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Correlations among morphological traits of sweet chestnut (*Castanea sativa* Miller) from Bosnia and Herzegovina

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Abstract

Sweet chestnut forests in Bosnia and Herzegovina are underrepresented in the total forest fund, and their area is declining today. The research aims to determine the correlation relations of morphological traits of chestnut leaves, fruit and cupule in the population of Bosnian Krajina (northwestern Bosnia and Herzegovina) to undertake selection and breeding activities. Eleven traits of fruits, leaves and cupules were measured, seven assessed and six calculated. A mutual correlation for the researched traits was calculated using the Pearson correlation coefficient *r*. When the fruit traits and cupule traits were compared, the needle length showed a statistically significant correlation with most of the fruit traits. The traits of the number of fruits in the cupule and needle length had a statistically significant correlation coefficient with leaf blade width and leaf petiole length. The correlated traits can be distinguished as important for further selection and breeding of sweet chestnuts. The research results indicate that the genetic material of sweet chestnut is a rich source of genetic diversity and can be used in selection to obtain new varieties and cultivars in Bosnia and Herzegovina and for the protection of the indigenous gene pool.

Key words

correlation coefficient, morphological traits, sweet chestnut

Introduction

The sweet chestnut (*Castanea sativa* Miller) spreads along the Mediterranean region, from the Caspian Sea to the Atlantic Ocean (Fernández-López and Alía 2003), and is a valuable species in the countries of southern Europe, including Bosnia and Herzegovina.

The species is thought to have survived in several refuges during the last ice age in southern Europe, northeastern Turkey and the Caucasus (Pereira-Lorenzo et al. 2010; Lang et al. 2007; Conedera et al. 2004).

In Bosnia and Herzegovina, this species has a small disjunctive areal. It can be found in the northwestern part (around Cazin, Velika Kladuša, Bužim, Bosanska Kostajnica), south (Neretva and Rama valleys, around Jablanica, Konjic, Prozor, Mostar and Trebinje) and northeastern part (around Srebrenica, Goražde and Bratunac).

There are not much studies on this species in Bosnia and Herzegovina so far. Mujić et al. (2010) and Skender (2010) studied pomological traits, Mujić et al. (2006) researched chemical composition of the fruit, Treštić et al.

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(2009) studied diseases, and Mujagić-Pašić and Ballian (2012, 2013a, 2013b), Ballian et al. (2012–2013), Daničić et al. (2018) and Tuğ et al. (2020) researched morphological traits.

Several authors used correlation analysis to determine the correlation among traits of sweet chestnut. Atar and Turna (2018) obtained high values of correlation coefficient for fruit traits from eight natural populations of sweet chestnut in Turkey. Grygorieva et al. (2018) investigated 28 genotypes of sweet chestnut in Ukraine in a sample of 800 fruits. The obtained results indicated a high correlation of the investigated traits. Also, Serdar et al. (2006) researched the width, length and surface of leaves in sweet chestnut populations in Turkey. The obtained results showed a high correlation among these leaf traits. Ertan (2007) found significant correlations among the pomological traits of fruits and among the characteristics of leaves of the sweet chestnut populations in western Turkey. Yıldız et al. (2009) studied the correlations among length, width, thickness and weight of the chestnut fruit from the populations of the Black Sea region of Turkey and found correlations among them. There have been no studies on the correlations among the morphological traits of fruits, leaves and cupules of sweet chestnut in Bosnia and Herzegovina so far.

This research aims to determine the correlation of the analysed morphological traits in a significant Bosnia–Herzegovinian sweet chestnut population. It is expected that the results of this research would contribute to the knowledge of morphological traits of sweet chestnut in Bosnia and Herzegovina and help in the choice of activities for the selection, breeding and protection of its indigenous gene pool.

