>Host Species</b>	<b>Reference or source</b>
		Collected from field plant in		C.A. Young, The Samuel Roberts Noble Foundation, Oklahoma
<i>E. typhina</i>	OR10	Oregon	<i>Dactylis glomerata</i>	
<i>E. typhina</i> subsp. <i>poae</i> BlaTG-1	NFe671	BRLA-671	<i>Bromus laevipes</i>	(12) C.A. Young, The Samuel Roberts Noble Foundation, Oklahoma
<i>E. amarillans</i>	NFe-708	ELCA-708	<i>Elymus canadensis</i>	C.A. Young, The Samuel Roberts Noble Foundation, Oklahoma
<i>E. elymi</i>	NFE728	ELVI-728	<i>Elymus virginicus</i>	Foundation, Oklahoma
<i>E. elymi</i>	NFE741	ELVI-741	<i>Elymus virginicus</i>	C.A. Young, The Samuel

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				Roberts Noble Foundation, Oklahoma C.A. Young, The Samuel Roberts Noble
<i>E. festucae</i> var. <i>lolii</i>	NFe243	LOPE-243	<i>Lolium perenne</i>	Foundation, Oklahoma
<i>E. cabralii</i> BlaTG-2	NFe661	BRLA-661	<i>Bromus laevipes</i>	(12)
<i>E. cabralii</i> BlaTG-2	NFe688	BRLA-688	<i>Bromus laevipes</i>	(12)
<i>E. sp.</i> BlaTG-3 (G1)	NFe79	BRLA-79	<i>Bromus laevipes</i>	(12)
<i>E. sp.</i> BlaTG-3 (G2)	NFe82	BRLA-82	<i>Bromus laevipes</i>	(12)
<i>E. sp.</i> BlaTG-3 (G3)	NFe83	BRLA-83	<i>Bromus laevipes</i>	(12) C.A. Young, The Samuel Roberts Noble
<i>E. canadensis</i>	NFe699	ELCA-699	<i>Elymus canadensis</i>	Foundation, Oklahoma C.A. Young, The Samuel
<i>E. amarillans</i>	NFe715	ELCA-715	<i>Elymus canadensis</i>	Roberts Noble

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NFe1001 =

*E. canadensis* NFe727 ELCA-727 *Elymus canadensis* (13)

*E. canadensis* NFe716 ELCA-716 *Elymus canadensis* (13)

e19 = ATCC

*E. coenophiala* 90664 LOAR-00437 *Lolium arundinaceum* (4, 14)

*E. coenophiala* AR584 LOAR-00405 *Lolium arundinaceum* (15, 16)

LOAR-00245

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*E. coenophiala* E34 Barenbrug *Lolium arundinaceum* Foundation, Oklahoma

*E. coenophiala* NFe45078 LOAR-00190 *Lolium arundinaceum* (17)

*E. sp. FaTG-2 G2* NFe45079 LOAR-00193 *Lolium arundinaceum* (17)

*E. sp. FaTG-2 G3* NFe45115 LOAR-00210 *Lolium arundinaceum* (17)

*E. sp. FaTG-3* NFe1100 LOAR-00488 *Lolium arundinaceum* (6, 16)

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<i>E. sp. FaTG-4</i>	e4305	4305	<i>Lolium sp.</i>	(6, 16)
	e167 = CBS			
<i>E. uncinata</i>	102646	FEPR-00400	<i>Lolium pratense</i>	(6, 18-20)
<i>E. siegelii</i>	e915	955	<i>Lolium pratense</i>	(20, 21)
				C.L. Schardl, University
<i>E. sp.</i>	e4678	4678	<i>Poa alsodes</i>	of Kentucky
				C.L. Schardl, University
<i>E. sp.</i>	e4742	4742	<i>Poa autumnalis</i>	of Kentucky
				C.L. Schardl, University
<i>E. sp.</i>	e4755	4755	<i>Poa autumnalis</i>	of Kentucky
<i>E. sp. PauTG-1</i>	e55		<i>Poa autumnalis</i>	(4)
				C.L. Schardl, University
<i>E. sp.</i>	e4427	4427	<i>Sphenopholis sp.</i>	of Kentucky
				C.L. Schardl, University
<i>E. sp.</i>	e4768	4768	<i>Festuca versuta</i>	of Kentucky

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<i>E. sp.</i>	e4672	4672	<i>Agrostis hyemalis</i>	C.L. Schardl, University of Kentucky
<i>E. sp.</i>	e4676	4676	<i>Agrostis hyemalis</i>	C.L. Schardl, University of Kentucky

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**Table S3. PCR primers used in this study**

