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Ploidy level in the genus *Leucanthemum* correlates with resistance to a specialist herbivore

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Abstract. Polyploidy is considered to be a major source of genetic diversity in plants. Genome duplication has been shown repeatedly to be associated with changes in biotic interactions, but little is known about whether species traits such as herbivore resistance consistently change with increasing ploidy level among closely related plant species. We tested whether larval survival and performance of the specialist root-mining moth *Dichrorampha aeratana* are influenced by the ploidy level of plant species in the genus *Leucanthemum* by experimentally infesting 16 different taxa with ploidy levels ranging from diploid to dodecaploid. We found that survival of *D. aeratana* larvae consistently decreased with increasing ploidy level, irrespective of whether phylogenetic distance among taxa was taken into account or not. The mass of larvae and the proportion of adults emerging from last-instar larvae, however, did not consistently change with increasing ploidy level. Root biomass and dry matter content of the *Leucanthemum* taxa were neither correlated with ploidy level nor correlated with survival or mass of *D. aeratana* larvae. In summary, our results provide evidence that in the genus *Leucanthemum,* resistance to the specialist root herbivore *D. aeratana* consistently increases with increasing plant ploidy level, but it remains unclear which characteristics associated with polyploidy account for the higher herbivore resistance.

Key words: *Dichrorampha aeratana;* herbivore resistance; *Leucanthemum;* plant–herbivore interactions; polyploidy.

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Introduction

Polyploidy, that is, the possession of more than two sets of chromosomes, is a common characteristic of plants and has played a major role in plant evolution (Soltis et al. 2009). Polyploidy can arise through intraspecific genome duplication (autopolyploidy) or through interspecific hybridization and subsequent genome doubling (allopolyploidy) and can lead to changes in chemical, physiological, morphological, or ecological properties of plants. Among other characteristics, polyploidy has been shown to be associated with increased cell size (Müntzing 1936, Stebbins 1971), increased biomass (Müntzing 1936), slower growth rates (Gottschalk 1976), increased water content (Stebbins 1971), as well as qualitative and quantitative changes in secondary metabolites (Levin 1983, Dhawan and Lavania 1996). Levin (1983) suggested that, because polyploids typically have increased concentrations of secondary metabolites, resistance to pathogens and herbivores should also be higher than in diploids. However, plant resistance toward insect herbivores has also been found to be associated with increasing toughness or its surrogate

dry matter content (Choong et al. 1992, Clissold et al. 2009, Johnson et al. 2010). The higher water content and consequently lower dry matter content of polyploids might therefore decrease mechanical resistance to herbivores. In addition, the increased biomass often associated with polyploids may also increase their attractiveness to herbivores in the field or enhance herbivore survival and performance (Price 1991).

Studies that compared the abundances of herbivorous insects in natural populations of diploid and polyploid cytotypes found that polyploids were either more, equally, or less often attacked by herbivores than diploids, depending on plant taxa, herbivore species, or even the population examined (Thompson et al. 1997, Nuismer and Thompson 2001, Münzbergová 2006, Halverson et al. 2008, Richardson and Hanks 2011, Münzbergová et al. 2015, Stutz et al. 2016). Similarly, laboratory experiments assessing herbivore resistance among closely related plant species differing in ploidy level revealed no consistent pattern between ploidy level and larval survival or performance (Lou and Baldwin 2003, Hull-Sanders et al. 2009, König et al. 2016).

Most of the previous studies on the effects of polyploidy on plant–herbivore interactions compared taxa of two different ploidy levels, rather than species complexes covering a range of ploidy levels. This is probably due to the fact that diploid–polyploid complexes of closely related species may at least partly consist of allopolyploids and hence harbor uncertainty about phylogenetic relationships. Recently, Ramsey and Ramsey (2014) argued for increased research focus on allopolyploid complexes, emphasizing that this common mode of genome duplication is largely unstudied in an ecological context. In contrast to pairwise comparisons, research on closely related plant taxa covering a range of ploidy levels may offer insight into whether phenotypic traits differ consistently with increasing ploidy levels. This approach has been adopted to assess the effects of polyploidy on breeding systems, ecological, and climatic niches or on biogeographic patterns (Guggisberg et al. 2006, Oberprieler et al. 2012, Theodoridis et al. 2013). However, little is known about whether and how plant–herbivore interactions vary among congeneric plant taxa differing in ploidy level.

