Flower formation in different taxa of *Lonicera* L. (Caprifoliaceae) in a culture in southern Sakhalin (Russia)

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**ABSTRACT**

The floral meristem of many orthotropic honeysuckles starts to form nearly a year before flowering. Such an early formation may serve to support the Nedoluzhko's hypothesis that honeysuckle phenorhythm types evolved from summer/fall to early summer, passing through the stages of late autumn, winter and early spring. The relationship between flowering dates and the dates when floral meristems begin to form is distinct from that of the subgenus *Caprifolium* and orthotropic honeysuckles. This difference allows us to deduce that there is a special evolutionary pathway for phenorhythm types in the *Caprifolium* subgenus: a shift from a summer/fall flowering period to an earlier — not later — period. The relationship between flowering dates and dates of floral meristem formation in species of the *Tataricae* series, related to the *Lonicera* section, is almost identical to that of the *Rhodanthae* subsection. This supplements previously established arguments that suggest *Rhodanthae* is the closest subsection to the *Lonicera* section.

**Key words:** *Lonicera*, Caprifoliaceae, floral meristem, flowering periods

Early-summer flowering is a characteristic feature of all species of the *Lonicera* section and many species of other sections (except for the *Nintooa* (Sweet) Maxim.) in the genus *Lonicera*. Nedoluzhko (1984) considered the early-summer flowering in the genus as evolutionary progressive trait that arose from summer-autumn flowering through the stages of winter and early spring flowering. One of the ways to test this hypothesis is a comparative study of the dynamics of the ontogenesis of flower primordia in different systematic groups. The calendar dates of laying the floral meristem and the duration of the development of flower primordia can serve as the simple criteria for determining the stages of ontogenesis. Differences in these indicators observed under identical conditions can be considered as a manifestation of specific characters inherent to the distant ancestors of these species. The comparison of the species of the *Lonicera* section with species of other sections according to these criteria is of particular interest, since there are a number of paradoxical combinations of archaic and progressive traits among the traits of this section.

The contradictions in the analysis of the morphology of species of the genus *Lonicera* are reflected in the molecular systematics of the genus based on the analysis of nuclear and chloroplast DNA.

The objective of this study was to obtain data on the laying time of the floral meristem and the duration of the development of flower primordia in different systematic groups of the genus *Lonicera* in the collection of living plants of the Botanical Garden-Institute FEB RAS. Comparison of these data for species of different systematic groups in the genus *Lonicera* will open the possibility of testing the Nedoluzhko's hypothesis.
MATERIAL AND METHODS

Forty species from the genus Lonicera from the collection of the Sakhalin Branch of the Botanical Garden-Institute FEB RAS were used in this study, in which we examined two characteristics: (1) the flowering start date, which was noted in the course of routine phenological observations recorded during the 2000–2018 period; (2) the dates of floral meristem formation in the generative buds, which were determined with a binocular microscope in microphenological studies during periods of 2004–2006 and 2017–2018. We examined buds from various sections from shoots of different types. The formation was set as earliest date in a particular season. In certain instances, it was impossible to establish the earliest appearance of floral meristem. We were only able to record a later development stage of the flower bud. In these cases, we studied the length of time between the appearance of floral meristem and that particular development stage in other (delayed) flower buds. These buds were selected either from plants of the same species or from closely related species with a similar phenorhythm. After determining the length of this period, we calculated an estimated date for the appearance of floral meristem in the species of interest. The years of 2004 and 2018 both saw abnormally cool summers. Since our microphenological observations lasted only five seasons, and occurrence rate for abnormally cool summers in the southern part of Sakhalin Island is less than 40%, the data for these years were not included in the calculation of means. The average delay in floral meristem formation during such years was about 10 days. Therefore, the dates for these years shifted by 10 days. Given that the flower development period were determined over several months in most cases, any errors resulting from this correction could not have significantly affected the findings.

RESULTS

Table 1 presents the collected data on flowering start dates, floral meristem formation dates and the duration of the flower development period in 40 species and one hybrid of the Lonicera genus. The species in the table are grouped according to their systematic position (after Rehder 1903). Supraspecific taxa, including Lonicera xylosteum L., were treated as generic taxa. Other supraspecific subdivisions examined:

Subgenera: Caprifolium (Adans.) Dipp. 1889 according to G. Krüssmann (1977).

