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FLOWERING PHENOLOGY MAY SHAPE HYBRIDIZATION PATTERNS OF HAWTHORN (*CRATAEGUS* L.) SPECIES

Thomas KUHN, Eszter RUPRECHT
Babeș-Bolyai University, Hungarian Department of Biology and Ecology, Republicii str. 42,
RO-400015 Cluj-Napoca, Romania
e-mail: kuhnthomas89@yahoo.com

Abstract: Asynchronous flowering phenology is an important prezygotic barrier to hybridization, especially in the case of sympatric species, while the degree of overlap in flowering can influence hybridization odds and shift introgression. In our study, we investigated the flowering phenology of three sympatric *Crataegus* species and their hybrids in Transylvania, Romania. *C. laevigata* flowered first at the end of April, followed by *C. rhipidophylla* and the hybrid taxa *C.* × *subsphaerica* and *C.* × *media*, *C.* × *macrocarpa* 8 days later, while *C. monogyna* flowered last, 3 days later. The parental species of the most frequent hybrid taxa *C.* × *subsphaerica* have been found to have the greatest overlap in their flowering, while hybrids of *C. laevigata*, which overlap narrowly in their flowering with the other two species, are rare. Interestingly, all three hybrid taxa overlapped almost perfectly in their flowering phenology with that of *C. rhipidophylla*, and except for *C.* × *media*, hybrid taxa are not intermediate in the timing of their flowering relative to their parents. Our results provide evidence that distribution patterns and frequency of *Crataegus* hybrids in the landscape are at least partially shaped by the parental species' overlap in flowering phenology, which influences hybridization odds and may shift introgression towards one of the parental species.

Keywords: *Crataegus monogyna*, *Crataegus rhipidophylla*, *Crataegus laevigata*, hybrid, flowering asynchrony, flower opening, protogynous flowers

Introduction

Interspecific hybridization is widespread among Angiosperms and more common in certain families such as Betulaceae, Onagraceae, Orchidaceae, Rosaceae and Salicaceae [1]. However, for genetically diverging populations to become or remain isolated, either prezygotic or postzygotic barriers are required. Prezygotic barriers prevent genetic exchange between previously interbreeding populations, but if fertilization does happen, postzygotic barriers can prevent hybrids from reproducing. Prezygotic barriers usually impede fertilization between species and include several pre-pollination barriers, e.g. mechanical, ecogeographical, temporal, or the isolation of pollinators, all preventing pollen transfer. However, if transfer of pollen does occur, fertilization is still not guaranteed if gametic incompatibilities act as post-pollination prezygotic barriers [27]. While the cumulative effect of various reproductive barriers can lead to almost complete impairment of interspecific gene-flow, early acting barriers will contribute more to reproductive isolation [26, 27]. Thus, asynchrony in flowering phenology can be considered the most important prezygotic barrier to prevent interspecific hybridization of sympatric, closely related species. This mechanism can impede cross pollination almost completely if there is no overlap in opened, receptive flowers, or it is insignificant between the species [22, 27]. Sequential, non-overlapping flowering reduces competition for pollinators and promotes speciation. Asynchronous flowering is reported to influence introgression, which represents the gene-flow from one species to another by repeated backcrossing of an interspecific hybrid with one of its parental species [18, 35]. The effect of asynchronous flowering phenology on the likelihood of interspecific hybridization has also been investigated and discussed in numerous studies, and the degree of overlap in flower-opening is expected to correlate positively with the odds of hybrid formation [24, 22, 27]. In the case of hybridizing protandrous species, the species that flowers earlier is usually the maternal parent, and if hybrids are intermediate in their flowering phenology between parent taxa, introgression is expected to shift the flowering phenology of offspring towards the earlier flowering species [20, 36]. In contrast, in the case of hybridizing protogynous species, in the short period of flowering overlap, the later flowering species has a greater chance of receiving pollen from the earlier flowering species, as the anthers do not yet release pollen, while the stigma is already receptive [28, 23].

Large-scale interspecific gene-flow, as a result of frequent hybridization and introgression, has long been acknowledged as an important characteristic of the genus *Crataegus* L. The frequency and wide distribution of putative F1-hybrids and introgressant backcrosses has confused taxonomists for decades, and the variety of apomictic microspecies in existence has created numerous nomenclatural and taxonomical problems [7, 8]. The resulting genetic and morphologic gradient has blurred species boundaries, and the exact number of species is still debated, and estimated between 150 and 1500 taxa worldwide [7, 8]. Reasons for this increased hybridization potential and the persistence of hybrid swarms can be multiple; however, most experts suggest that land-use change and habitat fragmentation in the past centuries, combined with the effect of climate change and weakened ecogeographical barriers between sympatric or allopatric species, facilitated the genetic exchange between more or less distinct genetic lineages [8, 14]. Beside numerous morphometric studies focusing on hybrid identification and the study of introgression [3, 13, 5, 6, 8, 15], genetic research also provided evidence of introgression between taxa [12, 11, 2], while cytological research shed light on the complexity of the breeding system in *Crataegus* [31, 34]. Studies evidenced that diploid species such as *C. monogyna* and *C. laevigata* are obligate outcrossers, with selfing being extremely rare in their case, while hybrids being mostly polyploid, which are predominantly pseudogamous apomicts reproducing asexually [30, 34]. Thus, fertilization of an individual egg cell belonging to a species with hybrid pollen is expected to result in more viable offspring than the opposite. Considering that all *Crataegus* species have protogynous flowers, with female sexual organs reaching maturity earlier than the male, the likelihood of interspecific pollen transfer from earlier flowering (being the pollen donor) towards later flowering species (being the mother plant) may shift introgression, favouring later flowering species as the maternal parent [28, 23].

