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Karyotype analysis of a natural *Lycoris* double-flowered hybrid

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Abstract. A putative natural double-flowered hybrid in *Lycoris* Herb. was found on Mt. Zhangjiajie in Hunan, China. The putative natural hybrid had a chromosome number of $2n = 18$ and was karyotypically formulated as $2n = 4m + 6st + 5t + 3T$. The karyotype of the putative natural hybrid was classified as 2B type according to the degree of asymmetry and Stebbins' criteria. According to the gross morphology, phenology and karyotype of the putative natural hybrid, it was suggested that this taxon was probably from the natural hybridization between *L. aurea* and *L. radiata*.

Keywords. Karyotype, *Lycoris* Herb., natural hybrid.

INTRODUCTION

The genus *Lycoris* Herb., a small group of the family Amaryllidaceae, comprises approximately 20 species, distributed in warm temperate and subtropical zones of East Asia, with a few extending to northern Indochina and Nepal (Liu and Hsu 1989; Hsu et al. 1994; Shi et al. 2006). The *Lycoris* species are very popular bulb flowers characterized by their plentiful colors and beautiful and varied shapes (Hsu et al. 1994; Zhou et al. 2007). The hybridization frequently happens in the genus *Lycoris* and causes a number of the presently established hybrid taxa, such as *L. houdyshelii* Traub, *L. straminea* Lindl., *L. squamigera* Maxim., *L. incarnata* Comes ex C. Sprenger and *L. × hubeiensis* K. Liu (Kurita 1987; Hsu et al. 1994; Shi et al. 2006; Meng et al. 2018). During our field investigations of the wild populations of *Lycoris* in Hunan Province, China, a mixed population of three taxa, namely *L. aurea* (L'Her.) Herb., *L. radiata* (L'Her.) Herb., and a putative natural hybrid was found. The putative natural double-flowered hybrids attracted our more attention, which were discovered for the first time in *Lycoris* in the wild. To illustrate the origin of the natural double-flowered hybrids, some bulbs were successfully transplanted and cultivated in the experimental garden, together

with their putative parents. The karyotypes of the three *Lycoris* taxa were analyzed in this study, indicating that the double-flowered taxon might be from the hybridization between *L. aurea* and *L. radiata*.

MATERIALS AND METHODS

The plants were collected from Mt. Zhangjiajie, Hunan, China (110°17'E, 29°19'N), and then transplanted to the experimental garden of Anhui Normal University, Wuhu, China. The flowers of each taxon were shown in Fig. 1. For chromosome observation, actively growing root tips were pretreated in p-Dichlorobenzene solution at 4 °C for 5 h before they were fixed in Carnoy I (glacial acetic acid : absolute ethanol = 1:3) at 4 °C for 20 h. Then they were macerated in 1 mol L⁻¹ hydrochloric acid at 60 °C for 3 minutes, stained in Phenol-Fuchsin for 20 h, and squashed in 45% acetic acid.

The karyotype formula was based on the measurements of metaphase chromosomes taken from photographs. For each taxon, measurements were taken from at least five well-spread metaphase cells in no fewer than three different individuals. For the description of karyotypes, the symbols had been adapted according to Levan et al. (1964): m for median-centromeric chromosome with arm ratio of 1.01-1.70; st for subterminal-centromeric chromosome with arm ratio of 3.01-7.00; t for terminal-centromeric chromosome with arm ratio of over 7.00; T for terminal-centromeric chromosome with no short arm. Karyotypes were classified on the basis of their degrees of asymmetry according to Stebbins (1971) and Li and Chen (1985). The intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2) were calculated using Romero Zarco' equations (1986).

