Nastic corolla movements of nine *Gentiana* species (*Gentianaceae*), presented in the Bulgarian flora

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Abstract. The corolla movements promote partial self-pollination in the perennial *Gentiana pneumonanthe*, *G. cruciata* and *G. pyrenaica,* as well as in the annual *G. utriculosa* and *G. nivalis*. There is a threshold flower temperature for opening/closing movements. Both the infrared and ultraviolet rays are of importance for flower closure. The flowers of some gentians – the homogamous *G. verna, G. utriculosa* and *G. nivalis* – gradually lose their extremely high sensitivity during their lifespan. The flowers of protogynous *G. punctata* also lose their flower sensitivity. Flower sensitivity does not correlate with flower age in the strongly protandrous *G. cruciata* and *G. pyrenaica*.

Key words: corolla movements, *Gentiana,* nastic reactions, self-pollination

Introduction

Genus *Gentiana* L. contains 361 species divided amongst 15 sections (Ho & Liu 1990). *Gentiana* s.str. is equivalent to *Eugentiana* Kuzn. (Kuznetzov 1894) and has been accepted widely by most 20th century authors on *Gentianaceae*, incl. Tutin (1972).

There are some data available on the floral mechanisms and breeding systems of *G. lutea* subsp. *lutea*, *G. punctata* L., *G. asclepiadea* L., *G. pneumonanthe* L., *G. cruciata* L., *G. pyrenaica* L., *G. verna* L., *G. utriculosa* L., and *G. nivalis* L. (Müller 1881; Petanidou & den Nijs 1991; Petanidou & al. 1995a, b; Kery & al. 2000), and some specificities of the flower mechanisms such as anthesis, dichogamy, hercogamy, and lifespan have been described, as most of these papers provide data on the corolla movements too (Weber 1924; Scharfetter 1953; Petanidou & al. 1995a, b).

This is a review paper dealing with a comparative analysis of the nastic corolla movements of *G. lutea* subsp. *symphyandra* (Murb.) Hayek, *G. punctata*, *G. asclepiadea*, *G. pneumonanthe, G. cruciata, G. pyrenaica*, *G. verna*, *G. utriculosa,* and *G. nivalis* occurring in Bulgaria. It is based primarily on our published data on *gentian* reproductive biology: floral mechanisms, nastic corolla movements, lifespan, nectar production, species composition of pollinators, pollinator activity and behaviour, and fruit and seed set (Kozuharova 1994, 1998, 1999, 2004; Kozuharova & Anchev 2001, 2002; Kozuharova & al. 2005). Some additional, still unpublished data are also presented.

The aim of this study is: i) to investigate the nastic corolla movements within species and sections; ii) to analyse the expected correlations and evolutionary trends on different taxonomic levels; iii) to offer a hypothesis on the adaptive significance of the nastic corolla movements.

Material and methods

Eleven *Gentiana* species occur in the Bulgarian mountains (Kozhuharov & Petrova 1982). They pertain to six sections, namely sect. *Gentiana* (sect. *Coelanthe* Froelich), sect. *Pneumonanthe* (Gled.) Gaudin, sect. *Cruciata* Gaudin (sect. *Aptera* Kuzn.), sect. *Chondrophyllae* Bunge, and sect. *Calathianae* Froelich (sect. *Cyclostigma* Griseb.). This study includes nine of the species that belong to five sections (Table 1). *Gentiana acaulis* L. and *G. frigida* Haenke are not included. They are very rare in Bulgaria.

Study sites

The observations for this study were conducted in the period from May to September in 1990, 1991, 1992, 1994, 2003 and 2004, in the mountains of SW Bulgaria (Table 2).

Observations of the floral mechanism and corolla movements

Flowers and inflorescences of the gentians were individually labelled as follows: *Gentiana lutea* subsp. *symphyandra* ($N_{fl} = 200$, $N_{infl} = 15$), *G. punctata* ($N_{fl} = 190$, $N_{infl} = 23$), *G. asclepiadea* ($N_{fl} = 176$, $N_{infl} = 22$), G. *pneumonanthe* ($N_{fl} = 340$, $N_{infl} = 34$), G. *cruciata* ($N_{fl} = 264$, $N_{infl} = 7$) *G. pyrenaica* ($N_{fl} = 20$, $N_{clones} = 20$), *G. verna* ($N_{fl} = 30$, $N_{clones} = 25$), *G. utriculosa* ($N_{fl} = 45$, $N_{infl} = 15$), and *G. nivalis* ($N_{fl} = 25$, $N_{infl} = 15$). Their development was periodically recorded for the entire day, in order to study the floral mechanisms and corolla movements. Observations were conducted under different weather conditions. The temperature of the air was measured (in shade) and wind was taken into account (no wind, mild or strong wind). The clouds covering the sky were recorded on a 0–10 scale (standard method of the National Hydro-Meteorological Institute, BAS).