Data about flowering phenology in European *Crataegus* species are scarce, and most studies include only *C. monogyna* [19], providing little detail about the length and dynamics of flowering in other *Crataegus* species and no data about their natural hybrids is available [10, 32]. The aim of our study was to compare the flowering phenology of three sympatric *Crataegus* species (*C. laevigata* (Poir.) DC., *C. monogyna* Jacq., *C. rhipidophylla* Gand.) and their natural hybrids (*C.* × *subsphaerica* Gand., *C.* × *media* Bechst., *C.* × *macrocarpa* Hegetschw.), which might give us some insight about the formation and subsistence of *Crataegus* hybrids within a landscape of highly heterogeneous structure in Western Transylvania, Romania.

Based on the known distribution and genetic patterns of European *Crataegus* populations [8] and the effect of flowering phenology on interspecific hybridization of protogynous species

[27], we formulated the following hypothesis: (1) flowering time between *Crataegus* species and their natural hybrids partially overlap; (2) the degree of overlap in flowering time between species correlates with the known frequency of their putative hybrids across the landscape.

Material and Methods

Study area and selection of specimens

For the study of flowering phenology, *Crataegus* specimens belonging to three species and three hybrids (see below) were selected in the proximity of Florești ("Cetatea Fetei" hill, Cluj county, Transylvania, Romania) in April 2020. Our study site included a mosaic of semi-natural grasslands (basiphilous dry grasslands belonging to the *Cirsio-Brachypodion pinnati* alliance) and oak-hornbeam forests (*Lathyro hallersteinii-Carpinenion* Boşcaiu et al. 1982, and *Galio schultesii-Carpinenion* Täuber 1992 alliances) situated on a predominantly south-facing hillside characterized by slopes with moderate inclination (0 – 15°), and altitudes between 380 – 550 m. The study site is surrounded by rural and urban settlements and agricultural lands (Figure 1). The study area is characterized by a temperate continental climate with a mean annual temperature of 8.6 °C, mean annual precipitation of 568 mm and maximum precipitation occurring during summer months (based on data between 1923 and 2014, Cluj-Napoca Meteorological Station; http://www.ecad.eu).

