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Research Article

Comparative Analysis of Growth, Genome Size, Chromosome Numbers and Phylogeny of *Arabidopsis thaliana* and Three Cooccurring Species of the Brassicaceae from Uzbekistan

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Contrary to literature data *Arabidopsis thaliana* was rarely observed in Middle Asia during a collection trip in 2001. Instead, three other Brassicaceae species were frequently found at places where *A. thaliana* was expected. To reveal reasons for this frequency pattern, we studied chromosome numbers, genome sizes, phylogenetic relationships, developmental rates, and reproductive success of *A. thaliana, Olimarabidopsis pumila, Arabis montbretiana*, and *Arabis auriculata* from Uzbekistan in two temperature treatments. There are little but partially significant differences between phenotypes. All studied species have very small genomes. The 1Cx-values of different genotypes within the sampled species are correlated with altitude. Developmental rates are also correlated with 1Cx-values. In our growth experiments, *Arabidopsis* had high seed sterility at higher temperature, which might be one reason for the rarity of *A. thaliana* in Middle Asia.

1. Introduction

Arabidopsis thaliana is a model plant in many aspects of plant biology. Naturally occurring variability of the species may be a valuable source for biochemical and genetic analyses (reviewed in [1, 2]). Usually, *A. thaliana* is investigated separately or with congeners in studies dealing with ecologically relevant characters. Here, we compare *A. thaliana* with related species of other genera growing in the same or similar ecological niche in Middle Asia, particularly Uzbekistan. The starting point for this comparative analysis were observations on an expedition to Uzbekistan in spring 2001 where we studied *A. thaliana* in the field at its eastern distribution boundary [3]. Literature data suggested *A. thaliana* to be quite common in different types of habitat and occurring in wide altitudinal ranges of Middle Asian mountains (e.g., [4–7]). We also studied all available vouchers in the herbarium in Tashkent (TASH, Uzbekistan) for localities where the species were collected and for detailed ecological data. *Arabidopsis thaliana* was, in contrast to literature data, very rare or absent in the field in comparison to three other annual species of the Brassicaceae growing at same places or observed in the following years at these places (R. Fritsch, pers. obs.). The three other species investigated *Olimarabidopsis pumila*, *Arabis montbretiana,* and *Arabis auriculata* are of similar growth, vegetative structure, and ecology and were found in high abundances at places where we expected *A. thaliana.* The reasons for the absence of *A. thaliana* in 2001 were not obvious in the field, but we suspect that climate in conjunction with morphological and DNA 2C-value may provide an explanation for this observation.

Arabidopsis thaliana, *O. pumila*, *Arabis montbretiana*, and *A. auriculata* are annuals having distinct rosettes of leaves

but also several cauline leaves. The fruit of all taxa are siliques. *Olimarabidopsis pumila* (syn. *Arabidopsis pumila*) is a yellow flowering species mostly observed in the lowlands and on riverbanks. The two white flowering *Arabis* species have a tendency to occur more frequently in foothills and mountains. Rather contradictory taxonomic treatments of the species can be found in older literature. Using molecular analysis of the ribosomal internal transcribed spacer (ITS) sequences, the distinctness of the species became apparent. Sometimes the species intermingle locally but are readily distinguishable from *A. thaliana*, which has no clasping cauline leaves. All taxa are selfing, and interspecific hybridisation is hardly possible (e.g., [8]).

The aim of this study is to compare the four species with respect to chromosome numbers and genome sizes in relation to their phenological behaviour under controlled conditions. Growth chamber conditions that simulate constraints and competition of the plants in natural habitats are difficult to design. Temperature is considered to be one of the major environmental factors influencing plant distribution (e.g., [9]). Therefore, we decided to study the developmental rates and the reproductive success of the four species in two controlled temperature conditions, that is, 14°C and 22°C. The following questions will be addressed by our experiments: (1) are there different developmental rates of the co-occurring plant species in two different temperature treatments and does temperature significantly favour or retard species? (2) How do the developmental rates and responses correlate with the chromosome numbers and genome sizes at different temperatures?

