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Morphological variability of the rare species *Linaria cretacea* in the conditions of the chalk hills in North-Western Kazakhstan

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ABSTRACT

Morphometric techniques are crucial when studying rare plant species as they provide a comprehensive dataset on individual plants and the status of specific populations under various ecological conditions and different levels of human impact. *Linaria cretacea*, an endemic plant species, is listed as rare and can be found exclusively in the chalk hills in North-Western Kazakhstan (NWK). Over the span of 2020 to 2022, we conducted a study on four populations of this species located in the chalk hills in NWK. Through the process of inventorying sampling plots, we assessed the elevation range occupied by the species, while also identifying the age structure and population density. The results of our study provided further evidence of the limited population counts of *Linaria cretacea*, as reported previously. Additionally, we conducted an investigation into the biometric traits of virgin and generative *Linaria cretacea* individuals across different populations, including an analysis of their seed parameters. The morphometric parameters exhibited notable variations among the populations under investigation, with the exception of the seed measurements, which showed minimal to negligible variability. The species' coenopopulations are currently in a stable state, but the absence of adequate protection measures is a cause for concern. Therefore, it is crucial to undertake regular monitoring of their habitats and enhance environmental measures to safeguard their well-being.

Keywords: *Linaria cretacea*, North-Western Kazakhstan, Sampling plots.
Article type: Research Article.

INTRODUCTION

The natural landscape of North-Western Kazakhstan (NWK) spans from the western banks of the Volga River to the eastern slopes of the Mugodzhar Mountains and from the northern reaches of the Obshchy Syrt to the Caspian Sea coast, encompassing a latitude range of 48° to 52° N and a longitude range of 46 ° to 58° E (Boukouvala *et al.* 2022; Ospangaliyev *et al.* 2023). The prevailing climate in this area is continental, marked by unpredictable

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and meager rainfall, limited snowfall easily swept away by gusty winds, arid soil and air, an abundance of direct sunlight during, and significant evaporation the growing period (Yunussova *et al.* 2017). These exceptional soil and weather conditions converge to give rise to distinct chalk formations (Darbayeva & Ramazanova 2012). Among them, chalk hills emerge as a prominent and exclusive feature in this transitional zone between Europe and Asia. The delineations demarcate the boundaries between the arid northern Caspian desert and the turf-grass steppes regions. The emergence of plant life native to the Northern Europe and the Mediterranean within the chalk hills has been subject to influences from not just by indigenous Turanian flora but also the propagation of European and Eurasian botanical taxa (Golovanov & Abramova 2019; Jasprica *et al.* 2023). The botanical composition of chalk hill ecosystems represents a complex amalgamation of species, resulting from the interplay of diverse plant assemblages from various origins over distinct historical epochs (Karimova *et al.* 2017). This convergence unfolded through protracted battles against challenging climatic circumstances and reciprocal acclimatization among native groupings. Consequently, unique plant assemblages, adapted to specific, localized environmental settings or ecotopes, emerged. *Linaria cretacea* Fisch. ex Spreng. is a plant species of scientific interest within the family Plantaginaceae. This perennial herbaceous plant exhibits several distinctive morphological features and has ecological significance due to its restricted geographic distribution in the Mediterranean region, primarily on the island of Crete (Izbastina *et al.* 2018). Morphologically, *Linaria cretacea* is characterized by its small stature, typically reaching heights of 8 to 57 cm. The leaves are lance-shaped and arranged alternately along the stems, displaying a grayish-green hue. However, the most striking feature of this species is its inflorescence. The flowers of *Linaria cretacea* are characterized by a vibrant violet-blue coloration, although pink and white variants can occasionally be observed. They possess a unique snapdragon-like morphology with a prominently spurred lower lip and two smaller upper lips (Sergaliev *et al.* 2023). This floral structure is adapted to its pollination ecology, attracting specific pollinators like bees and butterflies (Fig. 1).

Fig. 1. *Linaria cretacea* inflorescence. [*Source*: species.wikimedia.org].

The primary focus of this investigation revolved around the plant species *Linaria cretacea* inhabiting the chalk hills found within the Ural, Aqtobe, Kirghiz steppe, and Emba. We encountered populations comprising several hundred individuals on just two occasions, with none of these groups exceeding a count of 370 plants. Population numbers exhibit notable fluctuations owing to the biennial life cycle and a preference for habitats that are frequently disrupted, such as rocky cliffs, mudflows, and pebble-covered cascades. The decrease in *Linaria cretacea* populations is chiefly a consequence of habitat destruction and the unmonitored extraction of flowering plants for ornamental purposes (Simanchuk & Sultangazina 2023). To ensure the protection of the species within its native habitat, it is imperative to conduct comprehensive appraisals and ongoing surveillance of all extant populations, alongside the development of conservation strategies. Achieving this objective necessitates a comprehensive knowledge of the species' morphology and biology, alongside a thorough assessment of the well-being of its populations. The novelty embedded in our research is founded upon the revelation and subsequent examination of four newly discovered *Linaria cretacea* populations, one of which notably exists at an exceptionally low elevation of 1632 meters above sea level (MASL). Our findings now afford a finer estimation of the species' altitudinal preferences, previously documented within the narrow spectrum of 1800 to 2100 MASL.

