Article

Cytogeography of the East Asian Tulips (Amana, Liliaceae)

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Abstract: Amana Honda (Liliaceae), known as ‘east Asian tulips’, is a spring ephemeral genus endemic to Sino-Japanese Floristic Subregion, mainly distributed in eastern and central China, Japan and the Korean peninsula. Chromosome numbers are reported here for the first time from 89 populations of Amana (representing all seven accepted species, two new species about to be published, and two suspected new species). Three ploidy levels are found in this genus. These are diploid (2n = 2x = 24) and tetraploid (2n = 4x = 48) in the widespread A. edulis, while all the narrow endemics are diploid, except for one population of A. tianmuensis, which is triploid (2n = 3x = 36). The northernmost and southernmost populations of A. edulis are diploid and tetraploid, respectively, while diploids and tetraploids coexist in between, with gradual transition to diploids as the latitude increases. This may indicate polyploids have an advantage in tolerance of environmental stress and are more adaptable to high-temperature environment in subtropical regions than diploids. The species and cytotype distributions in Amana are complex, and these results provide hypotheses about the origins of the genus and the polyploid cytotypes.

Keywords: chromosome; cytotype; ploidy; polyploid; spring ephemeral; widespread

1. Introduction

Liliaceae belong to Liliales of the monocotyledons (APG IV, 2016 [1]) and consist of 15 genera with more than 600 species (Lu et al., 2021 [2]). The family is organized into six tribes, and Amana Honda, Erythronium L., Gagea Salisb. and Tulipa L. are included in tribe Tulipeae (Peruzzi, 2016 [3]).

Among these, Amana, commonly known as ‘east Asian tulips’, is a genus endemic to Sino-Japanese Floristic Subregion, mainly distributed in eastern and central China, Japan and the Korean peninsula (Honda et al., 1935 [4]; Tan et al., 2005 [5]; Li et al., 2017 [6]). All the species are spring ephemerals, which occur in temperate deciduous or subtropical mixed forests. Spring ephemerals refer to the perennial plants that appear quickly in the early spring and die after a short period of growth and reproduction (Struik, 1965 [7]). It is a common survival strategy in understory herbs in temperate deciduous forests. Before woody plants form a canopy, spring ephemerals effectively use strong sunlight that reaches the forest understory to complete their life cycle. Then, in the summer, when the canopy begins to close, the aboveground parts of spring ephemerals die, and the underground parts enter a dormant period (Mckenna and Houle, 2000 [8]). Therefore, spring ephemerals are closely linked with temperate deciduous forests and are usually found in temperate regions. However, the genus Amana spreads to subtropical regions (such as Zhejiang, southern Anhui and northern Jiangxi, China), unlike classic spring ephemerals, such as

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tulips, trout lilies, crocuses, daffodils, and hyacinths. The mechanism of such a spring ephemeral genus adapting to the subtropical climate is intriguing.

Currently, seven species are recognized in the genus *Amana*, namely *A. anhuiensis* (X.S. Shen) D.Y. Tan and D.Y. Hong, *A. baohuaensis* B.X. Han, Long Wang and G.Y. Lu, *A. edulis* (Miq.) Honda, *A. erythronioideis* (Baker) D.Y. Tan and D.Y. Hong, *A. kuo-cangshanica* D.Y. Tan and D.Y. Hong, *A. latifolia* (Makino) Honda, and *A. wanzhensis* L.Q. Huang, B.X. Han and K. Zhang (Ohwi and Kitagawa, 1992 [9]; Chen and Mordak, 2000 [10]; Shen, 2001 [11]; Tan et al., 2007 [12]; Han et al., 2014 [13]; Wang et al., 2019 [14]). Within the genus, *A. edulis* is the most common and widespread species, covering the entire distribution range of the genus, while the other six species are narrow endemics. Although the range of the narrow endemics overlaps with that of *A. edulis*, their habitats often differ in altitude. Therefore, admixed populations of two or more species in the same place are not common (Li et al., 2017 [6]). With extensive fieldwork since 2014, we found that there may be four more cryptic species in the genus. *Amana sp.1* occurs in the northeastern part of Zhejiang Province; *Amana sp.2* is mainly distributed along the Dabie Mountains in Henan and Hubei provinces; *A. nanyuensis* (Wang et al., 2022 [15]) is only found in Mt. Heng in Hunan Province; and *A. tianmuensis* (Wang et al., 2022 [15]) appears from northern Zhejiang to southern Anhui provinces.

Geographical isolation results in restricted gene flow between populations and promotes genetic differentiation (Yuan, 2013 [16]). Habitat heterogeneity also changes genetic composition, which is reflected in the chromosome morphology and numbers. Therefore, ploidy is an important basis for studying the inheritance and evolution of species. Previous studies have shown that the basic chromosome number of *Amana* is *x* = 12 (Peruzzi, 2009 [17]), and there are two ploidy levels in *A. edulis*: diploid (2*n* = 2*x* = 24 in the northernmost populations, such as Liaoning and Henan provinces, China) and tetraploid (2*n* = 4*x* = 48 in the southernmost population, such as Zhejiang Province, China) (Zhu et al., 2002 [18]; Deng, 2016 [19]). This gives us a hint that polyploidization might be the reason for its adaptation to subtropical climate. However, there are very few karyotypic studies on the other *A. edulis* populations or the other *Amana* species (only *A. latifolia* was reported to be diploid), and thus, a comprehensive analysis is needed.