2. Materials and Methods

2.1. Molecular Phylogenetics. The following taxa were included in our analysis (species name and GenBank accession numbers are given, resp.): *Aethionema arabicum* (AY254539), *Arabidopsis thaliana* (AJ232900), *A. cebennensis* (AF137545), *A. lyrata* (AJ232889), *A. lyrata* ssp. *petraea* (AJ232891), *A. lyrata* ssp. *kamchatica* (U96266), *A. croatica* (AF137546), *A. neglecta* (U52186), *A. arenosa* (U52188), *A. halleri* ssp. *gemmifera* (AF137544), *A. halleri* ssp. *halleri* (AF137541), *Olimarabidopsis korshinskyi* (AJ232931), *O. pumila* (AF137549), *O. griffithiana* (AJ232911), *Capsella bursa-pastoris* (AF055196), *Crucihimalaya wallichii* (AJ-131396), *Arabis pendula* (AF137572), *A. lignifera* (AJ-232899), *A. holboellii* (AY457932), *A. lyallii* (AF137561), *A. drummondii* (AF137575), *A. parshii* (AJ232902), *A. turrita* (AJ232906), *A. hirsuta* (AJ232886), *A. procurrens* (AJ232917), *A. nuttallii* (AF137562), *A. flagellosa* (AF-137560), and *A. alpina* (AF137559).

The following taxa were sequenced (the acronym HAL refers to vouchers in the herbaria of Halle, Germany, GAT for Gatersleben, Germany): *A. auriculata*: Austria, Bruck, Niederdonau (HAL 30014); Spain, Burgos (HAL 84268); Bulgaria, Golo Bardo (HAL 72898); Germany: Kyffhäuser (GAT 6359). DNA isolation and sequencing of the ribosomal ITS region were done as described in [10]. The plants of *A. auriculata* and *A. montbretiana* from Uzbekistan were grown from collected seed material in the greenhouse in Gatersleben.

The analysis was run using PAUP 4.0* beta version [11] following the settings by Koch et al. [12]: HEURISTIC, TBR, STEEPEST DESCENT. Insertions and deletions were treated as one event each. Characters and character states were weighted equally (Fitch parsimony). The bootstrap option of PAUP 4.0* (1000 replicates) was used to assess relative support. An estimate of the phylogenetic signal present within the ITS data was obtained using the Random Trees option. One thousand bootstrap samples were analysed to assess the significance of nodes on the original neighbour joining tree.

2.2. Flow Cytometry. For preparation of suspensions of nuclei, about 30 mg of fresh leaf tissue was chopped with a razor blade together with material from the reference plant in 1 ml ice-cold staining buffer in a Petri dish and analysed according to the protocol of Barow and Meister [13]. A FACStar^{PLUS} flow cytometer (Becton Dickinson, San José, CA, USA) equipped with two argon lasers INNOVA 90-5 (Coherent, Palo Alto, CA, USA) was used (excitation at 514 nm, emission at 630 nm). The data were analysed with the program CellQuest (Becton Dickinson). DNA content of the nuclei was estimated by the fluorescence of the nuclei of samples stained with propidium iodide relative to the internal standards *Raphanus sativus* (2C = 1.10 pg, for *Arabidopsis thaliana)* and *Glycine max* (2C = 2.25 pg, for the other species) [14]. Usually 10,000 nuclei were measured.

2.3. Chromosome Counting. Root tips (from plants grown in pots or from seedlings) were treated for 45 minutes with saturated paradichlorobenzene solution in water and were fixed in a mixture of methanol and formic acid (vols. 3 : 1) under room temperature overnight. After maceration in n-HCl (7 minutes, 37°C) root tips were squashed in a drop of acetic acid (45 vol. %), and chromosomes were analysed under a light microscope using phase contrast.

2.4. Growth Chamber Experiment. Single seeds of each species' original collection in Uzbekistan (Table 1) were placed in 6.5 × 6.5 cm pots filled with the autoclaved Klassmann Substrat2 substrate (Klassmann-Deilmann GmbH, Geeste, Germany), moistened and stratified in the dark at 4°C for 7 days. Thereafter, the plants were transferred to growth chambers at constant 14°C and 22°C, respectively. Long-day conditions (20 hours light, 4 hours dark) were maintained during the experiment. In total, up to five plants per accession were cultivated for *A. montbretiana*, *A. Auriculata,* and *O. pumila.* For *A. thaliana* more plants were grown. In order to minimise microenvironmental effects, trays containing the pots were randomly rearranged on the shelves every second day. Pots were not allowed to dry out.

