Apomixis Allows the Transgenerational Fixation of Phenotypes in Hybrid Plants

Sailer, Christian ; Schmid, Bernhard ; Grossniklaus, Ueli

Abstract: The introduction of apomixis-asexual reproduction through seeds-into crop plants is considered the holy grail of agriculture, as it would provide a mechanism to maintain agriculturally important phenotypes [1, 2]. Apomicts produce clonal offspring, such that apomixis could be used to transgenerationally fix any genotype, including that of F1 hybrids, which are used in agriculture due to their superior vigor and yield [3-9]. However, traits (phenotypes) do not only result from a complex combination of genetic and environmental variation but can also be influenced by epigenetic variation, which can be transgenerationally heritable in plants [10-15]. Hence, it is far from clear whether genetic fixation by apomixis suffices to fix the agriculturally relevant phenotypes of F1 hybrids, in particular because hybridization was recently shown to induce epigenetic changes [16, 17]. Here, we show that the phenotypes of Hieracium pilosella hybrids can be fixed across generations by apomixis. Using a natural apomict, we created 11 hybrid genotypes (lines). In these and a parental line, we analyzed 20 phenotypic traits that are related to plant growth and reproduction. Of the 20 traits, 18 (90%) were stably inherited over two apomictic generations, grown at the same time in a randomized design, in 11 of the 12 lines. Although one hybrid line showed phenotypic instability, our results provide a fundamental proof of principle, demonstrating that apomixis can indeed be used in plant breeding and seed production to fix complex, quantitative phenotypes across generations.

DOI: https://doi.org/10.1016/j.cub.2015.12.045

Apomixis allows the transgenerational fixation of phenotypes in hybrid plants

Christian Sailer[1,3], Bernhard Schmid[2], Ueli Grossniklaus[1]

[1]Department of Plant and Microbial Biology & Zürich-Basel Plant Science Center, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

[2]Department of Evolutionary Biology and Environmental Studies & Zürich-Basel Plant Science Center, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

[3]current address: Department of Cellular and Developmental Biology, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK. ORCID ID: 0000-0003-0070-0317

Correspondence to: **Ueli Grossniklaus**
Department of Plant and Microbial Biology
University of Zürich
Zollikerstrasse 107
CH-8008 Zürich
Switzerland
Tel: +41 (0)44 634 82 40
Fax: +41 (0)44 634 82 04
Email: grossnik@botinst.uzh.ch

Summary

The introduction of apomixis — asexual reproduction through seeds — into crop plants is considered the holy grail of agriculture, as it would provide a mechanism to maintain agriculturally important phenotypes [1,2]. Apomicts produce clonal offspring, such that apomixis could be used to transgenerationally fix any genotype, including that of F1 hybrids, which are used in agriculture due to their superior vigor and yield [3-9]. However, traits (phenotypes) do not only result from a complex combination of genetic and environmental variation, but can also be influenced by epigenetic variation, which can be transgenerationally heritable in plants [10-15]. Hence, it is far from clear whether genetic fixation by apomixis suffices to fix the agriculturally relevant phenotypes of F1 hybrids, in particular since hybridization was recently shown to induce epigenetic changes [16,17]. Here we show that the phenotypes of *Hieracium pilosella* hybrids can be fixed across generations by apomixis. Using a natural apomict, we created 11 hybrid genotypes (lines). In these and a parental line we analyzed 20 phenotypic traits that are related to plant growth and reproduction. Of the 20 traits, 18 (90%) were stably inherited over two apomictic generations, grown at the same time in a randomized design, in 11 of the 12 lines. Although one hybrid line showed phenotypic instability, our results provide a fundamental proof-of-principle, demonstrating that apomixis can indeed be used in plant breeding and seed production to fix complex, quantitative phenotypes across generations.

Results and Discussion

Apomictic reproduction does not require a paternal contribution, hence offspring are maternal clones [1, 2]. Thus, in theory, any genotype and the corresponding traits (phenotypes) will become transgenerationally fixed [1, 3-5]. The fixation of F1 hybrids would have a tremendous impact on breeding programs and the welfare of subsistence farmers [6, 7]. However, traits are the result of a combination of genetic, environmental, and epigenetic variation [8-13]. Hence, it is far from clear whether the fixation of the genotype through apomixis is sufficient for the stable propagation of the agriculturally relevant phenotypes of F1 hybrids.