In this study, we included 94 populations (89 from present study and 5 from previous studies) representing all 11 species (including 2 suspected new species) and performed an investigation of the cytogeography. We aim to find out the geographical distribution pattern of cytotypes in *Amana*, which is helpful for clarifying the phylogenetic relationships within this genus and providing support for subsequent taxonomic revisions. Moreover, it will deepen our understanding on the evolutionary history and polyploidization event of spring ephemerals.

2. Materials and Methods
2.1. Field Observations and Sampling

Extensive field surveys and sample collections were conducted in Anhui, Jiangsu, Jiangxi, Henan, Hubei, Hunan, Shandong, Shanxi, and Zhejiang provinces in China since 2014. We also collected a population of *A. edulis* from the Korean peninsula, which is the only species that grows there. The vertical dropper (about 5 mm) that germinates for asexual reproduction in spring was used as the material for subsequent observation of chromosomes (Figure A1). A total of 89 populations were collected to analyze the geographical distribution pattern of ploidy levels (Table 1). Voucher specimens were deposited at the Herbarium of Zhejiang University (HZU).
Table 1. The populations information and ploidy levels found in *Amana*. A, B, C refer to different collections from the same population.

| Pop. | Species       | Voucher Number | Chromosome Numbers | Latitude | Longitude | Elevation | Locality                                                                 |
|------|---------------|----------------|--------------------|----------|-----------|-----------|---------------------------------------------------------------------------|
| 1    | *A. edulis*   |               | 24                 | 40.1161  | 124.3584 |           | China, Liaoning Province, Dandong City, Zhenxin District (Deng, 2016)     |
| 2    | *A. edulis*   |               | 24                 | 39.0989  | 121.7955 |           | China, Liaoning Province, Dalian City, Mt. Dabei (Deng, 2016)             |
| 3    | *A. edulis*   | LP161230      | 24                 | 37.4546  | 121.5833 | 45        | China, Shandong Province, Yantai City, Yangma Island                     |
| 4    | *A. edulis*   | LP161195      | 24                 | 36.6298  | 117.0413 | 283       | China, Shandong Province, Jinan City, Mt. Fohsi                         |
| 5    | *A. edulis*   | LP161194      | 24                 | 36.2551  | 117.0746 | 749       | China, Shandong Province, Taian City, Mt. Tai                           |
| 6    | *A. edulis*   |               | 48                 | 36.2200  | 127.2400 |           | South Korea, Daejeon                                                    |
| 7    | *A. edulis*   |               | 48                 | 35.9167  | 140.4333 |           | Japan, Tokyo, Koshikawa Botanical Garden (Sato, 1943)                   |
| 8    | *A. edulis*   |               | 48                 | 35.7783  | 139.4968 | 56        | Higashimurayama (Noguchi and Kowano, 1974)                               |
| 9    | *A. edulis*   | LP161180      | 24                 | 35.6164  | 116.9902 | 73        | China, Shandong Province, Qufu County, Cemetery of Confucius             |
| 10A  | *A. edulis*   | LP161174      | 24                 | 35.6017  | 117.1556 | 335       | China, Shandong Province, Sishui County, Mt. Ge                         |
| 10B  | *A. edulis*   | LP161175      | 24                 | 35.6013  | 117.1583 | 240       | China, Shandong Province, Sishui County, Mt. Tai                         |
| 11A  | *A. edulis*   | LP161176      | 24                 | 35.5972  | 117.1950 | 226       | China, Shandong Province, Sishui County, Mt. Matou                       |
| 11B  | *A. edulis*   | LP161177      | 24                 | 35.5969  | 117.1975 | 302       | China, Shandong Province, Sishui County, Mt. Matou                       |
| 11C  | *A. edulis*   | LP161178      | 24                 | 35.5964  | 117.2050 | 330       | China, Shandong Province, Sishui County, Mt. Matou                       |
| 11D  | *A. edulis*   | LP161179      | 24                 | 35.5930  | 117.2016 | 349       | China, Shandong Province, Sishui County, Mt. Matou                       |
| 12   | *A. edulis*   |               | 24                 | 35.3000  | 113.9000 |           | China, Henan Province, Xinxiang City (Deng, 2016)                         |
| 13   | *A. edulis*   | LP161123      | 24                 | 35.3133  | 117.4130 | 198       | China, Shandong Province, Zaozhuang City, Yundengshan Village             |
| 14   | *A. edulis*   | LP173025      | 24                 | 34.9805  | 111.4382 | 910       | China, Shanxi Province, Xia County, Wujiangmiao                           |
| 15   | *A. edulis*   | LP173027      | 48                 | 34.7155  | 119.4267 | 183       | China, Jiangsu Province, Liangyugang City, Mt. Yuntai                    |
| 16A  | *A. edulis*   | LP207907      | 24                 | 34.4333  | 117.1736 | 104       | China, Jiangsu Province, Xuzhou City, Mt. Yulin                           |
| 16B  | *A. edulis*   | LP173071      | 24                 | 34.2433  | 117.1737 | 104       | China, Jiangsu Province, Huai'an City, Mt. Bochi                         |
| 17   | *A. edulis*   | LP173072      | 24                 | 33.9899  | 119.0568 | 7         | China, Henan Province, Wugang County, Dengaota                           |
| 18   | *A. edulis*   | LP173055      | 48                 | 33.1717  | 113.0545 | 295       | China, Henan Province, Nanyang City, Mt. Du                              |
| 19A  | *A. edulis*   | LP173057      | 48                 | 33.0600  | 112.0579 | 354       | China, Henan Province, Nanyang City, Mt. Du                              |
| 19B  | *A. edulis*   | LP207916      | 48                 | 33.0600  | 112.0579 | 354       | China, Jiangsu Province, Nanjing City, Mt. Guizi                          |
| 20   | *A. edulis*   | LP196198      | 48                 | 32.4635  | 118.9308 | 15        | China, Henan Province, Tongbai County, Shiziling                           |
| 21   | *A. edulis*   | WMZ1490       | 48                 | 32.3954  | 113.2989 | 249       | China, Henan Province, Tongbai County, Taiyang Temple                    |
| 22   | *A. edulis*   | WMZ1493       | 48                 | 32.3067  | 113.4549 | 161       | China, Henan Province, Tongbai County, Huangjialaozhuang                 |
| 23   | *A. edulis*   | WMZ1422       | 48                 | 32.2956  | 118.2864 | 87        | China, Anhui Province, Chuzhou City, Mt. Angya                           |
| 24   | *A. edulis*   | WMZ1416       | 48                 | 32.1251  | 119.0896 | 129       | China, Jiangsu Province, Jurong County, Mt. Baohua                       |
| 25   | *A. edulis*   | WMZ1418       | 48                 | 32.1008  | 118.5875 | 102       | China, Jiangsu Province, Nanjing City, Mt. Lao                           |
| 26   | *A. edulis*   | WMZ1420       | 48                 | 32.0625  | 118.5233 | 87        | China, Jiangsu Province, Nanjing City, Mt. Lao                           |
| 27   | *A. edulis*   | WMZ1408       | 24                 | 31.8154  | 119.3089 | 269       | China, Jiangsu Province, Jurong County, Mt. Mao                          |
| 28   | *A. edulis*   | WMZ1488       | 24                 | 31.7980  | 114.0873 | 394       | China, Hubei Province, Guangshui County, Heilongtan                      |
| 29   | *A. edulis*   | WMZ1482       | 24                 | 31.7153  | 115.5012 | 379       | China, Henan Province, Shangnan County, Lishuocheng Village              |
| 30   | *A. edulis*   | WMZ1424       | 24                 | 31.6710  | 118.0846 | 41        | China, Anhui Province, Hanshan County, Mt. Baohan                       |
| 31   | *A. edulis*   | WMZ1486       | 24                 | 31.5727  | 114.6192 | 660       | China, Hubei Province, Hong'an County, Mt. Tianzai                       |
### Table 1. Cont.