Importantly, this marks the inaugural acquisition of data encompassing age distribution, population density, morphological features of both vegetative and generative plants, and their inherent variability.

MATERIALS AND METHODS

Within NWK the chalk hills of the Ural, Aqtobe, Kirghiz steppe, and Emba stand out. In these regions, we identified and subjected four newfound *Linaria cretacea* populations to scrutiny. Population 1 was discovered inhabiting the northwestern slopes of the Mugodzhar Hills. This population occupies elevations ranging from 1967 to 2098 MASL, precisely situated at coordinates N 51° 07.116′ and E 59° 05.193′. Population 2 was discovered at coordinates N 39° 05.840′, E 67° 53.432′, situated at an elevation of 2236 MASL. This population was located on the south side of the Aqtobe. Population 2 was observed at coordinates N 43° 04.790′, E 76° 59.512′, situated at an elevation of 1806 MASL, specifically on the north-oriented incline of Kazmunaigaz region. Population 3 was observed at coordinates N 41° 10.530′, E 64° 18.496′, located at an elevation of 1632 MASL. This population was situated on the right side of the Karasu area, specifically on the western side of the Zhem River. Population 4 was observed at coordinates N 48° 10.530′, E 37° 18.496′, located at an elevation of 1886 MASL. This population was situated on the Gornyy area (Fig. 2).

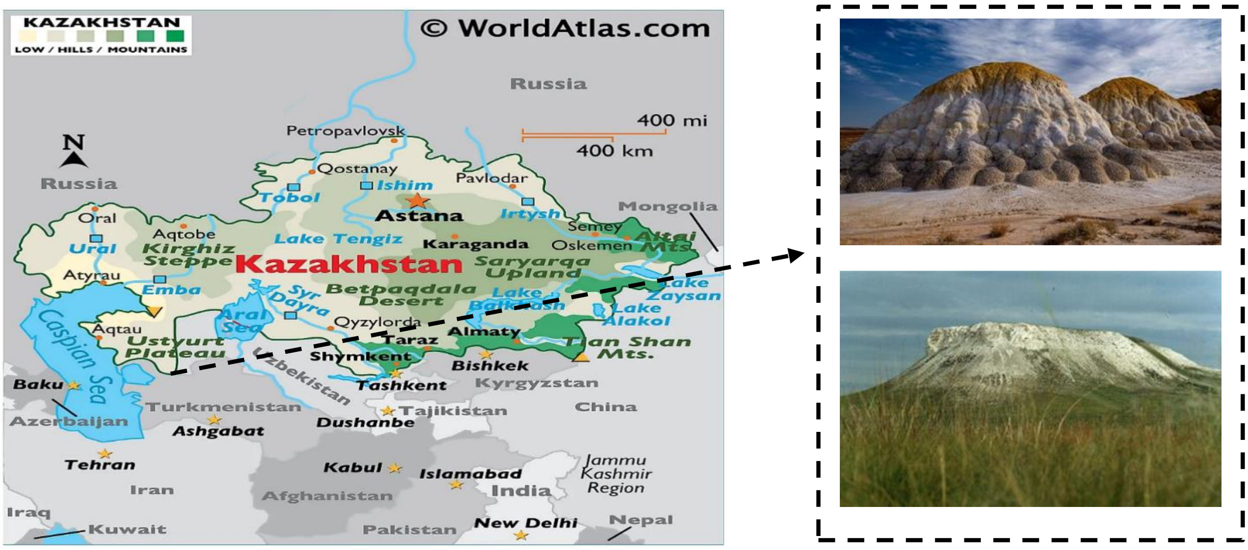

Fig. 2. The map of the research area depicts the geographical arrangement of the four examined *Linaria cretacea* populations. To the right, you can see the Akzhar chalk mountains and the vicinity of the Ishkargan mountain.