The methods used to measure floral mechanisms, nectar production, species composition of pollinators, pollinator activity and behaviour, and fruit and seed set were described earlier, together with the detailed results (Kozuharova 1994, 1998, 1999, 2004; Kozuharova & Anchev 2001, 2002; Kozuharova & al. 2005).

Table 1. Life form, inflorescence, flower morphology and flower colour of *Gentiana* species

Taxa	Life form	Inflorescence	Flower morphology	Flower colour
Sect. *Gentiana* (*Coelanthe* Froelich)				
G.lutea susbsp. *symphyandra*	Rhizomatous perennial	Numerous flowers sitting in cymose clusters at the base of the upper leaves	Five-lobed wide opened with short corolla tube	Bright yellow
Gentiana punctata	Rhizomatous perennial	1-3 flowers sitting at the base of each of the upper leaves	Seven-lobed funnel shaped	Pale- yellow with purple nectar guides
Sect. *Pneumonanthe* (Gled.) Gaudin				
G. asclepiadea	Rhizomatous perennial	1-3 flowers sitting at the base of the upper leaves	Five-lobed funnel shaped	Blue
G. pneumonanthae	Rhizomatous perennial	1-10 flowers sitting at the base of the upper leaves	Five-lobed funnel shaped	Purple-blue
Sect. *Cruciata* Gaudin (*Aptera* Kuzn.)				
G. cruciata	Rhizomatous perennial	8-20 flowers sitting in cymose clusters at the base of the upper leaves	Four-lobed funnel shaped	Blue
Sect. *Chondrophyllae* Bunge				
G. pyrenaica	Rhizomatous perennial	Solitary, terminal on short stems	Narrow-funnel shaped	Purple
Sect. *Calathianae* Froelich (*Cyclostigma* Griseb.)				
G. verna	Rhizomatous perennial	Solitary, terminal on short stems	Rather narrow-funnel shaped	Bright blue
G. utriculosa	Tiny annual	Either solitary, terminal on short stems, or several and clustered in a loose cymose panicle	Rather narrow-funnel shaped	Bright blue
G. nivalis	Tiny annual	Solitary, terminal on short stems, or several and clustered in a loose cymose panicle	Rather narrow-funnel shaped	Bright blue

Table 2. Study sites.

Sections / Species	Time	Mountain	Altitude	Habitats
Sect. *Gentiana*				
G. lutea subsp. *symphyandra*	August	Mt Vitosha	1900 м	subalpine grasslands
G. punctata	June, July	Mt Vitosha	2000 м	subalpine grasslands
Sect. *Pneumonanthe*				
G. asclepiadea	August, September	Mt Vitosha Mt Vitosha	1500 m 1900 m	coniferous forest belt – open woodlands subalpine grasslands
G. pneumonanthe	August, September August, September	Mt Lyulin W Balkan Range	1000 m 1450 m	meadows open wet woodland in coniferous forest belt
Sect. *Cruciata*				
G. cruciata	July	Mt Vitosha		limestone stony places, and grasslandss
Sect. *Chondrophyllae*				
G. pyrenaica	July	Rila Mts Pirin Mts	2300 m 2100 m	wet subalpine grasslands wet subalpine grasslands
Sect. *Calathianae*				
G. verna	May	Mt Vitosha	1900 m	subalpine grasslands
G. utriculosa	June	Mt Vitosha	1000 m	wet grasslands
G. nivalis	August	Mt Vitosha	1900 m	wet subalpine grasslands

Results and discussion

Section *Gentiana*

Gentiana lutea subsp. *symphyandra*

Floral mechanism. Our observations revealed that the flowers were protandrous and self-compatible, with no spontaneous self-pollination (Table 3, Fig. 1a) (Kozuharova 1994, 1998). The insects (flies and bumblebees) actively cross-pollinated them (Kozuharova 1994, 1999). The P/O ratio (mean = 13147.1, min = 9471.0, max = 21623.6) indicated obligate xenogamy (Kozuharova & Hadzieva 2004). The typical subspecies has homogamous flowers (Müller 1881). They were obligate out-crossers (Bucher 1987) and self-incompatible (Kery & al. 2000). This species had the highest pollinator activity (Kozuharova 1999).

Table 3. Dichogamy (protandry or protogyny) and homogamy observed on labeled plants. Self-compatibility and spontaneous self-pollination.