<b>Target<sup>1</sup></b>	<b>Primers used</b>	<b>Product size (bp)<sup>2</sup></b>
<i>dfsA</i>	<i>dfsA</i> _F: ACGATGGATTGGTCTTCTC <i>dfsA</i> _R: GCAGACGAGCAACGCAACG	1362
<i>qcrA</i>	<i>qcrA</i> _F: TTCCGAGATTGTGCGAGG <i>qcrA</i> _R: TCACTCTCCTGCTGCTG	639
<i>perA</i> A1-domain	<i>perA</i> -5'_F3: ATGACGAGCTCGGAGCGAGTTG <i>perA</i> -A1_R: AGACTTCCATCTGCACAGTATC	1691
<i>perA</i> T1-domain	<i>perA</i> 1_4: TCGGAAAGGTCGGCTGTAC <i>perA</i> 1_R: TTGCTTCATCCCAGTCAGC	1073
<i>perA</i> C-domain	<i>perA</i> 2_F: ATCCAAGACGCATATCCC <i>perA</i> -C_R: ATCATCTCGGCGGCTTCC	878
<i>perA</i> A2-domain	<i>perA</i> 2_1: ACAGCTTTGCCACTCCAAG <i>perA</i> 2_R: ATCCACGCCTATGTAGCTC	2363
<i>perA</i> M-domain	<i>perA</i> 3_F: GCTTGCTGCGTTTGTAC <i>perA</i> -M_R: TGGGAAATCGGAACAAGG	1298
<i>perA</i> T2-domain	<i>perA</i> -T2_F: TCTTCAGGCATCGCAGGAAC <i>perA</i> -T2_R: TCGGCCACCTCCAGCCTGATG	600
<i>perA</i> R*-domain	<i>perA</i> 3_3: AGGAAGGCATCAGGCTGG <i>perA</i> 3_R: CTAGCCTCCAGATCTTGTG	1376
<i>perA</i> R*-domain	<i>perA</i> -T2_F: TCTTCAGGCATCGCAGGAAC	742
deletion	<i>perA</i> -17bp_R: GTACGGATAACCTCAAC	(E2368)

<i>perA-1</i>	perA1_F: ATGGACGCGGAGCCTTTTG	2905
	perA1_R: TTGCTTCATCCCAGTCAGC	
	<b>Additional sequencing primers:</b>	
	perA1_1: TTTGCAGTCCGGCGAAGC	
	perA1_2: ACGCCTAGAGTTCTGCAT	
	perA1_3: ATACTCACGCACACCAAC	
	perA1_4: TCGGAAAGGTCGGCTGTAC	
<i>perA-2</i>	perA2_F: ATCCAAGACGCATATCCC	2973
	perA2_R: ATCCACGCCTATGTAGCTC	
	<b>Additional sequencing primers:</b>	
	perA2_1: ACAGCTTTGCCACTCCAAG	
	perA2_2: AGATTTGGCAGTGGAACG	
	perA2_3: GTCTCCATCAGCAACATC	
<i>perA-3</i>	perA3_F: GCTTGCTGCGTTTGTAC	3058
	perA3_R: CTAGCCTCCAGATCTTGTG	
	<b>Additional sequencing primers:</b>	
	perA3_1: TATCTCTTCAACATCATCCAG	
	perA3_2: ATCGCAGGAACACTCATCG	
	perA3_3: AGGAAGGCATCAGGCTGG	
	perA3_4: ATTCCGCCAGGATGTAGAG	
<i>perA-3ΔR*</i>	perA3_F: GCTTGCTGCGTTTGTAC	1864
	perA-17bp_R: GTACGGATAACCTCAAC	(E2368)
	<b>Additional sequencing primers:</b>	
	perA3_1: TATCTCTTCAACATCATCCAG	

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	perA3_2: ATCGCAGGAACACTCATCG	
	perA3_3: AGGAAGGCATCAGGCTGG	

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<i>perA-qcrA</i>	perA-mid2_F2: CATCAGGCTGGAGGTGGCCGA	1451
intergenic region	Ef104_R: CTAAGCTTTGGTCCAAGCTGCG	(E2368)

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<sup>1</sup> Abbreviations: A = adenylation, T = thiolation, C = condensation, M = methylation and R\* = reductase\*.

<sup>2</sup> Product fragment sizes are for amplification of *E. festucae* Fl1 genomic DNA unless *E. festucae* E2368 is indicated in brackets.