In order to maximize data collection, we implemented a systematic approach by setting up 15 sampling plots with dimensions of 1.5×1.5 m along each transect, specifically targeting the habitats inhabited by populations of *Linaria cretacea*. Within each sampling plot, we conducted a comprehensive assessment by quantifying the abundance of individuals belonging to the targeted species within the same age group and obtaining precise measurements of their biometric traits. In cases where the population density of *Linaria cretacea* was low, a thorough enumeration and measurement of all species individuals within the site were conducted. The calculation of the mean weight of 800 seeds was accomplished by averaging the results obtained from three separate measurements. All statistical analyses were carried out using Microsoft Excel 2019, encompassing the necessary data manipulations and interpretations.

RESULTS

The assessment of the age distribution demonstrated that the relative abundance of virgin and generative individuals showcased diversity across populations. The prevalence of generative individuals was most notable in Population 1 (73.96%), whereas Population 2 showcased a pronounced presence of virginal individuals (87.64%). In Population 3, the proportions of generative (51.64%) and virginal (48.36%) individuals were relatively balanced. Population 4 also showed a pronounced presence of virginal individuals (83.28%). This pattern can potentially be attributed to the biennial life cycle of *Linaria cretacea*, as reported by Izbastina *et al.* (2018), which suggests considerable year-to-year variability in the population sizes of different age classes. The computation of population density posed challenges due to the heterogeneous nature of the study sites. In certain locations, sampling plots measuring 2.25 m² were successfully established, enabling accurate calculations. However, in other areas, a comprehensive enumeration of all individuals was necessary.

Table 1. Morphometric parameters of the studied Populations.

	Population	1		2		3		4	
		Mean ± SD	Cv (%)	Mean ± SD	Cv (%)	Mean ± SD	Cv (%)	Mean ± SD	Cv (%)
Virginal individuals	Height, cm	3.49 ± 0.39	64.6	1.84 ± 0.29	139.78	5.63 ± 0.39	50.12	1.16 ± 0.23	127.14
	Number of leaves	13.19 ± 0.87	40.53	6.11 ± 0.29	51.12	13.48 ± 1.16	58.1	5.11 ± 0.24	49.11
	Leaf length size (cm)	2.91 ± 0.10	61.79	1.75 ± 0.10	101.66	2.91 ± 0.10	54.48	1.87 ± 0.11	99.45
	Leaf width size (cm)	0.58 ± 0.02	40.43	0.49 ± 0.01	50.05	0.58 ± 0.01	52.09	0.41 ± 0.01	49.13
Generative individuals	Height (cm)	44.58 ± 1.75	26.2	48.31 ± 3.49	24.02	35.41 ± 2.52	46.1	44.21 ± 2.88	23.15
	Number of stem leaves	21.34 ± 1.36	42.6	14.89 ± 0.97	21.5	22.12 ± 1.75	49.8	13.94 ± 0.74	19.78
	Leaf length size (cm)	3.69 ± 0.10	28.6	6.01 ± 0.19	19.2	4.18 ± 0.10	44.5	5.98 ± 0.21	18.3
	Leaf width size (cm)	0.87 ± 0.02	32.8	1.36 ± 0.05	25.9	0.78 ± 0.02	51	1.19 ± 0.15	24.7
Reproductive organs	Flowers per individual	13.39 ± 1.07	44.63	593.68 ± 94.23	77.93	7.28 ± 0.10	9.86	549.67 ± 89.25	69.83
	Seeds per individual	11.16 ± 1.45	37.97	NA	NA	NA	NA	NA	NA
	Length of a seed pod (cm)	36.34 ± 6.86	103.18	644.36 ± 80.41	41.86	6.01 ± 0.19	19.72	609.39 ± 88.45	39.78
Seeds from Population 1 and	Length size (cm)	1.82 ± 0.02	6.92	-	-	1.81 ± 0.02	4.41	-	-
	Width size (cm)	0.89 ± 0.01	7.27	-	-	0.80 ± 0.02	9.60	-	-
	Weight of 800 seeds (cm)	0.54 ± 0.01	0.86	-	-	0.55 ± 0.01	4.24	-	-

