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Ploidy level interacts with population size and habitat conditions to determine the degree of herbivory damage in plant populations

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Recently it has been suggested that ploidy level of a plant population may have important effects on plant-animal interactions. Plant–animal interactions can also be strongly altered by factors such as plant population size and habitat conditions. It is, however, not known how these factors interact to shape the overall pattern of plant-animal interactions.

I studied the interaction between a perennial plant, *Aster amellus*, and a monophagous herbivorous moth, *Coleophora obscenella,* and investigated the effect of ploidy level of the plant population, plant population size, isolation and habitat conditions on density of the insect, damage by the insect, and plant performance.

Ploidy level, plant population size and habitat conditions, but not isolation, strongly influence plant–herbivore interactions. Furthermore, there are significant interactions between effects of ploidy level and plant population size and between ploidy level and isolation. Hexaploid plants suffer higher seed damage by the herbivore, but their seed production is still higher than that of diploids. Herbivores thus partly limit the evolutionary success of the hexaploid plants. Plant–animal interactions are also strongly determined by plant population size. Small populations of *A. amellus* (below forty flowering ramets) host no *C. obscenella* larvae, indicating a minimum *A. amellus* population size that can sustain a viable *C. obscenella* population. Negative and positive effects of plant population size balance and result in no relationship between plant population size and number of developed seeds per flower head. The results also show a significant interaction between ploidy level and plant population size, indicating that the increase in density of *C. obscenella* larvae with plant population size is greater in hexaploid than in diploid populations. The results also indicate that the effect of ploidy level on plant–herbivore interactions can be altered by plant population size, which suggests that plant–herbivore interactions are driven by a complex of interactions among different factors. Studying each factor separately could thus lead to biased conclusions about patterns of interactions in such systems.

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Polyploidy, a state of having more than two complete chromosome sets per nucleus, has played a key role in the evolution and diversification of the plant kingdom. It has been estimated that between 47 and 70% of flowering plants are descendants of polyploid ancestors (Masterson 1994). Polyploid complexes have recently become the subject of many studies, and there is an increasing amount known about the evolution of these complexes. Many of these studies deal with origins of polyploid species (Ramsey and Schemske 2002, Ainouche et al. 2004), effects of polyploidy on species' habitat requirements and their geographical distribution

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(Rothera and Davy 1986, Felber 1988, Lumaret 1988, Bayer 1991, Van Dijk and Bakx-Schotman 1997), and evolution of their breeding systems (Cook and Soltis 1999, Pannell et al. 2004).

In contrast, effects of polyploidy on plant–animal interactions are largely unexplored. This is surprising since the pattern of interactions with animals strongly influences performance of many plant species (Crawley 1983, 1989, Doak 1992, Louda and Potvin 1995, Eubanks et al. 2005, Juenger et al. 2005, Stinchcombe 2005) and may thus shape evolutionary dynamics of polyploid species (Thompson et al. 2004). In a pioneering study, Thompson et al. (1997) have shown that specialist moths differentially attack diploid and tetraploid individuals of *Heuchera grossularifolia*. Similarly, Husband (2000) showed that bumblebees and honeybees more frequently visit diploid than tetraploid *Chamaerion angustifolium* plants. These studies were followed by a larger number of additional studies on both systems (Segraves and Thompson 1999, Nuismer and Thompson 2001, Janz and Thompson 2002, Husband et al. 2004, Nuismer and Cunningham 2005). Both of these study systems suggest that animals can differentiate between ploidy levels and that such patterns may be common. If so, herbivores can act as important selective factors and determine the relative success of different ploidy levels in natural systems.

Plant–animal interactions have been shown to depend on many different environmental factors (Levine et al. 1998, Veteli et al. 2003, Willmot et al. 2004, Kersch and Fonseca 2005, Ostergard and Ehrlén 2005) at different spatial scales (Johnson and Agrawal 2005). Since the effect of different factors on various interactions in natural communities are often not independent (Fritz 1999, de Mazancourt and Loreau 2000, Barrett and Agrawal 2004), it is expected that the effect of ploidy level on plant–animal interactions will likewise depend on other factors known to affect relationships between plants and animals.

The probability of occurrence of monophagous insects may depend on plant population size and isolation (Rausher and Feeny 1980, Johannesen and Loeschke 1996, Kruess and Tschartke 2000, Colling and Matthies 2004). Similar to environmental factors, population size and isolation may interact with ploidy level. Plant–insect interactions can thus be influenced by complex interactions among a large number of different factors. When studying effects of ploidy level it is thus important to take other factors into account.

Here I studied interactions between two different ploidy levels of a perennial plant, *Aster amellus*, and a monophagous seed predator moth, *Coleophora obscenella*. In plant populations with different ploidy levels, I measured herbivore density and its effect on seed production. I also explored how the interaction between the herbivore and plant was affected by habitat conditions, plant population size and isolation. Specifically, I addressed the following questions: (1) what is the effect of ploidy level on the occurrence of the monophagous herbivore in populations of *Aster amellus*? (2) How is this relationship modified by habitat conditions (aboveground biomass) and by the size and isolation of the local plant populations? (3) How do the different factors (ploidy level, population size and isolation, and habitat conditions) affect plant performance?

The study was performed in the western part of the Czech Republic (Bohemia), a contact zone of diploid and hexaploid populations of *Aster amellus*. I investigated herbivore density and its effect on seed production in 15 populations of each ploidy level over three years. The studied populations ranged in size from 1 to 2600 flowering ramets. The studied localities also greatly varied in overall productivity, measured as aboveground biomass.