***Hieracium pilosella*, a natural aposporous model system.** Apomixis comprises the alteration or omission of meiosis (apomeiosis), the initiation of embryogenesis in the absence of fertilization (parthenogenesis), and functional endosperm development [18]. Clones produced by gametophytic apomixis (apomicts) can be generated by two types of apomeiosis (apospory or diplospory) and the endosperm either develops autonomously or requires fertilization (pseudogamy). Although epigenetic reprogramming may occur at any developmental transition, with respect to transgenerational inheritance the focus lies on germline specification and fertilization[19]. Therefore, diplospory and pseudogamy are likely accompanied by epigenetic changes because they involve the specification of a megaspore mother cell (MMC, the first cell of the female germline [20]) and fertilization of the central cell, respectively, when epigenetic reprogramming has been demonstrated [21, 22]. Similarly, synthetic clones in *Arabidopsis thaliana*, which are generated by combining meiotic mutants with paternal chromosome elimination [23], require the specification of an MMC and fertilization. Consequently, transgenerational phenotypic stability is best addressed in a natural aposporous apomict with autonomous endosperm development such as *Hieracium pilosella* L. [24]. In apospory, the

specification of a MMC is bypassed as the female gametophyte develops from a sporophytic (somatic) unreduced cell in the ovule (aposporous initial cell, AIC). Furthermore, hybridization is circumvented as both embryo and endosperm develop without fertilization. Therefore, we assume that offspring do not only maintain the maternal genomic constitution [3,9], but that the epigenome also stays unaltered.

The existence of microspecies in apomicts (e.g. [25]), implies that phenotypic traits are stably inherited. However, these microspecies are the result of natural selection and do not correspond to an F1 hybrid. Because hybridization can alter epigenetic states and/or other maternal carry-over effects – mediated by maternally deposited, bioactive molecules, including proteins, RNAs, polysaccharides, and other compounds – in the offspring [16,17,20,26], such epigenetic changes induced by hybridization could influence the phenotype and affect its stability in subsequent generations. Thus, hybrids of *H. pilosella* offer a system to test the fundamental question whether the phenotype of superior F1 hybrids can indeed be fixed by apomixis, as originally suggested in the 1930s [4-6].

Generation of apomictic hybrid lines, propagation, and phenotyping. In *H. pilosella*, apomictic reproduction depends on two loci, one controlling apospory (*LOSS OF APOMEIOSIS, LOA*) and one controlling parthenogenesis (*LOSS OF PARTHENOGENESIS*, *LOP*) [27], both being necessary for producing maternal clones. Male gametophytic development is usually unaffected in apomicts [3,18], enabling outcrossing of apomixis and segregation of *LOA* and *LOP* through the male. Outcrossing leads to four possible offspring types [28]: (1) sexual (*loa lop*; meiosis and fertilization occur), (2) B_{III} hybrid-makers (*LOA lop*; apomeiosis with fertilization leads to increased ploidy), (3) polyhaploid-makers (*loa LOP*; meiosis with parthenogenesis leads to decreased ploidy), and (4) apomicts (*LOA LOP*; apomeiosis with parthenogenesis leads to maternal clones) (Figure 1).

To generate hybrid genotypes, four sexual mothers were crossed with two apomictic fathers, resulting in F1 hybrids that segregate for the *LOP* and *LOA* loci, resulting in four different offspring

types (Figure 1). Since *H. pilosella* is an obligate outcrosser, the parental plants are heterozygous and therefore each individual F1 hybrid is a new genotype. Parthenogenetic F1 hybrids (types (3) and (4)) were identified by decapitation, which removes stigmas and anthers, disabling pollination [7], and propagated to generation A1 (apomictic generation 1, Figure 1), in which they were further tested for apomeiosis by measuring the ploidy of A1 individuals and, by ensuring apomictic reproduction through decapitation, they were propagated to A2. We started with 51 parthenogenetic hybrid lines from seven families, but only 11 of these new apomictic hybrid lines from one family and one of the apomictic parents had a sufficiently high fitness to be used throughout the experiment. The excluded lines either produced too few apomictic seeds or their germination rate was too low for sufficient replication. Because apomixis is a dominant trait, this means that a single set of *LOA* and *LOP* alleles conferring apomixis was present in the 12 lines. Plants of the 12 lines of generations A1 and A2 were grown from seeds at the same time in a fully randomized design (season 4 in Figure 1). We confirmed hexaploidy of all plants used for our analyses in season 4, when the phenotypic data were collected (Figure 1). Growing the plants of different generations at the same time greatly reduces the effect of environmental variation (Figure 1). This, together with ecological statistical analysis, allowed us to efficiently test for the transgenerational fixation of hybrid phenotypes relevant to generative and vegetative propagation, as well as plant growth (Table 1).