The average population density across the established sampling plots was determined to be 6.4±0.3 individuals per m² in Population 1, while Population 3 exhibited an average density of 7.9±0.8 individuals per m². Comprehensive measurements of morphological parameters were conducted for *Linaria cretacea* in all populations. Detailed biometric characteristics of virginal and the corresponding data for generative individuals can be found in Table 1. Significant fluctuations were evident in the morphometric parameters of plants originating from distinct populations. Among virginal individuals, Population 3 showcased the highest recorded maximum height, while Population 2 displayed the lowest recorded minimum height. Individuals from Populations 1 and 3 demonstrated comparable values for the number of leaves in the rosette, as well as the length and width of the leaf. Conversely, Population 2 exhibited the lowest values for these parameters. Population 2 demonstrated the highest recorded maximum height among generative individuals, while Population 3 exhibited the lowest recorded minimum height. The greatest number of stem leaves was observed in Population 3, whereas Population 2 displayed the fewest. In terms of leaf size, Population 2 recorded the largest values for both length and width, whereas the average leaf sizes in the other two populations were similar (Table 1). The degree of variability in the morphometric parameters of both virginal and generative individuals, as observed in our study, can be described as high or very high based on the Mamaev scale of variation in characteristics (Shoman *et al.* 2021). All three populations were subjected to a comprehensive analysis of the morphometric characteristics of the generative organs. Key parameters such as the number of flowers and seeds per individual were quantified, and precise measurements were taken for seed pod length (Table 1). However, it is worth noting that seed collection in Population 2 was not accomplished successfully. Both populations displayed substantial variability in the number of flowers and seeds, with fruit length demonstrating moderate variability in Population 1 and high variability in Population 3. Populations 1 and 3 exhibited nearly identical size and weight values for 800 seeds (Table 1). The variability levels in seed size and weight were either low or very low. Consequently, these attributes exhibited the least degree of fluctuation.

DISCUSSION

The results of our study affirm earlier indications pointing to a constrained population size within *Linaria cretacea* (Ageeva *et al.* 2012; Onyshchenko *et al.* 2017; Shustov *et al.* 2023). We were solely able to calculate the mean population density for Populations 1 and 3, with the discrepancy between these populations being minimal. This suggests the overall population density of *Linaria cretacea* is consistently low across its habitat range. Substantial discrepancies were evident in the ratios of virginal and generative plants within each examined population, largely stemming from the biennial life cycle. The variability in age structure has significant implications for population stability and reproductive success. Generative individuals produce seeds for future recruitment, so populations skewed heavily towards virginal plants may suffer declines. Monitoring age ratios over time will indicate demographic trends for conservation. Morphometric attributes of both virginal and generative specimens displayed considerable plasticity, manifesting either a high or very high degree of variability. Such extensive morphological diversity could be attributed to phenotypic plasticity in adapting to microhabitat conditions. However, genetic diversity between populations may also play a role. Further genetic analysis is warranted to elucidate the relative contributions of phenotypic plasticity versus heritable traits to the observed morphological variability. The elevated number of flowers in Population 3 was primarily attributed to the prevalence of branching inflorescence, a trait concurrently marked by a significant level of variation. This finding reveals the potential for certain populations to achieve far greater reproductive output through higher flower numbers. From a conservation perspective, propagated individuals from highly fecund populations could boost seed production in small, struggling populations. Population 1 showed a minimal difference in seed pod length when contrasted with Population 3. The variability level for this attribute ranged from low in Population 1 to moderate in Population 3. Pod size often correlates with number of seeds, suggesting Population 3 may have greater fecundity overall. Among all the features examined, the size and weight of 800 seeds exhibited the least variation, affirming these traits of *Linaria cretacea* as the most stable. Conservation efforts should focus on preserving populations that maintain robust seed sets with adequate viability.

CONCLUSION

Our study has enriched previous morphological accounts of the species (Fernandez-Mazuecos & Vargas 2011; Shustov *et al.* 2021). Based on our findings, the stem length now spans 74–85 cm, representing a substantial deviation from the previously stated 57 cm. Additionally, leaf length measurements encompass 7-10 cm, in contrast to the earlier range of 4–8 cm. Similarly, leaf width measurements now extend to 1.8–2.2 cm, exceeding the previous range of 1.4–1.9 cm. Furthermore, seed pod length has been established to range from 1.3–7.4 cm, surpassing the prior range of 2.8–6.5 cm. We have also verified several seed characteristics, including seed length at 1.2–1.9 mm, seed width at 0.5–0.9 mm, and the weight of 800 seeds at 0.53–0.56 g. Our findings reveal that this rare endemic species occupies a wider elevation range (1632-2236 meters above sea level) than previously reported. We also uncovered substantial differences in the proportions of virgin and generative plants across populations, likely stemming from the biennial life cycle. Notable plasticity was evident in most vegetative and generative morphometric traits, with plant height, leaf size, flower count, and seed pod length exhibiting high to very high variability. In contrast, seed dimensions and weight were the most stable parameters. Overall, our results provide an expanded perspective on the morphology and status of *Linaria cretacea* in its native habitat. The populations appear stable currently, but habitat loss and unmonitored extraction are concerns. Continuous monitoring and strengthened conservation measures are imperative to safeguard the species. This study establishes a baseline for tracking morphological variability across populations over time in this unique chalk hill endemic.

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