# Ecological risk assessment of genetically modified strawberries – the hybridization potential between cultivated and wild strawberries

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## General introduction

### *Hybridization and genetically modified economic plants*

Hybridization is a widespread phenomenon in many plant and animal species complexes (Arnold 1997). Generally, hybridization refers to crosses between individuals from different taxa but also to crosses between genetically differentiated populations or subspecies within a species (Bresinsky et al. 2008). According to a definition of natural hybridization of Arnold (1997) '*a natural hybrid individual derives from crosses in nature between individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters*'.

Plant scientists have studied hybridization to understand sytematics of particular plant groups (e.g., Ellis 1962; Gillett 1966; Huskins 1931; Mangelsdorf and East 1927) and natural hybridization has been acknowledged as an important evolutionary process that can lead to new evolutionary lineages (e.g., Arnold 1992; Arnold 1997; Brokaw and Hufford 2010; Rieseberg et al. 1995; Widmer and Baltisberger 1999). In the last decades, natural hybridization processes became an increasingly important subject in conservation biology. It has been recognized that hybridization may contribute to the demise of rare species, especially in the wake of continuous loss of natural habitats and the introduction of non-native species (Levin et al. 1996; Rieseberg 1991). Furthermore, the introduction of genetically modified (GM) economic plants has raised questions about the potential for transgene escape from GM plants into populations of wild or weedy relatives via hybridization (Colwell et al. 1985; Ellstrand 2003; Ellstrand et al. 1999). To date, numerous studies have shown the potential of GM economic plants to hybridize with wild species (Belanger et al. 2003; Jorgensen and Andersen 1994; Simard et al. 2006; Spencer and Snow 2001; Warwick et al. 2003) or the potential for introgression of transgenes from GM economic plants into wild relatives (Laughlin et al. 2009; Schoenenberger et al. 2006; Snow et al. 2003). There is general consensus that regulatory frameworks for GM economic plants should be based on rational scientific analysis, however, there has been vigorous public campaigning for and against the cultivation of GM crops by interest groups (Dale 2005). Also within the scientific community studies on the ecological effects of GM economic plants are discussed with much controversy, e.g. the debate on the transgene introgression into maize landraces in Mexico (Editor

2002; Metz and Futterer 2002; Quist and Chapela 2001; Quist and Chapela 2002) or the debate on the effects of transgenic insecticidal corn on non-target species such as the monarch butterfly (e.g., Obrycki 2001; Obrycki et al. 2001; Ortman et al. 2001). In Europe, as compared to other continents, reservations against cultivation of GM crops have been pronounced and GM crop regulations have been relatively strict (Davison 2010). In Switzerland, voters accepted a five-year moratorium on the commercial use of GM plants in 2005 (Schläpfer 2008). As a reaction to this vote the Swiss Federal Council requested that the Swiss National Science Foundation should implement the National Research Programme NRP 59 'Benefits and Risks of the Deliberate Release of Genetically Modified Plants' (NRP 59, 2007).

The present thesis has been carried out as a project within the NRP 59. Its goal was to assess the hybridization potential between cultivated garden strawberries (*Fragaria* x *ananassa* Duch.) and wild relatives in Switzerland, as a basis for estimating the risks of a potential future cultivation of transgenic garden strawberries. Strawberries are a high-value niche crop in Switzerland with yearly production quantities of 6000 – 9000 tons during the last 20 years (FAO, 2011) and there is a need for investigating possible effects of transgenic strawberries on natural habitats. Furthermore, the *Fragaria* species complex is insofar special as *Fragaria* species are perennials that reproduce clonally (Darrow 1966) and also sexually infertile species hybrids may persist locally through formation of clonal offspring (Bringhurst and Khan 1963). This is a characteristic that is not often found in crop plants and strawberries may serve as a model system for clonal perennial plants.

### *Hybridization in the genus* Fragaria

The genus *Fragaria* (Rosaceae) contains 24 perennial herbaceous species, including well defined hybrid species such as *F.* x *ananassa* (Staudt 2009). Ploidy levels of the different species range from di- to octoploid. Natural hybrids between *Fragaria* species have been reported repeatedly (Bringhurst and Khan 1963; Staudt et al. 2003; Westman et al. 2004). Furthermore, numerous experimental attempts to produce hybrids between species with similar or different ploidy levels have been made to date to study their phylogenetic relationships or to introduce novel traits into cultivars (Evans 1974; Mangelsdorf and East 1927; Marta et al. 2004; Noguchi et al. 2002; Olbricht et al. 2006; Yarnell 1931a; Yarnell 1931b). In general, it is possible to cross

species with similar ploidy levels and their progeny are fertile. Yet it is far more difficult to breed hybrids between species of different ploidy levels, which usually are odd-ploid (e.g. 2n x 8n = 5n). They exhibit high mortality at early developmental stages and hybrids are generally highly sterile due to chromosome imbalances, but can be vigorous with high clonal reproduction rates.