| Pop. | Species       | Voucher Number | Chromosome Numbers | Latitude | Longitude | Elevation | Locality                                      |
|------|---------------|----------------|--------------------|----------|-----------|-----------|-----------------------------------------------|
| 32   | *A. edulis*   | WMZ1485        | 48                 | 31.5719  | 114.6154  | 590       | China, Hubei Province, Hong'an County, Mt. Tianlai |
| 33   | *A. edulis*   | WMZ1426        | 24                 | 31.4650  | 117.7859  | 132       | China, Anhui Province, Wuzhou County, Loulialong |
| 34A  | *A. edulis*   | LP207905       | 48                 | 31.2615  | 119.7530  | 82        | China, Jiangsu Province, Xingyang County, Haoshan Village |
| 34B  | *A. edulis*   | WMZ1404        | 48                 | 31.2614  | 119.7528  | 88        | China, Jiangsu Province, Xingyang County, Haoshan Village |
| 35   | *A. edulis*   | LP172908       | 24                 | 31.0643  | 119.2967  | 322       | China, Jiangsu Province, Lyang County,          |
| 36   | *A. edulis*   | WMZ1477        | 24                 | 30.4191  | 117.2035  | 90        | China, Anhui Province, Chizhou City,           |
| 37   | *A. edulis*   | LJK54          | 48                 | 30.3866  | 118.2291  | 473       | China, Anhui Province, Huangshan County,        |
| 38   | *A. edulis*   | WMZ1471        | 48                 | 30.3846  | 118.2460  | 614       | China, Anhui Province, Huangshan County,        |
| 39   | *A. edulis*   | WMZ1494        | 48                 | 30.1979  | 115.1092  | 158       | China, Hu Bei Province, Hu Bei City,             |
| 40   | *A. edulis*   | WMZ1496        | 24                 | 29.9485  | 114.7491  | 85        | China, Zhejiang Province, Yuyao County,         |
| 41A  | *A. edulis*   | LP150069       | 48                 | 29.7446  | 121.0833  | 892       | China, Zhejiang Province, Yuyao County, Mt. Yuyao |
| 41B  | *A. edulis*   | WMZ1432        | 48                 | 29.7426  | 121.0842  | 880       | China, Zhejiang Province, Yuyao County, Mt. Yuyao |
| 41C  | *A. edulis*   | WMZ1430        | 48                 | 29.7398  | 121.0869  | 861       | China, Zhejiang Province, Yuyao County, Mt. Yuyao |
| 42A  | *A. edulis*   | WMZ1434        | 48                 | 29.6488  | 121.5570  | 571       | China, Zhejiang Province, Yuyao County, Mt. Yuyao |
| 42B  | *A. edulis*   | LP184953       | 48                 | 29.6458  | 121.5575  | 564       | China, Zhejiang Province, Yuyao County,           |
| 42C  | *A. edulis*   | WMZ1436        | 48                 | 29.6386  | 121.5596  | 458       | China, Zhejiang Province, Yuyao County, Mt. Yuyao |
| 43A  | *A. edulis*   | WMZ1456        | 48                 | 29.5147  | 120.2411  | 564       | China, Zhejiang Province, Ningbo City, Mt. Yuyao |
| 43B  | *A. edulis*   | LP184930       | 48                 | 29.5138  | 120.2438  | 447       | China, Zhejiang Province, Ningbo City, Mt. Yuyao |
| 44   | *A. edulis*   | WMZ1459        | 48                 | 29.4565  | 120.2906  | 234       | China, Zhejiang Province, Zhejiang County,       |
| 45   | *A. edulis*   | LP184939       | 48                 | 29.3811  | 121.6190  | 263       | China, Zhejiang Province, Hangzhou City,         |
| 46A  | *A. edulis*   | LP184936       | 48                 | 29.3773  | 121.5875  | 628       | China, Zhejiang Province, Hangzhou City, Mt. Hangzhou |
| 46B  | *A. edulis*   | WMZ1440        | 48                 | 29.3772  | 121.5875  | 671       | China, Zhejiang Province, Hangzhou City, Mt. Hangzhou |
| 47   | *A. edulis*   | WMZ1438        | 48                 | 29.3428  | 121.7588  | 268       | China, Zhejiang Province, Xiangyang County,      |
| 48   | *A. edulis*   | WMZ1453        | 24                 | 29.2085  | 119.6260  | 558       | China, Zhejiang Province, Xiangyang County,      |
| 49   | *A. edulis*   | LP184860       | 48                 | 29.0454  | 120.2871  | 331       | China, Zhejiang Province, Yuanlinchang            |
| 50   | *A. edulis*   | LP161162       | 48                 | 28.8559  | 121.1082  | 561       | China, Zhejiang Province, Xiangyang County,      |
| 51A  | *A. nanquensis* | WMZ1443    | 24                 | 27.2881  | 112.6932  | 1067      | China, Hubei Province, Hengyang City, Mt. Heng    |
| 51B  | *A. nanquensis* | LP196219   | 24                 | 27.2881  | 112.6932  | 1055      | China, Hubei Province, Hengyang City, Mt. Heng    |
| 52   | *A. nanquensis* | WMZ1464    | 24                 | 27.2767  | 112.6746  | 1063      | China, Anhui Province, Qingyang County, Mt. Tianmu |
| 53   | *A. tianmuensis* | WMZ1473  | 24                 | 30.4728  | 117.8345  | 736        | China, Anhui Province, Hangzhou City, Yuxiang    |
| 54A  | *A. tianmuensis* | WMZ1470   | 24                 | 30.3884  | 118.2182  | 629        | China, Anhui Province, Hangzhou City, Yuxiang    |
| 54B  | *A. tianmuensis* | LJK51      | 24                 | 30.3878  | 118.2169  | 692        | China, Anhui Province, Hangzhou City, Yuxiang    |
| 54C  | *A. tianmuensis* | LP173012  | 24                 | 30.3878  | 118.2169  | 692        | China, Anhui Province, Hangzhou City, Yuxiang    |
| 55A  | *A. tianmuensis* | WMZ1504   | 24                 | 30.3497  | 119.4262  | 1437       | China, Zhejiang Province, Hangzhou County, Mt. Tianmu |
| 55B  | *A. tianmuensis* | LJK42     | 24                 | 30.3425  | 119.4332  | 1104       | China, Zhejiang Province, Hangzhou County, Mt. Tianmu |
| 55C  | *A. tianmuensis* | WMZ1465  | 24                 | 30.3423  | 119.4333  | 1108       | China, Zhejiang Province, Hangzhou County, Mt. Tianmu |
| 56A  | *A. tianmuensis* | LJK45     | 24                 | 30.3085  | 119.1215  | 1120       | China, Zhejiang Province, Hangzhou City, Zheutianchi |