Transgenerational phenotypic stability across lines. In order to identify which factors (generation, line) significantly affect the measured traits we examined our data using an analysis of variance (ANOVA). In case of a transgenerationally fixed phenotypic trait, we would expect no effect of generation, an effect of lines (lines are genetically different and are therefore expected to be phenotypically different), and no 2-way interaction between these factors. While the factor generation tests for genetic and epigenetic contributions to the phenotypic trait, the 2-way interaction separates the

genetic from the epigenetic component and indicates non-genetic contributions to the phenotypic trait. Inspecting our analyzed data for these signatures, we found 2-way interactions of generation and line for 14 of 20 traits, showing that different lines behaved differently across generations for these 14 traits. This means that for these 14 traits some of the 12 lines increased, some decreased, and some did not change their phenotypic trait values across generations. For the remaining 6 traits, all lines behaved similarly across generations, that is all 12 lines either increased, decreased, or did not change trait values from generation A1 to A2.

Separate analyses of each trait showed that one line, 198-7, was always the main contributor to the 'generation x line' interaction term for these 14 traits, suggesting that this line is different from the other 11 lines and not stable across generations. To test whether line 198-7 behaves differently than the other 11 lines thereby causing the significant 'generation x line' 2-way interaction term, we split (partitioned) the lines into two groups: line 198-7 and the 11 remaining lines. These two groups represent two levels of the new factor L198-7, which places line 198-7 in contrast to the 11 remaining lines. Therefore, this type of partitioning is also referred to as contrast, and is a powerful tool for in-depth analysis. We further partitioned our data into another contrast, 'father vs. offspring' (FvO) to test whether the apomictic parent was phenotypically different from its F1 offspring. A significant 'father vs. offspring' term would constitute an indication for heterosis.

The refined ANOVA tested for significant effects of the factors 'generation', 'L198-7', 'FvO', and 'line' (the remaining 10 lines, each line being one level) and contained two 2-way interactions: 'generation x L198-7' and 'generation x line'. For 7 traits, the apomictic father was different from its 11 offspring lines. However, a conservative test evaluating the variance of the father against the variance of its offspring was not significant (manual F in Table S1-3). This means that the apomictic

parent was phenotypically indistinguishable from its offspring, which is not unexpected for traits related to heterosis given that both parent and offspring were highly heterozygous.

For all but one trait (total seed mass), 'generation x line' was no longer significant even with a liberal test using $\alpha = 0.1$ as the significance level (Table S1-3). This shows that line 198-7 was the sole reason for the significant 'generation x line' interaction term of the first analysis. Thus, line 198-7 is an exception and has transgenerationally labile phenotypes, whereas the other 10 lines have transgenerationally stable phenotypes in apomictic offspring. Furthermore, we found in this refined ANOVA that the factor 'line' was significant, showing that the 10 remaining lines were phenotypically different from each other.

Since we used uniform environmental conditions for all plants in this experiment, and had confirmed apomictic reproduction and hexaploidy of all individuals, we speculate that the high variability in hybrid line 198-7 might be due to (1) the reactivation of transposons or other genome instabilities caused by hybridization, (2) large epigenetic changes influencing the phenotypes, (3) a combination of the former, or (4) a high sensitivity to maternal carry-over effects. This hints towards an interdependency of genome and epigenome, an interesting observation that will require future studies.