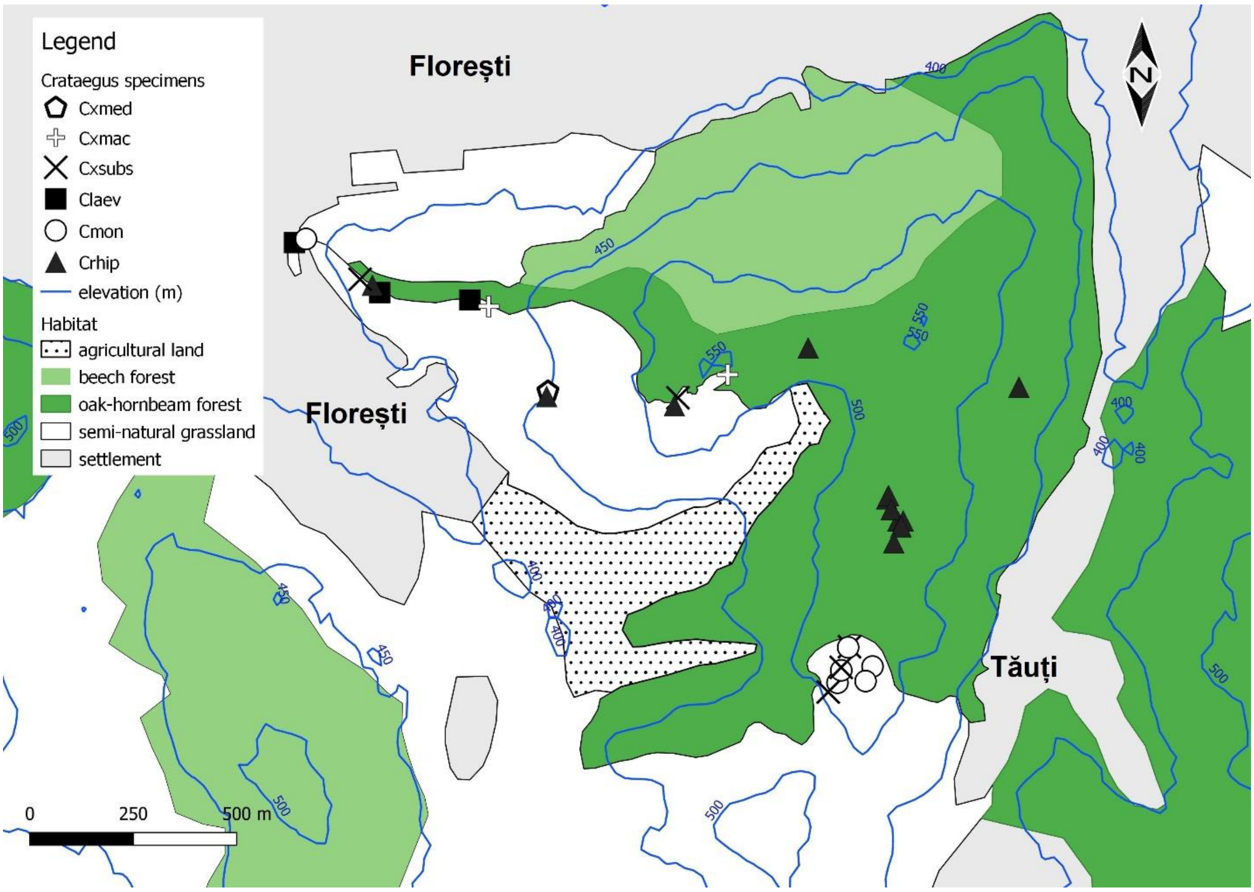

Fig. 1: Distribution of *Crataegus* specimens included in the study of flowering phenology, at Florești ("Cetatea Fetei" – hill, Cluj county, Romania. Cmon – *Crataegus monogyna*, Crhip – *Crataegus rhipidophylla*, Claev – *C. laevigata*, Cxsubs – *Crataegus* × *subsphaerica*, Cxmac – C. × *macrocarpa*, Cxmed – *C.* × *media*. Habitat classification is based on field observations and aerial photographs

Because of the difficulty of precise identification of *Crataegus* taxa before flowering [7, 8], data collection was based on precisely identified and marked specimens belonging to different taxa in a previous study [15]. Identification was based on generative shoots with intact leaves, stipules and ripe fruits with sepals, and some individuals were identified in late May 2020. For the study of flowering phenology, only mature, 2-3 m high, fertile specimens were selected. Taxa included in the present study were *Crataegus monogyna* (6 specimens), *C. rhipidophylla* (11 specimens), *C. laevigata* (3 specimens), *C.* × *subsphaerica* (syn., *C.* × *kyrtostyla* - primary hybrid of *Crataegus monogyna* and *C. rhipidophylla*) (5 specimens), *C.* × *macrocarpa* (2 specimens) (primary hybrid *C. laevigata* and *C. rhipidophylla*) and *C.* × *media* (1 specimen) (primary hybrid of *C. monogyna* and *C. laevigata*).

The frequency and distribution of the enumerated *Crataegus* taxa has been investigated in Western Romania, including a detailed survey of the study site and its surroundings in a previous study [15]. From lowland to foothill broad-leaved forests surrounding the Apuseni Mountains, *C. rhipidophylla* is the most frequent *Crataegus* species, while *C. monogyna* × *C. rhipidophylla* hybrids (nothospecies *C.* × *subsphaerica*) is sporadic. In grasslands and other open habitats in the same region, the hybrid taxon *C.* × *subsphaerica* is the most frequent taxon, although most specimens are highly introgressed forms with *C. monogyna*, while 'pure' *C. monogyna* is only approximately half as frequent as the hybrid. 'Pure' *C. monogyna* is most common in grasslands on the Transylvanian Plain and the Western-Hills surrounding the Apuseni Mountains. It should be noted that *C. rhipidophylla* is also sporadically present in grasslands surrounded by broad-leaved forests. *C. laevigata*, *C.* × *media* and *C.* × *macrocarpa* are comparatively rare in the Transylvanian Basin including our study site; however, *C. laevigata* is much more frequent in the forests of the lowland areas and hills situated west of the Apuseni Mountains. [15].

Data collection

For the study of flowering phenology, selected *Crataegus* specimens belonging to six taxa were visited on six occasions from 24 April to 21 May 2020 (Appendix A1). Flowering phenology was scored on an ordinal scale ranging between 1–7, similar to the classification of flowering phenology applied by Mijnsbrugge *et al.* [19] (Table 1, Appendix A1). Score 1 represented flower buds fully enclosed by the sepals while score 7 mostly overblown flowers. A phenological score was assigned to a specimen if more than half of the flowers clearly presented the given phenological score.