Material and methods

Within the natural populations of sweet chestnut (*C. sativa* Miller) in Bosnian Krajina (northwestern Bosnia and Herzegovina), in the year 2009, 16 subpopulations and 140 trees, as well as six individuals of the cultivar 'Pelasore' at three localities, Bužim, Cazin and Velika Kladuša, were selected (Tab. 1). By the method of random selection, in 2010, 30 healthy fruits, 30 leaves and 10 cupules per tree were collected (leaves in July and fruits and cupules in October at full maturity). A total of 3,631 fruits, 4,020 leaves and 1,315 cupules were collected and analysed. All measurements were based on the methods used worldwide for the morphological analyses of sweet chestnut (UPOV, 1989). The methods of Furonez-Pérez and Fernández-López (2009), Serdar et al. (2011) and Pereira-Lorenzo et al. (1996) were used to calculate the ratio of individual characteristics of fruits, leaves and cupules.

Table 1. List of researched subpopulations

Location	Subpopulation label	Longitude	Latitude	Altitude (m)
Vrnograč (Velika Kladuša)	V1	45°10′11″	15°56′07″	268.80
	V2	45°09′56″	15°58′13″	262.10
	V3	45°09′54″	15°58′12″	252.10
	V4	45°10′06″	15°58′13″	258.60
	V5	45°10′02″	15°58′19″	285.20
Zaradostovo (Bužim)	B6	45°04′44″	16°03′42″	282.60
	B7	45°04′44″	16°03′53″	271.10
	B8	45°04′40″	16°03′43″	319.60
	B9	45°04′35″	16°03′38″	323.30
Gornja Koprivna (Cazin)	C10	44°58′47″	15°57′35″	503.00
	C11	44°58′44″	15°57′40″	490.10
	C12	44°58′49″	15°57′39″	484.10
	C13	44°58′59″	15°57′45″	498.80
	C14	44°59′08″	15°57′46″	486.40
	Ck15	44°58′52″	15°57′41″	498.60
	Ck16	44°58′47″	15°57′35″	492.00

Morphological traits of sweet chestnut fruits

In this research, the following traits of sweet chestnut fruits were measured: fruit length (FL; in mm), fruit width (FW; in mm), fruit thickness (FT; in mm), hilum width (HW; in mm), hilum length (HL; in mm) and fruit weight (FWG; in g). The following traits were assessed according to the international descriptor for sweet chestnut (UPOV, 1989): fruit shape (FS); fruit embryony (FE) and penetration of seed coat into embryo (PSCiE). The following parameters were calculated (according to the method given by Furonez-Pérez and Fernández-López 2009): FL/FW ratio, relative size of the hilum (RSH): HW × HL.

FWG was measured in a fresh condition immediately after fruit collection, using an analytical balance

with an accuracy of 0.01 g. Other traits were measured using a digital caliper with an accuracy of 0.01 mm.

Morphological traits of sweet chestnut leaves

The following traits of sweet chestnut leaves were measured: leaf blade length (LBL; in mm), leaf blade width (LBW; in mm) and leaf petiole length (LPL; in mm). The following traits were assessed according to the international descriptor for sweet chestnut (UPOV, 1989): leaf blade shape (LBS) (sharp, blunt, serrated) and leaf edge shape (LES) (mucronate, dentate). The following parameters were calculated according to method given by Serdar et al. (2011): total leaf length (TLL) (TLL = LBL + LPL), LBW/LBL ratio and LBW/TLL (blade + petiole).

Measurements were performed using a graph paper with an accuracy of 1/10 mm.

Morphological traits of sweet chestnut cupules

The following traits of sweet chestnut cupules were measured: cupule width (CW; in mm) and cupule height (CH; in mm). The following traits were assessed according to the method given by Pereira-Lorenzo (1996): needle length (NL) (long, medium, short) and number of fruits in cupule (NfiC). The following parameters were calculated according to the method given by Serdar et al. (2011): relative size of cupule (RSC) (CW × cupule height CH).

The measurement of these traits was performed using a digital caliper with an accuracy of 0.01 mm.

Statistical data processing

A linear correlation was calculated using Pearson correlation coefficient *r*. All investigated traits of fruits, as well as all investigated traits of leaves and all investigated traits of cupules were mutually compared. Data processing was done using the packages XLSTAT 2011 and STATISTICA 10.

Results

In this paper, the investigated traits of fruits, leaves and cupules of sweet chestnut were analysed to determine the existence or absence of significant correlations and to find whether the regression factor was positive or negative at the level of significance (Sig. < 0.0001) for most analysed traits.