We set out to assess whether increasing polyploidy in the genus *Leucanthemum* Mill. (Compositae, Anthemideae) consistently affects larval performance or survival in the tortricid moth *Dichrorampha aeratana* Pierce & Metcalfe. The genus *Leucanthemum* is a large polyploid complex comprising 42 perennial species with a ploidy level ranging from 2*x* to 22*x* (Greuter 2006+). Most of the species are considered as allopolyploids (Greiner et al. 2012). The diploid *Leucanthemum vulgare* (Vaill.) Lam. (ssp. *vulgare*) and the closely related tetraploid *L. ircutianum* DC. (ssp. *ircutianum*) are distributed all over Europe and western Asia, while the other taxa have more narrow distribution ranges (Greuter 2006+). The root-miner *D. aeratana* is widely distributed across Europe, and its larvae are specialized on *Leucanthemum* species (Razowski 2003). The adults of this univoltine species emerge in spring and lay their eggs on the underside of leaves or on stems. The young larvae move to the roots where they feed and overwinter. This species is presently also being studied in view of its use as a potential biological control agent against *L. vulgare* (ssp. *vulgare*), which has become invasive in North America (McClay et al. 2013).

We experimentally infested plants from 16 *Leucanthemum* taxa with six different ploidy levels ranging from diploid to dodecaploid with larvae of *D. aeratana* to assess whether ploidy level and/or species traits such as root biomass and root dry matter content affect larval survival or larval performance. Specifically, we asked whether the survival and performance of *D. aeratana* correlate with increasing ploidy level in the *Leucanthemum* genus. Furthermore, we hypothesized that root biomass is positively and root dry matter content negatively correlated with increasing ploidy level in the *Leucanthemum* genus and that these physiological traits may at least partly explain the potential differences in the survival and performance of *D. aeratana* among ploidy levels. As traits of related taxa are not independent due to their shared evolutionary history (Felsenstein 1985), we also incorporated information on the phylogenetic distances among the different taxa into our analyses.

Materials and Methods

Plant material

We grew plants from 16 *Leucanthemum* taxa comprising 13 species with a ploidy level of 2*x*,

Table 1. Populations of *Leucanthemum* taxa included in the study.

Code	*Leucanthemum* species	Ploidy level	Country collected	Longitude (°N)	Latitude (°E)	Altitude (m)
vul1	*L. vulgare* (Vaill.) Lam. ssp. *vulgare*	2*x*	France	43.892	3.247	750
vul2	*L. vulgare* (Vaill.) Lam. ssp. *vulgare*	2*x*	Romania	47.475	26.270	375
vup1	*L. vulgare* ssp. *pujiulae* Sennen	2*x*	Spain	42.394	2.742	478
vup2	*L. vulgare* ssp. *pujiulae* Sennen	2*x*	Spain	41.613	1.781	670
gal	*L. gallaecicum* Rodr. Oubiña & S. Ortiz	2*x*	Spain	42.860	−7.987	397
vir	*L. virgatum* (Desr.) Clos	2*x*	France	43.840	7.460	719
irc1	*L. ircutianum* DC. ssp. *ircutianum*	4*x*	France	43.393	2.411	733
irc2	*L. ircutianum* DC. ssp. *ircutianum*	4*x*	Czech Republic	50.699	15.097	470
ica1	*L. ircutianum* ssp. *cantabricum* (Sennen) Vogt	4*x*	France	42.819	−0.558	1111
ica2	*L. ircutianum* ssp. *cantabricum* (Sennen) Vogt	4*x*	France	43.076	−0.555	615
ile	*L. ircutianum* ssp. *leucolepis* (Briq. & Cavill.) Vogt & Greuter	4*x*	Croatia†			
mop1	*L. monspeliense* (L.) H. J. Coste	4*x*	Spain	42.412	2.750	1015
mop2	*L. monspeliense* (L.) H. J. Coste	4*x*	France‡			
adu1	*L. adustum* (W. D. J. Koch) Gremli	6*x*	Switzerland	46.607	10.039	1797
adu2	*L. adustum* (W. D. J. Koch) Gremli	6*x*	Switzerland	46.444	7.396	1727
atr	*L. atratum* (Jacq.) DC.	6*x*	Austria	47.77	15.830	1900
cot	*L. coronopifolium* ssp. *tenuifolium* (Guss.) Vogt & Greuter	6*x*	Italy‡			
pal1	*L. pallens* (Perreymon.) DC.	6*x*	Spain	42.690	−0.634	1220
pal2	*L. pallens* (Perreymon.) DC.	6*x*	Spain	42.504	2.960	410
fav	*L. favargeri* Vogt	8*x*	Spain	42.527	−0.556	1080
het	*L. heterophyllum* (Willd.) DC.	8*x*	Italy†			
mon	*L. montserratianum* Vogt	10*x*	Spain	41.611	1.813	711
max1	*L. maximum* (Ramond) DC.	12*x*	France	43.076	−0.555	615
max2	*L. maximum* (Ramond) DC.	12*x*	Spain	43.284	−2.310	168