Sections / species	Corolla colour	Reactivity	Dichogamy / homogamy	Lifespan	Self-compatibility	Spontaneous self-pollination
Sect. *Gentiana*						
G. lutea subsp. *symphyandra* .	Y	–	1 day – protandry[5]	4-5 days	self-compatible[5] obligate xenogamy[4]	no[5]
G. lutea subsp. *lutea* 1	Y		homogamy[1, 2]		self-incompatible[2]	no[2]
G. punctata	Y	+	1 day protogyny[5]	3-4 days	facultative/obligate xenogamy [4,*]	no[5]
Sect. *Pneumonanthe*						
G. asclepiadea	B	++	2-4 days protandry[6]	6-11 days	self-compatible[6] decreases during the life span	no[6]
G. pneumonanthe	P-B	+++	2-4 days protandry[6]	7-10 days	self-compatible[6]	partially[6]
Sect. *Cruciata*						
G. cruciata	B	+++	3-4 days protandry[7]	5-9 days	self-compatible[7]	partially[7]
Sect. *Chondrophyllae*						
G. pyrenaica	P	+++	3-4 days protandry[5]		facultative xenogamy[4]	partially[?] [*]
Sect. *Calathianae*						
G. verna	B	+++	homogamy[8]	7-10 days	self-compatible[8]	no[8]
G. utriculosa	B	+++	homogamy[8]	6-8 days	self-compatible[8]	yes[8]
G. nivalis	B	+++	homogamy[8]	7-9 days	self-compatible[8]	yes[8]

Legend
+++ rapidly reactive flowers; ++ medium reactive flowers; + slowly reactive, – non reactive; *excluded plants destroyed by larvae.
Data sources: [1]Müller, 1881; [2]Kery & al. 2000; [3]Bucher 1987; [4]Kozuharova & Hadzieva 2004 (P/O ratio method); [5]Kozuharova 1994; [6]Kozuharova 2004; [7]Kozuharova & Anchev 2001; [8]Kozuharova & Anchev 2002.