Table 1: Scoring used for the classification of flowering phenology in the studied *Crataegus* taxa

Phenological score	Description
1	flower buds visible, petals not visible, enclosed by sepals
2	opening sepals, petals visible, green
3	opening sepals, petals visible, white
4	less than 50% of flower buds with opening petals, anthers and stamens visible
5	more than 50% of flower buds with opening petals, anthers and stamens visible
6	all flowers opened with less than 50% of flowers overblown
7	more than 50% of flowers overblown

Statistical analysis

To model the relationship between flowering phenology, time of observation and taxa identity, due to the ordinal nature of phenological score as the dependent variable, we used cumulative logistic regression with a log-link function. Phenological score represented the dependent variable, while taxa identity and number of days since first observation (included as integer number, with 1 as lowest value representing the first day of observation) were included as independent variables in the model. We additionally included altitude and the interaction term between taxa identity and days since first observation as independent terms in the model, however, these variables did not decrease the AIC-value (Akaike information criterion) significantly, so we decided to exclude them from the model. We decided not to include the habitat (forest, forest-edge grassland), in which the specimens were identified as an independent factor in the analysis, because certain taxa were found only in a particular habitat and only in the case of *C. rhipidophylla* was it possible to include specimens from both forests and grasslands and there was no difference between the phenology of forest and grassland specimens (results not shown). Because the presence of nonproportional odd structures was encountered in the data via scale-test, two restrictions were imposed on the model: Thresholds were set to be equidistant or equally spaced ($\theta_j - \theta_{j-1}$ = constant; j = 2 ⇾ J-1; θ - the threshold coefficient; J - phenological score) while the scale was allowed to differ between the taxa. With these settings, the assumption of proportional odds was met.

The time lag in days between different taxa reaching a given phenological score was calculated with the following formula:

$$D_{t1} - D_{t2} = (\beta_{t2} - \beta_{t1})/\beta_D$$

D is the day counting since day 1 of observation, *t1, t2* are the taxa, β_t is the estimated coefficient for the fitted model for a given taxa and β_D is the estimated coefficient for the number of days since first observation [19].

We performed post-hoc comparations on the cumulative logistic regression model to estimate if differences in phenological scores between pairs of *Crataegus* taxa where significant [9].

To predict and plot probabilities of reaching a given phenological score for the taxa in a certain point of time, the ggpredict-function was used from the “ggeffects”-package [17], and probabilities for each taxa reaching a given phenological score were plotted for 28 time-points (days). Other important functions used were the clm-function for the cumulative logistic regression analyses, the scale test-function and nominal test-function to test the proportional odds assumption, all from the “ordinal” package [9]. All statistical analyses were performed in R [25].

Results

Phenological score of flowering was significantly influenced both by taxa identity and the number of days since observations started, with phenological scores increasing with days since first observation (Table 2).

Table 2: Estimated parameters of the cumulative logistic regression model. All values, with the exception of p-values, represent log-odds ratios. Estimate – estimated beta coefficient, SE – standard error, z-value – the ratio of estimate and SE, p-values are defined on a 95% confidence interval, Day – the number of days since observation started, Cmon – *Crataegus monogyna*, Crhip – *Crataegus rhipidophylla*, Cxsubs – *Crataegus* × *subsphaerica*. Cxmac (*C.* × *macrocarpa*) and Cxmed (*C.* × *media*) were not included in the analysis

Coefficients	**Estimate**	**SE**	**z-value**	**p-value**
Cmon	-11.5973	3.9672	-2.923	0.00346
Crhip	-8.2547	2.8051	-2.943	0.00325
Cxsubs	-8.727	3.0237	-2.886	0.0039
Day	1.0541	0.3794	2.778	0.00546
log-scale coefficients				
Cmon	-0.226	0.495	-0.457	0.648
Crhip	0.5281	0.3842	1.375	0.169
Cxsubs	0.653	0.4109	1.589	0.112
Threshold coefficients				
threshold	-6.247	2.106	-2.966	
spacing	5.068	1.825	2.777	

**Crataegus laevigata* (Claev) is the standard (estimated parameter for Claev = 0) to which the other taxa are compared

Based on the model`s predictions, in the case of all seven phenological scores, the highest probability of a specimen being in the particular phenological stage peaks earliest for *C. laevigata*, followed by *C. rhipidophylla* and *C.* × *subsphaerica*, which are comparatively close in timing of their flowering, with *C. monogyna* being the last taxa reaching the particular stage (Figure 2). The relative differences between the taxa in reaching with the highest probability a particular phenological score is comparatively similar for all seven phenological stages (Figure 2). The highest probability for the first flowers to open (for a specimen to reach the 4^{th} phenological score) is around the sixth day (corresponding to 29 April 2020) for *C. laevigata*, the 13^{th} and 14^{th} day (corresponding to 6 May 2020 and 7 May 2020, respectively) for *C. rhipidophylla* and *C.* × *subsphaerica*, and the 17^{th} day (10 May 2020) for *C. monogyna* (Figure 2). Furthermore, the highest probability for most of the flowers to be open (for a specimen to reach the 5^{th} phenological score) is around the 12^{th} day (corresponding to 5 May 2020) for *C. laevigata*, the 18^{th} and 19^{th} day (corresponding to 11 May 2020 and 12 May 2020, respectively) for *C. rhipidophylla* and *C.* × *subsphaerica* , and the 22^{nd} day (corresponding to 15 May 2020) for *C. monogyna* (Figure 2).