Sections: Insufolactea Rehd. 1903, Isska Rehd. 1903, Nintooa (Sweet) Maxim. 1903, according to A. Rehder (1903).

Subsections: Pteroparasite Rehd., Pheateae Rehd., Gerochae Rehd. 1903, Vescarum Kom. 1901, Dictygeae Rehd., Alpigenae Rehd., Rhodanthar Rehd., Phenanthi (Rafin.) Rehd., Cypholae Raf. according to A. Rehder (1903); Fragmentissimae Rehd. emend. Dedoluzh., Bracteatae Hook. f. et Thoms. 1858 emend. Dedoluzh. 1986 according to V.A. Dedoluzhko (1986).

Series: Pseudoflaurae (Nakaj) Dedoluzh. 1984, Nigrae Pojark. ex Dedoluzh. 1984, Orienteae Pojark. ex Dedoluzh. 1984, Maximowiczianae Pojark. ex Dedoluzh. 1984, according to V.A. Dedoluzhko, (1984 a); Tataricae (Rehd.) Dedoluzh. 1983, Ruprechtianae Pojark. ex Dedoluzh. 1983, Maackiana Pojark. ex Dedoluzh. 1983, according to V.A. Dedoluzhko (1983) and the Hesperidae Pojark. series (1985, descr. ross.), Arphenidiae Pojark. (1958, descr. ross.), Almannaianae Pojark. (1958, descr. ross.), Almannaianae Pojark. (1958. descr. ross.), Heterophyllae Pojark. (1958. descr. ross.) according to Poyarkova (1958).

In most orthotropic honeysuckles, the floral meristems form 1–1.5 months after flowering begins. It may occur, although infrequently, two weeks earlier or later. For climbing honeysuckle, the situation is slightly different in that floral meristem starts forming at a minimum of more than two months after flowering begins. Most often, it occurs around four months after flowering, i.e. toward the end of autumn and sometimes even in spring of the following year. The latest recorded floral meristem formation was found in climbing Lonicera henryi from the Nintooa section. Therefore, different systematic groups of honeysuckle displayed different ranges in flowering periods (hereafter referred to as RFS) and in the lengths of the flowers’ development periods (hereafter, RDFD). RFS in the Caprifolium subgenus is relatively small and significantly inferior its considerably more variable RDFD (Fig. 1). The Lonicera subgenus has a wider RFS and RDFD than Caprifolium. However, this difference in RDFD is due solely to the species from the Nintooa section. If we exclude this section from the Lonicera subgenus analysis, i.e. evaluate only orthotropic honeysuckles, then we find the opposite, where the RDFD is inferior to the RFS.

When comparing the same values in lower-ranking taxa, we see that the RFS within the Caprifolium subgenus is generally the same in the Cypholae and Caprifolium subsections. Meanwhile, the RDFD is significantly higher in the Caprifolium subsection than in Cypholae (Fig. 1). The RDFD exceeds the RFS in both subsections. The situation is different for orthotropic honeysuckle of the Lonicera subgenus. Within the Isska section, the genus’s largest, both values are higher than in Lonicera. However, the RFS in the Isska section is larger than the RDFD, while this relationship is reversed in Lonicera (Fig. 1).

The relationship of these values for various subsections within the Isska section shows that the RDFD does not exceed the RFS. It also does not exceed the RFS for those series in the Lonicera section for which there is sufficient RDFD data (Fig. 1). A large RDFD for a section generally ensures variation among the series (for some of which there is insufficient RDFD data).

If we graphically represent the relationship between the length of the flower development period and the flowering dates for each of the 40 species (Fig. 2), then a large number of points for certain supraspecific taxa fall outside the main array. This is especially true for the subgenus Caprifolium and among the section Nintooa in the subgenus Lonicera.

Analyzing the relationship between flowering dates and the dates of floral meristem formation in those supraspecific taxa for which there is sufficient data yields the graph shown in Figure 3. Clearly, this relationship in the subgenus Caprifolium differs than that in the variety of subsections and series of this genus’s orthotropic members.

An attempt to link flowering dates with the development stage observed in the plants at the beginning of winter did not reveal any connection with the species’ systematic position.