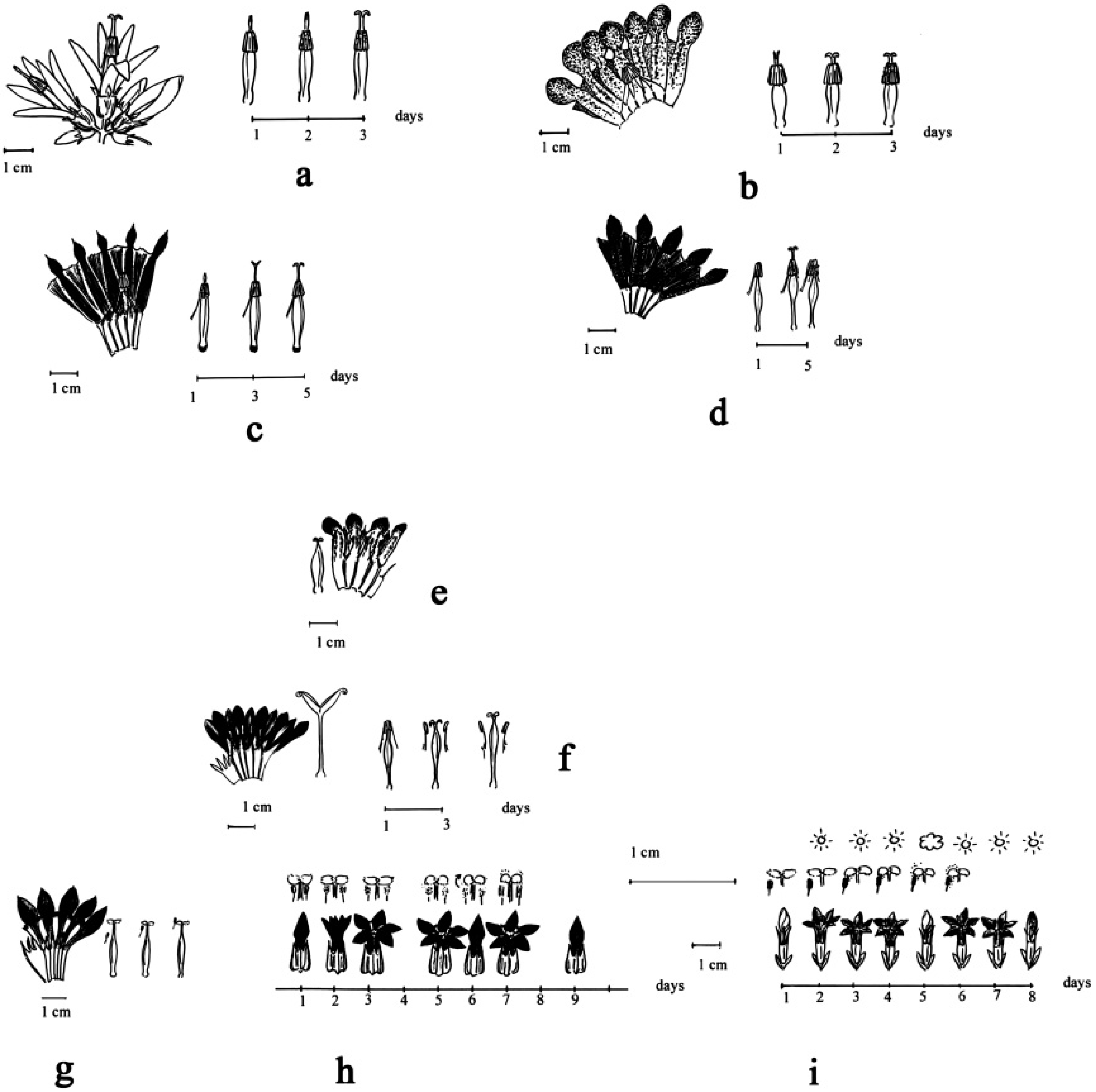

Fig. 1. Corolla morphology and floral mechanisms of:
a, *G. lutea* subsp. *symphyandra*; **b**, *G. punctata*; **c**, *G. asclepiadea*; **d**, *G. pneumonanthe*; **e**, *G. cruciata*; **f**, *G. pyrenaica*; **g**, *G. verna*; **h**, *G. utriculosa*; **i**, *G. nivalis*.

Corolla movements. The flowers remained open until the end of their anthesis. They never closed again either at night, or during the day. The lowest temperature recorded for the period of observations was 11 °C, clouds completely covered the sky and there was no wind (Table 4). Weber (1924) described *G. lutea* as a species with no seismonastic and thermonastic reactions.

Lifespan. The flowers pollinated during the first day remained open for 3–4 more The lifespan was 4–5 days (Table 3). The stigmas of most observed flowers had pollen within the next day after maturation.

Gentiana punctata

Floral mechanism. The investigated flowers were protogynous, with no spontaneous self-pollination (Table 3, Fig. 1b) (Kozuharova (1994, 1998) and the insects (flies and bumblebees, Kozuharova 1994, 1999) actively cross-pollinated them. The P/O ratio (mean = 2026.3, min = 1373.6, max = 2801.5) indicated facultative/obligate xenogamy (Kozuharova & Hadzieva 2004).

Corolla movements. The flowers reacted only to very drastic deterioration of meteorological conditions, when temperature fell down to 13 °C and prolonged rain and fog. The younger flowers were slightly more sensitive but even they did not close completely. At night the flowers typically remained unclosed, but slightly folded. Near sunset (8:30 PM), at a temperature of 11 °C there were some completely open flowers. In the morning unfolding was slower: it started at 9:30 AM (summer time, 3 hours after sunrise), at air temperature of 14–15 °C (Table 4).

Lifespan. The labelled flowers remained open 2–3 days after their pollination. The lifespan was 3–4 days (Table 3).

Section *Pneumonanthe*

Gentiana asclepiadea

Floral mechanism. The populations of *G. asclepiadea* inhabiting Mt Vitosha were strongly protandrous (Kozuharova 2004), as well as those investigated by Müller (1881). The P/O ratio (mean = 4262.2, min = 3322.0, max = 6309.7) indicated obligate xenogamy (Kozuharova & Hadzieva 2004). The tested flowers were self-compatible, with no spontaneous self-pollination. They were actively

Table 4. Nastic movements of the flowers of *Gentiana* species.

Sections / species	**Morning / evening changes** time / air temperature			**Closure during the day**
	movements	Sunny	Cloudy	Temperature decrease
Sect. *Gentiana*				
G. lutea subsp. *symphyandra*	remain open	once opened the flowers remained like that until the end of their life		
G. punctata	remain open	9:30 am/14-15 °C 8:30 pm/11 °C		partial closing 13 °C rain - fog
Sect. *Pneumonanthe*				
G. asclepiadea	opening closing	10-10:30 am/10.5 °C 7 pm/10.5 °C	10-10:30am/10.5 °C 7 pm/10.5 °C	Cloudy/Sunny/5 °C
G. pneumonanthe	opening closing	10:30-11 am/12 °C 6 pm/16 °C		Cloudy 16.5 °C
Sect. *Cruciata*				
G. cruciata	opening closing fully closed	10-10:30 am/18-20 °C 7 pm/22 °C 8 pm/19 °C	10-10:30 am/18-20 °C	Sunny 17 °C Cloudy 20.5 °C
Sect. *Chondrophyllae*				
G. pyrenaica	opening closing	10-10:30 am/8 °C 7-7:30 pm/16 °C	10-10:30 am /16 °C 7-7:30 pm/16 °C	Sunny 12 °C Cloudy 16 °C
Sect. *Coelanthe*				
G. verna	opening closing fully closed	8:30-9 am/13°-14 °C ?	? 7:30 pm/14 °C	Cloudy 13-15 °C Sunny 11 °C
G. utriculosa	opening closing	8:30 am/21 °C 6:15 pm/22 °C	? ?	Sunny 11 °C Cloudy 18 °C
G. nivalis	opening closing	? ?	? ?	Sunny 15 °C Cloudy17 °C