Note: Seeds were collected directly in the field unless stated otherwise.
† Seeds from plants cultivated in Botanical Garden Berlin-Dahlem, Germany.
‡ Seeds from plants cultivated in Botanical Garden Nantes, France.

4*x*, 6*x*, 8*x*, 10*x*, or 12*x* from seed. Whenever possible, we included two populations of each taxon and several taxa of each ploidy level (see Table 1). The majority of seeds were sampled from field populations; seeds from four populations (see Table 1) were obtained from plants cultivated in botanical gardens. Because some of the taxa are similar in morphology, flow cytometric analyses were used to confirm the ploidy level of each plant population (see Appendix S1 for more information on the method applied). On 22 January 2013, seeds from a total of 24 populations were sown in seedling trays filled with garden soil (Selmaterra, Eric Schweizer AG, Thun, Switzerland) in a glasshouse set to 20°C and 16-h photoperiod at CABI in Delémont, Switzerland (47.3731° N, 7.3253° E). Three weeks later, seedlings were transplanted into individual cells (4 cm × 4 cm) of seedling trays filled with a mixture of garden soil, sand, and vermiculite (14:3:1) with 1 g/L of slow-release NPK fertilizer (Hauert Tardit 6M) added. At the same time, glasshouse temperature was reduced to 10°C during the night and the photoperiod was reduced to 12 h. In mid-April, 12–18 plants per population (12 plants for infestation with larvae in spring and up to six plants for infestations with larvae found during plant dissections in autumn) were potted in plastic pots (diameter 14 cm, height 17 cm) filled with the same mixture of soil as described above and moved outside into gauze-covered field cages (2 m × 2 m × 1.6 m) to keep them protected from herbivores occurring naturally in the garden.

Insect rearing

The *D. aeratana* larvae used in the experiment originated from a rearing colony which had been established from larvae collected from a natural population of *L. ircutianum* ssp. *ircutianum* in Sonogno, Ct. Ticino, Switzerland, in winter 2011. *Dichrorampha aeratana* was reared for two generations on potted *L. vulgare* ssp. *vulgare* and

L. ircutianum ssp. *ircutianum* plants kept outdoors in gauze-covered field cages (2 m × 2 m × 1.6 m). In spring 2013, plants were individually covered with gauze bags and emerging adults were regularly collected. Adults were placed in transparent plastic cylinders (1.3 L) and provided with rosettes of *L. vulgare* or *L. ircutianum* for mating and oviposition. Leaves with eggs were kept in Petri dishes at room temperature until the larvae hatched.

Experimental setup

Five larvae, not older than 24 h, were transferred with a fine-hair paintbrush on the shoot base of each of 12 potted plants per population. From 17 to 20 May 2013, a total of 282 plants were infested with larvae. After infestation, the plants were left for one day in the laboratory and then transferred to the gauze-covered field cages and embedded in sawdust. Between 7 and 24 October 2013, the roots and rhizomes of all surviving plants ($n = 268$) were dissected under a stereo microscope and the number of larvae was counted. All live larvae were weighed, and the maximum width of their head capsule was measured to determine larval instar. Because most of the larvae were found in the main root or in larger rhizomes, we collected the main root and any rhizomes with a diameter of at least 5 mm to estimate the root biomass suitable for larval development. The collected root parts were dried at 80°C for 24 h and then weighed. In addition, we counted the number of rhizomes with a diameter of at least 5 mm. To estimate the dry matter content of the roots, a small (approximately 1 cm^3) undamaged piece of the central part of the main root was weighed immediately after dissection and again after drying at 80°C for 24 h, and the ratio of these two measurements was calculated. Larvae found during dissection were transferred onto potted plants of the respective population (except for five taxa where no plants were available in autumn). Each plant was infested with up to five larvae, and the plants were moved outside and embedded in sawdust for overwintering. In spring 2014, plants were individually covered with gauze bags and adult emergence was recorded from 11 April to 2 June 2014.