| 56B  | *A. tianmuensis* | WMZ1505  | 24                 | 30.3082  | 119.1214  | 1118       | China, Zhejiang Province, Hangzhou City, Zheutianchi |
| 57   | *A. tianmuensis* | WMZ1472  | 24                 | 30.2008  | 118.1846  | 629        | China, Anhui Province, Huangshan City, Yanzhuchang |
| Pop. | Species          | Voucher Number | Chromosome Numbers | Latitude | Longitude | Elevation | Locality                                      |
|------|------------------|----------------|-------------------|----------|-----------|-----------|-----------------------------------------------|
| 58   | A. tianmuensis   | WMZ1506        | 24                | 30.1426  | 118.1704  | 1620      | China, Anhui Province, Huangshan City, Mt. Huang |
| 59A  | A. tianmuensis   | LJK48          | 24                | 30.1097  | 118.9013  | 902       | China, Zhejiang Province, Hangzhou City, Qiandongfeng Botanical Garden |
| 59B  | A. tianmuensis   | WMZ1502        | 36                | 30.1097  | 118.9014  | 872       | China, Zhejiang Province, Hangzhou City, Qiandongfeng Botanical Garden |
| 60A  | A. tianmuensis   | LJK19          | 24                | 29.2085  | 119.6263  | 577       | China, Zhejiang Province, Jinhu City, Shuanglong Scenic Area |
| 60B  | A. tianmuensis   | LP207913       | 24                | 29.2085  | 119.6263  | 577       | China, Zhejiang Province, Jinhu City, Shuanglong Scenic Area |
| 60C  | A. tianmuensis   | LP173009       | 24                | 29.2085  | 119.6263  | 577       | China, Zhejiang Province, Jinhu City, Shuanglong Scenic Area |
| 59   | A. erythronioides| WMZ1429        | 24                | 29.7398  | 121.0869  | 577       | China, Zhejiang Province, Yuyao County, Mt. Jin |
| 59A  | A. erythronioides| LP184934       | 24                | 29.3735  | 121.5855  | 492       | China, Zhejiang Province, Ninghai County, Mt. Cha |
| 59B  | A. erythronioides| WMZ1439        | 24                | 29.3735  | 121.5854  | 509       | China, Zhejiang Province, Ninghai County, Mt. Cha |
| 65   | A. kuocangshanica| WMZ1414        | 24                | 29.3752  | 121.5909  | 460       | China, Zhejiang Province, Linhai County, Mt. Kuocang |
| 66   | A. kuocangshanica| WMZ1443        | 24                | 28.8150  | 120.9432  | 868       | China, Zhejiang Province, Wenzhou City, Jinbao Village |
| 67   | A. kuocangshanica| WMZ1448        | 24                | 28.5512  | 120.7998  | 865       | China, Zhejiang Province, Wenzhou City, Jinbao Village |
| 68A  | A. latifolia     | WMZ1445        | 24                | 27.9379  | 120.5080  | 401       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 68B  | A. latifolia     | LP173010       | 24                | 27.9423  | 120.5061  | 400       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 69   | A. latifolia     | WMZ1444        | 24                | 27.9079  | 120.6970  | 324       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 70   | A. latifolia     | LP172995       | 24                | 27.8950  | 120.7445  | 364       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 71   | A. latifolia     | WMZ1446        | 24                | 27.8273  | 120.3295  | 336       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 72A  | Amana sp. I      | LJK10          | 24                | 29.5070  | 120.4370  | 438       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 72B  | Amana sp. I      | WMZ1461        | 24                | 29.5068  | 120.4370  | 430       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 73A  | Amana sp. I      | LP184959       | 24                | 29.4501  | 120.2861  | 432       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 73B  | Amana sp. I      | WMZ1458        | 24                | 29.4500  | 120.2860  | 413       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 74A  | Amana sp. I      | LP150073       | 24                | 29.3517  | 120.0251  | 588       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 74B  | Amana sp. I      | WMZ1451        | 24                | 29.3514  | 120.0251  | 629       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 74C  | Amana sp. I      | LP161171       | 24                | 29.3513  | 120.0251  | 636       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 75A  | Amana sp. I      | WMZ1450        | 24                | 29.2525  | 120.0977  | 990       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 75B  | Amana sp. I      | LP161169       | 24                | 29.2523  | 120.0959  | 962       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 76   | Amana sp. I      | WMZ1449        | 24                | 28.9799  | 120.5393  | 747       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 77   | A. wanzhensis    | WMZ1427        | 24                | 31.0871  | 119.3349  | 307       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 78A  | A. wanzhensis    | LJK44          | 24                | 30.3487  | 119.2298  | 720       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 78B  | A. wanzhensis    | WMZ1466        | 24                | 30.3480  | 119.2298  | 720       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 79   | A. wanzhensis    | LJK46          | 24                | 30.3065  | 119.3163  | 1120      | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 80   | A. wanzhensis    | WMZ1469        | 24                | 30.3064  | 119.1196  | 1126      | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 81   | A. wanzhensis    | WMZ1455        | 24                | 29.5148  | 120.2410  | 572       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 82   | A. wanzhensis    | WMZ1460        | 24                | 29.4565  | 120.2096  | 244       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
| 83   | A. baohuaensis   | WMZ1413        | 24                | 32.1385  | 119.2762  | 259       | China, Zhejiang Province, Pan’an County, Mt. Donghai |
Table 1. Cont.