Based on the model β – coefficients, the time lag in reaching the same phenological score was 11 days between *C. laevigata* and *C. monogyna*, 7.8 days between *C. laevigata* and C. *rhipidophylla* and 8.3 days between *C. laevigata* and *C.* × *subsphaerica* , with *C. laevigata* flowering the earliest compared to all the other taxa. In addition, the time lag between *C. monogyna* – *C. rhipidophylla* was 3.2 days with *C. rhipidophylla* flowering first, *C. rhipidophylla* – *C.* × *subsphaerica* 0.5 days with *C.* × *subsphaerica* flowering first, *C. monogyna* – *C.* × *subsphaerica* 2.8 days with *C.* × *subsphaerica* flowering first.

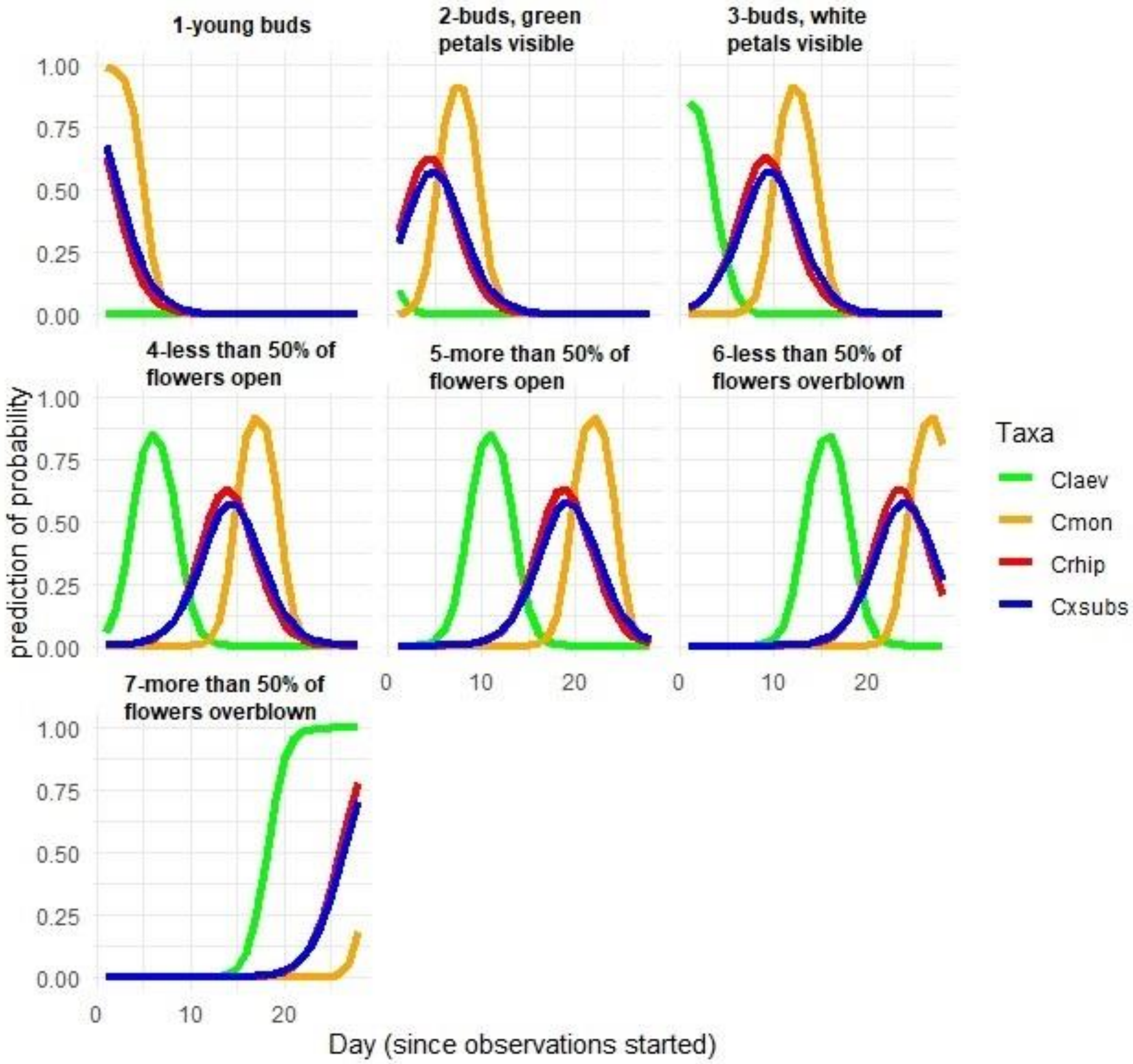

Fig. 2: Predicted probabilities for the seven (1-7) phenological scores of flowering, of four *Crataegus* taxa (abbreviations are given in Table 2), estimated for 28 time points of observation (1 - 28 days since observation started)

Post-hoc pairwise comparations on the cumulative logistic regression model revealed that there was a significant difference between the phenological scores of all the *Crataegus* taxa, except between *C. rhipidophylla* and *C.* × *subsphaerica* (Appendix A2).