*cloud cover less than 5/10 of the sky; ** cloud cover over 5/10 of the sky.

cross-pollinated by flies and bumblebees (Fig. 1c, Table 3) (Kozuharova (2004). Furthermore, self-compatibility tests revealed that 77 % of the flowers that were hand-selfed at an early female stage set fruits ($N_{fl} = 22$), as compared to only 40 % of the flowers hand-selfed at a late female stage that set fruits ($N_{fl} = 25$). Their seed set was respectively 62 % and 50 % of the seed set of free pollinated flowers (Kozuharova 2004).

Corolla movements. Both male and female stage flowers on a stem closed simultaneously at about 7 PM and at air temperature of 10.5 °C (near sunset). The flowers opened in the morning at about 10 AM and at air temperature above 10.5 °C (about three hours after sunrise, Table 4). Daytime temperatures below 4–5 °C caused closing of the flowers. However, they did not close totally but only the appendices of the petals folded to cover the entrance of the corolla tube. The lobes of the petals folded slightly and never put their tips together before final wilting. Even air temperature of 0 °C during some nights did not cause full corolla closure. The younger (functionally male) flowers were more reactive. Weber (1924) and Scharfetter (1953) described similar flower closure behaviour, with slow seismonastic and thermonastic reactivity of *G. asclepiadea* flowers.

Lifespan. Labelled flowers remained open several days after the pollen was deposited on the stigma (Table 3).

Gentiana pneumonanthe

Floral mechanism. The populations of *G. pneumonanthe* inhabiting Mt Lyulin and the Western Balkan Range (Kozuharova 2004) were strongly protandrous and more or less cross-pollinated, similarly to those in the Netherlands (Petanidou & al. 1995b). Spontaneous self-pollination of *G. pneumonanthe* in the Netherlands was strongly limited (Petanidou & al. 1995b), while almost half of the bagged flowers in the Balkan Range set fruits (Table 3, Fig. 1d) (Kozuharova (2004). Comparatively low bumblebee activity was recorded (Kozuharova 1999, 2004).

Corolla movements. Unlike the other investigated species in this section, the flowers of *G. pneumonanthe* were rather sensitive. Both male and female stage flowers of a plant closed simultaneously within 30 minutes at about 6 PM and air temperature of 16 °C (clouds covering less than 1/10 of the sky). They opened in the morning at about 10:30–11:00 AM and at air temperature above 12 °C, with clouds covering less than 1/10 of the sky (Table 4). Temperature beneath 16.5 °C combined with clouds (8/10 of the sky) caused complete closing of the flowers during the day. All flowers closed completely, resembling buds. The younger (functionally male flowers) were insignificantly more reactive. Weber (1924) regarded the marsh gentian as a rapidly reactive species, seismonastically and thermonastically. Relative humidity and temperature were significantly correlated with the daily opening pattern. The flowers opened in good weather between 9 AM and 4 PM (Petanidou & al. 1995b).

Lifespan. Labelled flowers remained open several days after the pollen was deposited on the stigma (Table 3).

Section *Cruciata*

Gentiana cruciata

Floral mechanism. The flowers were strongly protandrous (Table 3, Fig. 1e). The pollen was usually removed during the male stage by insects. The flowers were self-compatible and spontaneous self-pollination was possible, but limited. They were predominantly cross-pollinated by bumblebees (Kozuharova & Anchev 2001). There were certain specificities of the Dutch populations and of the Bulgarian population of this species (Petanidou & al. 1995a; Kozuharova & al. 2005).

Corolla movements. According to Weber (1924) and Scharfetter (1953), *G. cruciata* demonstrated slow seismonastic and thermonastic reactiveness. However, our study showed that the corolla movements of *G. cruciata* were rapid. Both male and female stage flowers on a stem closed simultaneously near sunset. The process started at 7 PM and t 22 °C and completed at 8 PM, and t 20 °C. The closing began at higher air temperature than the opposite process in the morning. Then the plants exposed to the sun for a couple of hours opened their flowers simultaneously with those shaded by nearby hills. During the day the flowers closed rapidly (2–5 min) in cloudy weather, even at high temperature (20.5 °C). On the contrary, they remained open at lower air temperature (17 °C), if the sun shone directly on them (Table 4).