| Pop. | Species       | Voucher Number | Chromosome Numbers | Latitude  | Longitude  | Elevation | Locality                                      |
|------|---------------|----------------|--------------------|-----------|------------|-----------|-----------------------------------------------|
| 84   | A. baohuaensis| WMZ1415        | 24                 | 32.1251   | 119.0896   | 216       | China, Jiangsu Province, Jurong County, Mt. Baohua |
| 85   | A. baohuaensis| WMZ1419        | 24                 | 32.0559   | 118.5476   | 346       | China, Jiangsu Province, Nanjing City, Shiziling   |
| 86A  | A. baohuaensis| WMZ1407        | 24                 | 31.8160   | 119.3090   | 333       | China, Jiangsu Province, Jurong County, Mt. Mao    |
| 86B  | A. baohuaensis| LP172906       | 24                 | 31.8156   | 119.3090   | 235       | China, Jiangsu Province, Jurong County, Mt. Mao    |
| 87   | A. baohuaensis| WMZ1409        | 24                 | 31.7883   | 119.2961   | 107       | China, Jiangsu Province, Jurong County, Mt. Mao    |
| 88   | A. baohuaensis| WMZ1423        | 24                 | 31.6714   | 118.0888   | 74        | China, Anhui Province, Hanzhan City, Mt. Baohua   |
| 89A  | A. anhuiensis | LJK62          | 24                 | 30.7412   | 116.4531   | 1183      | China, Anhui Province, Qianshan County, Mt. Tianzhu |
| 89B  | A. anhuiensis | CMQ2015075     | 24                 | 30.7410   | 116.4526   | 1207      | China, Anhui Province, Qianshan County, Mt. Tianzhu |
| 90   | A. anhuiensis | WMZ1480        | 24                 | 30.7235   | 116.4537   | 708       | China, Anhui Province, Qianshan County, Mt. Tianzhu |
| 91A  | A. anhuiensis | WMZ1499        | 24                 | 29.0968   | 115.5768   | 711       | China, Jiangsu Province, Yongxiu County, Mt. Yanju   |
| 91B  | A. anhuiensis | LP173014       | 24                 | 29.0963   | 115.5767   | 703       | China, Jiangxi Province, Yongxiu County, Mt. Yanju  |
| 92   | A. mania sp.2 | WMZ1489        | 24                 | 32.3954   | 113.2989   | 248       | China, Henan Province, Tongbai County, Taiyuan Temple |
| 93   | A. mania sp.2 | WMZ1487        | 24                 | 31.7973   | 114.0872   | 529       | China, Hubei Province, Guangshui County, Heliugang   |
| 94   | A. mania sp.2 | WMZ1483        | 24                 | 31.7212   | 115.5038   | 496       | China, Henan Province, Shangnan County, Lihuocheng Village |