Although *C.* × *macrocarpa* and *C.* × *media* were not included in the analysis because of the low number of specimens identified in their case, both nothospecies followed closely the flowering phenology of *C. rhipidophylla* in the study site (Appendix A1).

Discussion

Based on our results, there is a significant difference in the peak of flowering between the investigated species and their putative hybrids, however, some temporal overlap between opened and not yet overblown flowers exists between each taxon (*C. laevigata*, *C. monogyna*, *C.*

rhipidophylla, *C.* × *subsphaerica*). Thus, both our raw data and model predictions confirm our first hypotheses about the existence of overlap in flowering, which offers an opportunity for gene exchange between each of the studied taxa. *Crataegus laevigata* flowers first, followed by both *C. rhipidophylla* and *C.* × *subsphaerica* approximately eight days later, and ultimately *C. monogyna* approximately 11 days after *C. laevigata*. This ranking of flowering time is in concordance with the findings of Eriksson and Ehrlén [10], who estimated the start of flowering for *C. laevigata* earlier in the year compared to *C. rhipidophylla* (referred to as *C. curvisepala*) and followed, ultimately, by *C. monogyna*. Furthermore, according to Thomas *et al.* [32], *C. laevigata* comes into flower around 8-14 days earlier relative to *C. monogyna*, with our day estimate falling within this range [16, 29]. In contrast, according to van Vliet *et al.* [33], both *C. laevigata* and *C. monogyna* first flower synchronously in the Netherlands. However, this estimate should be treated with care, because their multispecies analysis was based on large databases, with independent observations from multiple volunteers, spanning from 1868 till 2010, across a comparatively large area. Thus, these phenological observations cover various environments across an altitudinal gradient of approximately 300 m [33] and misidentification of *Crataegus* specimens is also likely, considering the numerous taxonomic and nomenclatural changes for this genus in the past centuries [8].

Furthermore, our results provide a potential explanation for the observed frequency and distribution patterns of hybrids/introgressants between the studied *Crataegus* species in Northwestern Romania. We expected the likelihood of pollen transfer and thus interspecific hybridisation to be higher between species with a wider overlap in flowering. The results of the present study confirmed our second hypothesis. Frequency of the investigated hybrids in the landscape correlate positively with the overlap in flowering of their parental taxa. *C.* × *media* hybrid is the rarest in the study region [15], with only one such hybrid specimen confidently identified in the study site, which equally incorporated both *C. monogyna* and *C. laevigata* characters (if highly introgressant forms are not counted). This pattern matches with our findings on flowering phenology, as the two parental taxa, *C. laevigata* and *C. monogyna*, overlap in their flowering for a very short period of time, and when the first *C. monogyna* flowers open, most of the *C. laevigata* flowers are overblown (Figure 3). Furthermore, *C.* × *macrocarpa* hybrids are also rare in the study region, however, they are more frequent in the larger region (Transylvania) relative to *C.* × *media* hybrids, if highly introgressed *C. monogyna*-like specimens are not accounted for [15]. Accordingly, the overlap in flowering between *C. laevigata* and *C. rhipidophylla*, the two parental taxa, was found to be narrow as well, although not to the extent as between *C. laevigata* and *C. monogyna*. Thus, the likelihood of pollen transfer thus hybridization between *C. laevigata* and *C. rhipidophylla* is also expected to be small. The overlap in flowering is the highest between *C. rhipidophylla* and *C. monogyna*, and correspondingly, their hybrid *C.* × *subsphaerica* is the most common *Crataegus* hybrid both in the study site and across Eastern and Central Europe [7, 8, 14, 15].