Methods

Study species and sites

Aster amellus is a perennial herb growing in open xerothermic habitats. Its European distribution ranges from northern Italy to Lithuania; the southern distribution limit crosses northern Italy and Macedonia (Merx and Schreiber 1976). Outside Europe the range extends to the Black see and northern Caucasus (Meusel and Jäger 1992). Throughout its range it is known to occur in three ploidy levels (di-, tetra- and hexa-, Merx and Schreiber 1976). In the study area, the western part of the Czech Republic (Bohemia), only diploid individuals were previously reported (Holub et al. 1970, Kovanda 1984, Krahulcová 1990). However, we have recently shown that both diploid and hexaploid populations occur in the region (Mandáková and Münzbergová, in press). We suggest that the distribution of *Aster amellus* in the Czech Republic is a result of secondary contact. This conclusion is among others based on the fact that we did not find any intermediate tetraploid cytotype during the extensive field survey in the Czech Republic (Mandáková and Münzbergová, in press).

Kovanda (2005) separated the different cytotypes occurring in the Czech Republic into two separate species, *Aster amellus* (2n) and *Aster scepusiensis* (6n). However, our finding of hexaploid plants in central Bohemia, and results indicating no clear morphological and genetical differences between the two ploidy levels suggest that the species distinction is not well supported (T. Mandáková and Z. Münzbergová, unpubl.). Consequently, in this study I use the name *Aster amellus* for both ploidy levels.

Because the two ploidy levels never co-occur in a population, ploidy level could be used as a population characteristic in the analyses. The estimates of the ploidy

level come from our previous study (Mandáková and Münzbergová, in press). In the cited study, we have also shown that there are no differences in habitat conditions between localities of the two ploidy levels, but still that different ploidy levels can occur at localities in close proximity (0.5 km).

The species is self incompatible (unpubl.). Our observations indicate that this is true for both diploid and hexaploid plants.

Coleophora obscenella (Lepidoptera: Coleophoridae) is a monophagous moth, whose larvae feed on seeds of *Aster amellus* (Baldizzone and Tabell 2002). Pupae develop in the ground and adults emerge in July and August (Vávra 2004). In the Czech Republic it is common in most populations of its host plant (Vávra 2004).

This study was carried out in northern Bohemia, the western part of the Czech Republic. I used all populations (15 diploid and 15 hexaploid) of the plant species in an area delimited by the towns Úštek, Roudnice nad Labem, Lovosice and Bílina. The exact positions, ploidy level and size of the populations are provided in Table 1. From these data it is clear that populations of the two ploidy level are not well mixed. No habitat conditions associated with this distribution pattern were, however, detected (Mandáková and Münzbergová, in press).

Table 1. List of populations used in the study.

Ploidy level	Population size	Geographical position	
		N	E
Diploid	1	50°31′59.2″	14°19′39″
	8	50°29′55.9″	14°18′16″
	145	50°33′26.6″	14°08′40.6″
	180	50°33′3.2″	14°05′15.9″
	226	50°31′56.8″	13°48′3″
	280	50°32′48″	14°05′23.3″
	290	50°32′58.3″	14°5′23.7″
	350	50°31′33.7″	14°20′06.1″
	550	50°51′46.3″	14°15′33.0″
	630	50°32′02.1″	14°14′07.4″
	810	50°31′40.8″	14°13′47.8″
	850	50°33′04.3″	14°05′44.4″
	930	50°33′23.2″	14°7′51.6″
	1800	50°32′36.9″	14°05′22.9″
	2600	50°33′38.5″	14°07′53.8″
Hexaploid			
	5	50°30′56.7″	14°23′20.7″
	20	50°29′57.8″	14°19′5.6″
	40	50°28′9.5″	14°18′29.8″
	66	50°29′43.2″	13°58′26.7″
	84	50°28′13″	14°18′25.4″
	110	50°28′07.4″	14°18′07.2″
	110	50°29′49.7″	14°21′18.2″
	180	50°29′36.4″	14°18′40.6″
	180	50°31′37.8″	14°20′27″
	210	50°29′46.3″	14°18′08.7″
	220	50°27′56″	14°18′23.8″
	290	50°30′08.7″	14°18′50.9″
	330	50°30′02.4″	14°18′58.5″
	920	50°30′14.2″	14°17′50.7″
	1150	50°30′15.1″	14°17′55.4″

Occurrence of *C. obscenella* larvae and plant performance

The density of *Coleophora obscenella* larvae in each population was estimated as mean number of larvae per flower head. To do this, 100 flowering ramets were selected at each locality in each year of the study and one randomly selected flower head per flowering ramet was sampled. If there were fewer than 100 flowering ramets in the population, then all ramets were sampled. The ramets were selected in a grid of 1.5 m. If the area of the population was smaller than the size of the sampling grid, the distance between two neighboring sampling points was reduced. The shape of the sampling grid depended on the area covered by the population. The number of *C. obscenella* larvae in the last instar from the total sample from each population was then determined. The density of flowering ramets did not differ between the two ploidy levels (pers. obs.).

To estimate plant performance, I counted the total number of developed seeds, the number of seeds damaged by *C. obscenella* and the number of all the other undeveloped seeds from twenty flower heads in each population in each year. To estimate seed germination rates, a mixture of 1000 developed undamaged seeds from each population $year^{-1}$ were split equally among ten replicate petri dishes. The number of germinated seeds was recorded until all seeds germinated or rotted (approximately 6 months, 10/20°C, 12/12 h). Sampling was performed at the time of fruiting in September 2001, 2002 and 2003, and all populations were sampled each year.

In 2002, I counted the number of flowering ramets in each population and used it as an estimate of plant population size. In larger populations, where it was difficult to count all the ramets individually, the number was estimated by counting groups of ten ramets. In all populations there is quite a high number of non-flowering ramets each year. The number of flowering ramets, however, does not vary much between years (pers. obs.). I therefore used population sizes estimated in 2002 for all years. Although a single genet of the species can consist of multiple ramets, it is often difficult to recognize, which ramets belong to the same genet. Population size was thus expressed as the number of flowering ramets.