DISCUSSION

Dedoluzhko (1984 b) considered the summer/autumn phenorhythm inherent in all species of the Nintooa section to be the primary flowering phenorhythm for the Lonicera
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While some species in this sections are common in the tropics, most are found in the subtropics. It is the opinion of this author that honeysuckle in the tropical climates of previous geological ages initially flowered during the late summer and in autumn. This autumnal flowering was replaced by a late autumnal one as a result of the differentiation by altitudinal belts in vegetation during orogenic processes, with a likely winter flowering period acting as an intermediate stage. Further climatic cooling led to dormancy in winter and early springtime flowering. In modern honeysuckles, winter flowering is typically found in the Eastern Chinese Lonicera fragrantissima Lindl. et Paxt. (Yang et al. 2011). It is perhaps the most archaic member of its evolutionary branch, which includes an eponymous subsection and the Bracteatae subsection. The winter phenorhythm eventually transformed into a late spring period.

Table 1. Dates of flowering, formation of floral meristem and duration of the development of flowers

| Subgenus Lonicera | Section | Subsection | Series | Species | Date of flowering start | Date of floral meristem formation | Duration of flower development (days) |
|------------------|---------|------------|-------|---------|-------------------------|----------------------------------|-------------------------------------|
| Lucilo steum     | L. mirtillus Hook. f. et Thoms. |         |       | 5.06    | 12.07*                  | 327                              |
| L. mirtillus     | Hook. f. et Thoms. | 15.05    | 20.05  | 336                              |
| L. canadensis    | Marsh. | 16.06**    | 332                              |
| L. gracilipes    | Miq. var. glandulosa Maxim. | 29.05    | 6.07* | 324                              |
| L. tangutica     | Maxim. | 20.07    | 6.08   | 346                              |
| L. caerulea      | L. caerulea L. | 18.05    | 16.06  | 334                              |
| Perforatusentes  | 12.07** | 332                              |
| L. pileata Ov.   | 27.06    | 21.08* | 308                              |
| Vesicariae       | L. vesticaria Kom. | 25.06    | 10.08  | 317                              |
| Fragrantissimae  | L. praeflorae Batal. | 3.05    | 8.06** | 327                              |
| s.l.             | L. praeflorae Batal. | 20.05    | 16.06  | 336                              |
| Almannotae       | L. alminnii Regel. et Schmalh. | 30.05    | 16.06  | 346                              |
| Braucteates s.   | L. hispida Pall. ex Roem. et Schult. | 3.06    | 16.06* | 350                              |
| str.             | L. olge Regel. et Schmalh. | 15.05    | 15.06* | 322                              |
| Distigiæ         | L. involucrata Banks ex Spreng. | 23.05    | 28.06  | 327                              |
| Alpigenæ         | L. glehni Fr. Schmidt | 25.05    | 25.06* | 332                              |
| L. alpigena L.   | 2.06    | 1.07   | 332                              |
| Alpigenæ         | L. webbian Wall. | 28.05    | 1.07* | 328                              |
| Heterophyllæ     | L. webbian Wall. | 28.05    | 1.07* | 328                              |
| Rhodantheæ       | L. chanimuni Bunge | 3.06    | 10.07** | 326                              |
| Nigrae           | L. nigra L. | 7.06    | 20.07* | 320                              |
| Orientales       | L. canasica Pall. | 23.06    | 15.08* | 310                              |
| L. disolor Lindl. | 26.06    | 10.08  | 318                              |
| Macimovicznæ     | L. schuttlenii Fr. Schmidt | 16.06    | 1.08   | 318                              |
| L. macimoviczi Fr. Schmidt | 16.06    | 1.08* | 318                              |
| L. macimoviczi Fr. Schmidt | 5.06    | 5.07   | 332                              |
| L. chrysantha  | Turcz. ex Ledeb. | 14.06    | 12.07** | 335                              |
| Ruprechoizænæ   | L. morrowii A. Gray | 17.06    | 6.08   | 313                              |
| L. ruprechoizænæ | L. ruprechoizænæ Regel | 14.06    | 5.08** | 311                              |
| Tatariae         | L. karataviensis Pavl. | 7.06    | 16.07  | 324                              |
| L. tatarica L.  | 18.06    | 7.08** | 313                              |
| L. floribunda  | Boiss. et Buhse | 22.06    | 15.08* | 309                              |
| Maaikinaæ        | L. prostrata Rehd. | 10.06    | 15.08* | 297                              |
| L. maackii Herd. | 22.06    | 15.08  | 309                              |
| hybrid           | L. quinquefloralis Hardw. × L. maackii | 25.06    | 7.09* | 289                              |
| Nintoua          | L. henryi Hemsl. | 28.07    | 10.05  | 79                                 |