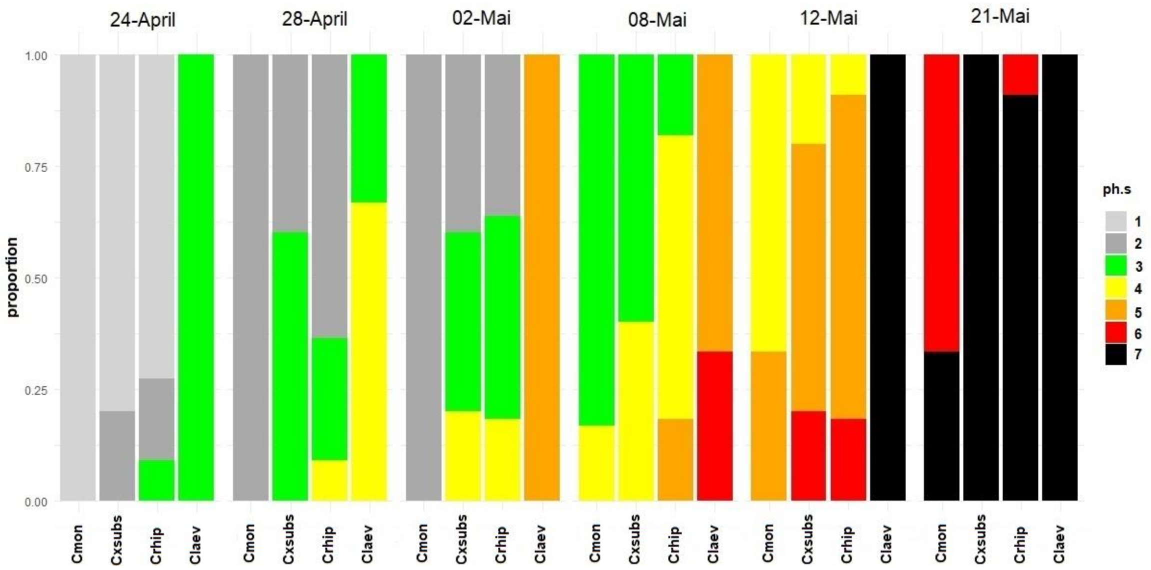

Fig. 3: Proportion of specimens of four *Crataegus* taxa with given phenological score of flowering, on six observation dates. ph.s – phenological score of flowering with first flowers opening at score 4 (yellow), species abbreviations are given in Table 2, the meaning of each score is described in Table 1

Considering the presumed effect of flowering asynchrony on the direction of gene-flow between protogynous species, *C. rhipidophylla* is expected to be more often the maternal parent in the case of *C.* × *macrocarpa* hybrids, while *C. monogyna* is expected to be more often the maternal parent in the case *C.* × *subsphaerica* and *C.* × *media* hybrids. Interestingly, the timing of flowering in the case of *C.* × *subsphaerica* and *C.* × *macrocarpa* hybrids was not intermediate between their parental species as expected, and in the case of both hybrids, it followed the flowering phenology of their *C. rhipidophylla*-parent, suggesting a dominant genetic control of flowering on behalf of this parent. Thus, pollen transfer and introgression from both *C.* × *subsphaerica* and *C.* × *macrocarpa* hybrids towards *C. rhipidophylla* as a maternal species should be more likely due to their synchronous flowering. In contrast, based on the intermediate flowering time of *C.* × *media* relative to its parental taxa *C. monogyna* and *C. laevigata*, the odds should favour the gene-flow from *C.* × *media* towards *C. monogyna* rather than in the opposite direction. In a previous morphometric study of *Crataegus* species and hybrids in Transylvania, Kuhn et al. [15] suggested a shift in introgression towards *C. monogyna* for *C.* × *media* based on morphometric evidence: the vast majority of *C.* × *media* specimens were *C. monogyna*-like in their morphology, and similar results were indicated by Byatt [3], Byatt [4], and Christensen [5] as well. However, it should be mentioned that only three *C. laevigata* specimens were identified in the present study, and *C. laevigata* being a suboceanic element is considered rare in Transylvania [8, 14, 15]. This distribution pattern would also favour a shift in introgression towards *C. monogyna* in the case of *C.* × *media* as suggested by Byatt [3]. Furthermore, in the case of the hybrid *C.* × *macrocarpa,* the maternal parent appears to be *C. rhipidophylla,* while introgression appears to shift towards this parent, especially in regions where *C. laevigata* is rare (personal observation), thus, their similar flowering phenology appears to determine this gene-flow pattern. In the case of *C.* × *subsphaerica* hybrids, while phenology should shift introgression towards *C. rhipidophylla,* numerous studies contradict this, and evidence that introgression is directed predominantly towards *C. monogyna*, with *C. monogyna* being the maternal parent in the case of backcrosses [5, 6, 7, 15]. Thus, in the case of *C.* × *subsphaerica*'s hybrid zone, other factors besides phenology may potentially influence backcrossing of hybrids. Some of these factors that have to be considered are: (1) distribution patterns (lower frequency in the landscape of *C. rhipidophylla*), habitat differentiation [15], lower abundance of flowers per specimen and thus lower pollen availability of *C. rhipidophylla*, and directional movement of pollinators, which may all shift the likelihood of pollen transfer from the hybrid towards *C. monogyna*; (2) introgression that is more intensive towards *C. rhipidophylla*, as generally assumed; however, *C. rhipidophylla*-like introgressants are more difficult to identify [15]; (3) *C. rhipidophylla* has a more effective, yet unidentified pre- or postzygotic hybridization barrier than *C. monogyna*.