The following phenological characters (phenological stages) were recorded: days until germination, days until bolting, days until opening of the first flower, and days until opening of the first fruit (siliqua).

Table 1: Overview of Uzbek species grown in the experiment including geographical coordinates and altitudes. Grouping of genome size values within the species based on the Tukey test is indicated by superscript letters. Accessions with the same letter are *not significantly different at 5% level.*

Location	North	East	Altitude	DNA 2C-values (pg DNA) Mean	SD	*N*	Chromosome number (2n)	1Cx DNA amount (Mbp)
Olimarabidopsis pumila								
Buchara	39°48′	64°25′	225 m	0.708[a]	0.015	4	32	173
Jizzakh	40°10′	67°40′	500 m	0.737[b]	0.007	4	32	180
Mirzaaul	40°38′	68°47′	400 m	0.755[bc]	0.018	4	32	185
Shur-Ob	38°12′	66°58′	1100 m	0.772[c]	0.010	4	32	189
Arabidopsis thaliana								
Sidzhak	41°44′	70°05′	1100 m	0.342	0.004	10	10	167
Arabis auriculata								
Khumsan	41°41′	69°57′	850 m	0.451[a]	0.008	4	16	221
Sidzhak	41°44′	70°05′	1100 m	0.459[a]	0.008	4	16	224
Arabis montbretiana								
Galabasaj	41°33′	69°52′	1100 m	0.562[a]	0.001	4	16	275
Belder-Saj	41°30′	69°55′	1100 m	0.568[ab]	0.008	4	16	278
Sukok	41°15′	69°49′	1500 m	0.575[ab]	0.004	4	16	281
Aksakata-Saj	41°23′	69°54′	1100 m	0.576[ab]	0.005	4	16	282
Sangardak	38°33′	67°33′	1500 m	0.579[bc]	0.010	4	16	283
Aman-Kutan	39°17′	66°54′	1650 m	0.596[cd]	0.008	4	16	291
Chetsuv	41°12′	70°15′	1800 m	0.601[d]	0.011	4	16	294

The proportion of ripe seeds per locus was calculated using the formula: (number of ripe seeds − number of sterile ovules)/(total number of ovules). The result of the formula is between +1, that is, all ovules developed into ripe seeds, and −1 indicating that all ovules are sterile. Due to this procedure the relative reproductive success of the different species in the treatments can be compared.

2.5. Statistics. Statistics were calculated using the program SPSS (SPSS for Windows. 1999. Chicago: SPSS Inc.). After testing for normality, analysis of variance (ANOVA) was performed. Tukey post hoc tests were calculated to find significantly different groups of species or accessions.

3. Results

3.1. Taxonomy, Chromosome Numbers, Genome Size, and Molecular Analyses. The taxonomy of the two *Arabis* species included in the analysis caused several problems. The plants collected in Uzbekistan were identified as *A. montbretiana* and *A. auriculata*, respectively [6]. Titz [15] considers both taxa to be conspecific and lists *A. montbretiana* as a synonym of *A. auriculata*, which is a very widespread taxon in his treatment ranging from Europe to Middle Asia. The Middle Asian *A. montbretiana* appears, however, to be a species rather well distinguished from *A. auriculata* in having seeds >1 mm and leaves with rounded and coarse lobes. *Arabis auriculata* has small seeds (<1 mm long) and more or less entire leaves with a partly dentate margin.

Despite that Flora Europaea [16] considers *A. auriculata* Lam. (1783) to be a synonym of the widespread taxon *A. recta* Vill, (1788) the nomenclature of *A. auriculata* is unambiguous. The plants are very similar having a sepal length of less than 4 mm, fruit pedicel of <4 mm and a fruit diameter of <1 mm. They can be distinguished from *A. nova* Vill., which is larger in all parts.

Chromosome numbers were counted for plants collected in Uzbekistan (Table 1). *Olimarabidopsis pumila* was confirmed to have $2n = 32$ chromosomes, the two *Arabis* species have $2n = 16$ chromosomes each. Determination of chromosome morphology was difficult because of the small size between 0.5 and 1.6 μm length and less than 0.3 μm width. Only very few well spread metaphase plates showed centromeres but were not sufficient for statistical analyses of karyotypes. Both *Arabis* species shared two long chromosomes having possibly satellites. Six medium-sized and four short chromosomes were apparently metacentric. No conspicuous karyotype differences were found between these taxa. Most chromosomes of *Olimarabidopsis* were somewhat smaller, but the length of one pair reaches nearly 2 μm. The other chromosomes differed little in length showing metacentric and sub-metacentric centromere positions. These data widely agree with the figure of a metaphase plate given by Manton [17].