**Table S4. Details of sequenced *perA* alleles**

<b>Species</b>	<b>Isolate</b>	<b>Allele progenitor<sup>a</sup></b>	<b><i>perA</i> type</b>	<b>Notes<sup>b</sup></b>	<b>Accession #<sup>c</sup></b>
<b>Nonhybrid species</b>					
<i>E. amarillans</i>	E57	<i>E. amarillans</i>	<i>perA</i>		<b>JN640285</b>
<i>E. amarillans</i>	E4668	<i>E. amarillans</i>	<i>perA</i>		
<i>E. baconii</i>	As6	<i>E. baconii</i>	<i>perA</i> -ΔR		KP347847
	E357/E1031	<i>E. baconii</i>	<i>perA</i>		KP347874
	E424	<i>E. baconii</i>	<i>perA</i> -ΔR		KP347851
<i>E. brachyelytri</i>	E4804	<i>E. brachyelytri</i>	<i>perA</i>		<b>JN613323</b>
<i>E. bromicola</i>	AL0434	<i>E. bromicola</i>	<i>perA</i>		KP347845
	AL0426_2	<i>E. bromicola</i>	<i>perA</i> -ΔR	START	KP347846, KP719965
	E501	<i>E. bromicola</i>	<i>perA</i>		KP347854
	E502	<i>E. bromicola</i>	<i>perA</i>		<b>JX441995</b>
	E799	<i>E. bromicola</i>	<i>perA</i> -ΔR	STOP@7051	KP347855
<i>E. elymi</i>	E56	<i>E. elymi</i>	<i>perA</i>		<b>JX402755</b>
<i>E. festucae</i>	E189	<i>E. festucae</i>	<i>perA</i> -ΔR		KP347868
	E2368	<i>E. festucae</i>	<i>perA</i> -ΔR		<b>JN640287</b>
	Fg1	<i>E. festucae</i>	<i>perA</i>	-236@3652	KP347869
	Fl1	<i>E. festucae</i>	<i>perA</i>		<b>AB205145</b>
<i>E. mollis</i>	E3601	<i>E. mollis</i>	<i>perA</i>	+1@1412,	KP347873

				+2@3470, +4@6153, +7@6162	
<i>E. sylvatica</i>	E354	<i>E. sylvatica</i>	<i>perA</i> -ΔR	+1@992, -259@1891, +2@4200	KP347850, KP719966
	E503	<i>E. sylvatica</i>	<i>perA</i> -ΔR	+1@992, -259@1891, +2@4200	KP347852
<i>E. typhina</i>	E8	<i>E. typhina</i>	<i>perA</i>		<b>JX402754</b>
	E505	<i>E. typhina</i>	<i>perA</i> -ΔR	STOP@2116	KP347853, KP719967
	E1022	<i>E. typhina</i>	<i>perA</i> -ΔR		KP347856
	ORE04	<i>E. typhina</i>	<i>perA</i> -ΔR	STOP@7045	KP347848, KP719969
	ORE06	<i>E. typhina</i>	<i>perA</i> -ΔR	STOP@7045	KP347849
	OR10	<i>E. typhina</i>	<i>perA</i> -ΔR	incomplete	KP347870
<i>E. typhina</i> subsp. <i>clarkii</i>	Holcus 3	<i>E. typhina</i> subsp. <i>clarkii</i>	<i>perA</i> -ΔR	STOP@6628	KP347859, KP719968
<i>E. typhina</i> subsp. <i>poae</i>	BlaTG-1	<i>E. typhina</i> subsp. <i>poae</i>	<i>perA</i>	incomplete	KP347875
	E5819	<i>E. typhina</i> subsp. <i>poae</i>	<i>perA</i> -ΔR		<b>JN640289</b> , KP719970
<b>Hybrid species</b>					
<i>E. cabralii</i>	BlaTG-2	<i>E. amarillans</i>	<i>perA</i>	incomplete	KP347876

	BRLA-00661				
		<i>E. typhina</i>	<i>perA</i>	incomplete	KP347877
<i>E. siegelii</i>	e915	<i>E. festucae</i>	<i>perA</i> -ΔR		KP347857, KP719971- KP719973
		<i>E. bromicola</i>	<i>perA</i> -ΔR	STOP@2839	KP347858
<i>E. uncinata</i>	e167	<i>E. bromicola</i>	<i>perA</i>	-1@5044	KP347860
		<i>E. typhina</i>	<i>perA</i>	+1@7972	KP347861
<i>E. sp. FaTG-2</i> G2	NFe45079	LAE	<i>perA</i>	+1@1031, -621@1251, -328@3970	KP347862
		<i>E. festucae</i>	<i>perA</i>		KP347863
<i>E. sp. FaTG-2</i> G3	NFe45115	LAE	<i>perA</i>	+1@1031, -621@1251, -328@3970	KP347864
		<i>E. festucae</i>	<i>perA</i>	STOP@525	KP347865
<i>E. sp. FaTG-3</i>	NFe1100	LAE	<i>perA</i>	+1@1031, -621@1251, -328@3970	KP347866
		<i>E. typhina</i>	<i>perA</i>		KP347867
<i>E. sp.</i>	e4768	<i>E. festucae</i>	<i>perA</i> -ΔR	incomplete	KP347871
		<i>E. typhina</i>	<i>perA</i>	incomplete	KP347872