Because the localities appeared to strongly differ in productivity (pers. obs.), habitat conditions of each locality were described by measuring habitat productivity, expressed as total aboveground biomass per area. The data come from our previous study, where we describe the details of their collection (Mandáková and Münzbergová, in press). The variation in productivity of sites between years seems to be relatively low so the estimates are representative for all years (pers. obs.).

There was no significant relationship between population size and productivity ($F_{1,29} = 0.20$, $P = 0.65$) and

between population size and ploidy level ($F_{1,28} = 3.25$, $P = 0.08$). There was also no relationship between ploidy level and habitat productivity, even though hexaploid populations occupy wider range of productivities that do diploid populations (Mandáková and Münzbergová, in press). It was thus possible to separate the effect of population size, habitat productivity and ploidy level in the study. All the sites are abandoned, and there is thus no variation in current land use between sites.

To investigate whether the differences between the ploidy levels correlated with differences in their morphology, I used data on stem height and number of flower heads per stem collected in a previous study (T. Mandáková and Z. Münzbergová, unpubl.). The data are based on 20 flowering ramets randomly selected over the locality, with the condition that the next flowering ramet is at least two meter far from the previous. This was done to ensure that the two selected ramets belong to different genets; the maximum distance between mother and daughter ramets is about eight cm (pers. obs.). The single clone is expected to be never larger that 15 cm (pers. obs.). Due to difficulties with recognizing genets, the morphology of the plant was studied only at the ramet level.

Data analysis

I used a generalized linear model with type III sum of squares including plant population size, ploidy level, site productivity, isolation, and year, as well as interactions of population size, ploidy level, site productivity and isolation with year and interactions of population size, site productivity and isolation with ploidy level as independent variables. In the first tests I also included all the higher-level interaction terms. Since only three of these were marginally significant over all analyses, all these were dropped from the subsequent results and are not discussed further.

Density of *Coleophora obscenella* larvae, number of initiated seeds (sum of all seeds in the flower head), number of developed seeds, proportion of damaged seeds and percentage germination of developed seeds were used as dependent variables. The dependent variables were normally distributed. All of the independent variables except for ploidy level were treated as continuous; ploidy level was included as a fixed factor. Population size was log transformed to gain normality. Square root transformation, another possible transformation of population size, provided qualitatively similar results. Locality code was used as one of the error terms in the analyses of the effect of predictors measured at the locality level in order to take into account that measures from the same population from multiple years are not independent.

Isolation of each target population was calculated as

$$I_j = -\Sigma\,(S_k/d_{jk}^2), \quad j \neq k \tag{1}$$

where I_j is isolation of population j, S_k is size of one of another population (k), and d_{jk} is distance between population j and k. The I_j is sum over all k, where $j \neq k$. Isolation of a population is thus calculated as a product of distance from each patch and its size. In this way nearby larger populations are weighted more heavily than small ones, and the isolation measure thus represent a measure of amount of *Aster amellus* in surroundings of the target population. All populations occur in isolated patches within an agricultural landscape covered mainly by large arable fields. There are no clear corridors in the landscape that could represent migration routes for the herbivores. Consequently, measuring isolation of a population using the linear distance between populations is the most reasonable approach in this case.

Absence of *C. obscenella* from small populations may be just due to random sampling. To see how likely it is that no *C. obscenella* larvae will be found in small populations due to random processes I calculated a probability of finding no *C. obscenella* larvae using the mean *C. obscenella* density per population and assuming a binomial distribution of occurrences. All tests were performed using S-PLUS (2000).

Effects of ploidy and other factors on population size

All the above results deal with effects of ploidy level, population size, isolation and habitat productivity on single life-history traits. Ehrlén (2003) and Münzbergová (2005) demonstrated that the effect of herbivores on single life history traits cannot be easily translated into demographic effects. To estimate the effect of herbivory on future population size, I thus used a parameterized matrix population model and projected development of populations of different size. Specifically, I used my data on population dynamics of the species coming from both diploid and hexaploid populations from three transition periods (2002–2005). These data were collected in 9 different populations (three diploid and six hexaploid) of the species in the study area. The life cycle of the species was divided into three life history stages (seedlings, vegetative ramets, flowering ramets); at least 30 ramets per stage, per population, per transition period were recorded (Z. Münzbergová, unpubl.).

For the purpose of this study I combined the data from all populations in each transition period to create one transition matrix for that period. This resulted in three transition matrices, each describing the mean performance of the species. Merging data between the two ploidy levels is justified by the absence of significant

differences in population dynamics between the two ploidy levels (Z. Münzbergová, unpubl.).

The transition from reproduction to seedlings in the matrix model consists of information on seed germination in the field, number of flower heads per ramet and number of seeds per flower head. In the current study I collected data on seed production per flower head. To explore the effect of ploidy level and population size on population dynamics of the species, I thus combined this ploidy level and population size specific data with mean data on number of flower heads per plant and on seed germination. Specifically, I multiplied the product of mean number of flower heads per plant and mean field germination by the expected numbers of developed and initiated seeds per flower head in diploid and hexaploid populations of 10, 100 and 1000 flowering ramets. These values were estimated from the regressing the number of developed/initiated seeds against the size of each population.

I used these matrices to project growth of a hypothetical population, initiated with 10, 100 or 1000 flowering ramets, over 20 years. In each projection, one of the three matrices was drawn in each time step. Each projection was replicated 100 times. Projections were performed using Matlab, version 5.3.1 (The MathWorks, Inc., Natick, Massachusetts, USA).

Results

Density of *Coleophora obscenella* larvae is affected by the ploidy level of the *Aster amellus* population (it explains 8% of the total variation in the dataset, Table 2) with hexaploid populations hosting higher densities of *C. obscenella* (0.3 larvae/flower head) than diploid (0.2 larvae/ flower head, Fig. 1). The results also show that density of *C. obscenella* ramets is driven primarily by population size (it explains 21% of the variation). This is because small populations of *A. amellus* host no or very few *C. obscenella* larvae, whereas large populations can host both high and low densities (Table 2, Fig. 2A). The relationship between population size and number of *C. obscenella* larvae is significant even after the smallest populations are removed (not shown). Below 11 flowering ramets, the probability of absence of *C. obscenella* just due to random processes is higher than 5%. The absence of *C. obscenella* from populations larger than this is, however, unlikely due to random processes only.