2.2. Chromosome Observation and Geographical Distribution Analysis

Droppers collected in the field were washed and pretreated with 0.1% Colchicine for 4.5 h, then fixed in Carnoy’s solution (1 glacial acetic acid: 3 ethanol, v/v) for 12–24 h. Finally, they were transferred to absolute ethanol and stored in the refrigerator at −20 °C for later use. Before observation, front tip (about 2 mm) was taken and hydrolyzed in 1 mol/L HCl for 10–15 min, then washed with distilled water, dyed with Carbol fuchsin and squashed for observation. Cells in metaphase of mitosis were found using a 10-fold objective lens, and chromosome numbers were counted using a 100-fold objective lens. Photographs were taken using the SOPTOP DMCX40 microscope (SOPTOP, China). The latitude, longitude and chromosome number of each collection were used to create geographical distribution maps of cytotypes through ArcMap 10.2 (Minami et al., 2000 [20]). The Elevation data (30 s) was downloaded from WorldClim v2.1 (https://worldclim.org/ (accessed on 21 February 2022)). Phylogenomic analyses revealed three clades within the genus Amana. Clade I included 3 spp. (A. edulis, A. tianmuensis, and A. nanyueensis); Clade II consisted of 4 spp. (A. erythronioides, A. kuocangshanica, A. latifolia, and Amana sp.1); and Clade III was also composed of 4 spp. (A. baohuaensis, A. wanzhensis, A. anhuiensis, and Amana sp.2) (our unpublished data). To show cytogeography of closely related species more clearly, we created different maps according to three different clades, except for the widespread A. edulis, for which we created a separate map.

3. Results

3.1. Chromosome Number and Ploidy Levels

According to chromosomal observation, there are two ploidy levels in A. edulis: diploid (2n = 2x = 24) and tetraploid (2n = 4x = 48) (Table 1, Figure 1). All the populations of the other ten species are diploid (2n = 2x = 24), except for one population (Population 59, A. tianmuensis), which is both diploid and triploid (2n = 3x = 36) (Table 1, Figure 1).
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![Figure 1. Chromosome numbers of *Amana*.](image-url)