2C-values [18] were determined (Table 1) revealing a twofold difference among the species. *Arabidopsis thaliana* has the smallest genome [19] followed by *A. auriculata* and *A. montbretiana*. Mean 2C-values of species are highly correlated with chromosome numbers ($n = 4$, Spearman's

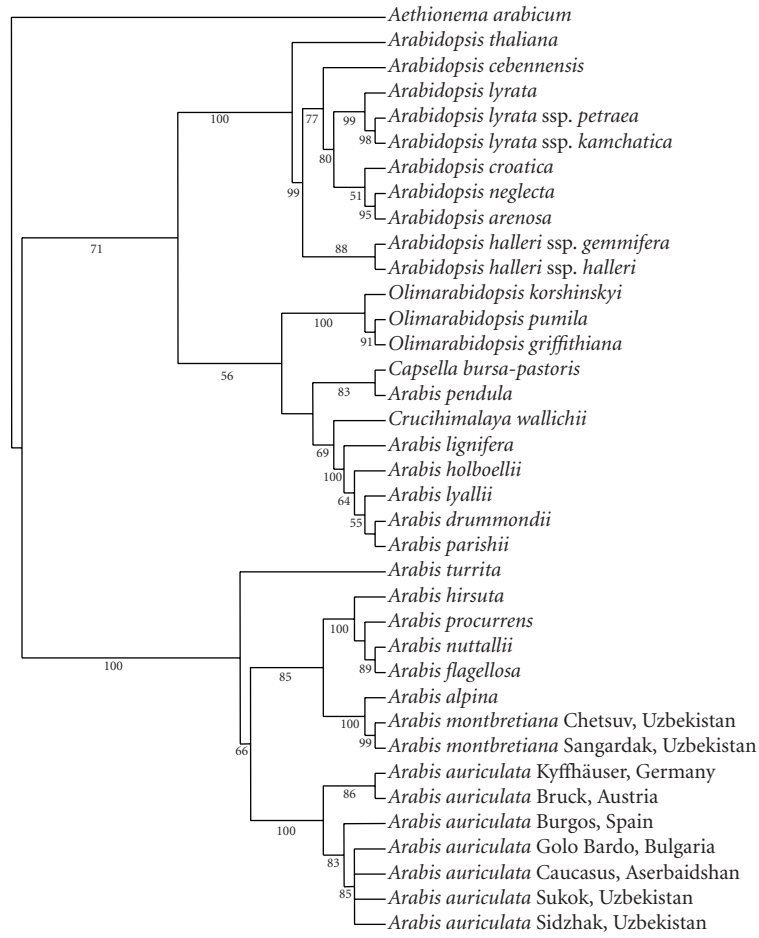


Figure 1: Strict consensus tree of 15 most parsimonious trees from the ribosomal ITS data set. Bootstrap values in percent are indicated below the branches. The *Boechera*-clade comprises, for example, *A. lyallii*. The *Arabis*-clade is the large clade at the bottom of the cladogram.

correlation coefficient = 0.949). However, this correlation is not significant at the 0.05 level ($P = .0833$) because of the small number of species. The *Arabis* species are well distinguished by their different 2C-values. The largest genome was observed in *O. pumila*, which is about twice as large as that of *A. thaliana*. We observed a number of significant differences of 2C-values among the populations of *A. montbretiana* and *O. pumila*. (Table 1) despite the rather low sample size used for comparison. The same was observed for *A. thaliana* [19]. The two populations of *A. auriculata* do not significantly differ in their 2C-values. A significant positive correlation between 1C-value and altitude was found within the species *O. pumila* ($r = 0.728$, $P = .0011$) and *A. montbretiana* ($r = 0.803$, $P < .001$). *A. auriculata* gives also a clear correlation ($r = 0.546$), but because of the low amount of data (only 2 different altitudes) it is not significant ($P = .139$). For *A. thaliana,* correlation cannot be calculated because all plants are collected at the same altitude.