Habitat productivity also significantly affects the density of *C. obscenella* larvae (4% of the total variation), with more productive sites hosting more *C. obscenella* larvae. There is also high inter annual variation in the density of *C. obscenella* larvae (0.24, 0.35 and 0.17 larvae plant^{-1}, respectively) and an interaction between year and productivity (Table 2).

Table 2. Results of GLMs testing the effect of plant population size, isolation, ploidy level, biomass (a measure of habitat productivity) at the locality, and year on the number of *C. obscenella* larvae per flower head, proportional seed damage, numbers of developed and initiated seeds per flower head, and percent seed germination. DF = 1, DF error = 16 for terms not including year and DF = 2, DF error = 38 for year and interactions with year. Parenthetical expressions indicate the direction of the relationship between the specified variables. R^2 values are provided only for significant effects with $p < 0.05$. Locality was used as one of the error terms in the analyses of the effect of predictors measured at the locality level (but it is not included in the table).

	No. of larvae per flower head		Proportion of damaged seeds		No. of developed seeds per flower head		Number of initiated seeds		Germination (%)	
	F	R^2	F	R^2	F	R^2	F	R^2	F	R^2
Biomass	6.88	0.04 (+)	9.97	0.03 (+)	0.96	–	0.01	–	1.05	–
Population size	32.95	0.21 (+)	39.82	0.11 (+)	0.58	–	120.21	0.44 (+)	0.16	–
Ploidy level	12.48	0.08 (+)	14.90	0.04 (+)	9.37	0.07 (+)	17.29	0.06 (+)	0.05	–
Isolation	1.72	–	0.06	–	2.81	–	4.08	0.02 (−)	0.07	–
Year	8.84	0.11	61.40	0.33	24.44	0.37	16.94	0.13	10.02	0.21
Year × biomass	3.43	0.04	9.24	0.05	0.14	–	1.92	–	0.39	–
Year × population size	0.69	–	1.53	–	0.83	–	0.25	–	0.62	–
Year × ploidy level	1.67	–	2.75	–	5.15	0.08	2.36	–	2.81	–
Year × isolation	0.30	–	4.63	0.03	2.57	–	0.04	–	0.36	–
Ploidy level × biomass	0.02	–	0.46	–	0.01	–	0.69	–	3.03	–
Ploidy level × population size	5.30	0.03	0.39	–	2.38	–	21.12	0.08	0.03	–
Ploidy level × isolation	2.87	–	20.17	0.05	0.00	–	1.45	–	5.05	0.05

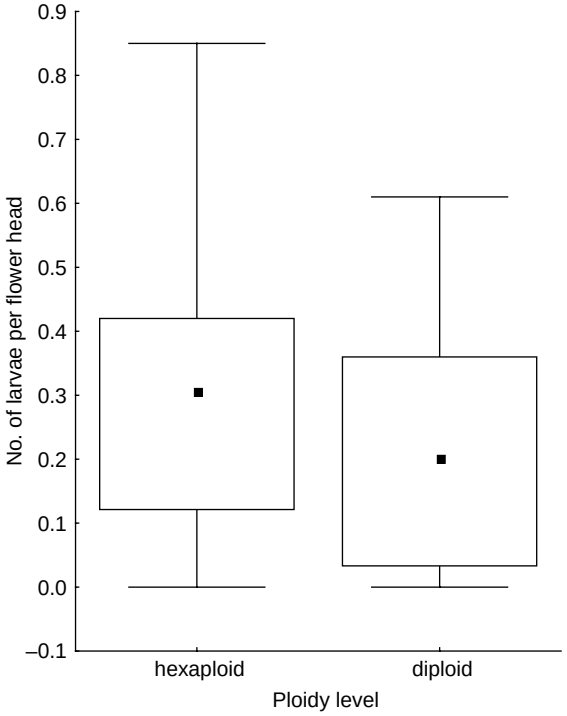

Fig. 1. Effect of ploidy level on the number of larvae per flower head. Median, upper and lower quartiles, and non-outlier minimum and maximum are shown. Since the interaction between ploidy level and year was not significant, data from the different years are merged.

The response to productivity is the strongest in the wettest year (2002).

Furthermore, the results show a significant interaction between ploidy level and population size (3%), indicating that the increase in density of *C. obscenella* larvae with population size is stronger in hexaploid than diploid populations (Table 2, Fig. 2A).

Similarly to *C. obscenella* larval density, the proportion of damaged seeds is affected by population size (it explains 11% of the total variation), ploidy level (4%; the proportion of damaged seeds is 43% and 52% for the diploid and hexaploid plants, respectively), habitat productivity (3%) and year (33%). Additionally, there are significant interactions between year and productivity (explaining 5% of the total variation) and year and isolation (3%). The response to both productivity and isolation is the strongest in the wettest year (2002). Also there is a significant interaction between ploidy level and isolation (5%, Table 2). Specifically, proportional damage of seeds decreases with habitat isolation more in hexaploids than in diploids.