RESULTS

1. The putative natural *Lycoris* double-flowered hybrid (Table 1, 2; Figure 2A, 2D) – The chromosomes were counted to be $2n = 18$, consisting of 4 large median-centromeric (m), 6 subterminal-centromeric (st), 5 terminal-centromeric (t) and 3 Terminal-centromeric (T). The karyotype was formulated as $2n = 4m + 6st + 5t + 3T$. The average length of chromosome complement was 122.3 μm . The ratio of the length of the largest chromosome to that of the smallest was 3.39, and the proportion of chromosomes with arm ratio $>2:1$ was 77.8%. The karyotype was therefore of 2B type according to the degree of asymmetry and the chromosomes ranged from

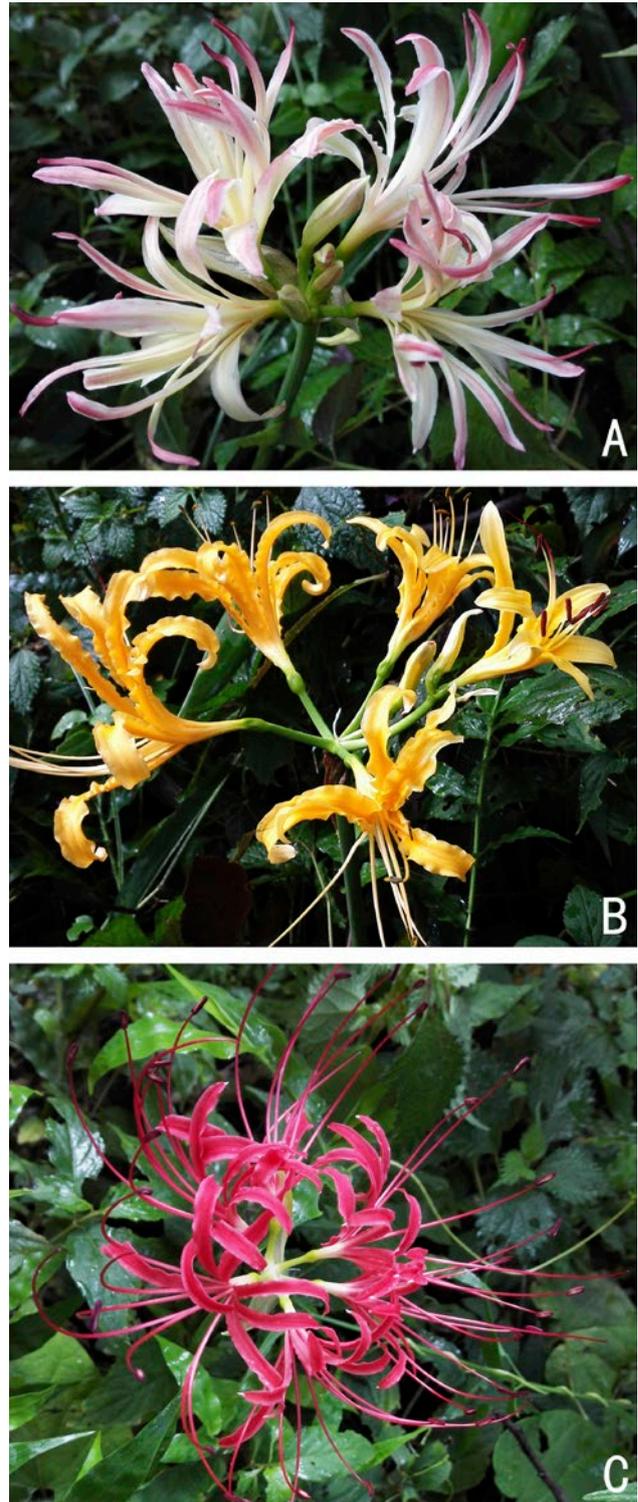


Fig 1. The flowers of the three taxa. A: the putative natural hybrid; B: *L. aurea*; C: *L. radiata*.

Table 1. Karyotype characteristics of the studied *Lycoris* taxa.

Species	Karyotypic formula	Stebbins' type	A ₁	A ₂	TCL (μm)
Hybrid	2n = 4m + 6st + 5t + 3T	2B	0.72	0.50	122.3
<i>L. aurea</i>	2n = 8m + 6T	2B	0.47	0.44	138.6
<i>L. radiata</i>	2n = 12st + 10t	4A	0.86	0.10	111.2

A₁: intrachromosomal asymmetry index, A₂: interchromosomal asymmetry index, TCL: average length of total chromosome complement.

Table 2. Measurements of somatic chromosomes of the putative natural hybrid.