Although future commercialisation of GM garden strawberries is very likely (Qin et al. 2008) knowledge about the hybridization potential of garden strawberries with wild relatives under natural conditions is limited. I am aware of only one study on natural gene flow from cultivated *F.* x *ananassa* to its wild American parent species *F. virginiana* Mill. in south-eastern USA (Westman et al. 2004). Westman et al. (2004) found significant gene flow from *F.* x *ananassa* to *F. virginiana*, which is not surprising as both species are octoploids and are closely related. The situation is different in Europe, where cultivated *F.* x *ananassa* is the only octoploid *Fragaria* species present (Staudt 1989) and hybridization with wild relatives seems less likely. In Switzerland, three wild strawberry species can be found, i.e. the diploid *F. vesca* L., the diploid *F. viridis* Duch. and the hexaploid *F. moschata* Duch. (Lauber and Wagner 1996). The distribution of *F. viridis* and *F. moschata* is relatively sparse in Switzerland and the most likely wild candidate species for hybridization seems to be the common *F. vesca* (Lauber and Wagner 1996). Therefore, this thesis was focused on the hybridization potential between *F. vesca* and *F.* x *ananassa*.

***The study species***

The octoploid *F.* x *ananassa* emerged from accidental hybridization between the wild octoploid American species *F. chiloensis* Mill. and *F. virginiana*, and was first described by Duchesne in the 18$^{th}$ century from botanical gardens in Europe (Darrow 1966). Many of the morphological traits found in modern *F.* x *ananassa* cultivars are still intermediate to its parent species, but considerable segregation has occurred (Hancock 1999). There are self-compatible monoecious and dioecious varieties and plants reproduce clonally via formation of stolons.

The diploid *F. vesca*, the woodland strawberry, is the only *Fragaria* species that occurs throughout the northern hemisphere and it is the most common wild *Fragaria* species (Hancock 1999). In Europe, it is distributed all over the British Isles and continental Europe, including parts of Scandinavia and parts of the Iberian

peninsula. It is a self-compatible monoecious plant and generally reproduces clonally via formation of stolons.

***Main research aims and methodological approaches***

The main research aims of this thesis were:

(1) Assessment of the hybridization potential between *F.* x *ananassa* and *F. vesca*
(2) Assessment of fitness of hybrid plants and the potential effects of hybridization on natural *F. vesca* populations

The studies that were carried out to address aim (1) are presented in Chapters I and II and studies dealing with aim (2) make up Chapters III and IV. In the following, I give a short outline of the studies presented in Chapters I-IV.

Chapter I:

To study the natural hybridization potential between *F.* x *ananassa* and *F. vesca*, a hybrid survey was conducted in the surroundings of farms in Switzerland and southern Germany, where garden strawberries have been cultivated for at least ten years and wild *F. vesca* plants occur in the close vicinity. Based on reference samples of wild *F. vesca* plants and *F.* x *ananassa* cultivars I selected seven microsatellite markers that yielded species-specific alleles. Samples of wild *F. vesca* plants were collected at farm survey sites in 2007 and 2008 and were analysed with microsatellite markers. All survey sites were revisited in 2010 and morphological traits of wild *F. vesca* plants were inspected. Morphologically deviating plants were sampled and ploidy levels of plants were estimated by flow cytometry to identify putative hybrids. Furthermore, I carried out experimental hand-crosses between *F.* x *ananassa* and *F. vesca* plants in a greenhouse to study the hybridization potential under controlled conditions. Hybrid plants from hand-crosses were used to test the potential of microsatellite analysis and flow cytometry to detect first generation hybrids.

Chapter II:

Solitary bees are most important and effective pollinators that visit both *F.* x *ananassa* and *F. vesca* plants in the field. However, it is unknown whether these animals show a

preference for either plant species. To assess whether natural hybridization between *F.* x *ananassa* and *F. vesca* is promoted by the behaviour of pollinators I studied the flower choice behaviour of solitary bees in a greenhouse experiment. I presented blocks of *F.* x *ananassa* and *F. vesca* plants to marked red mason bees (*Osmia rufa* L.) and recorded flower visits and flower handling during forage bouts of individual bees.

Chapter III:
The biology of *F. vesca* can serve as a referential framework in any attempt to compare the fitness of *F. vesca* x *F.* x *ananassa* hybrids and *F. vesca* plants. To date, there are only limited demographic data on *F. vesca*. Therefore, the demography of *F. vesca* was studied at natural sites in northwestern Switzerland from spring 2008 to spring 2010. *Fragaria vesca* plants were marked and mapped and different demographic parameters were measured during four yearly censuses, i.e. plant survival, plant size, sexual reproduction and clonal reproduction. Demographic data were used to parameterise periodic matrix population models and population growth rates were calculated for the different sites. The importance of different growth parameters for population growth was assessed using prospective (elasticity analyses) and retrospective (life table response experiments) matrix analysis methods.

Chapter IV:
Growth of different *F. vesca* clones and *F. vesca* x *F.* x *ananassa* hybrid clones was compared in a competition experiment in a greenhouse. Single *F. vesca* or hybrid plants were grown with flanking *F. vesca* plants (competition treatment) or alone (control treatment) from July 2009 until September 2010. During this time I regularly recorded sexual and clonal reproduction of plants. At the end of the experiment, plant biomass was harvested. I tested for differences in total plant biomass and allocation of biomass to vegetative plant structures and sexual and clonal reproductive structures between *F. vesca* and hybrid plants. Furthermore, fruit and runner plant production was compared. I interpreted these results based on the findings of the importance of different growth parameters for *F. vesca* population growth (Chapter III) and estimated general fitness of *F. vesca* x *F.* x *ananassa* hybrids.