Statistical analyses

To examine whether increasing polyploidy, root biomass, the number of rhizomes, or root dry matter content influenced larval survival and larval mass, we used generalized linear mixed models (GLMMs, for larval survival) with binomial error distributions and linear mixed models (LMMs, for larval mass). Population nested within plant taxa was included as random factor, and polyploidy (as an integer variable), root biomass, the number of rhizomes and root dry matter content were individually included as fixed effects. To investigate whether larvae found in plants dissected earlier were lighter than those found in plants dissected later and whether the potential increase in larval mass during the dissection period differed between ploidy level, we included the date when the plants had been dissected for larvae (as a fixed effect) and its interaction with ploidy level in the analyses on larval mass. To investigate whether the proportion of adults that emerged in spring from larvae that had been transferred in autumn was influenced by increasing ploidy level, we used a GLMM with binomial distribution. To analyze whether increasing ploidy level was correlated with root biomass, the number of rhizomes, or root dry matter content, we used LMMs (for root biomass and root dry matter content) and GLMMs with Poisson distribution (for the number of rhizomes). Root biomass was square-root-transformed to increase normality of residuals.

To correct for a potential correlation between polyploidy and phylogenetic distance in the genus *Leucanthemum,* we established a phylogenetic distance matrix for the 16 *Leucanthemum* taxa included in this study (Appendix S2: Table S1) as well as Euclidean distance matrices for mean taxon values of larval survival and ploidy level and compared the matrices using Mantel tests (Legendre and Legendre 1998). The phylogenetic distance matrix was based on yet unpublished sequence variation at nine nuclear DNA loci and at five concatenated intergenic spacer regions of the chloroplast genome (K. Konowalik, *unpublished data*; see Appendix S2 for a detailed description for the calculation of the phylogenetic distance matrix). Simple Mantel tests were conducted to test for correlations between the three distance matrices, and a partial Mantel test was conducted to test for correlations between the distance matrices of larval survival and ploidy level while controlling for phylogenetic distances. Although more sophisticated techniques

than Mantel tests are available to account for the statistical non-independence of taxa (Felsenstein 1985, Grafen 1989), these phylogenetic regression methods are all based on phylogenetic trees. However, the majority of the polyploid taxa included in our study are expected to be allopolyploids (Greiner et al. 2012) and their phylogenetic relationship cannot be accurately represented by a phylogenetic tree. Therefore, pairwise phylogenetic distances among the taxa involved using the above-mentioned methods were used as a proxy summarizing these reticulate relationships.

All calculations except for the phylogenetic distance matrix were made with the software R version 3.1.2 (R Core Team 2014), LMMs were performed with the lme function in the nlme package (Pinheiro et al. 2014), and GLMMs were performed with the glmer function in the lme4 package (Bates et al. 2014). Distance matrices were calculated using the dist function in core R, and Mantel tests were calculated using the mantel function in the ecodist package (Goslee and Urban 2007).

Results

Larval survival until autumn was negatively affected by increasing ploidy level (z = 6.0, P < 0.0001, Fig. 1a), but was not affected by root biomass, the number of rhizomes, or root dry matter content (all P > 0.1). Larval mass was not affected by increasing ploidy level (t = −0.9, P = 0.4; Appendix S3: Fig. S1), but it was strongly influenced by the dissection date (t = 7.7, P < 0.0001; Appendix S3: Fig. S2). Larvae found on plants dissected earlier were on average 50% lighter compared to those found 17 days later, but there was no significant interaction between ploidy level and dissection date (P > 0.1). Larval mass was not influenced by root biomass, the number of rhizomes, or root dry matter content (all P > 0.1). Although larval mass was very variable (Appendix S3: Fig. S2), head-capsule measurements revealed that 98% of the larvae (n = 227) were in their final instar (Appendix S3: Fig. S3). The proportions of adults that emerged in spring from larvae that had been transferred in autumn were not influenced by increasing ploidy level (z = −1.1, P = 0.3; Appendix S3: Fig. S4). Root biomass, the number of rhizomes, and root dry matter content were not influenced by increasing ploidy level (all P > 0.1, Fig. 1b, c). Polyploidy and larval survival were both not correlated with phylogenetic distances (Mantel test r = 0.2, P = 0.08 and r = 0.1, P = 0.11, respectively), and larval survival was still significantly correlated with polyploidy when controlling for phylogenetic distances (partial Mantel test r = 0.6, P = 0.001).