Correlation among the investigated fruit traits

The correlation coefficient (*r*) was determined by mutually comparing each investigated fruit trait with eachThe obtained results are shown in Table 2. Statistically significant and highest values of the correlation coefficient are marked in red. The values of the correlation coefficient for the fruit traits range from $r = -0.521$ to 0.923 for individual compared traits. Statistically significant positive and high values of the coefficient of variability were found in all measured fruit traits (FL, FW, FT and FWG, as well as HW and HL), which is very important in the breeding of sweet chestnut. In contrast to the measured traits, the assessed trait of FE showed a statistically significant positive correlation only with the trait FS ($r = 0.070$) and did not represent a significant trait for sweet chestnut selection and breeding. The highest values of correlation coefficient were found in most of the investigated fruit traits. For the compared traits, the highest values of the correlation coefficient were found for the following: relative hilum size and HW ($r = 0.923$) and relative hilum size and HL ($r = 0.915$). Statistically significant negative correlations with the highest value of the correlation coefficient were found in the following compared traits: FS with FW ($r = -0.521$) and FS with HL ($r = -0.403$). Traits that were neither in positive nor in negative statistically significant correlation were: FS and HL ($r = -0.009$), FE and FL ($r = 0.007$), FE and FW ($r = -0.014$), FE and FT ($r = -0.031$), FE and HW ($r = -0.012$), FE and HL ($r = -0.012$), FE and fruit mass ($r = -0.023$), PSCiE and FL ($r = -0.006$), PSCiE and FW ($r = -0.029$), PSCiE and HL ($r = -0.028$), FS and FE ($r = 0.030$) and relative hilum size and FE ($r = -0.015$).

Correlation among the investigated leaf traits

The correlation coefficient (*r*) for leaf traits was determined by comparing the investigated leaf traits with each other, and the obtained results are shown in Table 3. Statistically significant and the largest positive and negative values obtained are marked in red. The values of the correlation coefficient for the leaf ranged from −0.621 to 0.989 for individual compared leaf traits. The highest positive and statistically significant value of the correlation coefficient was shown by the traits of LBL and LBW ($r = 0.616$), and LBW/LBL and LBW/TLL, where the correlation coefficient was 0.989. The highest negative value of the correlation co-

Table 2. Correlation coefficients and labels of significant differences for the fruit traits

Variables	FL	FW	FT	HW	HL	FWG	FS	FE	PSCiE	FL/FW	RSH
FL	1	0.766****	0.567****	0.557****	0.531****	0.794****	−0.173****	0.007	−0.006	0.139****	0.575****
FW		1	0.617****	0.666****	0.725****	0.877****	−0.054***	−0.014	−0.029	−0.521****	0.734****
FT			1	0.651****	0.582****	0.796****	−0.120****	−0.031	−0.042*	−0.198****	0.652****
HW				1	0.723****	0.699****	−0.047**	−0.012	−0.033**	−0.278****	0.923****
HL					1	0.694****	−0.009	−0.012	−0.028	−0.403****	0.915****
FWG						1	−0.103****	−0.023	−0.045**	−0.290****	0.748****
FS							1	0.070****	0.418****	−0.149****	−0.033**
FE								1	0.133****	0.030	−0.015
PSCiE									1	0.035*	−0.033**
FL/FW										1	−0.351****
RSH											1

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. FL, fruit length; FW, fruit width; FT, fruit thickness; HW, hilum width; HL, hilum length, FWG, fruit weight; FS, fruit shape; FE, fruit embryony; PSCiE, penetration of seed coat into embryo.

Table 3. Correlation coefficients and labels of significant differences for the leaf traits

Variables	LBL	LBW	LPL	LBS	LES	TLL	LBW/LBL
LBL	1	0.616****	0.294****	−0.035	−0.001	0.989****	−0.366****
LBW		1	−0.177****	0.036	−0.012	0.553****	0.492****
LPL			1	−0.041	−0.114****	0.434****	−0.527****
LBS				1	0.185****	−0.039	0.083
LES					1	−0.019	−0.022
TLL						1	−0.427
LBW/LBL							1

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. LBL, leaf blade length; LBW, leaf blade width; LPL, leaf petiole length; LBS, leaf blade shape; LES, leaf edge shape; TLL, total leaf length.

efficient (−0.621) was shown by the traits of LBW/TLL and LPL.