The phylogenetic tree of the ribosomal ITS data (strict consensus tree of 15 maximum parsimony trees) is given in Figure 1. The tree topology is similar to the ITS data set of Koch et al. [12] and the combined data set of the *matK-Chs* data set [20], particularly for *Arabidopsis* and *Olimarabidopsis*. *Arabis* is a polyphyletic genus in these trees. One clade is named *Boechera* and the other *Arabis*. The two *Arabis* species included in our data set group together with other *Arabis* species not included in *Boechera*. *Arabis montbretiana* is most closely related to *A. alpina* and belongs to a larger clade that is a sister group to a clade comprising *A. auriculata*.

Three subclades with considerable bootstrap support can be distinguished within the *A. auriculata* clade. The two plants from the Kyffhäuser mountains (Germany) and from Bruck (Austria), respectively, are basal in this clade. Finally, a plant from Burgos (Spain) is basal to a group of plants from south-eastern Europe (Bulgaria), the Caucasus (Azerbaidzhan), and Middle Asia (Uzbekistan).

3.2. Growth Chamber Experiments. The species performed unequally well in the growth chambers experiments. Olimarabidopsis, *A. thaliana,* and *A. montbretiana* grew almost without problems. *Arabis auriculata* performed less well, that is, some plants died at several stages of the development. Maybe some cold period was missing for a proper development in our controlled environment experiment.