Discussion

In our study, we found that increasing ploidy level in the genus *Leucanthemum* was negatively correlated with the survival of the specialist herbivore *D. aeratana,* irrespective of whether the phylogenetic distance among the *Leucanthemum* taxa was taken into account or not. As far as we know, this is the first evidence for a consistent shift in plant–herbivore interactions with increasing polyploidy among closely related plant taxa. In contrast, the mass of the last-instar larvae and the survival from the last larval instar to adult moths were not influenced by increasing polyploidy, indicating that characteristics associated with increasing ploidy level may primarily affect the development of early-instar larvae. When dissecting the plant roots in autumn, only three of 405 larvae were found to be dead and only few empty feeding mines were found. Hence, larvae which were not found back had most likely died early in their development. Potential reasons for the observed reduction in larval survival might be that first-instar larvae were not able to penetrate the roots and initiate feeding or that mechanical or chemical properties of the roots affected survival of those early-instar larvae that started feeding on the roots.

Only a few other studies have investigated the influence of plant ploidy level on herbivore survival or performance, and the results reveal no consistent pattern (Lou and Baldwin 2003, Hull-Sanders et al. 2009, König et al. 2016). In contrast to our results, larval survival of the generalist herbivore *Spodoptera exigua* (Hübner) was higher on tetraploid and hexaploid than on diploid cytotypes of *Solidago gigantea* Aiton while larval mass was reduced on tetraploid compared to diploid and hexaploid cytotypes (Hull-Sanders et al. 2009). However, *S. gigantea* is not a natural host of *S. exigua* and the survival