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# Chapter I

## Searching for gene flow from cultivated to wild strawberries in Central Europe

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# Searching for gene flow from cultivated to wild strawberries in Central Europe

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• *Background and Aims* Experimental crosses between the diploid woodland strawberry (*Fragaria vesca* L.) and the octoploid garden strawberry (*F.* × *ananassa* Duch.) can lead to the formation of viable hybrids. However, the extent of such hybrid formation under natural conditions is unknown, but is of fundamental interest and importance in the light of the potential future cultivation of transgenic strawberries. A hybrid survey was therefore conducted in the surroundings of ten farms in Switzerland and southern Germany, where strawberries have been cultivated for at least 10 years and where wild strawberries occur in the close vicinity.
• *Methods* In 2007 and 2008, 370 wild *F. vesca* plants were sampled at natural populations around farms and analysed with microsatellite markers. In 2010, natural populations were revisited and morphological traits of 3050 *F. vesca* plants were inspected. DNA contents of cell nuclei of morphologically deviating plants were estimated by flow cytometry to identify hybrids. As controls, 50 hybrid plants from interspecific hand-crosses were analysed using microsatellite analysis and DNA contents of cell nuclei were estimated by flow cytometry.
• *Key Results* None of the wild samples collected in 2007 and 2008 contained *F.* × *ananassa* microsatellite markers, while all hybrids from hand-crosses clearly contained markers of both parent species. Morphological inspection of wild populations carried out in 2010 and subsequent flow cytometry of ten morphologically deviating plants revealed no hybrids.
• *Conclusions* Hybrid formation or hybrid establishment in natural populations in the survey area is at best a rare event.

**Key words:** *Fragaria vesca*, *Fragaria* × *ananassa*, hybridization, microsatellite markers, genetically modified organisms, gene flow.

## INTRODUCTION

The genus *Fragaria* (Rosaceae) contains 23 reported herbaceous species, including well defined hybrids (Folta and Davis, 2006). The different species show various ploidy levels ranging from di- to octoploid. Today, the diploid woodland strawberry (*Fragaria vesca* L.) is the only *Fragaria* species that occurs throughout the northern hemisphere (Hancock, 1999). Tetraploid species are confined to East Asia and the hexaploid *F. moschata* L. to Europe. The octoploid species, which are generally interpreted as the phylogenetically most advanced, are distributed in the Americas.

Numerous experimental attempts to produce hybrids between species with the same or different ploidy levels within the genus *Fragaria* have been made to date to investigate the genetic compatibility of species and their phylogenetic relationship or to introduce novel traits into cultivars (Mangelsdorf and East, 1927; Yarnell, 1931*a*, *b*; Evans, 1974; Noguchi *et al.*, 2002; Marta *et al.*, 2004; Olbricht *et al.*, 2006). Generally, species with the same ploidy level can be crossed successfully and their progeny are fertile. Hybrids between species of different ploidy levels are far more difficult to breed. They show high mortality at early developmental stages and plants reaching maturity are usually highly sterile, but can be vigorous and vegetatively prolific. Gene flow between *Fragaria* species with the same ploidy level in the field has been reported repeatedly (Staudt *et al.*, 2003; Westman *et al.*, 2004). In addition, a tetraploid clone that originated either from autopolyploidization of *F. vesca* or from polyploidization of a *F. vesca* × *F. viridis* hybrid has been described from Finland (Ahokas, 1999). However, the only report of naturally occurring hybrids between *Fragaria* species of different ploidy levels that we are aware of comes from Bringhurst and Khan (1963). It describes two occurrences of pentaploid hybrids between octoploid *F. chiloensis* Mill. and diploid *F. vesca* in coastal California. These hybrids were described as infertile but competing well with their co-occurring parental species due to superior stolon productivity. Bringhurst and Khan (1963) assumed that interspecific hybrids arise fairly often in nature. Furthermore, they hypothesized that in the case of *F. chiloensis* × *F. vesca* hybrids the next fertile species level of decaploid hybrids may already have been reached by somatic chromosome doubling or the functioning of unreduced gametes.

Subsequently, Bringhurst and Senanayake (1966) continued the survey and reported >20 other pentaploid hybrid individuals as well as a nonaploid and a partially fertile hexaploid hybrid from seven sites in coastal California. These findings confirmed their assumption of widespread occurrences of hybrids.

However, reports of hybrids in wild populations have not triggered any surveys on *Fragaria* populations in Europe, although surveys are increasingly important with a growing availability of genetically modified (GM) crop plants and the outlook for GM strawberries (Qin *et al.*, 2008). Besides the above-mentioned studies (Bringhurst and Khan, 1963; Bringhurst and Senanayake, 1966), we are only aware of one systematic survey on hybridization between *Fragaria* species. This is a survey on hybridization between the cultivated octoploid garden strawberry (*Fragaria* × *ananassa* Duch.) and one of its two wild parent species, the octoploid *F. virginiana* Mill. in south-eastern USA (Westman *et al.*, 2004). Not unexpectedly, this study showed substantial gene flow from cultivated strawberries to wild *F. virginiana*.