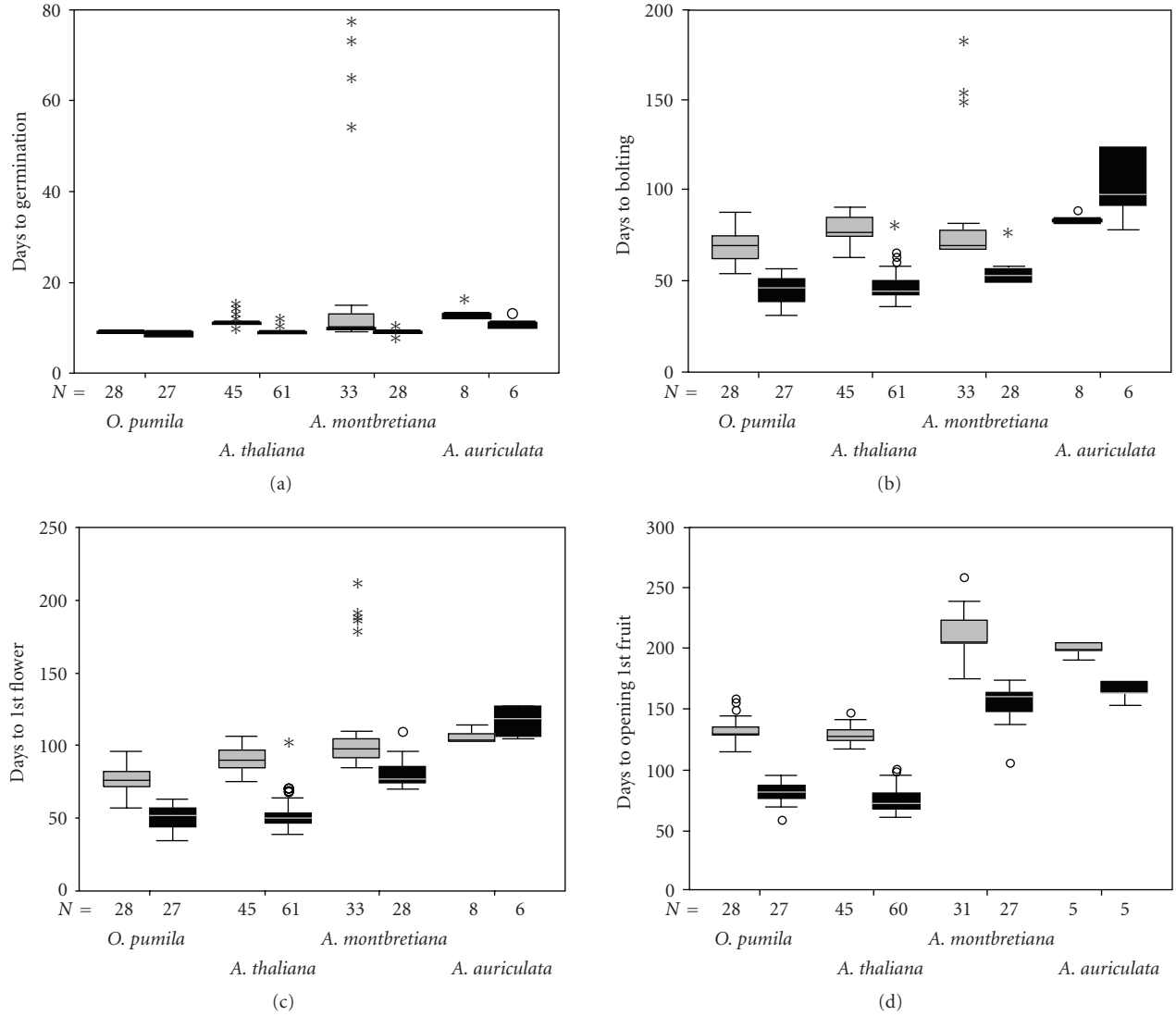


Figure 2: Boxplots showing the phenological stages of the studied species. The two treatments (14 and 22°C) are plotted together: the grey boxes demarcating the 25th and 75th percentile (interquartile range), with the black median line referring to the 14°C treatment, the black boxes with the grey median to the 22°C treatment. The error bars mark the largest and smallest value, respectively. An open circle marks outliers, extreme values are marked by an asterisk. The number of studied plants is indicated below the columns. The number may decline from one stage to another due to death of plants.

Analysis of variance (ANOVA) revealed significant differences between the developmental rates among the species within each of the treatments (data not shown). These differences may also be inferred from the boxplots shown in Figure 2. However, some species did not differ significantly in their phenological phases. Groups of species not significantly differing from one another are shown in Table 2.

The numbers of days until germination (Figure 2(a)) are comparable among the species in the treatments. *Arabis auriculata* and *A. thaliana* did not differ significantly between the treatments. In the 22°C treatment all taxa germinated earlier than in the 14°C treatment. *Arabis montbretiana* revealed a remarkable pattern that consisted apparently of two different plant types: one with early and one with late germination. These late germinating seeds were collected among early germinating seeds in Sukok and Chetsuv. The reason for this difference is unknown. It could be either different genotypes growing together in the wild or perhaps epigenetic modifications that regulate dormancy. These germination types were not visible in other species of the 14°C experiment. In the 22°C treatment these differences were not observed.

The numbers of days until bolting (Figure 2(b)) were similar between the species in the 14°C treatment; the fastest developing species were *O. pumila* and *A. montbretiana*. All species bolt earlier in the 22°C treatment with the exception of *A. auriculata*. *Arabidopsis thaliana* got the strongest growth acceleration in higher temperatures. It needed the same time to this phenological stage as *O. pumila*. In the following stage, that is, the opening of the first flower

Table 2: Groups of similarly developing species in the 14°C (above diagonal) and 22°C (below diagonal) treatment as revealed from ANOVA and subsequent Tukey's significant difference test. For example, in the 22°C treatment *A. thaliana* and *O. pumila* developed similarly in the phenological stages: B-days until bolting, F-days until flowering, and Fr-days until opening of the first fruit. Further abbreviation: G-days until germination. Nonoverlapping groups of species from the Tukey's test are marked in bold italics letters. For example in the 14°C treatment, *O. pumila* and *A. thaliana* are not significantly different from one another in the days to opening of the first fruit, but they differ significantly from the *Arabis* species that are not significantly different from one another.

	A. thaliana	*O. pumila*	*A. montbretiana*	*A. auriculata*
A. thaliana	—	G, B, Fr	B	G, B, F
O. pumila	B, F, Fr	—		G, B
A. montbretiana			—	G, B, F, Fr
A. auriculata				—

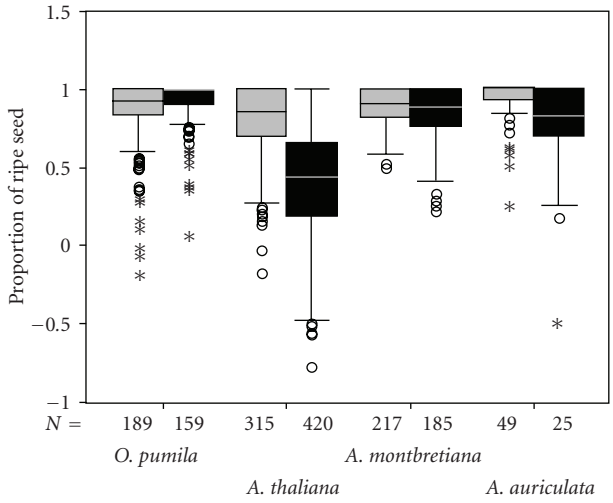


Figure 3: Boxplots showing the proportion of developed seeds to the total number of ovules. For further explanation see Figure 2.