In spite of the higher proportion of damaged seeds in the hexaploid populations, the number of developed seeds per flower head is still higher in the hexaploid populations (Table 2, Fig. 3). This is attributable to the higher number of initiated seeds in the hexaploids (63 vs

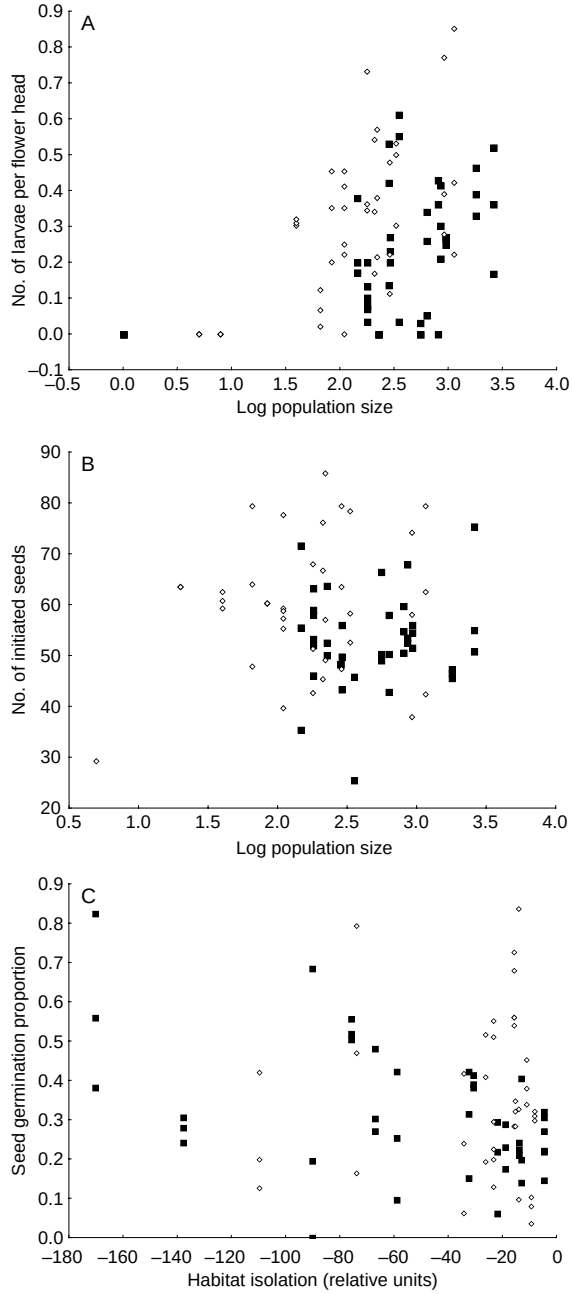

Fig. 2. Effect of plant population (A) size on the number of larvae per flower head, (B) size on number of initiated seeds and (C) isolation on seed germination. Each data point represents one population in one year and the years are not distinguished in the graphs. For the statistical significance of the patterns see Table 2. The patterns with habitat size stay significant even after removing populations below 40 flowering ramets. The black squares represent diploid and the white diamonds hexaploid populations.

57 in diploids). There is also a significant interaction between population size and ploidy level in the effect on number of initiated seeds (Table 2, Fig. 2B). In contrast,

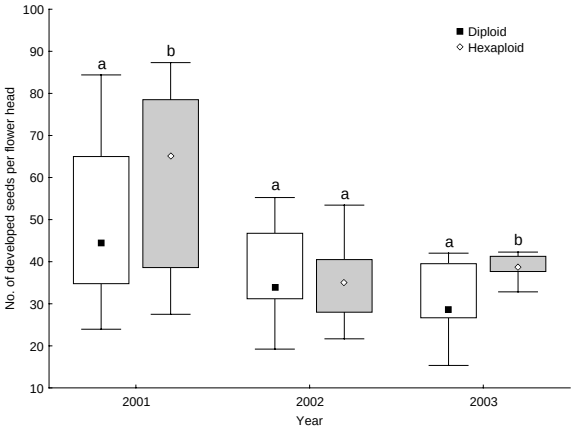

Fig. 3. Effect of ploidy level and year on the number of developed seeds per flower head. Median, upper and lower quartiles, and non-outlier minima and maxima are shown. Different letters above single boxes indicate significant differences between the ploidy levels within year. There is a significant effect of the ploidy level as well as an interaction between ploidy level and year.

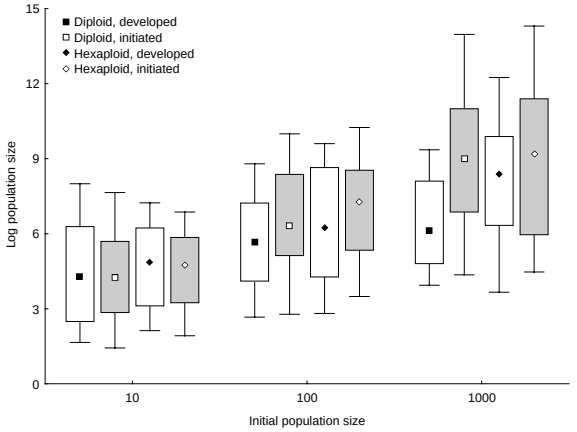

Fig. 4. Effect of ploidy level and plant population size on predicted population size in 20 years. The results use transition matrices based on combined data from both diploid and hexaploid populations over three transition periods. The matrices are combined with predicted values of the number of initiated and developed seeds from diploid and hexaploid populations of 10, 100 and 1000 flowering ramets, and are used to project the growth of populations with the given initial population size. The result is based on 100 simulations. Median, upper and lower quartiles, and non-outlier minima and maxima are shown.

the higher number of initiated seeds in larger populations does not lead to significant relationship between number of developed seeds and population size (Table 2). Both numbers of initiated and developed seeds also vary between years (Table 2); the lowest seed production was in 2003. Also there is a significant interaction between ploidy level and year for number of developed seeds (Table 2), with the 2003 decrease being stronger in hexaploids than in diploids.

Proportional germination is the only parameter unaffected by ploidy level; it was only affected by the year (Table 2, highest germination in 2002) and by interaction between isolation and ploidy level (Table 2). Specifically, diploid plants germinated less in more isolated populations whereas the hexaploids were not affected by habitat isolation (Fig. 2C).