Transgenerational phenotypic stability across traits. Separating the exceptional line 198-7 from the remaining lines (data partitioning) enabled us to investigate the effect of generation in the remaining 11 lines, i.e. to test whether the phenotypic traits changed from generation A1 to A2. Since there is no 2-way interaction 'generation x line', we know that these 11 lines react equally across generations. As a consequence, we were able to explicitly test for transgenerational phenotypic fixation in genetically fixed lines by scanning ANOVA results for the signature combinations listed in table 2. In particular, a phenotypic trait is transgenerationally fixed if there is no significant generation term. We found no significant effect of generation for 13 of 20 traits (65%), indicating transgenerational

phenotypic stability (Table 2). For a further five traits (25%), we did find a main effect of generation; however, the term generation includes all 12 lines. This means that a strong change from generation A1 to A2 in the aberrant line 198-7 could cause the generation term to be significant. If L198-7 is indeed the cause, then the 2-way interaction 'generation x L197-8' should be significant, which was indeed the case. This was further supported by estimating the broad-sense heritability H^2 from Kendall's τ [29] by correlating the remaining lines' median values between generation A1 and A2 (Table S4). This analysis supports transgenerational stability of phenotypic differences between genotypes, i.e. genetic or epigenetic heritability. In total, we observed phenotypic stability for 18 of the 20 phenotypic traits we assessed (90%, Table S1-4, Figures S1-3).

Only for 2 of 20 traits (10%) we did not find transgenerational phenotypic stability. For one trait, age at flowering, the generation effect could also be explained by the 2-way interaction. However, the lines' median values across generations did not correlate ($\tau = H^2 = 0.04$, $p = 0.876$), indicating that heritable differences in age at flowering between lines were labile and thus phenotypic stability across generations absent. This likely reflects differences in the degree of epigenetic changes or loss of maternal carry-over effects between lines.

For the second trait, the number of leaves at bolting, we found a generation effect (mean ± 2 standard deviations: increase from 12.7 ± 2.3 in A1 to 13.4 ± 2.2 in A2, Table S3), and no 2-way interactions. Since the plants of generations A1 and A2 are genetic clones due to ensured apomictic reproduction, and we randomized environmental variation in our experimental design, this increase can only be explained by a consistent epigenetic change or a consistent loss of maternal carry-over effects in all 12 lines, because changes in this phenotypic trait across generations were correlated among lines ($\tau = H^2 = 0.66$, $p = 0.005$). We speculate that this change in one direction is due to seed age, as seeds of generation A1 were a few months older than the seeds of generation A2. This could have led to a

consistent epigenetic change or a consistent difference in maternal carry-over effects between the two generations, resulting in the observed increase in leaves at bolting. We conclude that these two phenotypic traits, both related to flowering time which is known to be influenced epigenetically [30], are not stably inherited despite apomictic fixation of the genotype.

Selected examples of stable phenotypes. For a more detailed discussion of our results, we present four selected phenotypic traits of agricultural interest that relate to generative propagation, vegetative propagation, and growth. The first trait we describe is apomictic fertility, quantified as the number of apomictic seeds/number of ovules, a measure of how efficiently a line can be maintained and propagated. This trait varied widely among lines (Figure 2A), indicating that apomictic fertility is a complex (polygenic), and quantitative trait. We found the factor 'generation' to be significant, but this result was driven by the aberrant line 198-7 mentioned above (2-way interaction 'generation x L198-7', $F_{1, 119} = 92.5$, $p < 0.001$, Table S1). That line 198-7 caused the generation effect was further supported by the significant correlation between the line's median values and the high broad-sense heritability ($\tau = H^2 = 0.75$, $p = 0.001$, Table S4). Taken together, these results indicate the transgenerational stability of phenotypic differences between the remaining lines. In other words, if generation did influence apomictic fertility, this effect was weak and consistent among all lines but 198-7 (change from 0.50 ± 0.14 in A1 to 0.51 ± 0.14 in A2), supporting the conclusion that apomictic fertility can be transgenerationally fixed by apomixis.

A second phenotype relevant to generative propagation is the agronomically important trait total seed mass, corresponding to yield in grain crops. Like apomictic fertility, seed mass is a quantitative trait and varied widely among lines (Figure 2B). There was no effect of the factor 'generation' (Table S1), suggesting transgenerational phenotypic stability. However, significant 2-way interaction terms were discovered for 'generation x L198-7' and 'generation x line' ($F_{1, 109} = 6.8$, $p = 0.010$ and

$F_{10, 109} = 1.74$, $p = 0.082$, respectively, $\alpha = 0.1$, Table S1), indicating that the trait values changed inconsistently (up, down, equal) from generation A1 to A2. Our interpretation of this result is that generations do not differ in general, but that the epigenome can significantly affect the phenotype in certain genomic contexts. Together with the absence of a global generation effect and the high heritability ($H^2 = \tau = 0.60$, $p = 0.010$, Table S4) this indicates that total seed mass slightly increases in some and decreases in other lines; thus, transgenerational phenotypic stability depends on the genomic context. Nonetheless, lines that have transgenerationally fixed yields can be selected in breeding programs.