(A) *A. edulis* (2n = 2x = 24, WMZ1408); (B) *A. edulis* (2n = 2x = 24, WMZ1486); (C) *A. edulis* (2n = 4x = 48, WMZ1494); (D) *A. edulis* (2n = 4x = 48, Daejeon, South Korea); (E) *A. tianmuensis* (2n = 2x = 24, WMZ1504); (F) *A. tianmuensis* (2n = 3x = 36, WMZ1502); (G) *A. nanyueensis* (2n = 2x = 24, WMZ1464); (H) *A. anhuiensis* (2n = 2x = 24, WMZ1480); (I) *A. baohuaensis* (2n = 2x = 24, WMZ1407); (J) *A. wanzhensis* (2n = 2x = 24, WMZ1460); (K) *A. erythronioides* (2n = 2x = 24, WMZ1435); (L) *A. kuocangshanica* (2n = 2x = 24, WMZ1443); (M) *A. latifolia* (2n = 2x = 24, WMZ1445); (N) *Amana sp.1* (2n = 2x = 24, WMZ1458); (O) *Amana sp.2* (2n = 2x = 24, WMZ1489).
3.2. Geographical Distribution Pattern of Cytotypes

The distribution of cytotypes for 50 A. edulis populations was shown in Figure 2. This species is widespread in eastern and central China, the Korean peninsula and Japan. In China, the northernmost (such as Liaoning, Shandong and northern Henan provinces) and southernmost populations of A. edulis (such as Zhejiang and southeastern Hubei provinces) are diploid and tetraploid, respectively, while diploid and tetraploid coexist in between, with gradual transition to diploid as the latitude increases. The only population we sampled from the Korean peninsula (Daejeon, South Korea) is tetraploid.

![Figure 2. Distribution of ploidy levels of Amana edulis in China, Japan and the Korean peninsula based on present data. The red color represents tetraploid (2n = 4x = 48), and the black color represents diploid (2n = 2x = 24). The five populations centered with a white dot are based on previous studies (Deng, 2016 [19]; Sato, 1943 [21]; Noguchi and Kowano, 1974 [22]).](image)

Within Clade I, Amana nanyueensis is only found at several peaks of Mt. Heng in Hunan Province, China. It mostly grows in moist deciduous broadleaf forests on the mountain slope, at elevations of 950–1150 m. Two populations of A. nanyueensis that we sampled are both diploid (Table 1, Figure 3). Additionally, Amana tianmuensis is essentially distributed in southeastern Anhui and western Zhejiang Province, China (Figure 3). It grows in evergreen-deciduous broadleaved mixed forests or moist-deciduous broadleaf forests on the mountain slope, at elevations of 600–1500 m. Most populations (7/8) of
A. tianmuensis are all diploid, but a population from Qingliangfeng Botanical Garden, Lin’an District, Hangzhou City, Zhejiang Province, is both diploid and triploid (Table 1, Figure 3).

**Figure 3.** Distribution of ploidy levels of *Amana nanyueensis* and *A. tianmuensis* in Anhui province and Zhejiang province. The green color represents triploid ($2n = 3x = 36$) and the black color represents diploid ($2n = 2x = 24$).

The species of Clade II (*A. erythronioides, A. kuocangshanica, A. latifolia* and *Amana sp.1*) are all found in Zhejiang Province but rarely coexist (Figure 4). We detected four populations of *A. erythronioides* (at elevations of 120–860 m, northeastern Zhejiang), three of *A. kuocangshanica* (at elevations of 400–1380 m, eastern Zhejiang), four of *A. latifolia* (at elevations of 80–700 m, southeastern Zhejiang) and five of *Amana sp.1* (at elevations of 400–1000 m, central to northeastern Zhejiang). All the populations that we examined are diploid.
Figure 4. Distribution of ploidy levels of *Amana erythronioides*, *A. kuocangshanica*, *A. latifolia* and *Amana sp.1* in Zhejiang Province. All four species are diploid (2\(n = 2x = 24\)).

The species of Clade III (*A. wanzhensis*, *A. baohuaensis*, *A. anhuiensis* and *Amana sp.2*) show a much wider and parapatric distribution (Figure 5). We detected six populations of *A. wanzhensis* (at elevations of 300–1130 m, central Zhejiang to southeastern Anhui), six of *A. baohuaensis* (at elevations of 70–350 m, southwestern Jiangsu to eastern Anhui), three of *A. anhuiensis* (at elevations of 700–1200 m, southwestern Anhui and northwestern Jiangxi) and three of *Amana sp.2* (at elevations of 160–530 m, along Mt. Dabie bordering Henan and Hubei). All the populations that we examined are diploid.
4. Discussion

Increasing relative DNA content by polyploidization has proven to be an important mechanism of speciation in angiosperms and is a key driver of diversity (De Bodt et al., 2005 [23]; Soltis et al., 2009 [24]; Tank et al., 2015 [25]; Soltis and Soltis, 2016 [26]; Landis et al., 2018 [27]). Recent research shows that the ancestral haploid chromosome number for angiosperms is $n = 7$, with the inferred ancestral diploid status and with the low ancestral genome size, which highlights the importance of WGD events later in the evolution of angiosperms (Carta et al., 2020 [28]). Relationships between genome size and environmental variables suggest that DNA content might be adaptive and of evolutionary importance in plants, and extreme environmental pressures may have facilitated repeated whole-genome duplication events (Vidal-Russell et al., 2021 [29]). Related research shows that the genome size increase in Liliaceae is constrained by climate seasonality, which has a negative correlation with altitude and precipitation (Carta and Peruzzi, 2016 [30]). Polyploidy can potentially contribute to the acquisition of new morphological, genetic and/or physiological features, which may enhance the competitive ability, fitness or ecological tolerance of polyploids compared to the diploid parents. These events, which have occurred at temporal scales from ancient to contemporary times, are thought to have a fundamental role in plant adaptation and range expansion (Levin 1983 [31]; Udall and Wendel 2006 [32]). This hypothesis has been supported by several investigations confirming that polyploidy has obvious advantages in adaptation and tolerance to environmental stress (McArthur and Sanderson 1999 [33];