There are no significant differences in stem height ($F_{1,27}=0.82$, $P=0.38$) or in the number of flower heads per stem ($F_{1,27}=0.11$, $P=0.74$) between the two ploidy levels in the study region.

Population size projections over 20 years using data on population dynamics of the species show that seed herbivory reduces population growth rate of both ploidy levels. In absolute terms the reduction is larger in hexaploid than in diploid populations, this is true especially in smaller populations (Fig. 4, note that y axis of the graph is logarithmic).

Discussion

Ploidy level, population size, habitat productivity as well as year all influence the occurrence of the herbivore in the study populations. This confirms the expectation that these factors may play a role in determining the pattern of plant–animal interactions in the study system. There are also significant interactions between plant-population size and ploidy level and population isolation and ploidy level, indicating that the effects of these factors are not independent.

Hexaploid populations host more herbivores and suffer higher herbivore damage than do diploid populations, supporting the prediction that ploidy level affects plant–animal interactions (Segraves and Thompson 1999, Husband 2000, Nuismer and Thompson 2001, Janz and Thompson 2002, Husband et al. 2004, Thompson et al. 2004, Nuismer and Cunningham 2005). While I assumed that the different effects of the two ploidy levels might be due to differences in plant size, the analysis of sizes of randomly selected flowering ramets from the populations did not confirm this. Hexaploid plants, however, clearly have higher number of initiated seeds, which could make the plants more attractive to the herbivores. Such larger seed production in higher ploidy levels has also been found in other studies (Lindner and Garcia 1997, Burton and Husband 2000). Also Segraves and Thompson (1999) have shown that morphology can explain differential response of insects to plants of different ploidy level.

In spite of higher seed damage, seed production in the hexaploid populations is still a bit higher than in the diploid populations. Herbivores thus limit the fitness of hexaploids, which otherwise would have much larger potential seed production than diploids as indicated by higher number of initiated seeds. Without herbivores the

seed production and thus the potential of the hexaploids to spread would be far higher than that of the diploids (Fig. 4).

I expected that ploidy level would not be the only factor affecting plant-animal interactions in the system and that other factors would interact with ploidy level. Both of these assumptions were correct. Population size and habitat productivity, but not population isolation, affected the plant-animal relationship. There was also a significant interaction between ploidy level and population size and between ploidy level and population isolation.

Population size was overall the most important factor affecting the plant–herbivore interactions in this system. Larger populations host higher densities of *C. obscenella* larvae and suffer higher proportional herbivore damage than do small populations. This agrees with findings of many previous studies that the occurrence of monophagous insects in populations of their host plants can be affected by host plant population size (Johannesen and Loeschke 1996, Kruess and Tscharntke 2000, Colling and Matthies 2004). In the case of *C. obscenella,* the minimum plant population size that can host a viable *C. obscenella* population is about forty flowering ramets. *A. amellus* populations smaller than this seem not to be able to support this species. This statement should, however, be interpreted cautiously since there are only 4 small (below 40 flowering ramets) populations in the system.

In spite of the strong effect of population size on the occurrence of *C. obscenella* larvae and on the proportion of damaged seeds, plant performance, measured as the proportion of developed seeds, was not affected by population size. This contrasts with commonly reported positive relationships between population size and plant performance (Eisto et al. 2000, Dinnetz and Nilsson 2002, Sæther et al. 2002, Snyder 2003, Münzbergová 2006). In this system, the positive effect of population size on plant performance can be seen in higher number of initiated seeds in flower heads of plants from larger populations. This positive effect of population size is, however, compensated for by the negative effect of the monophagous herbivore (see Kéry et al. 2001 for a similar pattern).

Habitat productivity was another significant factor in the system. Populations on more productive habitats hosted higher numbers of *C. obscenella* larvae and suffered higher proportional seed damage. There was, however, no effect of habitat productivity on the number of developed and initiated seeds. The inconsistency in the results here (no differences in seed production despite higher damage) can be explained by the low percentage of variation in *C. obscenella* density explained by habitat productivity.

Population isolation had no direct effect on the plant-herbivore interaction. Plants from less isolated populations had, however, higher numbers of initiated seeds. This is consistent with the expected negative effect of population isolation on plant performance (Menges and Dolan 1998, Murren 2002).

The results also show that occurrence of *C. obscenella* in populations of *A. amellus* is affected by an interaction between ploidy level and population size. This interaction can be seen for the density of *C. obscenella* larvae but not for the proportion of damaged seeds. The absence of the latter pattern can be explained by the significant effect of this interaction on the number of initiated seeds. The number of initiated seeds increases faster with population size in hexaploids than in diploids, and the slope of this increase is larger than the increase of density of *C. obscenella* larvae.

Ploidy level also interacts with population isolation. Specifically, proportional damage of seeds decreases with habitat isolation in hexaploids whereas no such trend can be found for diploids. This is in contrast with the above conclusions that hexaploid plants are more attractive to the herbivores. Obviously they cannot find them so well if the plant populations are isolated. A significant interaction between ploidy level and isolation was also found for seed germination. Here the effect is stronger in diploid populations. Since germination rate can be consequence of pollinator behavior, it may mean that pollinators sense *A. amellus* plants in a different fashion to the herbivores. Alternatively, this pattern could be explained by the fact, that plants with higher ploidy level suffer less from inbreeding since they possess more copies of a single gene within an individual plant. Patterns of differential relationship of pollinators to different ploidy levels was demonstrated also by Segraves and Thompson (1999), Husband (2000) and (2004).

The results vary markedly between years. Year as main effect has a significant effect on all dependent variables indicating that both the plant–herbivore interaction and plant performance depend on year. Year also modifies the effect of habitat productivity on the plant–herbivore interaction. The likely explanation is that in the wettest year of the study (2002) the effects of habitat productivity were weaker. During 2002, there were also reduced differences in seed production between the two ploidy levels, causing a significant year by ploidy level interaction effect on seed production. Also, the effect of habitat isolation on seed damage seems to have been reduced in 2002.