(Figure 2(c)), *A. montbretiana* developed slower in the 14°C treatment than *O. pumila*. In the 22°C treatment *O. pumila* and *A. thaliana* did not significantly differ from one another and developed faster than *Arabis*. *Arabis auriculata* had a still slower development at higher temperatures.

The seeds of *A. thaliana* and *O. pumila* ripened very rapidly (number of days until opening of the first fruit, Figure 2(d)). The fruit of *A. auriculata* developed also very fast, especially in the 22°C treatment where the above-mentioned pattern of slow development was reversed. The late germinating plants of *A. montbretiana* were still delayed to the bulk of the other species but not as strong as during the early phenological stages.

The proportion of ripe seeds was significantly different among all species and treatments (Figure 3). In the 22°C treatment *O. pumila* produced a higher proportion of ripe seeds than the other species. Most apparent was the pattern of higher seed sterility in *A. thaliana*, which produced considerably less ripe seeds in the 22°C treatment.

Correlations between the 2C-value genome sizes of the species and developmental rates were statistically not significant (data not shown). Correlating the 1Cx-value with the developmental rates of the two growth conditions yielded

Table 3: Correlations between 1Cx-value of the species and developmental rates, that is, days until germination, bolting, flowering, and fruiting. Spearman correlation coefficients are given for the two treatments ($n = 14$). Asterisks indicate significant correlations ($P < .05$) after treatment-wise sequential Bonferroni correction.

	14°C	22°C
Germination	0.534	0.052
Bolting	0.218	0.622
Flower	0.631*	0.579
Fruit	0.789*	0.656*

significant results (Table 3). In both treatments the days until opening of the first fruit were significantly correlated with the 1Cx-value. The two *Arabis* species having larger 1Cx-values developed slower than the two other species.

4. Discussion

The cruciferous species co-occurring with *A. thaliana* in Uzbekistan belong to different tribes of the Brassicaceae. The taxonomy of *Arabis auriculata* raises some interesting issues. Although morphologically similar, the individuals of *A. auriculata* from Europe and Middle Asia are distantly related from one another and form sister clades in our phylogenetic analysis with high bootstrap support. Either *A. auriculata* may comprise several cryptic species (e.g., reviewed by Brochmann and Brysting [21]) or is one quite polymorphic species. If *A. auriculata* turns out to be polymorphic, the basal clade comprises plants from northern areas of Europe, while the plants of the sister clade are from the Mediterranean region, the Caucasus, and Middle Asia. This might point to a migration of the plants from Europe to Middle Asia. Taking the scenario of cryptic species, the Southern taxa may have diverged from a taxon distributed in the North. *Arabis montbretiana* is in our analysis a sister clade to *A. alpina*, a rather widespread species of the northern hemisphere having isolated occurrences also in the East African mountains.

The species included in our analysis differ largely in genome size and chromosome numbers, but still belong to plant species having very small genome sizes. Genome size is potentially tied to all features of cell division rates,