The fact that *H. pilosella* also reproduces vegetatively enabled us to assess vegetative reproduction by counting the number of stolons. Similar to the two traits described above, this trait was also quantitative (Figure 2C). There was no effect of the factor 'generation' but a significant 2-way interaction term 'generation x L198-7' ($F_{1, 135} = 0.95$, $p = 0.332$, and $F_{1, 124} = 17.2$, $p < 0.001$, respectively, Table S2). Furthermore, the remaining lines showed a high heritability ($H^2 = \tau = 0.60$, $p = 0.021$, Table S4). Together, these results indicate transgenerational stability of vegetative reproduction, at least to the extent that our test could not detect instability at the $\alpha = 0.1$ significance level. In addition, it is worth noting that while line 198-7 decreased in apomictic fertility, it increased in the number of stolons, pointing to a trade-off between generative and vegetative propagation in this line.

We could not measure biomass to assess growth performance in the hybrid lines because plants had to be grown until senescence for seed harvest. As a surrogate measure, we used the diameter of the rosette at flowering [31], which also showed a continuous distribution across a wide range of values (Figure 2D). We did not find an effect of 'generation', but did find a significant 2-way interaction term 'generation x L198-7' ($F_{1, 95} = 2.1$, $p = 0.141$, and $F_{1, 95} = 8.1$, $p = 0.005$, respectively, Table S3). We

also found a high heritability in all other lines ($H^2 = \tau = 0.80$, $p < 0.001$, Table S4). Again, this phenotype and its differences between the 11 remaining lines were transgenerationally stable, suggesting that it can be fixed by apomixis.

Conclusion

In conclusion, we report a proof-of-principle for the fixation of complex, quantitative phenotypes by apomixis across generations, and thus demonstrate its potential applicability in plant breeding and seed production, which would have a tremendous impact on agriculture and the welfare of subsistence farmers.

Experimental Procedures

Accessions and generation of new apomictic lines. Two apomictic hexaploid lineages (aP6, apomictic Pilosella 6-ploid) were isolated from two populations in New Zealand (MwR1, Molesworth Road, latitude: -42.00933, longitude: 172.95406, and LaP1, Lake Pukaki, latitude: -44.15848, longitude: 170.22020). LaP1 has a low, MwR1 a high apomictic fertility. Four sexual hexaploid lineages (sP6, sexual Pilosella 6-ploid) were isolated from two populations from the Morteratsch glacier foreland, Upper Engadin, Switzerland (MoK5-4: 791849, 145561; MoG20-2, MoG20-8, MoG23-8: 792087, 148071; GPS, Swiss Grid).

The apomictic plants were used as fathers and both were crossed to all four (except for one case) sexual mother plants, creating seven families. Parthenogenetic lines were selected among the F1 by seed set of decapitated flower heads. In A1, apomeiotic parthenogenetic lines were selected by flow cytometry to identify the hybrid lines that produce maternal clonal offspring in the absence of a genotyping system for apomixis. Two different apomictic generations (A1 and A2) of these hybrid lines were then grown in a fully randomized design in the same environment at the same time. These plants

were grown from seeds. Only 11 lines from one family had a fitness high enough to be used throughout the experiment.

Plants were grown in a greenhouse cabin with an automated watering system as described previously [30].

Ploidy analysis. Ploidy analysis was performed by flow cytometry as described previously [32].

Statistical analysis. ANOVA on linear models was used for interval data, and ANOVA on generalized linear models with the family function "quasipoisson" (due to overdispersion of the data) with the canonical link function "log" was used for count data. For the proportion data of fertility, ANOVA on a generalized linear model with the family function "quasibinomial" (overdispersed data) and the canonical link function "logit" was used. We created two contrasts, father vs. offspring and line 198-7 vs. other offspring lines (L198-7). If a global generation effect together with an interaction was found ($\alpha = 0.1$), we used Kendall's τ as a measure of broad sense heritability H^2 to interpret the generation effect [29]. The conservative test of generation against the interaction is not allowed in our case, since it would favor our conclusion. A generation effect tested for genetic and epigenetic stability, whereas interaction effects of 'generation x line' tested for epigenetic stability. All the analyses were done in R[33], graphs were drawn using the packages ggplot2 [34] and grid [35].