Conclusions

Ploidy level, plant population size and habitat productivity affect the occurrence of the herbivore, and these factors strongly interact. For plant population size, the negative and positive effects counteract each other and

result in no relationship between plant population size and the number of developed seeds per flower head. In contrast, hexaploid plants have higher seed damage but nevertheless retain higher seed production. Furthermore, the effect of plant population size on herbivore damage depends on the ploidy level of the population. Herbivores thus exert differential selection pressure on individuals of the two ploidy levels, and this pressure is modified by plant population size.

The importance of both ploidy level and plant population size thus suggest that not recognizing the two ploidy levels could bias conclusions concerning the effects of plant population size on plant–herbivore interaction and on plant performance. Conversely, not recognizing the effect of plant population size could limit our ability to understand the effect of different ploidy levels. It is, therefore, very important to study all the potential determinants of plant–animal interactions together since only studies considering multiple factors can provide a thorough understanding of the evolutionary pressure in such systems.

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References

Ainouche, M. L., Baumel, A. and Salmon, A. 2004. *Spartina anglica* C. E. Hubbard: a natural model system for studying early evolutionary changes that affect allopolyploid genomes. – Biol. J. Linn. Soc. 82: 475–484.

Baldizzone, G. and Tabell, J. 2002. *Coleophora obscenella* Herrich-Schäffer, 1855, *C. virgaureae* Stainton, 1875 and *C. cinerea* Toll, 1953, three distinct species (*Lepidoptera: Coleophoridae*). – SHILAP Rev. Lepidopt. 30: 15–26.

Barrett, R. D. H. and Agrawal, A. A. 2004. Interactive effects of genotype, environment, and ontogeny on resistance of cucumber (*Cucumis sativus*) to the generalist herbivore, *Spodoptera exigua*. – J. Chem. Ecol. 30: 37–51.

Bayer, R. J. 1991. Allozymic and morphological variation in *Antennaria* (Asteraceae, Inuleae) from the low arctic of northwestern North America. – Syst. Bot. 16: 492–506.

Burton, T. L. and Husband, B. C. 2000. Fitness differences among diploids, tetraploids, and their triploid progeny in *Chamerion angustifolium*: mechanisms of inviability and implications for polyploid evolution. – Evolution 54: 1182–1191.

Colling, G. and Matthies, D. 2004. The effects of plant population size on the interactions between the endangered plant *Scorzonera humilis*, a specialized herbivore, and a phytopathogenic fungus. – Oikos 105: 71–78.

Cook, L. M. and Soltis, P. S. 1999. Mating systems of diploid and allotetraploid populations of *Tragopogon* (Asteraceae) I. Natural populations. – Heredity 82: 237–244.

Crawley, M. J. 1983. Herbivory. – Blackwell Scientific Publications.

Crawley, M. J. 1989. Insect herbivores and plant population dynamics. – Annu. Rev. Entomol. 34: 531–564.

de Mazancourt, C. and Loreau, M. 2000. Grazing optimization, nutrient cycling, and spatial heterogeneity of plant–herbivore interactions: should a palatable plant evolve? – Evolution 54: 81–92.

Dinnetz, P. and Nilsson, T. 2002. Population viability analysis of *Saxifraga cotyledon*, a perennial plant with semelparous rosettes. – Plant Ecol. 159: 61–71.

Doak, D. F. 1992. Lifetime impact of herbivory on a perrenial plant. – Ecology 73: 2086–2099.

Ehrlén, J. 2003. Fitness components versus total demographic effects: Evaluating herbivore impacts on a perennial herb. – Am. Nat. 162: 796–810.

Eisto, A. K., Kuitunen, M., Lammi, A. et al. 2000. Population persistence and offspring fitness in the rare bellflower *Campanula cervicaria* in relation to population size and habitat quality. – Conserv. Biol. 14: 1413–1421.

Eubanks, M. D., Carr, D. E. and Murphy, J. F. 2005. Variation in the response of *Mimulus guttatus* (Scrophulariaceae) to herbivore and virus attack. – Evol. Ecol. 19: 15–27.

Felber, F. 1988. Phenology of flowering in diploid and tetraploid populations of *Anthoxanthum alpinum* and *Anthoxanthum odoratum*. – Can. J. Bot. 66: 2258–2264.

Fritz, R. S. 1999. Resistance of hybrid plants to herbivores: genes, environment, or both? – Ecology 80: 382–391.

Holub, J., Mesícek, J. and Javůrková, V. 1970. Annotated chromosome counts of Czechoslovak plants (1–15). – Folia Geobot. Phytotax. 5: 339–368.

Husband, B. C. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. – Proc. R. Soc. Lond. B 267: 217–223.

Husband, B. C. 2004. The role of triploid hybrids in the evolutionary dynamics of mixed-ploidy populations. – Biol. J. Linn. Soc. 82: 537–546.

Janz, N. and Thompson, J. N. 2002. Plant polyploidy and host expansion in an insect herbivore. – Oecologia 130: 570–575.

Johannesen, J. and Loeschcke, V. 1996. Distribution, abundance and oviposition patterns of four coexisting *Chiastocheta* species (*Diptera: Anthomyiidae*). – J. Anim. Ecol. 65: 567–576.

Johnson, M. T. J. and Agrawal, A. A. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). – Ecology 86: 874–885.

Juenger, T., Morton, T. C., Miller, R. E. et al. 2005. Scarlet gilia resistance to insect herbivory: the effects of early season browsing, plant apparency, and phytochemistry on patterns of seed fly attack. – Evol. Ecol. 19: 79–101.

Kersch, M. F. and Fonseca, C. R. 2005. Abiotic factors and the conditional outcome of an ant–plant mutualism. – Ecology 86: 2117–2126.

Kéry, M., Matthies, D. and Fischer, M. 2001. The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. – J. Ecol. 89: 418–427.