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| Phenianthi       | L. sempervirens L. | 15.07    | 9.04 | 97                                 |
| Cypnodes         | L. dinica L. | 17.06    | 31.08 | 288                              |
| L. dinica×hirsuta | 6.07    | 27.10* | 250                              |
| L. prolifera Rehd. | 7.07    | 5.11* | 242                              |
| L. hirsuta Eaton | 13.07 | 5.11* | 248                              |
| Caprifolium      | L. caprifolium L. | 25.06    | 28.08* | 299                              |
| L. pericymenum L. | 22.07    | 26.04  | 87                                 |

Notes: * – adjustment in connection with the omission of the date of formation of the floral meristem; ** – adjustment due to cold summer
and, later still, early summer. Nedoluzhko did not distinguish between orthotropic shrubbery and climbing honeysuckle in his analyses on the phenorhythm shift in the Lonicera genus. The formation of the floral meristem almost a year before flowering can be considered as preserving the ontology of the traits inherent in the ancestors of these Lonicera species. A completely different relationship in Caprifolium between the flowering dates and floral meristem formation dates suggests a different evolutionary pathway for the phenorhythms in this subgenus. Moreover, there are no species in this subgenus with winter or early springtime flowering. This subgenus likely experienced a shift from a summer/fall flowering period to an earlier—not later—period.

Figure 3 shows the relationships between flowering dates and dates of floral meristem formation in Lonicera species. The species from the Tataricae series (L. floribunda,
L. tatarica, L. kuratovii) have nearly the same dates of meristem formation as species of the Rhodantha subsection. In this case, the correspondence can be viewed as additional confirmation of the Lonicera section's proximity to the Rhodantha subsection (part of the Irika section), as evidenced by chloroplast DNA sequencing (Theis et al. 2008, Nakaji et al. 2015). Theis et al. (2008) reported a conflicting findings from the chloroplast DNA and nuclear DNA analyses, regarding the Lonicera section. Some inconsistencies in opinions on the phylogenetic relationships may arise when analyzing the morphological features of this section's species and the fact that there is no polyploidy (unlike in other sections). Primitive characteristics also appear in large shrubbery (the largest for orthotropic honeysuckle), immature buds, and evanescent pistil (characteristic in relatives of the Symphoricarpos Duham. и Leyecestea Wall. genera). The origin of the this section could possibly be the result of introgression, but the morphological similarity, chloroplast DNA findings and the data from our microphenological studies suggests that the greatest contribution to this section's formation came from the Rhodantha subsection. For further investigation into this matter, it will be necessary to conduct chloroplast DNA testing on a larger species sampling from both the Lonicera section and the Rhodantha subsection. Most likely, an analysis of relatively ancient species in the Lonicera section, such as Lonicera quinquenervia Hardw., L. arborea Boiss., L. floribunda, and L. brevistipala Hsu et H.J. Wang (a widespread species to the west of the main habitats of the Ruprechtiana series), will be of particular interest. It would also be prudent to study the floral meristem formation periods in most of these species.

CONCLUSIONS

Research has found that the floral meristem of many orthotropic honeysuckles forms nearly a year before flowering, a feature that can be viewed as preserving the ontogeny of the character inherent in the distant ancestors of these Lonicera species. In turn, this may serve to support Nedoluzhko's hypothesis that honeysuckle phenorhythm types evolved from summer/fall to early summer, passing through the stages of late autumn, winter and early spring.

The relationship between flowering dates and the dates when floral meristems begin to form is distinct from that of the subgenus Caprifolium and orthotropic honeysuckles (species of the Lonicera subgenus, minus the Nintona section). This difference allows us to deduce that there is a special evolutionary pathway for phenorhythm types in the Caprifolium subgenus. It is likely that this subgenus experienced a shift from a summer/fall flowering period to an earlier—not later—period.

The relationship between flowering dates and dates of floral meristem formation in species of the Tataricae series, from the Lonicera section, is almost identical to that of the Rhodantha subsection. This supplements previously established arguments, based on morphological similarity and chloroplast DNA analysis that suggest Rhodantha is the closest subsection to the Lonicera section.

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