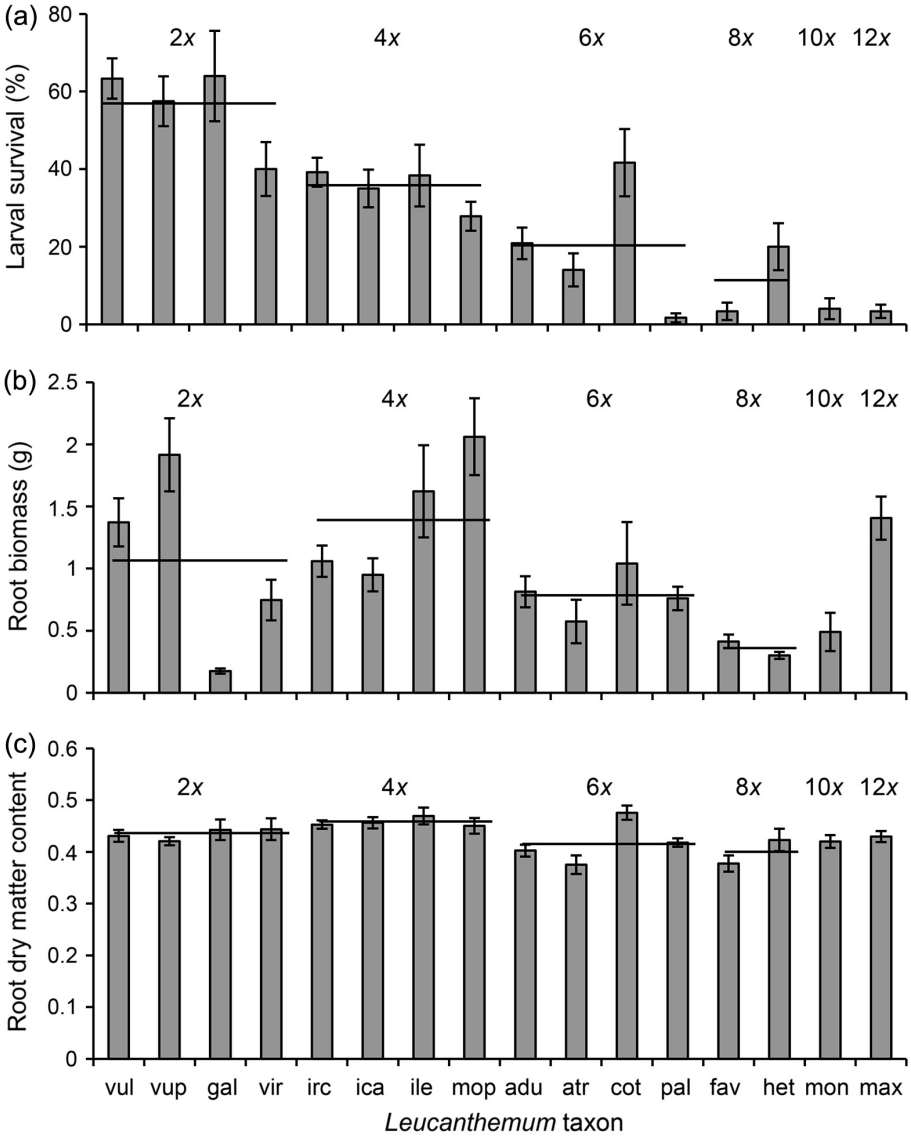

Fig. 1. (a) Percentage larval survival of *Dichrorampha aeratana* on 16 *Leucanthemum* taxa varying in ploidy level, (b) biomass of the main root and rhizomes with a diameter of at least 5 mm, and (c) root dry matter content of the central part of the main root of 16 *Leucanthemum* taxa varying in ploidy level. Mean values ± SE taxon are shown for each *Leucanthemum*, and horizontal lines indicate mean values for each ploidy level. vul = *L. vulgare* ssp. *vulgare*, vup = *L. vulgare* ssp. *pujiulae*, gal = *L. gallaecicum*, vir = *L. virgatum*, irc = *L. ircutianum* ssp. *ircutianum*, ica = *L. ircutianum* ssp. *cantabricum*, ile = *L. ircutianum* ssp. *leucolepis*, mop = *L. monspeliense*, adu = *L. adustum*, atr = *L. atratum*, cot = *L. coronopifolium* ssp. *tenuifolium*, pal = *L. pallens*, fav = *L. favargeri*, het = *L. heterophyllum*, mon = *L. montserratianum*, and max = *L. maximum*.

and performance of the specialist *Trirhabda virgata* LeConte were reported to be similar on all three cytotypes (Hull-Sanders et al. 2009, 2015 regarding incorrrect determination of cytotypes). Six-day-old larvae of the oligophagous *Manduca sexta* L. were lighter when fed on the diploid *Nicotiana attenuata* Torr. ex Watson than when fed on two derived allotetraploids (Lou and Baldwin (2003), while larval performance of the oligophagous butterfly *Anthocharis cardamines* (L.) was

similar on tetraploid and octoploid cytotypes of *Cardamine pratensis* L. (König et al. 2016).

In the field, larvae of *D. aeratana* have been found in the two most widespread taxa, the diploid *L. vulgare* ssp. *vulgare* and the tetraploid *L. ircutianum* ssp. *ircutianum* (S. Stutz, *personal observation*), but no data are available on which of the other taxa are included in the natural host range. In addition, we had reared *D. aeratana* on *L. vulgare* ssp. *vulgare* and *L. ircutianum* ssp. *ircutianum*. Potentially, selection pressure to survive on these two widespread taxa may have contributed to the higher survival on taxa with low ploidy levels. However, excluding *L. vulgare* ssp. *vulgare* and *L. ircutianum* ssp. *ircutianum* from the analysis on the influence of increasing polyploidy on larval survival did not change our results (data not shown). Our results could also be interpreted that *D. aeratana* occurring on taxa with low ploidy levels survive better on other taxa with similar ploidy levels than on taxa with different ploidy levels, but that herbivores occurring on taxa with high ploidy levels would reveal the opposite pattern. Further investigations taking a comparative approach with multiple closely related plant species differing in ploidy level are needed to elucidate whether higher ploidy levels in general tend to be more resistant to herbivory or whether herbivore species show a preference for the ploidy level similar to that of the host plant they have coevolved with. However, both explanations suggest that taxa with the same ploidy levels share more similar traits related to herbivore resistance than taxa with different ploidy levels.