Lifespan. Labelled flowers remained open several days after the pollen deposition on the stigma. The lifespan was 5–9 days (Table 3).

Section *Chondrophyllae*

Gentiana pyrenaica

Floral mechanism. The flowers were strongly protandrous (Table 3, Fig. 1f) (Kozuharova (1994, 1998). Facultative xenogamy was indicated by the P:O ratio (mean = 639.8, min = 169.8, max = 1184.2) (Kozuharova & Hadzieva 2004). Cross-pollination was restricted owing to a very low pollinator activity: mainly flies (Kozuharova 1994, 1999).

Corolla movements. The flowers of *G. pyrenaica* closed rapidly. Both male and female stage flowers closed simultaneously, resembling buds, within 15 minutes at 7–7:15 PM (about an hour before sunset) and air temperature beneath 16 °C. In the morning the flowers opened at about 10 AM (3 hours after sunrise) and at air temperature of 8 °C (measured in shade). The real temperature for the flowers, however, was higher as they were lightened directly up by the sun (no clouds). The air temperature measured near the flowers that were exposed to sunshine was 16 °C. During the day the flowers closed completely, resembling buds, if clouds covered more than half of the sky and the temperature was lower than 16 °C. This closure was also rapid (Table 4).

Lifespan. Labelled flowers remained open several days after pollen was deposited on the stigma (Table 3).

Section *Calathianae*

Gentiana verna

Floral mechanism. The flower was homogamous and, although self-compatible, did not self-pollinate spontaneously (Table 3, Fig. 1g) (Kozuharova & Anchev 2002). Facultative xenogamy to facultative autogamy was indicated by the P:O ratio (mean = 293.1, min = 176.2, max = 537.8) (Kozuharova & Hadzieva 2004). Cross-pollination was restricted owing to low pollinator activity (Kozuharova & Anchev 2002)

Corolla movements. The corolla, especially of the young flowers, closed rapidly when temperature and sunshine decreased. In the evening, closure occurred rapidly after 7:30 PM/14 °C and in the morning reopening was observed from 8:30–9:00 AM/13–14 °C. When flowers closed during the day, the air temperature was 13–15 °C in cloudy weather and below 11 °C in sunny weather. The corollas closed even at 15 °C, when clouds did not cover more than 5/10 of the sky, but humidity was high (Table 4). The flowers closed rapidly within a minute or two after mechanical treatment, such as hand self-pollination.

Lifespan. The labelled flowers lived 5 to 7 days, whether or not they had been pollinated (Table 3).

Gentiana utriculosa

Floral mechanism. The flower was homogamous, self-compatible and self-pollinated spontaneously (Table 3, Fig. 1h) (Kozuharova & Anchev (2002). Facultative autogamy was indicated by the P:O ratio (mean = 214.9, min = 65.1, max = 318.5) (Kozuharova & Hadzieva 2004). The pollinator activity was low (Kozuharova & Anchev 2002).

Corolla movements. The flowers, especially the young ones, were extremely sensitive to mechanical treatment, temperature and sunshine changes. In the evening, closure started after 6:15 PM at air temperature of 22 °C, with no clouds. In the morning, reopening started after 8:30 AM and 21 °C air temperature, with 2/10 cloud cover of the sky. When flowers closed during the day, the air temperature was 13–15 °C in cloudy weather and below 11 °C in sunny weather. They closed at air temperature of 18 °C in cloudy weather and were still open at 11–12 °C in sunny weather (Table 4). The flowers closed rapidly within a minute or two after mechanical treatment.

Lifespan. The marked flowers lived 6 to 8 days, depending on the weather conditions. They were all pollinated on the second day of their opening at the latest (Table 3).

Gentiana nivalis

Floral mechanism. The flower was homogamous, self-compatible and self-pollinated spontaneously (Table 3, Fig. 1i) (Kozuharova & Anchev (2002). The pollinator activity was very low (Kozuharova & Anchev 2002).

Corolla movements. The flowers, especially the young ones, were extremely sensitive like those of *G. utriculosa*. They were still open at 15 °C under sunshine (the lowest temperature recorded during the observations). These flowers closed within 2–3 minutes at 17 °C, when the sun was behind the clouds (Table 4). The flowers closed rapidly after mechanical treatment.

Lifespan. The anthesis was 7–9 days and depended on weather, but not on pollination as it was revealed by observation of the labelled flowers. They were all pollinated on the second day of their opening at the latest (Table 3).