In Europe, cultivated *F.* × *ananassa* is the only octoploid species. It is grown widely as a high-value fruit crop. *Fragaria* × *ananassa* emerged from hybridization between the wild American species *F. chiloensis* and *F. virginiana*, and was first described by Duchesne in the 18th century from botanical gardens in Europe (Darrow, 1966). Wild *Fragaria* species present in Europe are *F. vesca*, *F. viridis* and *F. moschata*. *Fragaria vesca* is the most abundant species and is distributed all over the British Isles and continental Europe, including parts of Scandinavia and parts of the Iberian peninsula (Hancock, 1999). It has bisexual flowers, is self-compatible and generally reproduces vegetatively through formation of stolons. Commercial strawberry fields can often be found in the close vicinity of wild *F. vesca*. This is particularly the case in landscapes with small-scale structures such as hedges, groves and forest edges providing a suitable habitat for *F. vesca*. The main flowering times of *F.* × *ananassa* and *F. vesca* can overlap during April, May and June in Switzerland. Honey bees are the most important pollinators for cultivated strawberries in open fields (Hancock, 1999), but relatively little is known about pollinators of *F. vesca* (Knuth, 1898). A study on pollinator overlap between *F.* × *ananassa* and *F. vesca* from north-western Switzerland suggested that solitary wild bees are the most important pollinators for *F. vesca* in that area (Gross, 2009). Furthermore, solitary wild bees were also frequently pollinating *F.* × *ananassa* flowers. Only honey bees were more important pollinators of cultivated strawberries in that study, but they rarely visited wild strawberries.

Given the combined occurrence of both wild and cultivated strawberry species, flowering time overlap and pollinator overlap, we hypothesized that there is potential for gene flow between cultivated strawberries and wild *F. vesca* that might lead to stable hybrid populations.

To assess the extent of hybrid formation between cultivated *F.* × *ananassa* and wild *F. vesca* a hybrid survey in populations of *F. vesca* was designed. In 2007 and 2008 wild *F. vesca* plants in the vicinity of strawberry cultures at farm sites in Switzerland and Baden-Württemberg, southern Germany, were sampled and samples were tested at microsatellite loci for *F.* × *ananassa* alleles. As no hybrids were detected, all farm sites were revisited in 2010 and wild *F. vesca* populations were screened. Morphologically conspicuous plants from *F. vesca* populations were sampled and their ploidy levels estimated by flow cytometry.

We expected most first-generation hybrids to be pentaploid, but also hexaploid, nonaploid or even decaploid hybrids could result from pairing of unreduced and normally reduced gametes or two unreduced gametes. We assumed that backcrossing of $F_1$ hybrids with *F. vesca* would be a rare event, as pentaploid $F_1$ hybrids derived from crosses between diploids and octoploids are highly sterile (Mangelsdorf and East, 1927; Yarnell, 1931*a*; Bringhurst and Khan, 1963; Senanayake and Bringhurst, 1967; Olbricht *et al.*, 2006). Senanayake and Bringhurst (1967) estimated the amount of functional pollen as below 1 % for pentaploids and somewhat over 5 % for hexaploids of different interspecific *Fragaria* crosses.

At the same time, we performed experimental crosses between *F. vesca* and *F.* × *ananassa* and used these experimental hybrids to test the power of our molecular analysis and flow cytometry to detect hybrids. Furthermore, we estimated germination rates and survival of hybrids.

The aim of the present study was to assess the extent of hybridization between wild *F. vesca* and cultivated *F.* × *ananassa* under natural conditions, and thus to assess the risk of transgene escape associated with a potential future cultivation of transgenic strawberry cultivars.

## MATERIALS AND METHODS

### *Plant reference samples*

To identify *Fragaria vesca*- and *F.* × *ananassa*-specific alleles four *F. vesca* reference populations were sampled at forest sites in northern and north-western Switzerland (Fig. 1, Table 1). These populations were situated within an altitudinal range representative for *F.* × *ananassa* cultures in Switzerland and, as far as we know, never had immediate contact with *F.* × *ananassa* cultures. Ten plants were sampled in each population along

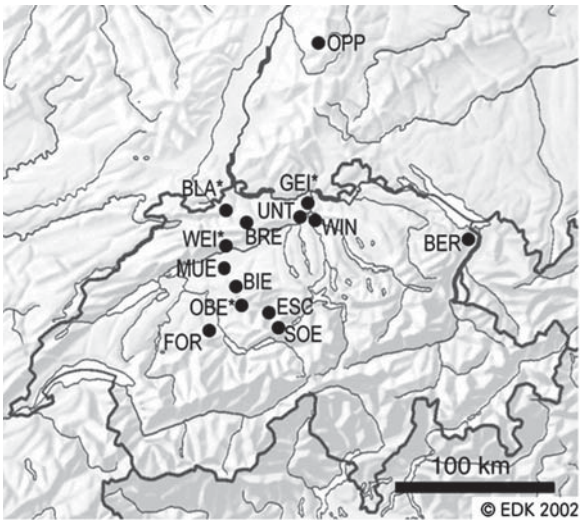


FIG. 1. Ten farm survey sites and four remote reference population sites (*) of woodland strawberries in Switzerland and southern Germany. BER, Berneck; BIE, Biembach; BLA, Blauen; BRE, Bretzwil; ESC, Escholzmatt; FOR, Forst; GEI, Geisberg; MUE, Muehledorf; OBE, Oberhueningen; OPP, Oppenau; SOE, Soerenberg; UNT, Unterboezberg; WEI, Weissenstein; WIN, Windisch.