Figure 5. Distribution of ploidy levels of *Amana anhuiensis*, *A. baohuaensis*, *A. wanzhensis* and *Amana sp.2* in Hubei, Henan, Jiangxi, Anhui, Jiangsu and Zhejiang provinces. All four species are diploid ($2n = 2x = 24$).
Brochmann et al., 2004 [34]; Hijmans et al. 2007 [35]). Areas with higher latitudes and altitudes are usually not good for the growth of plants due to the cold and dry climate, therefore, the percentage of polyploids generally increases when it goes from lower latitudes/altitudes to higher ones (Actinidia chinensis, Li et al., 2010 [36]; Claytonia perfoliata, Patrick, 2012 [37]; Eugenia, Silveira et al., 2016 [38]; Asparagus, Mousavizadeh et al., 2021 [39]; Peruzzi et al., 2012 [40]).

However, in the genus Amana, it is quite the opposite. The populations in the lower latitudes or altitudes are more likely to be tetraploid. Similar latitudinal distribution pattern has been reported in several other taxa (Solidago canadensis L., Li, 2011 [41]; Chamerion angustifolium L., Thompson et al., 2014 [42]; Andropogon gerardii, Mcallister et al., 2015 [43]; Callisia section Cuthbertia, Molgo et al., 2017 [44]). The correlation between ploidy level distribution and temperature indicates that polyploidy may be more adaptable to heterogeneous high-temperature environment than diploidy in low-latitude areas. In terms of altitude, some studies have also shown similar result (Pilosella officinarum, Mráz et al., 2008 [45]; Turnera sidoides subsp. pinnatifida, Elías et al., 2011 [46]). Diploids occur at higher elevations (Husband and Sabara, 2003 [47]; Schönswetter et al., 2007 [48]) and lower minimum temperatures (Pockman and Sperry, 1997 [49]) than polyploids, which suggests that ecological sorting based on cold tolerance can occur between cytotypes. Previous studies have shown that spring ephemerals inhabiting cool-temperate forests grow better under cool conditions, when under warm temperature, both vegetative and reproductive activities were negatively affected, resulting in less vegetative growth and lower seed-set (Sunmonu and Kudo, 2015 [50]). These previous findings together with our results imply that the ancestor of the genus Amana might be a diploid that originated in a temperate region and later migrated south to the subtropical region (perhaps during the Pleistocene glacial epoch). It then either moved up to higher altitudes to track suitable habitat or became a tetraploid and thus more adapted to warmer temperature in low latitudes/altitudes afterward. Although there are both diploids and triploids in an A. tianmuensis population, the latter could be a hybrid between historically sympatric tetraploid and diploid. Morphological and molecular analyses are needed to unravel the complex relationships among cytotypes of Amana, which will allow us to reveal the parentage and evolutionary history of all cytotypes within the genus.

The Sino-Japanese flora in east Asia has the most abundant temperate flora in the world (Liu, 1988 [51]; Ying, 2001 [52]). The Japanese archipelago was once part of Eurasia (Iijima and Tada, 1990 [53]). However, it began to separate from Eurasia during the Miocene period (about 20–16 million years ago) and gradually formed the prototype of a modern Japanese island (Maruyama et al., 1997 [54]; Otofuji et al., 1985 [55], 1996 [56]). Therefore, it is generally believed that Japanese plants originated from Eurasia (Iijima and Tada, 1990 [53]). Some recent studies have shown that the Japanese archipelago was connected to the east Asian continent during the period of 3.5 to 1.7 Ma (Kitamura et al., 2001 [57]; Kitamura and Kimoto, 2006 [58]), and after 1.7 Ma, the connection between the two was mainly achieved through unstable land bridges during the ice age (Tsuchiya et al., 2000 [59]; Shinozaka et al., 2004 [60]; Kawamura, 2007 [61]). Therefore, the separation of the Pleistocene land bridge and glacial refuges may have led to species formation and lineage differentiation between China and Japan. In our case, Amana populations dwelled at higher altitudes, such as sky islands, which might have further promoted species differentiation and formation in the genus. Two populations from Japan (one from Koishikawa Botanical Garden and the other from Akitsu) were reported to be tetraploid (Sato, 1943 [21]; Noguchi and Kowano, 1974 [22]). The Japanese populations of A. edulis may be most closely related with populations from southeastern China or the Korean peninsula, which explains why the Japanese populations are also tetraploid.

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Appendix A

Figure A1. A whole plant of Amana edulis. The red arrow points to the vertical dropper.
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