Conclusions

The intrageneric phylogeny and classification of genus *Gentiana* have been much debated (Serebryakova 1979; Yuan 1993; Yuan & al. 1996). A number of diagnostic characters, such as flower morphology, "membrana intracalicina", seed morphology, shooting type, phytochemical, karyological, etc., were used to build a phylogenetic hypothesis (Kuznetzov 1894; Scharfetter 1953; Serebryakova 1979; Müller 1982). According to this hypothesis, section *Gentiana* was primitive and ancestral, section *Pneumonanthe* was derived from it and, in turn, the other sections *Cruciata, Ciminalis*, *Frigidae, Chondrophyllae, Calathianae* originated from section *Pneumonanthe*. The inferences based on the DNA analysis, nucleotide sequences of the internal transcribed spaces (ITS) of nuclear ribosomal DNA, threw a new light on gentian phylogeny. According to Yuan & al. (1996), the ITS phylogeny was generally congruent with morphological classifications. However, there were several new points. Section *Gentiana* was not considered primitive as it was thought before, but rather had a derived status. *Gentiana asclepiadea* was found to be closely related to section *Gentiana*, instead of section *Pneumonanthe* (Yuan & al. 1996, Fig. 2 at page 649).

Floral mechanism

The corolla morphology and floral mechanisms of *G. lutea* subsp. *symphyandra*, *G. punctata*, *G. asclepiadea*, *G. pneumonanthe*, *G. cruciata*, *G. pyrenaica*, *G. verna*, *G. utriculosa*, *G. nivalis* are presented on Fig. 1. The spontaneous self-pollination was avoided by fusion of the anthers in a ring down beneath the stigma in the flowers of the whole populations of *Gentiana lutea* subsp. *symphyandra, G. punctata, G. asclepiadea* (Fig. 1a-c). Most of the observed plants of *G. pneumonanthe* had flowers with stigmas above the fused in ring anthers, but the stigmas of some flowers were into the anthers' ring and could be self-pollinated spontaneously (Fig. 1d). The anthers of *G. cruciata* and *G. pyrenaica* were free and at first they were close to each other in the centre of the flower, the stigma was closed and situated below the anthers; later the filaments gradually moved the anthers to the periphery of the flower and the style exerted the stigma above the anthers, where its two lobes opened. Most of the investigated plants had flowers with stigmas situated above the anthers. However some plants had flowers with stigma and anthers situated at one and the same level (Fig. 1e-f). The anthers of the homogamous flowers of *G. verna, G. utriculosa,* and *G. nivalis* were situated more or less below the stigma disc, which covered the entrance of the corolla tube (Fig. 1g-i). The stigma lobes and the ciliae on its margin of the two annuals *G. utriculosa* and *G. nivalis* were directed horizontally during the first day of the flowering. During the second, rarely on the third day, they bent down, touched the anthers and stuck autogamous pollen. This was prompted by periodical folding and unfolding of corolla in changeable weather, when the pollen was pushed upwards to the stigma by the recurrent contraction of the corolla tube, which was lavishly dusted with it (Fig. 1h-i).

The pattern dichogamy/homogamy was peculiar for each species but demonstrated certain trends on sectional level (Kozuharova 1998). Most sections contained protandrous species, except section *Gentiana.* The latter contained homogamous, protandrous and protogynous species. Species with homogamous flowers pertained to section *Calathianae*, which appeared to be related to section *Gentiana* (Yuan & al. 1996, Fig. 2 at page 649).

Flower reactivity

The pattern of reactivity also demonstrated some trends corresponding to the sectional delimitation of the species. The flowers of *G. lutea* subsp. *symphyandra* were not reactive and the flowers of *G. punctata* and *G. asclepiadea* were slowly reactive. The flowers of *G. pneumonanthe, G. cruciata, G. pyrenaica*, *G. verna*, *G. utriculosa,* and *G. nivalis* were rapidly to very rapidly reactive. The flowers of *G. straminea* Maxim. (sect. *Cruciata*) were rapidly reactive (He & al. 2006). In other words, the evolutionary trends were in two directions: i) lack of reactivity or slow reactivity (species of section *Gentiana* and *G. asclepiadea*); ii) rapid flower closure (sections *Pneumonanthe, Cruciata, Chondrophyllae*, and *Calathianae*). There was no correlation between the flowering period or habitat specificities and the nastic corolla reactions (Tables 2 & 3).

A pollination-induced flower senescence was observed in *G. saxosa* and *G. serotina.* Fresh female phase flowers closed at night and failed to reopen on the day following pollination (Webb & Littleton 1987).

These two species are distantly related members of *Gentianaceae* from New Zealand. Recently, they have been regarded as members of *Gentianella* and Chionogentias (Adams 1995; von Hagen & Kadereit 2001). Pollination-induced floral closure was observed in the flowers of *Gentiana straminea* in the eastern Qinghai-Tibetan Plateau (He & al. 2006). Such a pattern was not found in any of the nine investigated species. Pollination does not influence flower longevity. However, meteorological factors, such as high air temperature and low humidity, shorten the flower longevity, as was recorded most clearly for *G. punctata*.