TABLE 1. Fragaria vesca *reference populations, farm survey sites in Switzerland and Germany and information on sample sizes, genotype numbers and* F. ananassa *(*F. a.*) cultivation at survey sites*

| Site name | Site type* | Co-ordinates North/East | Height a.s.l. (m) | Period of *F. a.* cultivation (years) | Acreage (ha) | Sampling distance from cultivation centre (m) | Acreage shift around cultivation centre (m) | Sample size for molecular/ morphological analyses | No. of genotypes found at sites |
|---|---|---|---|---|---|---|---|---|---|
| Blauen | 1 | 47°26′48″/7°29′20″ | 600 | – | – | – | – | 10/– | 10 |
| Geisberg | 1 | 47°31′51″/8°11′12″ | 680 | – | – | – | – | 10/– | 9 |
| Oberhueningen | 1 | 46°52′05″/7°39′39″ | 980 | – | – | – | – | 10/– | 10 |
| Weissenstein | 1 | 47°14′31″/7°30′25″ | 780 | – | – | – | – | 10/– | 10 |
| Berneck | 2 | 47°25′29″/9°36′16″ | 460 | 51 | 0·6–1·2 | 70–300 | Approx. 275 | 39/360 | 8 |
| Biembach | 2 | 47°00′19″/7°37′56″ | 600 | 30 | Approx. 1 | 10–340 | Approx. 250 | 38/242 | 11 |
| Bretzwil | 2 | 47°23′34″/7°38′55″ | 700 | 17 | 0·3–0·4 | 130–210 | Approx. 200 | 34/185 | 8 |
| Escholzmatt | 2 | 46°56′24″/7°58′18″ | 850 | 28 | 0·1–0·3 | 140–170 | Approx. 100 | 33/247 | 8 |
| Forst | 2 | 46°46′07″/7°31′33″ | 770 | 12 | Approx. 0·4 | 80–90 | Approx. 100 | 35/416 | 9 |
| Muehledorf | 2 | 47°08′18″/7°29′18″ | 610 | 37 | 0·4–3 | 110–420 | Approx. 325 | 33/218 | 9 |
| Oppenau (Germany) | 2 | 48°30′26″/8°12′08″ | 630 | 38 | Approx. 4 | 50–150 | Approx. 175 | 37/315 | 8 |
| Soerenberg | 2 | 46°50′25″/8°01′01″ | 1080 | 20 | 0·5–1·5 | 40–250 | Approx. 325 | 43/213 | 13 |
| Unterboezberg | 2 | 47°28′47″/8°10′14″ | 470 | 40 | Approx. 0·05 | 80–120 | Approx. 225 | 40/310 | 7 |
| Windisch | 2 | 47°28′05″/8°13′11″ | 430 | 40 | 1–1·5 | 200–350 | Approx. 275 | 34/540 | 8 |

*1, reference population; 2, farm survey site.

forest tracks at spacings of 90–110 m. Additionally, single samples from a forest in Riehen, canton BS, and from the Morteratsch glacier forefield (2000 m a.s.l.), canton GR, were included in the analysis. Nineteen *F.* × *ananassa* cultivars that have been grown to a major extent at farm survey sites were obtained from nurseries and cultivar collections (Supplementary Data Table S1, available online). To reduce the possibility of confounding *F.* × *ananassa* with *F. moschata* or *F. viridis* alleles, two plants from two *F. moschata* populations in north-western Switzerland (Riehen and Dornach) as well as one *F. viridis* genotype (Niederau, Sachsen, DE) were included in the analysis (Supplementary Data Table S1).

### *DNA isolation, PCR conditions and analysis of PCR products*

All samples consisted of young leaf tissue, and were stored in plastic bags with Silicagel Rubin (Sigma-Aldrich) for drying immediately after collection. Samples were kept in the dark at room temperature until analysis.

DNA was isolated using the Dneasy Plant Miniprep Kit (Qiagen) for identification of species-specific alleles (see below) and the Dneasy 96 Plant Kit (Qiagen) for screening of *F. vesca* samples from farm survey sites according to the manufacturer's protocol. Sample DNA concentrations were measured with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific) and were diluted with water to a DNA concentration of 3–12 ng $\mu L^{-1}$.