<sup>a</sup>LAE = *Lolium*-associated endophyte



<sup>b</sup>The sign indicates if bases have been deleted (-) or inserted (+), the following number shows the number of bp affected and the number after the "@" symbol shows the coordinates at which, or immediately before which, the insertion or deletion begins, respectively. Small deletions or insertions that do not cause a frameshift mutation are not detailed here. The sizes of large deletions are given relative to the *perA* sequence from *E. festucae* F11. STOP indicates a nonsense mutation, with the number following "@" detailing the coordinate of the causative SNP. START indicates the ATG start codon has been disrupted, though a nearby ATG codon may fill this role.

<sup>c</sup>Accession numbers shown in bold are from previous publications (5, 22).

1. **Schardl CL, Leuchtman A, Tsai HF, Collett MA, Watt DM, Scott DB.** 1994. Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen, *Epichloë typhina*. *Genetics* **136**:1307-1317.
2. **Schardl CL, Leuchtman A, Chung KR, Penny D, Siegel MR.** 1997. Coevolution by common descent of fungal symbionts (*Epichloë* spp) and grass hosts. *Mol. Biol. Evol.* **14**:133-143.
3. **Schardl CL, Leuchtman A.** 1999. Three new species of *Epichloë* symbiotic with North American grasses. *Mycologia* **91**:95-107.
4. **Moon CD, Craven KD, Leuchtman A, Clement SL, Schardl CL.** 2004. Prevalence of interspecific hybrids amongst asexual fungal endophytes of grasses. *Mol. Ecol.* **13**:1455-1467.
5. **Schardl CL, Young CA, Hesse U, Amyotte SG, Andreeva K, Calie PJ, Fleetwood DJ, Haws DC, Moore N, Oeser B, Panaccione DG, Schweri KK, Voisey CR, Farman ML, Jaromczyk JW, Roe BA, O'Sullivan DM, Scott B, Tudzynski P, An Z, Arnaoudova EG, Bullock CT, Charlton ND, Chen L, Cox M, Dinkins RD, Florea S, Glenn AE, Gordon A, Gueldener U, Harris DR, Hollin W, Jaromczyk J, Johnson RD, Khan AK, Leistner E, Leuchtman A, Li C, Liu J, Liu J, Liu M, Mace W, Machado C, Nagabhyru P, Pan J, Schmid J, Sugawara K, Steiner U, Takach JE, Tanaka E, Webb JS, Wilson EV, Wiseman JL, Yoshida R, Zeng Z.** 2013. Plant-symbiotic fungi as chemical engineers: Multi-genome analysis of the Clavicipitaceae reveals dynamics of alkaloid loci. *PLoS Genet.* **9**.
6. **Schardl CL, Young CA, Pan J, Florea S, Takach JE, Panaccione DG, Farman ML, Webb JS, Jaromczyk J, Charlton ND, Nagabhyru P, Chen L, Shi C, Leuchtman A.** 2013. Currencies of mutualisms: Sources of alkaloid genes in vertically transmitted epichloae. *Toxins* **5**:1064-1088.
7. **White JF.** 1993. Endophyte-host associations in grasses .19. A systematic study of some sympatric species of epichloë in England. *Mycologia* **85**:444-455.
8. **Leuchtman A, Schardl CL.** 1998. Mating compatibility and phylogenetic relationships among two new species of *Epichloë* and other congeneric European species. *Mycol. Res.* **102**:1169-1182.
9. **Moon CD, Tapper BA, Scott B.** 1999. Identification of *Epichloë* endophytes *in planta* by a microsatellite-based PCR fingerprinting assay with automated analysis. *Appl. Environ. Microbiol.* **65**:1268-1279.
10. **Wilkinson HH, Siegel MR, Blankenship JD, Mallory AC, Bush LP, Schardl CL.** 2000. Contribution of fungal loline alkaloids to protection

from aphids in a grass-endophyte mutualism. *Mol. Plant-Microbe Interact.* **13**:1027-1033.

11. **Leuchtmann A.** 1994. Isozyme relationships of *Acremonium* endophytes from 12 *Festuca* species. *Mycol. Res.* **98**:25-33.
12. **Charlton ND, Craven KD, Afkhami ME, Hall BA, Ghimire SR, Young CA.** 2014. Interspecific hybridization and bioactive alkaloid variation increases diversity in endophytic *Epichloë* species of *Bromus laevipes*. *FEMS Microbiol. Ecol.*:doi: 10.1111/1574-6941.12393.
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