Author Contributions

Conceptualization, U.G.; Methodology, U.G., B.S., and C.S.; Formal Analysis, B.S., and C.S.; Investigation, C.S.; Writing – Original Draft, C.S.; Writing – Review & Editing, U.G., B.S., and C.S.; Supervision, U.G.; Funding Acquisition, U.G., and B.S.

Acknowledgements

Seeds from New Zealand were collected and kindly provided by Michael Beckman (Helmholtz Centre for Environmental Research). We thank Anja Herrmann, Anja Schmidt, Frederique Pasquer, Joana Bernades de Assis, Nina Chumak, Nuno Pires, and Sofia Nobre (University of Zürich) for fruitful discussions, and Klara Kropivsek, Nina Chumak, Nuno Pires, Roger Schmid (University of Zürich), as well as Jeffrey DaCosta, and Caroline Weissman (Harvard University) for critical reading of the manuscript. We further thank Alain Held, Anja Grossniklaus, Rita Galhano, Arturo Bolaños, and Karl Huwiler for assistance in greenhouse work. This work was supported by the University of Zurich and, in part, by a Syngenta PhD Fellowship of the Zurich-Basel Plant Science Center. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to UG (grossnik@botinst.uzh.ch).

References

[1] **Calzada, J., Crane, C.F., Stelly, D.M. (1996). Apomixis: The asexual revolution. Science *274*, 1322–1323.**

[2] **Spillane, C., Curtis, M.D., Grossniklaus, U. (2004). Apomixis technology development - virgin births in farmers' fields? Nat. Biotechnol. *22*, 687–691. doi:10.1038/nbt976.**

[3] **Asker, S.E., Jerling, L. (1992). Apomixis in Plants (Boca Raton: CRC Press).**

[4] **Navashin, M. (1933). New views in selection. Semenovodstvo *2*, 11–6.**

[5] **Karpenchenko, G.D. (1935). Experimental Polyploidy and Haploidy. In Theoreticheskie Osnovy Selekeii Raslenij, (Moskva-Leningrad: Obqaja selekcija), pp. 39–435.**

[6] **Solntzeva, M.P. (1978). Apomixis and hemigamy as one of its forms. Proc. Indian Natn. Sci. Acad. B *44*, 78–90.**

[7] **Koltunow, A.M.G., Bicknell, R.A., Chaudhury, A.M. (1995). Apomixis: Molecular strategies for the generation of genetically identical seeds without fertilization. Plant Physiol. *108*, 1345–1352.**

[8] **Grossniklaus, U., Moore, J.M., Gagliano, W.B. (1998). Molecular and genetic approaches to understanding**

and engineering apomixis: Arabidopsis as a powerful tool. In Advances in Hybrid Rice Technology, S.S. Virmani, E.A. Siddiq, K. Muralidharan, ed. (Manila, Philippines: Int. Rice Res. Inst.), pp. 187–211.

[9] **Hörandl, E., Grossniklaus, U., van Dijk, P.J., Sharbel, T.F. (2007). Apomixis. (Königstein: A. R. G. Gantner Verlag K.G).**

[10] **Jacobsen, S.E., Meyerowitz, E.M. (1997). Hypermethylated SUPERMAN epigenetic alleles in *Arabidopsis*. Science *277*, 1100–1103.**

[11] **Cubas, P., Vincent, C., Coen, E. (1999). An epigenetic mutation responsible for natural variation in floral symmetry. Nature *401*, 157–161. doi:10.1038/43657.**

[12] **Reinders, J., Paszkowski, J. (2009). Unlocking the *Arabidopsis* epigenome. Epigenetics *4*, 557–563.**

[13] **Hirsch, S., Baumberger, R., Grossniklaus, U. (2012). Epigenetic variation, inheritance, and selection in plant populations. Cold Spring Harb. Symp. Quant. Biol. *77*, 97–104. doi:10.1101/sqb.2013.77.014605.**