The M13(-21) method was used for labelling of PCR products (Schuelke, 2000). Forward primers of all primer pairs had an M13(-21) tail at their 5′ end. M13(-21) primer was labelled with fluorescent FAM, HEX or NED label (Applied Biosystems). PCR amplifications were carried out in 11 μL total volume of 1× PCR buffer (Colorless GoTaq Flexi Buffer; Promega), 2 mM MgCl, 0·2 mM of each of the four dNTPs, 0·05 μM of M13(-21) forward primer, 0·2 μM reverse primer, 0·2 μM M13(-21) primer$^{(FAM,\ HEX\ or\ NED)}$, 2 U of Go-Taq Flexi DNA Polymerase (Promega) and 3–12 ng of template DNA.

The following PCR conditions were used: an initial denaturation step of 94 °C (3 min), then 30 cycles of 94 °C (30 s), 60 °C annealing temperature (30 s) and 72 °C (30 s), followed by eight cycles of 94 °C (30 s), 52 °C (30 s) and 72 °C (30 s), and a final elongation step of 72 °C (5 min).

Fragments were separated by electrophoresis on an ABI PRISM 3130 × l Genetic Analyzer (Applied Biosystems). GeneScan-500 LIZ was used as internal size standard in each run. Data were analysed with Genemapper 3·7 software (Applied Biosystems).

For identification of species-specific alleles (see below), fragment length analysis was carried out for PCR products of every primer pair and every sample separately. For fragment length analysis of samples from survey sites, two to three differently labelled PCR products of the seven primer pairs that differed in fragment length range were grouped and analysed together (Table 2).

Ten per cent of *F. vesca* samples from survey sites were re-amplified with markers ARSFL 22, EMFv 27, EMFvi 108, EMFvi 109 and EMFvi 136 (Table 2), and the allele scoring error rate was calculated. No re-amplifications were made with markers ARSFL 27 and ARSFL 31 that were monomorphic for all *F. vesca* samples from survey sites.

### *Microsatellite primers and identification of* F. vesca- *and* F. ananassa*-specific alleles*

A microsatellite marker analysis of sampled plants was conducted. Primers for microsatellite loci are highly specific, therefore microsatellite analysis is less prone to erroneous results caused by accidental DNA contamination of samples than other techniques such as, for example, amplified fragment length polymorphism (AFLP; Selkoe and Toonen, 2006).

Many microsatellite markers are available for the genus *Fragaria* and they show high transferability between species

TABLE 2. *Overview of microsatellite markers and corresponding alleles in* F. vesca *(*F. v.*) reference samples and* F. ananassa *(*F. a.*) cultivars*

| Name | Source genome | Repeat motif* | Total no. of alleles | | Allele size range (bp) | | Alleles in *F. a.* cultivars (average) | Source† |
|---|---|---|---|---|---|---|---|---|
| | | | *F. v.* | *F. a.* | *F. v.* | *F. a.* | | |
| EMFvi 108 | Genomic library of *F. viridis* | $(ag)_n$ | 15 | 7 | 202–258 | 178–213 | 1–4 (3) | 1 |
| EMFvi 109 | Genomic library of *F. viridis* | $(tc)_n$ | 11 | 6 | 293–317 | 281–291 | 1–3 (2) | 1 |
| EMFvi 136 | Genomic library of *F. viridis* | $(tc)_n$ | 2 | 15 | 177–179 | 140–183 | 3–6 (5) | 1 |
| EMFv 27 | Genomic library of *F. v.* | Compound trinucleotide | 2 | 2 | 260–265 | 248–253 | 1–2 (1) | 2 |
| ARSFL 22 | Genomic library of *F. a.* | $(ga)_{11}$ | 30 | 17 | 200–286 | 150–206 | 3–8 (5) | 3 |
| ARSFL 27 | Genomic library of *F. a.* | $(ct)_{45-1}$ | 1 | 11 | 181 | 159–230 | 2–6 (4) | 3 |
| ARSFL 31 | GenBank sequence of *F. a.* | $(ag)_{10}$ | 1 | 11 | 223 | 187–246 | 2–7 (4) | 3 |

* Subscript number n–1 means the repeat was not perfect with either a base pair missing or a base pair substitution.
†1, Sargent *et al.* (2003); 2, Hadonou *et al.* (2004); 3, Lewers *et al.* (2005).

(Sargent *et al.*, 2003; Hadonou *et al.*, 2004; Lewers *et al.*, 2005; Davis *et al.*, 2006). Microsatellite markers were selected on the basis of a published linkage map for diploid *Fragaria* that contains seven linkage groups (LGs) (Sargent *et al.*, 2006). The linkage map for diploid *Fragaria* can be used as a reference map for the octoploid *F.* × *ananassa* (Sargent *et al.*, 2006) as diploid and octoploid species share a common genetic basis (Hancock, 1999). Transferability of the diploid reference map to *F.* × *ananassa* has been confirmed by a study of comparative genetic mapping between *F.* × *ananassa* and its diploid relatives, which showed that high levels of conserved macrosynteny and colinearity exist between octoploid homoeologous LGs and their corresponding LGs in the diploids (Rousseau-Gueutin *et al.*, 2008).