Factors affecting the nastic corolla movements

There is a threshold of flower temperature for opening/closing movements. Müller (1881) considered temperature a leading factor for the flower closure of *G. asclepiadea*, but not the light. He maintained that for these bright-blue flowers the air temperature and the infrared light spectra were the leading factors. In our opinion, the meteorological factors (temperature and sunshine) act as a complex factor. When direct sunlight heats the corolla, it may open even at lower air temperature. On the contrary, the flowers may close even at higher air temperature, if the sun is behind the clouds. This is the pattern of the corolla movements of *G. pneumonanthe*: closure at higher air temperate in cloudy weather and opening at lower air temperature in the sunshine indicate the synergetic effect of light and temperature in this case. The dark purple-blue colour of the corolla enhances the ability for heat absorption. The blue flowers of *G. cruciata* and the bright deep-blue flowers of *G. verna*, *G. utriculosa* and *G. nivalis* demonstrated similar behaviour. The populations of *G. cruciata* in the Netherlands show a negative correlation with humidity, and positive correlation with temperature. Relative humidity is the most important parameter (Petanidou & al. 1995a). Dependence on humidity was observed most obviously for *Gentiana verna:* the flowers closed at 2 PM in good weather (15 °C and 5/10 cloud cover of the sky) and at high air humidity.

In addition to infrared rays, ultraviolet rays are also important. In *G. utriculosa* the flowers closed at sunset, when temperature and cloudiness were still constant but ultraviolet rays were diffracted. In the evening, closure started after 6:15 PM and at 22 °C air temperature, without clouds, while the same flowers remained open during the day, in sunshine and at 11 °C.

Furthermore, we confirmed that the flowers of *G. verna*, *G. utriculosa* and *G. nivalis* close rapidly not only after lowering of temperature and sunshine, but also after mechanical treatment – a fact observed also by Weber (1924).

The flowers of some gentian species gradually lose their extremely high sensitivity during their lifespan. The young flowers of the homogamous *G. verna, G. utriculosa* and *G. nivalis* are slightly more sensitive than the older ones. These young flowers need higher temperature by 2–3 °C than the older ones to start opening. The situation is similar with the flowers of the protogynous *G. punctata*, though their reactivity in general is lower. The young (male) flowers of the strongly protandrous *G. pneumonanthe* and *G. asclepiadea* are also insignificantly more reactive than the old ones. The sensibility of the flowers do not correlate with their age in the strongly protandrous *G. cruciata,* and *G. pyrenaica.*

Positive feedbacks of the nastic corolla movements

Self-pollination. The lack of pollinators is considered as one of the main reasons for the shift from allogamy to autogamy, even in plants with highly co-adapted flowers in both annual and perennial species (Grant & Grant 1965; Stebbins 1974; Richards 1982, 1990).

In *Gentiana* the corolla movements promote the process of spontaneous self-pollination, by bringing together anthers and stigma in the centre of the flower. Thus, in bad weather when the insect activity is lower and the probability of cross pollination, pollen removal and distinct chronological separation of the sexes is reduced, the probability for self-pollination is enhanced. That is the case with the perennials with partial ability for spontaneous self-pollination (facultative xenogamy) *G. pneumonanthe*, *G. cruciata* and *G. pyrenaica*, as well as with the spontaneously self-pollinating annuals (facultative autogamy) *G. utriculosa* and *G. nivalis* (Kozuharova & Anchev 2002).

Nectar dilution. The flower closure prevents nectar dilution. The dew or rain dilution of the nectar is less in the rapidly reactive flowers of *G. cruciata* as compared to the slowly reactive flowers of

G. asclepiadea. In the flowers of *G. cruciata,* as a whole, the average quantity and sugar concentration of the nectar were higher during the female stage. The nectar volume [mg] during the male stage ($N_{flowers} = 20$) was 0.60±0.56 with concentration [%] 17.50±3.98. The nectar volume [mg] in the female stage ($N_{flowers} = 20$) was 0.98± 0.68 with concentration [%] 22.10±5.66 (Kozuharova & Anchev 2001). In the flowers of *G. asclepiadea* dew dilution was significant during the female stage. At the beginning of the male stage the nectar volume [mg] ($N_{flowers} = 28$) was 2.45±0.89 with concentration [%] 22.68±2.89. Later on, the nectar volume [mg] during the female stage ($N_{flowers} = 22$) was 2.76±0.67 with concentration [%] 19.36±6.07 (Kozuharova 2004).

Protection. Corolla movements protect the generative organs and pollen grains in *Gentiana.* The strong influence of the temperature-induced floral closure on reproductive fitness is well proven for *G. straminea* (He & al. 2006).

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