No.	Relative length			Arm ratio	Type
	LL	SL	TL		
1	5.71	5.67	11.38	1.01	m
2	5.81	5.38	11.19	1.08	m
3	6.11	4.65	10.76	1.31	m
4	4.39	4.09	8.48	1.07	m
5	4.63	0.37	5.00	12.51	t
6	4.63	0.33	4.96	14.03	t
7	3.97	0.65	4.62	6.11	st
8	4.53	0.00	4.53	∞	T
9	4.49	0.00	4.49	∞	T
10	3.90	0.46	4.36	8.48	t
11	4.34	0.00	4.34	∞	T
12	3.47	0.54	4.01	6.43	st
13	3.61	0.31	3.92	11.65	t
14	3.39	0.49	3.88	6.92	st
15	3.22	0.46	3.68	7.00	st
16	2.99	0.60	3.59	4.98	st
17	2.99	0.46	3.45	6.50	st
18	3.03	0.33	3.36	9.18	t

Note: LL, relative length of long arm; SL, relative length of short arm; TL, total relative length; LL + SL = TL. The same is below.

3.36~11.38 in relative length. The values of A₁ and A₂ were 0.72 and 0.50, respectively.

2. *Lycoris aurea* (Table 1, 3; Figure 2B, 2E) – The chromosome number was 2n = 14, consisting of 8 large median-centromeric (m) and 6 Terminal-centromeric (T). The karyotype formula was 2n = 8m + 6T. The average length of chromosome complement was 138.6 μm. The ratio of the length of the largest chromosome to that of the smallest was 3.32, and the proportion of chromosomes with arm ratio >2:1 was 42.9%. The karyotype type was 2B. The relative lengths of all chromosomes ranged from 3.49 to 11.59. The values of A₁ and A₂ were 0.47 and 0.44, respectively.

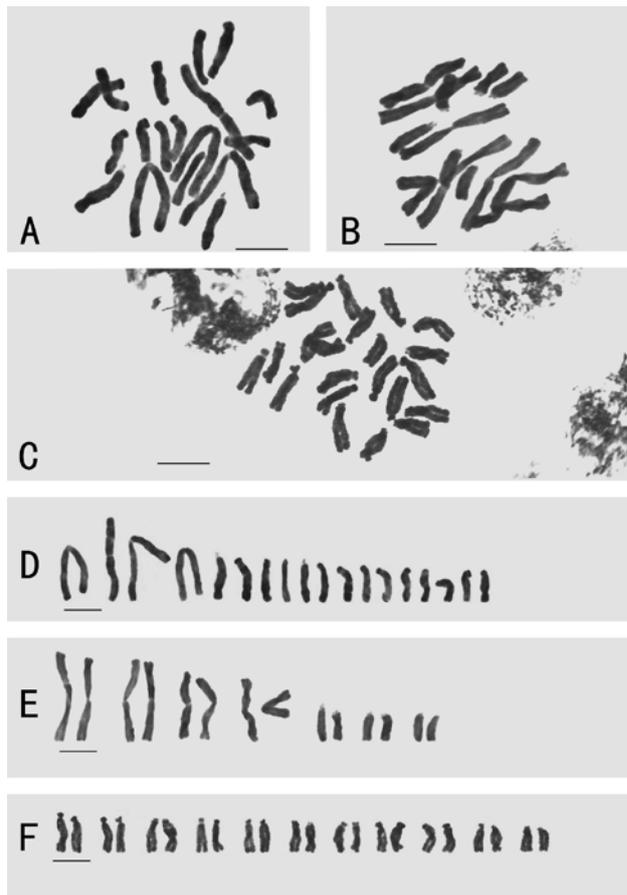


Fig 2. The metaphase chromosome morphology and karyotypes of the putative natural hybrid, *Lycoris aurea* and *L. radiata*. A, D: the putative natural hybrid; B, E: *L. aurea*; C, F: *L. radiata*. Scale bar = 10 μm.

Table 3. Measurements of somatic chromosomes of *L. aurea*.

No.	Relative length			Arm ratio	Type
	LL	SL	TL		
1	5.81	5.76	11.57	1.01	m
2	5.56	5.38	10.94	1.03	m
3	5.52	5.34	10.86	1.03	m
4	5.47	4.93	10.40	1.11	m
5	4.75	4.13	8.88	1.15	m
6	4.39	4.26	8.65	1.03	m
7	4.13	3.90	8.03	1.06	m
8	3.99	3.41	7.40	1.17	m
9	4.93	0.00	4.93	∞	T
10	3.81	0.00	3.81	∞	T
11	3.77	0.00	3.77	∞	T
12	3.68	0.00	3.68	∞	T
13	3.59	0.00	3.59	∞	T
14	3.49	0.00	3.49	∞	T

Table 4. Measurements of somatic chromosomes of *L. radiata*.