Eighteen microsatellite primers were tested in a subset of *F.* × *ananassa* cultivars, *F. vesca* samples from reference populations and on the *F. moschata* plants described above. Out of these, seven microsatellite loci with species-specific fragment lengths were chosen (Table 2). Because we wanted to use physically unlinked microsatellite loci with an even distribution throughout the genome, all seven loci were chosen from different linkage groups (Sargent *et al.*, 2006). Among them, four loci were monomorphic or diallelic for reference *F. vesca* samples, one of them being also diallelic for *F.* × *ananassa* (Table 2). The other three loci showed moderate to high variability, and the size ranges for the majority of species-specific alleles were different. The three variable loci regularly showed stutter peaks in *F. vesca* with peaks separated by 2–3 bp. This made scoring of alleles for these loci unreliable within a size range of $\pm$2–3 bp in *F. vesca*. Nevertheless, we included these loci in our study as they provided additional information to the low-variability loci, and stutter peak alleles did not overlap with the size range of the majority of well defined *F.* × *ananassa* alleles (Table 2).

### *Farm survey sites and sampling procedure*

In summer and autumn of 2007 and 2008 we located strawberry producers in Switzerland and Baden-Württemberg, Germany. About 90 producers were interviewed with regard to the duration and acreage of strawberry cultures and the vegetation surrounding the strawberry fields. Based on these interviews, ten farms were selected where (*a*) strawberries were grown for at least 10 years; (*b*) strawberry cultures were shifted within a relatively narrow range; and (*c*) wild *F. vesca* plants were growing in the close vicinity of strawberry cultures (Table 1). These farms are located in north-western, central and eastern Switzerland and one in Baden-Württemberg, Germany (Fig. 1). Information on the time span of strawberry cultivation, present and former acreage and location of strawberry cultures was obtained from farmers (Table 1). Furthermore, farmers provided lists of major strawberry cultivars that were used throughout the period of strawberry cultivation. The centre of strawberry cultivation was estimated as the centre of the shifting acreages used for strawberry cultures for each survey site. This centre of cultivation served as the reference point for calculations of mean distances between *F. vesca* sites and strawberry cultures (Table 1). We assume that all *F. vesca* sites were

established when strawberry cultivation was started at the respective farms.

In autumn 2007 and 2008 leaves of wild *F. vesca* were sampled at the farms. A transect was laid through each site of *F. vesca*, and plants closest to 1 m spaced markings on the transect line were sampled. End points of transects and the sampled plants were marked with wooden pegs, and co-ordinates of *F. vesca* sites were recorded with GPS in case a re-examination of individual plants would be necessary. Furthermore, we searched for *Fragaria* plants with morphological traits differing from common *F. vesca* traits. Attention was paid to sampling all *F. vesca* occurrences close to strawberry cultures. At each strawberry farm, 33–43 *F. vesca* individuals from 3–6 different sites were sampled. Altogether 370 plants were sampled.

In summer 2010 all *F. vesca* sites at all ten farms were revisited. Each site was screened for morphologically conspicuous plants, and the total numbers of *F. vesca* plants were counted, or estimated where plant density was very high. Many of the morphological traits of *F.* × *ananassa* cultivars are intermediate to those of their parent species. *Fragaria virginiana* leaves are relatively thick, medium to dark green and their shape is obovate to oblong, while *F. chiloensis* leaves are very thick and leathery, usually glossy, dark green and broadly obovate (Darrow, 1966). Leaves of different *F.* × *ananassa* cultivars exhibit a mixture of these characters. *Fragaria vesca* leaves, in contrast, are thin and light green and relatively narrow cuneate–ovate to rhombic–ovate (Darrow, 1966). It was observed that leaves of all vigorous *F. vesca* × *F.* × *ananassa* $F_1$ hybrids that originated from hand-crosses (see below) were either intermediate between the parental species with regard to thickness, colour and leave shape or showed a dominance of *F.* × *ananassa* traits. Consequently, we screened *F. vesca* sites for *Fragaria* plants differing from common *F. vesca* plants in one or more of the following characters: leaf thickness, leaf colour, leaf shape and plant size. At each farm, 190–540 *F. vesca* plants were inspected, resulting in a total of 3050 plants.

### *Flow cytometry*

Samples of ten morphologically conspicuous *Fragaria* plants collected from farm sites in 2010 were analysed by flow cytometry. Fresh young leaves of sampled plants were chopped together with leaves of *F.* × *ananassa* 'Calypso' as internal standard with a sharp razor blade in a Petri dish containing 0·8 mL of nuclei isolation buffer (Galbraith *et al.*, 1983) supplemented with 1 % polyvinylpyrrolidone K90. After 2 min of incubation the solution was filtrated through a 50 μm CellTrics filter (Partec) and 1·6 mL of 4′,6-diamidino-2-phenylindole (DAPI) staining solution (Cystain UV Precise P, Partec) was added. After 2 min of staining, fluorescence intensities of nuclei were measured with a CyFlow Ploidy Analyzer (Partec) equipped with a UV-LED of 365 nm emission wavelength.

As reference samples *F. vesca* × *F.* × *ananassa* hybrid plants from hand-crosses (see below) and their parental lines were used. Three experimentally produced hybrid individuals each of type *F. vesca* × *F.* × *ananassa* 'Calypso' and *F. vesca* × *F.* × *ananassa* AN93.231.53 were analysed. All measurements of reference samples were repeated three times.