[14] **Cortijo, S., Wardenaar, R., Colomé-Tatché, M., Gilly, A., Etcheverry, M., Labadie, K., Caillieux, E., Hospital, F., Aury, J.M., Wincker, P., et al. (2014). Mapping the epigenetic basis of complex traits. Science *343*, 1145–1148. doi:10.1126/science.1248127.**

[15] **Johannes, F., Porcher, E., Teixeira, F.K., Saliba-Colombani, V., Simon, M., Agier, N., Bulski, A., Albuisson, J., Heredia, F., Audigier, P., et al. (2009). Assessing the impact of transgenerational epigenetic variation on complex traits. PLoS Genet. *5*, e1000530–1000540. doi:10.1371/journal.pgen.1000530.**

[16] **Greaves, I.K., Groszmann, M., Ying, H., Taylor, J.M., Peacock, W.J., Dennis, E.S. (2012). Trans chromosomal methylation in *Arabidopsis* hybrids. Proc. Natl. Acad. Sci. USA. *109*, 3570–3575. doi:10.1073/pnas.1201043109.**

[17] **Chen, Z.J. (2013). Genomic and epigenetic insights into the molecular bases of heterosis. Nat. Rev. Genet. *14*, 471–482. doi:10.1038/nrg3503.**

[18] **Koltunow, A.M.G., Grossniklaus, U. (2003). Apomixis: a developmental perspective. Annu. Rev. Plant Biol. *54*, 547–574. doi:10.1146/annurev.arplant.54.110901.160842.**

[19] **Gutierrez-Marcos, J. F., and Dickinson, H. G. (2012). Epigenetic reprogramming in plant reproductive lineages. Plant Cell Physiol. *53*, 817–823.**

[20] **Grossniklaus, U. (2011). Plant germline development: a tale of cross-talk, signaling, and cellular interactions. Sex. Plant Reprod. *24*, 91–95. doi:10.1007/s00497-011-0170-3.**

[21] **She, W., Grimanelli, D., Rutowicz, K., Whitehead, M.W.J., Puzio, M., Kotlinski, M., Jerzmanowski, A., Baroux, C. (2013). Chromatin reprogramming during the somatic-to-reproductive cell fate transition in plants. Development *140*, 4008–4019. doi:10.1242/dev.095034.**

[22] **Baroux, C., Pien, S., Grossniklaus, U. (2007). Chromatin modification and remodeling during early seed development. Curr. Opin. Genetics Dev. *17*, 473–479. doi:10.1016/j.gde.2007.09.004.**

[23] **Marimuthu, M.P.A., Jolivet, S., Ravi, M., Pereira, L., Davda, J.N., Cromer, L., Wang, L., Nogue, F., Chan, S.W.L., Siddiqi, I., et al. (2011). Synthetic clonal reproduction through seeds. Science *331*, 876. doi:10.1126/science.1199682.**

[24] **Koltunow, A.M.G., Johnson, S., Bicknell, R.A. (1998). Sexual and apomictic development in *Hieracium*. Sex. Plant Repr. *11*, 213–30.**

[25] **Majeský, L., Vašut, R.J., Kitner, M., Trávníček, B. (2012). The pattern of genetic variability in apomictic clones of *Taraxacum officinale* indicates the alternation of asexual and sexual histories of apomicts. PLoS One. *7(8)*, e41868. doi: 10.1371/journal.pone.0041868.**

[26] **O'Connor, C.M., Norris, D.R., Crossin, G.T., Cooke, S.J. (2014). Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. Ecosphere *5*, art28. doi:10.1890/ES13-00388.1.**

[27] **Catanach, A.S., Erasmuson, S.K., Podivinsky, E., Jordan, B.R., Bicknell, R.A. (2006). Deletion mapping of genetic regions associated with apomixis in *Hieracium*. Proc. Natl. Acad. Sci. USA. *103*, 18650–18655. doi: 10.1073/pnas.0605588103.**

[28] **Rutishauser, A. (1948). Fortpflanzungsmodus und Meiose apomiktischer Blütenpflanzen (Berlin: Springer Verlag).**

[29] **Falconer, D.S., Mackay, T.F.C. (1996). Introduction to Quantitative Genetics, Fourth Edition (Essex: Longman Publishing Group).**