No.	Relative length			Arm ratio	Type
	LL	SL	TL		
1	4.60	0.75	5.35	6.13	st
2	4.43	0.67	5.10	6.61	st
3	4.38	0.70	5.08	6.26	st
4	4.25	0.70	4.95	6.07	st
5	4.63	0.27	4.90	17.15	t
6	4.23	0.62	4.85	6.82	st
7	4.25	0.57	4.82	7.46	t
8	4.05	0.67	4.72	6.05	st
9	4.18	0.52	4.70	8.04	t
10	3.81	0.85	4.66	4.48	st
11	4.08	0.55	4.63	7.42	t
12	4.01	0.60	4.61	6.68	st
13	4.08	0.51	4.59	8.00	t
14	3.91	0.67	4.58	5.84	st
15	4.11	0.38	4.49	10.81	t
16	3.73	0.55	4.28	6.78	st
17	3.81	0.45	4.26	8.47	t
18	3.86	0.30	4.16	12.87	t
19	3.38	0.55	3.93	6.15	st
20	3.41	0.52	3.93	6.56	st
21	3.38	0.47	3.85	7.19	t
22	3.21	0.35	3.56	9.17	t

3. *Lycoris radiata* (Table 1, 4; Figure 2C, 2F) – The chromosome number of this species was $2n = 22$. It consisted of 12 subterminal-centromeric (st) and 10 terminal-centromeric (t). The karyotype was formulated as $2n = 12st + 10t$. The average length of chromosome complement was 111.2 μm . The ratio of the length of the largest chromosome to that of the smallest was 1.43, and the proportion of chromosomes with arm ratio $>2:1$ was 100%. The karyotype type was 4A. The relative lengths of all chromosomes ranged from 3.56 to 5.10. The values of A_1 and A_2 were 0.86 and 0.10, respectively.

DISCUSSION

The karyotype of the putative natural double-flowered hybrid is formulated as $2n = 18 = 4m + 6st + 5t + 3T$. *Lycoris aurea* in this study has a chromosome number of $2n = 14$, in agreement with other reports (Liu and Hsu 1989; Hsu et al. 1994). *L. radiata* has a chromosome number of $2n = 22$, similar to some previous studies (Shao et al. 1994; Zhou et al. 2007; Liu et al. 2016). Based on the values of the two indices, A_1 and A_2 , *L. radiata* had the largest intrachromosomal asymmetry

and the smallest interchromosomal asymmetry among the three taxa. Considering the sympatric distribution of *L. aurea* and *L. radiata*, it was supposed that this putative natural hybrid might be a diploid between *L. aurea*, which produced the gamete having $4m + 3T$, and *L. radiata* with a reduced gamete having $6st + 5t$. According to our observation, the leaf of this putative natural hybrid emerged in September, the same as the putative parents. The sizes of leaf blade and bulb of this putative natural hybrid were intermediate between its two putative parents. So far, some *Lycoris* species were confirmed to be hybrids by karyotype or molecular sequence analysis, such as *L. houdyshelii*, *L. straminea*, and *L. incarnata* (Hsu et al. 1994; Shi et al. 2006; Liu et al. 2011), and these hybrids had the similar flower characteristics with their putative parents. Here, a putative natural double-flowered hybrid with no pistil and stamen was discovered and its karyotype was described for the first time. Based on the karyotype analysis, further molecular study was needed to uncover the origin of this natural hybrid and distinguish the paternal donor from the maternal donor.

Judging from the absence of seed, pistil and stamen, the putative natural hybrid could be sexually sterile. The *Lycoris* species all had considerable ornamental value. Compared with the normal *Lycoris* species, the double-flowered hybrid had more ornamental value. Because of the lack of seed, it was needed to propagate the double-flowered hybrid for the large application in landscaping in future by tissue culture and quick propagation technology.

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