Leaf traits which had a low value of the correlation coefficient (around zero) were LES and LBL ($r = -0.001$), LES and LBW ($r = -0.012$), LES and TLL ($r = -0.019$), LBW/LBL and LES ($r = -0.022$) and LBW/TLL and LES ($r = -0.001$).

Correlation among the investigated cupule traits

Table 4 shows the correlation coefficients (r) among all investigated traits of the cupule. Statistically significant correlations are marked in red. Statistically significant and high coefficients of positive and negative correlation of the compared traits of the cupule are shown in Table 4 and marked in red. The value of the correlation coefficient for the investigated traits of the cupule ranged from $r = -0.069$ to 0.935. All compared traits showed positive values of the correlation coefficient, which indicates that the increase of one variable affects the increase of another variable, for example, the size of the cupule is correlated with CW, CH and NfiC. The exception is the trait of NL, which was negatively correlated with CW and NfiC. The largest positive and statistically significant values of the correlation coefficient were found for the following traits: cupule size and CH ($r = 0.935$) and cupule size and CW ($r = 0.916$). The trait of NL showed a statistically significant negative correlation with NfiC ($r = -0.069$). The correlations

between NL and CW ($r = -0.042$) and cupule size and NL ($r = 0.007$) were not statistically significant.

Table 4. Correlation coefficients and labels of significant differences for the cupule traits

Variables	CW	CH	NfiC	NL	RSC
CW	1	0.750****	0.613****	−0.042	0.916****
CH		1	0.508****	0.076**	0.935****
NfiC			1	−0.069*	0.584****
NL				1	0.007
RSC					1

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. CW, cupule width; CH, cupule height; NfiC, number of fruits in cupule; NL, needle length.

Correlations among the investigated traits of fruit and leaf

The correlation coefficient (r) for fruit and leaf was determined by comparing the investigated traits of fruit and leaf with each other, and the obtained results are shown in Table 5. Statistically significant and highest values of the correlation coefficient are marked in red. The values of the correlation coefficient for fruit and leaf range from $r = -0.624$ to 0.989 for individual compared traits. The highest statistically significant values of the correlation coefficient were found between the measured fruit traits (FL, FW, FT, FWG, HL and HW) and measured traits of the leaf (LBW, LPL), while the shape of the leaf base showed a statistically significant correlation only with FS and FE. Statistically significant and highest positive correlations were found in the measured fruit traits and LPL, while statistically significant negative values of the correlation coefficient were shown by the measured fruit traits and LBW. Statistically significant negative correlations with the highest value of the correlation coefficient were found in the compared traits: HL and LBW ($r = -0.187$), FW and LBW ($r = -0.186$), relative hilum size and LBW ($r = -0.182$), FL and LBW ($r = -0.178$) and FWG and LBW ($r = -0.170$). A trait that did not show a statistically significant correlation for most of the compared fruit traits was the leaf base shape, except with the FS ($r = -0.053$) and FE ($r = -0.037$).

Correlation among the investigated traits of fruit and cupule

Table 6 shows the correlation coefficients (r) between the compared traits of fruit and cupule, with statistically significant correlations marked in red. The value of the correlation coefficient for the compared traits of the fruit and the cupule ranges from $r = -0.138$ to 0.210. The highest statistically significant values of correlation with the investigated traits of fruit showed the trait of the cupule NL, except with the trait of FE where no statistical significance was observed. The CH trait did not show statistically significant correlations with the

Table 5. Correlation coefficients and labels of significant differences for the traits of fruit and leaf