for example, the growth rates of plants [22–24]. Perennial plant species with larger genomes tend to have higher rates of shoot growth in cold conditions [23, 25]. Reduction in genome size in maize is correlated to earliness, that is, a fast development and finishing of the life cycle of the plants [26]. Similarly, a reduction in genome size is apparently a very important prerequisite for the evolution of annuals [27]. We observed remarkable differences in chromosome numbers and genome sizes and could relate these differences to developmental rates in the experiments. The species with the smallest genome (2C-value and 1Cx DNA amount) of our experiment (*A. thaliana*) develops in many phenological phases and stages similarly fast as *O. pumila* that has the largest 2C-value but a comparable 1Cx-value (Table 1). The *Arabis* species have intermediate 2C-values but higher 1Cx-values and develop almost always slower than the other two species. The 1Cx-value of the four species may thus explain phenological differences and similarities among the species at least for the later developmental stages. Chromosome numbers are not correlated with the developmental rates of the plants. The average DNA content per chromosome only partly correlates with developmental rates. *Olimarabidopsis pumila* has the lowest mean DNA content per chromosome that is followed by *A. auriculata* and the two remaining species. Considering the studied taxa in their phylogenetic context, that is, clade-wise, a partial compensation of the genome size by the chromosome number can be observed in the clade comprising *A. thaliana* and *O. pumila*. The more-than-threefold higher number of chromosomes in *O. pumila* compared to *A. thaliana* compensates partly the about twofold higher 2C-value of *O. pumila*. Thus, the 1Cx-value is approximately the same between the species. In the other clade including the *Arabis* species the mean DNA content per chromosome correlates with the genome size because the taxa have the same chromosome numbers.

To reveal reasons for the rarity of *A. thaliana* in Uzbekistan, we chose temperature as one of the major environmental factors to study the performance of co-occurring plants of apparently similar ecology and life pattern. Increasing temperature has a differential influence on the growth rates of plants. Morse and Bazzaz [28] observed a higher initial growth of plants due to higher temperatures but also an increased competition among the plants. Dunnett and Grime [25] made a similar observation that increasing temperatures had positive effects on the development of plant monocultures. However, in mixed cultures divergent responses of the species were observed. The plants studied in our experiment were found growing together in Middle Asia where climate for annual plants is most favourable from autumn to spring with a short disruption in winter. In general, the growing season is distinctly shorter than in northern area due to the rapid onset of the hot and dry summer. *Olimarabidopsis pumila* appears to be the species best adapted to a short growing season. This species develops very fast and has a high fertility in higher growing temperatures. *Arabidopsis thaliana* has a similarly fast development but at 22°C seed sterility increased. Increasing pollen sterility at higher temperatures may be the reason for an enhanced abortion of ovules [29]. Other reasons for a higher abortion rate may be developmental defects during the maturation of the seeds as observed in *Brassica* [30]. Temperature may, therefore, be a principal factor for the rarity or absence of *A. thaliana* from the hot and only shortly moist Middle Asian lowlands [3], where temperatures increase very rapidly in spring. The high temperatures might favour a rapid development of the species but the increasing seed sterility may exterminate the species over many generations. In this respect, *O. pumila* may be better adapted. However, *A. thaliana* may produce a soil seed bank [31, 32] from which plants can be recruited in favourable years with cooler springs.

The two species of *Arabis* develop significantly slower in most cases than the two above-mentioned species. However, their abundance in Uzbekistan point to the fact that they are also well adapted. We observed a distinct allocation of nutrients into root biomass in the experiment, that is, much more roots appeared at the bottom of the pots than in the other species. It might be possible that the roots of these species reach deeper and thus, in moister soil layers. Consequently, the slow development might not be a disadvantage because the plants can use a longer vegetation period than the other species having only a shallow root system. The differences between the two *Arabis* species are not very pronounced, which is in accordance with their frequent joint natural occurrence. However, for unknown reasons *A. auriculata* developed less well in the experiment as *A. montbretiana.*

Our experiments revealed distinct differences in the developmental rates of the species and their fertility in dependence of temperature. Although significantly different in our experiments the plants grow together in the field. The different response of the species to temperature, particularly observed in the vegetative development of the *Arabis* species and the seed set of *A. thaliana,* suggests that the species may be favoured differently in annually fluctuating weather conditions. This may balance their cooccurrence. It may be possible that instead of temperature the amount of precipitation may have been the reason for the observed rarity of *A. thaliana* in Uzbekistan in spring 2001. This would be in accordance with observations of Zavaleta et al. [33] that warming did not alter the species composition in a California annual grassland community but precipitation. Further studies are necessary to solve this question.

In our species set we observed correlations of 1Cx-values with some phenological characters as well as the altitudinal distribution of the species. Low 1Cx-values were observed in species of the dry and hot lowlands of Uzbekistan and species of the more mesic mountain habitats had higher 1Cx-values. This pattern may be in accordance with general observations that species with lower 2C DNA-values may have a wider distribution with respect to temperature and precipitation than those with high values [34].

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