[30] **Turck, F. G. C. (2014). Natural variation in epigenetic gene regulation and its effects on plant developmental traits. Evolution *68*, 620–631. doi:10.1111/evo.12286.**

[31] **Werner, P.A. (1975). Predictions of fate from rosette size in teasel (*Dipsacus fullonum* L.). Oecol. *20*, 197–201.**

[32] **Sailer, C., Schmid, B., Stöcklin, J., Grossniklaus, U. (2014). Sexual *Hieracium pilosella* plants are better inter-specific, while apomictic plants are better intra-specific competitors. Perspect. Plant Ecol. Evol. Syst. *16*, 43–51. doi:10.1016/j.ppees.2014.01.001.**

[33] **R Developmental Core Team 2. (2010). R: A Language and Environment for Statistical Computing.**

[34] **Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis. (Berlin: Springer)**

[35] **Murrell, P. (2005). R Graphics. (Boca Raton: CRC Press).**

Table 1 Measured phenotypic traits related to generative propagation, vegetative propagation, and growth

Generative Propagation	Vegetative Propagation	Growth
Fecundity (number of ovules)	Maximum length of stolons	Number of leaves at flowering
Number of seeds	Length of stolons	Diameter of capitulum at flowering
Total seed mass	Number of stolons	Length of stem
Number of empty achenes		Age at bolting
Total empty achenes mass		Age at seed set (generation time)
Individual seed mass		Diameter of rosette at bolting
Individual empty achene mass		Diameter of rosette at flowering
Apomictic fertility		Age at flowering
		Number of leaves at bolting

Table 2 Summary and interpretation of results. Lines were different from each other for all 20 phenotypic traits

Class	Generation effect	Generation x L198_7	Correlated generations	Interpretation	Observed for traits	Observed for n traits	Phenotype influenced by	Conclusion
1	no	no	no	Trait fixed, although trait value differences are not inherited.	Fecundity; Maximum length of stolons; Number of leaves at flowering; Diameter of capitulum at flowering	4	Genotype	Phenotype transgen-erationally fixed
2	no	no	yes	Trait fixed, trait value differences between lines are inherited.	Length of stolons	1		
3	no	yes	no	Trait fixed in general, but not in all genotypes. Interdependency of genome and epigenome.	Number of seeds; Length of stem; Age at bolting; Age at seed set	4	Genotype and Epigenotype	
4	no	yes	yes	Trait fixed in general, but not in all genotypes. Trait value differences between lines are inherited. Interdependency of genome and epigenome.	Total seed mass; Number of stolons; Diameter of rosette at bolting; Diameter of rosette at flowering;	4		
5	yes	yes	yes	Trait partially fixed. Generation effect is due to 'generation x L198-7'. Trait value differences between lines are inherited. Interdependency of genome and epigenome.	Number of empty achenes; Total empty achenes mass; Individual seed mass; Individual empty achene mass ; Apomictic fertility	5		

6	yes	yes	no	No fixation of trait. Generation effect is due to 'generation x L198-7', but trait value differences are not inherited. Interdependency of genome and epigenome.	Age at flowering	1	Genotype and Epigenotype	Phenotype not transgen-erationally fixed
7	yes	no	yes	No fixation of trait.	Number of leaves at bolting	1	Genotype	
8	yes	no	no	No fixation of trait.	-	0		

Figure Legends

Figure 1 Scheme of the experimental design. Segregating F1 hybrids were of four possible types (sexual, B_{III} hybrid-makers, polyhaploid-makers, apomictic). Apomictic, maternal clones were selected and propagated apomictically for two generations. Plants of apomictic generation A1 and A2 of hybrid lines were grown from seeds in a fully randomized design at the same time in the same environment (season 4). All presented data was collected in season 4. sP6 – sexual Pilosella hexaploid, aP6 – apomictic Pilosella hexaploid

Figure 2 Stability and change of four selected phenotypic traits between apomictic generations.

A, Apomictic fertility; **B,** total seed mass; **C,** number of stolons; and **D,** diameter of rosette at flowering. Dots represent the predicted mean value and the error bar the 95% confidence interval. Hybrid lines are ordered according to their value in generation A1, except for the apomictic parent MwR1, which is always on the left of each panel, and hybrid line 198-7, which is always on the right. Light grey bars are the results from generation A1, black bars from generation A2. See also Figures S1-S3 and Tables S1-S4