Appendix A1: Phenological scores of *Crataegus* specimens from the study site, near Florești (Romania, Cluj county). Cmon – *Crataegus monogyna*; Crhip – *Crataegus rhipidophylla*; Cxsubs – *Crataegus* × *subsphaerica*; Cxmac - *C.* × *macrocarpa*; Cxmed - *C.* × *media*

nr.	Taxa	Habitat	Date					
			Apr-24	Apr-28	Mai - 02	Mai - 08	Mai - 12	Mai - 21
1	Claev	grassland	3	4	5	6	7	7
2	Claev	ecotone	3	3	5	5	7	7
3	Claev	ecotone	3	4	5	5	7	7
4	Cmon	grassland	1	2	2	3	4	6
5	Cmon	grassland	1	2	2	4	4	7
6	Cmon	grassland	1	2	2	3	4	6
7	Cmon	ecotone	1	2	2	3	5	7
8	Cmon	grassland	1	2	2	3	4	6
9	Cmon	grassland	1	2	2	3	5	6
10	Crhip	forest	1	2	2	4	5	7
11	Crhip	forest	1	2	2	4	5	7
12	Crhip	forest	2	3	4	4	5	7
13	Crhip	forest	1	2	3	4	5	7
14	Crhip	forest	1	2	2	4	5	7
15	Crhip	forest	1	2	3	3	5	7
16	Crhip	forest	1	2	3	4	5	7
17	Crhip	forest	1	3	3	4	5	7
18	Crhip	ecotone	2	4	3	5	6	7
19	Crhip	grassland	3	3	4	5	6	7
20	Crhip	ecotone	1	2	2	3	4	6
21	Cxsubs	ecotone	1	3	4	4	6	7
22	Cxsubs	ecotone	2	3	3	4	5	7
23	Cxsubs	grassland	1	2	2	3	5	7
24	Cxsubs	grassland	1	2	2	3	4	7
25	Cxsubs	ecotone	1	3	3	3	5	7
26	Cxmac	ecotone	3	3	3	4	5	7
27	Cxmac	ecotone	3	3	3	3	5	7
28	Cxmed	grassland	1	2	3	4	5	7

1- flower buds visible, petals not visible, enclosed by sepals; 2 - opening sepals, petals visible, green; 3 - opening sepals, petals visible, white; 4 - less than 50% of flower buds with opening petals, anthers and stamens visible; 5 - more than 50% of flower buds with opening petals, anthers and stamens visible; 6 - all flowers opened with less than 50% of flowers overblown; 7 - more than 50% of flowers overblown

Appendix A2: The results of post-hoc pairwise comparations on the cumulative logistic model using an mvt-correction. (Claev – *Crataegus laevigata*; Cmon – *Crataegus monogyna*; Crhip – *Crataegus rhipidophylla*; Cxsubs – *Crataegus* × *subsphaerica*; *– statistically significant difference)

Species pair	**estimate**	**standard error**	**z-value**	**p-value**
Claev – Cmon	11.59	3.96	2.92	0.0124*
Claev – Crhip	8.25	2.80	2.94	0.0112*
Claev – Cxsubs	8.72	3.02	2.88	0.0134*
Cmon – Crhip	-3.34	1.32	-2.53	0.0380*
Cmon – Cxsubs	-2.87	1.30	-2.20	0.084
Crhip – Cxsubs	0.472	0.857	0.551	0.9005

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PERIOADA DE ÎNFLORIRE POATE MODIFICA TIPARELE DE HIBRIDIZARE A SPECIILOR DE PĂDUCEL (*CRATAEGUS* L.)

(Rezumat)

Înflorirea asincronă reprezintă o barieră prezigotică importantă pentru hibridizare, în special în cazul speciilor de plante cu tipar de răspândire simpatric. Gradul de suprapunere în perioada înfloririi poate influența probabilitatea hibridizării şi direcția introgressiei. În studiul prezent, s-a comparat fenologia de înflorire între trei specii de păducel și hibrizii interspecifici ai acestora în condiții de teren, în apropierea satului Florești din județul Cluj, România. Pe baza rezultatelor noastre, *C. laevigata* a înflorit cel mai devreme la sfârșitul lunii Aprilie, aproximativ 8 zile mai târziu au înflorit *C. rhipidophylla*, *C.* × *subsphaerica*, *C.* × *media*, *C.* × *macrocarpa*, iar *C. monogyna* a înflorit ultimul, aproximativ după încă 3 zile. Speciile parentale ale hibridului cel mai comun *C.* × *subsphaerica* au prezentat cea mai mare suprapunere în perioada de înflorire, iar hibrizii mai rari ai speciei *C. laevigata* s-au suprapus într-o măsură mult mai redusă în perioada înfloririi. Toți cei trei hibrizii s-au suprapus cu *C. rhipidophylla* în perioada de înflorire, iar cu excepția hibridului *C.* × *media*, nu s-au poziționat intermediar privind perioada înfloririi față de speciile lor parentale. Rezultatele studiului confirmă, că abundența hibrizilor de păducel este influențată cel puțin parțial de gradul de suprapunere a perioadelor de înflorire a speciilor parentale, deoarece ea influențează probabilitatea hibridizării și direcția introgresiei în cazul populațiilor mixte sau învecinate ale celor trei specii de păducel studiate.

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