Kovanda, M. 1984. Chromosomes numbers of selected Angiosperms (2). – Preslia 56: 289–301.

Kovanda, M. 2005. Aster. Flora of the Czech Republic. Vol. 7., Slavik, B. and Stepankova, J (eds.), Academia, Praha Academia, Praha, Czech Republic, pp.125–140, in Czech.

Krahulcová, A. 1990. Selected chromosomes counts of the Czechoslovak flora II. – Folia Geobot. Phytotax. 25: 380–388.

Kruess, A. and Tscharntke, T. 2000. Species richness and parasitism in a fragmented landscape: experiments and field studies with insects on *Vicia sepium*. – Oecologia 122: 129–137.

Levine, J. M., Hacker, S. D., Harley, C. D. G. et al. 1998. Nitrogen effects on an interaction chain in a salt marsh community. – Oecologia 117: 266–272.

Lindner, R. and Garcia, A. 1997. Genetic differences between natural populations of diploid and tetraploid *Dactylis glomerata* ssp. *izcoi*. – Grass Forage Sci. 52: 291–297.
Louda, S. M. and Potvin, M. A. 1995. Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. – Ecology 76: 229–245.
Lumaret, R. 1988. Adaptive strategies and ploidy levels. – Acta Oecol.-Oecol. Plant. 9: 83–93.
Mandáková, T. and Münzbergová, Z. in press. Distribution and ecology of *Aster amellus* agg. in the Czech Republic. – Ann. Bot.
Masterson, J. 1994. Stomatal size in fossil plants – evidence for polyploidy in majority of angiosperms. – Science 264: 421–424.
Menges, E. S. and Dolan, R. W. 1998. Demographic viability of populations of *Silene regia* in midwestern prairies: relationships with fire management, genetic variation, geographic location, population size and isolation. – J. Ecol. 86: 63–78.
Merx, H. and Schreiber, A. 1976. Aster. Flora Europaea, Vol. 4., Tutin, T. G., Heywood, V. H., Burges, N. A. et al. (eds). Cambridge Univ. Press.
Meusel, H. and Jäger, E. 1992. Vergleichende chorologie der Zentraleuropäischen flora. – Gustav Fischer Verlag, Jena.
Münzbergová, Z. 2005. Determinants of species rarity: population growth rates of species sharing the same habitat. – Am. J. Bot. 92: 1987–1994.
Münzbergová, Z. 2006. Effect of population size on prospect of species survival. – Folia Geobot. 41: 137–150.
Murren, C. J. 2002. Effects of habitat fragmentation on pollination: pollinators, pollinia viability and reproductive success. – J. Ecol. 90: 100–107.
Nuismer, S. L. and Cunningham, B. M. 2005. Selection for phenotypic divergence between diploid and autotetraploid *Heuchera grossulariifolia*. – Evolution 59: 1928–1935.
Nuismer, S. L. and Thompson, J. N. 2001. Plant polyploidy and non-uniform effects on insect herbivores. – Proc. R. Soc. Lond. B 268: 1937–1940.
Ostergard, H. and Ehrlén, J. 2005. Among population variation in specialist and generalist seed predation-the importance of host plant distribution, alternative hosts and environmental variation. – Oikos 111: 39–46.
Pannell, J. R., Obbard, D. J. and Buggs, R. A. 2004. Polyploidy and the sexual system: what can we learn from *Mercurialis annua*? – Biol. J. Linn. Soc. 82: 547–560.
Ramsey, J. and Schemske, D. W. 2002. Neopolyploidy in flowering plants. – Annu. Rev. Ecol. Syst. 33: 589–639.
Rausher, M. D. and Feeny, P. 1980. Herbivory, plant density and plant reproductive success: the effect of *Battus philenor* on *Aristolochia reticulata*. – Ecology 61: 905–917.
Rothera, S. L. and Davy, A. J. 1986. Polyploidy and habitat differentiation in *Deschampsia cespitosa*. – New Phytol. 102: 449–467.
Sæther, B.-E., Engen, S., Lande, R. et al. 2002. Density dependence and stochastic variation in a newly established population of a small songbird. – Oikos 99: 331–337.
Segraves, K. A. and Thompson, J. N. 1999. Plant polyploidy and pollination: floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. – Evolution 53: 1114–1127.
Snyder, R. E. 2003. How demographic stochasticity can slow biological invasions. – Ecology 84: 1333–1339.
S-PLUS 2000. Professional edition for Windows, Release 2. MathSoft, Inc.
Stinchcombe, J. R. 2005. Measuring natural selection on proportional traits: comparisons of three types of selection estimates for resistance and susceptibility to herbivore damage. – Evol. Ecol. 19: 363–373.
Thompson, J. N., Nuismer, S. L. and Merg, K. 2004. Plant polyploidy and the evolutionary ecology of plant–animal interactions. – Biol. J. Linn. Soc. 82: 511–519.
Thompson, J. N., Cunningham, B. M., Segraves, K. A. et al. 1997. Plant polyploidy and insect–plant interactions. – Am. Nat. 150: 730–743.
Van Dijk, P. and Bakx-Schotman, T. 1997. Chloroplast DNA phylogeography and cytotype geography in autopolyploid *Plantago media*. – Mol. Ecol. 6: 345–352.
Vávra, J. 2004. Classification of protected areas in the city of Prague using its butterfly fauna. – Natura Pragensis 16: 3–185, in Czech.
Veteli, T. O., Tegelberg, R., Pusenius, J. et al. 2003. Interactions between willows and insect herbivores under enhanced ultraviolet-B radiation. – Oecologia 137: 312–320.
Willmot, D. B., Hibbard, B. E., Darrah, L. L. et al. 2004. Effect of environment on resistance to the European corn borer (Lepidoptera: Crambidae) in maize. – J. Econ. Entomol. 97: 1745–1751.