Root biomass and the number of rhizomes varied considerably among plant taxa, but they were not correlated with increasing polyploidy. This is in contrast to other studies where polyploidy was associated with higher belowground biomass and a higher number of lateral rhizome buds in *Butomus umbellatus* L. (Hroudová and Zákravský 1993), a higher number of rhizomes in *S. gigantea* (Schlaepfer et al. 2010), and a higher belowground biomass in *Aster amellus* L. and in *Pimpinella saxifraga* L. (Sudová et al. 2010). Also, we found no evidence for a consistent association between root dry matter content and polyploidy. Polyploids generally have lower leaf dry matter content as a result of their larger cell size and thus an increase in the ratio of cell volume to cell surface (Stebbins 1971), but to our knowledge, the effect of polyploidization on cell size and dry matter content has not been assessed for roots so far.

We found no correlation between root biomass and larval survival or performance. This is in line with our observations that in most of the plants, only small parts of the roots were eaten and shows that the roots of all plants were large enough to support the five larvae that had been transferred on them. We did not measure initial root biomass of the plants, but as all plants had been sown 4 months before infestation, all of them were large enough when the larvae had been transferred. We also found no correlation between larval survival or larval mass and root dry matter content. As the mass of larvae found on the same plant individual varied considerably (up to 2.5-fold), it might have been influenced by differences in nutritional quality, root texture, or concentrations of defense chemicals within the root tissue rather than by differences among plants.

The higher herbivore resistance associated with increasing polyploidy might have been caused by higher levels of secondary metabolites in polyploids as proposed by Levin (1983). *Leucanthemum* species contain polyacetylenes and flavonoids (Wrang and Lam 1975, Wilcox 1984, Bellido et al. 1988, Christensen 1992), some of which have been shown to be toxic or act as feeding deterrents to generalist insect herbivores (Champagne et al. 1986). Yet, the effects of these or any other secondary metabolites on the survival of the specialist *D. aeratana* are unknown.

Following Greiner et al. (2012), the majority of the polyploid taxa included in our study are likely to have arisen through allopolyploidization. This assumption is based on sequence variation of the maternally inherited chloroplast DNA which revealed that most of the polyploid taxa included in our study (i.e., *L. ircutianum*, *L. adustum*, *L. pallens*, *L. favargeri*, *L. heterophyllum*, *L. montserratianum*, *L. maximum*) share haplotypes closely related to the haplotype realized in the diploid *L. virgatum* (the only diploid species in this haplotype group) and the fact that most of these species are morphologically similar to the diploid species belonging to another haplotype group which includes *L. vulgare* and other species not included in our study (Greiner et al. 2012). The allopolyploid origin of the tetraploid

L. ircutianum ssp. *ircutianum* and the hexaploid *L. adustum* has been confirmed by AFLP fingerprinting (Oberprieler et al. 2011). For allopolyploid species, differences in plant–insect interactions among different ploidy levels may also result from hybridization. As it is currently unclear which taxa contributed to the formation of the polyploid taxa, we cannot exclude hybridization as a potential factor contributing to the observed pattern in resistance of *Leucanthemum* species toward the specialist *D. aeratana.* Hybridization has repeatedly been shown to affect herbivore resistance, with hybrids usually revealing levels of herbivore resistance that are intermediate to that of their parental species, similar to the parental species with the lower resistance or lower than both of their parental species (Fritz et al. 1999, Cheng et al. 2011). Yet, increased resistance relative to their parents appears to be a rare phenomenon among hybrids (Fritz et al. 1999, Cheng et al. 2011), suggesting that hybridization may not be the main factor explaining the consistently lower susceptibility of the higher ploidy levels compared to the diploid species in the genus *Leucanthemum*.

In summary, our results provide evidence that in the genus *Leucanthemum,* resistance to the specialist root herbivore *D. aeratana* consistently increases with increasing plant ploidy level. We propose that comparative studies are a powerful tool to search for broad-scale patterns between ploidy variation among closely related plant taxa and species interactions. More detailed analyses, such as comparisons of allopolyploids with their parental species or the creation of neo-polyploids (Ramsey and Ramsey 2014), are needed to elucidate whether the observed pattern between ploidy level of *Leucanthemum* species and resistance to a specialist herbivore is indeed due to polyploidization *per se* or due to other factors such as hybridization.

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