Variables	LBL	LBW	LPL	LBS	LES	TLL	LBW/LBL	LBW/TLL
FL	−0.070	−0.178	0.225	0.011	−0.080	−0.031	−0.131	−0.165
FW	−0.092	−0.186	0.211	0.008	−0.062	−0.053	−0.119	−0.153
FT	−0.073	−0.156	0.161	−0.001	−0.033	−0.043	−0.107	−0.131
HW	−0.079	−0.167	0.170	0.030	−0.023	−0.048	−0.113	−0.140
HL	−0.099	−0.187	0.171	0.014	−0.057	−0.066	−0.113	−0.143
FWG	−0.060	−0.170	0.215	0.002	−0.031	−0.023	−0.133	−0.163
FS	−0.024	−0.001	−0.040	−0.053	0.039	−0.029	0.015	0.019
FE	−0.022	−0.039	−0.008	−0.037	0.001	−0.022	−0.015	−0.014
PSCiE	−0.002	−0.044	0.070	−0.007	−0.043	0.010	−0.055	−0.062
FL/FW	0.046	0.050	−0.029	0.006	−0.012	0.039	0.011	0.020
RSH	−0.086	−0.182	0.180	0.025	−0.030	−0.053	−0.122	−0.151

FL, fruit length; FW, fruit width; FT, fruit thickness; HW, hilum width; HL, hilum length, FWG, fruit weight; FS, fruit shape; FE, fruit embryony; PSCiE, penetration of seed coat into embryo; LBL, leaf blade length; LBW, leaf blade width; LPL, leaf petiole length; LBS, leaf blade shape; LES, leaf edge shape; TLL, total leaf length.

investigated fruit traits, except for the penetration of the seed coat into the cotyledon and the FL/FW ratio. The relative size of the hilum showed a statistically significant negative correlation ($r = -0.064$) only with the FL/FW ratio, while no statistically significant correlation was observed with other fruit traits.

Table 6. Correlation coefficients and labels of significant differences for the traits of fruit and cupule

Variables	CW	CH	NfiC	NL	RSC
FL	−0.087	−0.020	−0.077	0.134	−0.052
FW	−0.040	0.009	−0.031	0.210	−0.013
FT	−0.053	0.034	−0.058	0.180	−0.007
HW	−0.016	0.027	−0.055	0.174	0.018
HL	−0.012	0.002	−0.051	0.181	−0.001
FWG	−0.062	−0.004	−0.051	0.192	−0.032
FS	0.067	−0.030	0.023	0.153	0.013
FE	−0.046	−0.025	−0.025	−0.042	−0.033
PSCiE	−0.007	−0.069	−0.016	0.074	−0.040
FL/FW	−0.067	−0.056	−0.063	−0.138	−0.064
RSH	−0.022	−0.002	−0.057	0.177	−0.004

FL, fruit length; FW, fruit width; FT, fruit thickness; HW, hilum width; HL, hilum length, FWG, fruit weight; FS, fruit shape; FE, fruit embryony; PSCiE, penetration of seed coat into embryo; CW, cupule width; CH, cupule height; NfiC, number of fruits in cupule; NL, needle length.

Correlation among the investigated traits of leaf and cupule

The correlation coefficients (r) for leaf and cupule obtained by comparing each investigated leaf trait with each investigated cupule trait are shown in Table 7, with statistically significant correlations marked in red. The traits of LBL, leaf base shape, LES and TLL did not show a statistically significant correlation with cupule traits. Statistically significant and positive correlations were found for LBW with NfiC ($r = 0.064$) and LBW/TLL with NfiC. The trait LPL showed a negative and statistically significant correlation coefficient with CW, NfiC, cupule NL and relative hilum size.

Discussion

This paper presents the correlations between the investigated traits of fruit, leaf and cupule of sweet chestnut (*C. sativa* Miller) in the area of Bosnian Krajina (northwestern Bosnia and Herzegovina). Most of the investigated traits showed high values of the correlation coefficient, and the results are similar to previous studies of fruit, leaf and cupule morphology in sweet chestnut populations.

Atar and Turna (2018) studied the correlation of the traits of sweet chestnut fruit from eight natural populations (Adapazarı, Artvin, Aydın, Balıkesir, Bartın, Izmir, Kütahya and Sinop) in Turkey. They analysed four characteristics of the fruit: FL, FW, FT and shape of the fruit (length/width ratio). For the FL/FW ratio, they found a high value of the correlation coefficient, which varied between 0.8 and 0.9. Also, a statistically significant positive correlation was found at a significance level of 99% between the length, width, thickness and weight of 1,000 fruits.

Grygorieva et al. (2018) investigated 28 genotypes of 30-year-old sweet chestnut trees in Ukraine, which were produced from seeds from the Czech Republic, the Carpathians and Kyrgyzstan. In a sample of 800 fruits, the traits of fruit mass, FL, FW and FT, and HL and HW were measured. The obtained results indicated a high correlation between the following investigated traits: FW and FL ($r = 0.85$), FW and FWG ($r = 0.92$),

Table 7. Correlation coefficients and labels of significant differences for the traits of leaf and cupule

Variables	LBL	LBW	LPL	LBS	LES	TLL	LBW/LBL	LBW/TLL
CW	0.028	0.023	−0.081	−0.028	0.025	0.017	0.003	0.015
CH	−0.008	−0.009	−0.024	−0.036	−0.046	−0.010	−0.001	0.002
NfiC	0.031	0.064	−0.061	−0.007	0.022	0.022	0.045	0.054
NL	−0.036	−0.019	−0.143	0.021	−0.015	−0.052	0.000	0.017
RSC	0.014	0.010	−0.063	−0.031	−0.002	0.006	−0.002	0.008

CW, cupule width; CH, cupule height; NfiC, number of fruits in cupule; NL, needle length; LBL, leaf blade length; LBW, leaf blade width; LPL, leaf petiole length; LBS, leaf blade shape; LES, leaf edge shape; TLL, total leaf length.

FW and FT (r = 0.91), FW and HL (r = 0.68), FW and HW (r = 0.67), HL and HW (r = 0.94), FL and FWG (r = 0.94), FL and FT (r = 0.82), FL and HL (r = 0.70), FL and HW (r = 0.63), FWG and FT (r = 0.90), FWG and HL (r = 0.80), fruit mass and HW (r = 0.74) and FT and HL (r = 0.78). Similar results were obtained by Yıldız et al. (2009) on studying the correlations of length, width, thickness and weight of sweet chestnut fruit from the populations of the Black Sea region of Turkey, where they found statistically significant correlations of these traits. Ertan (2007) also found significant correlations between the pomological traits of the fruit (FL and FW, fruit height and FL, and fruit height and FW), as well as the morphological characteristics of leaves, LBL and LBW, LBW and LPL) in sweet chestnut populations in western Turkey. Serdar and Kurt (2011), based on the morphological parameters of the leaves, tried to determine the parameters important for distinguishing the genotypes of sweet chestnut in northern Turkey. In a study conducted by Zarfshar et al. (2010) in three chestnut populations in northern Iran, the variables leaf lamina length, leaf lamina width and LPL were measured, and leaf lamina length/width, leaf lamina length/LPL ratios were calculated. The traits of lamina length, lamina width and LPL showed higher values of the correlation coefficient compared to other measured traits. Statistically significant correlations of leaf traits were also found by Serdar et al. (2006) while examining the width, length and surface area of sweet chestnut leaves in populations in Turkey. The results of their research showed a high correlation between these traits, with the value of the correlation coefficient ranging from 0.95 to 0.98.

The most significant conclusion of this study is the high value of correlation coefficients for the traits of FL, FW, FT and FWG, HL and HW, leaf lamina length and width, TLL, CW and CH, and NfiC. Also, mutual correlations of traits of fruit, leaf and cupule showed statistically significant correlation values. Statistically most significant correlations were found between the measured fruit traits with leaf lamina width, LPL and NL on the cupule. High and statistically significant values of the correlation coefficient for the traits of NfiC and the length of the needles with the traits of leaf lamina width and petiole length were also observed. The highest positive correlation was shown by the measured traits of the fruit, and they can be used in further selection and breeding of this species in Bosnia and Herzegovina. The exception is the trait of FE, which was not statistically significantly related to other investigated traits of the fruit, and therefore, does not represent an important indicator of genetic variability of the studied populations. Since this is the first correlation analysis of the traits of the fruit, leaf and cupule of sweet chestnut in Bosnia and Herzegovina, it is necessary to expand the research to a larger number of samples in different habitats to draw more concrete conclusions.

The correlated traits can be distinguished as important for further selection and breeding of sweet chestnuts. The research results indicate that the genetic material of sweet chestnut is a rich source of genetic diversity and can be used in selection to obtain new varieties and cultivars in Bosnia and Herzegovina and for the protection of the indigenous gene pool.

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