### *Cloning and sequencing of overlapping alleles*

Alleles of two *F. vesca* samples from survey sites and one *F.* × *ananassa* cultivar with overlapping fragment length (see below) were cloned and sequenced. A 1·5 μL aliquot of each PCR product was ligated into the pJET1 vector using the GeneJet-PCR cloning kit (Fermentas). A 5 μL aliquot of ligation product was transformed into 50 μL of chemically competent *Escherichia coli* cells (SURE, Stratagene). *Escherichia coli* cells were grown on LB-ampicillin plates. Twenty-four clones from each *F. vesca* sample and 48 clones from the *F.* × *ananassa* sample were used as template for colony PCR with pJET1 vector primers. PCR products with the correct length were identified on agarose gels and 5 μL thereof purified with 10 U of exonuclease I (Fermentas) and 1 U of shrimp alkaline phosphatase (Promega) at 37 °C for 15 min. Inserts were cycle sequenced using BigDye Terminator v3·1 chemistry (Applied Biosystems) in combination with the pJET1 forward sequencing primer. Products were filtered through a Durapore filter plate (Millipore MSHVN4510) loaded with Sephadex-G50 (GE Healthcare) to remove unincorporated dyes, and resolved on an ABI PRISM 3130 × l Genetic Analyzer (Applied Biosystems).

Sequences were edited with the software Sequence Navigator 1·0 (Applied Biosystems). Sequences were collapsed with the software Collapse 1·2 (Posada, 2006) and the sequences flanking the microsatellite repeats were compared with one another. Haplotypes that were found only once and that differed from more common haplotypes by a single base pair substitution/indel were considered likely PCR or cloning artefacts and were discarded.

### *Defined crosses between* F. vesca *and* F. ananassa

Crosses were carried out in one direction with *F. vesca* plants from four different field sites as mother plants. As pollen donors two different *F.* × *ananassa* lines were used, *F.* × *ananassa* 'Calypso' and *F.* × *ananassa* AN93.231.53 (provided by B. Mezzetti, Marche Polytechnic University, Italy). From April to September 2008, hybrid seeds were generated by 100 controlled hand pollinations. Pollen was collected from closed *F.* × *ananassa* flowers. Anthers were placed in 2 mL tubes and were dried during 2–4 d in an exsiccator filled with Silicagel Rubin (Sigma-Aldrich). Pollen was used immediately after drying, or was stored in a fridge at 5 °C for up to 5 weeks prior to use. *Fragaria vesca* flowers were emasculated 2–3 d before opening. Anthers, sepals and petals were removed with a circular cut through the receptacle using a scalpel. This cutting treatment can be performed more quickly than removal of anthers with forceps and seems not to affect the following development of fruits negatively (pers. comm. from breeders). Furthermore, mechanical contact with anthers can be reduced. Prior to emasculation all redundant flowers were cut off and plants were rinsed with water to wash off pollen adhering to the plants. After emasculation plants were isolated in a polyester mesh tent to avoid accidental pollinations by insects.

Depending on the availability of suitable flowers, 1–4 flowers per *F. vesca* plant were pollinated. Flowers were pollinated twice, on day 1 or 2 and on day 3 or 4 after emasculation. Pollen was applied to flowers with a marten-hair brush that was washed with 96 % ethanol before and after pollination. After pollination plants were again isolated in a polyester mesh tent for 14 d. A total of 100 crosses of the type *F. vesca* × *F.* × *ananassa* were carried out. Sixty crosses were made with pollen from *F.* × *ananassa* 'Calypso' and 40 with pollen from *F.* × *ananassa* AN93.231.53. Ripe strawberries were cut in half and dried on blotting paper.

For germination, dishes (10·5 × 13 cm) were used; seeds were put on a moist 1:1 mixture of quartz sand and soil for germination (Ricoter, Aarberg, Switzerland) and were covered with a thin layer of quartz sand. This seed bed was covered with moist blotting paper and wrapped with plastic foil to avoid drying out. Seeds were then kept in the dark at 5 °C in a cold storage room for 2 weeks. Thereafter, dishes were placed in a greenhouse and all germinated seedlings were recorded for a period of 7 weeks. Seedlings that germinated within this period were transplanted to small pots after they reached the one- or two-leaf stage. All seedlings were treated with fungicide Previcur N (Bayer CropScience AG) after transplantation.

We could raise 67 and 55 seedlings from *F. vesca* × *F.* × *ananassa* 'Calypso' and *F. vesca* × *F.* × *ananassa* AN93.231.53 hybrid seeds, respectively (see below). From both crossing types 25 plants together with their parental lines were randomly sampled. Molecular analysis of these samples was performed with the same methods as described above for *F. vesca* samples from farm survey sites.

## RESULTS

### Fragaria vesca *sampling at farm survey sites in 2007 and 2008*

A total of 368 *F. vesca* plants were sampled from transects through *F. vesca* sites. None of them showed any morphological indications of hybrid identity. In addition, two morphologically conspicuous plants were sampled at Berneck at the margin of a former strawberry field that is now an apple orchard. These had thick, leathery leaves typical for *F.* × *ananassa* but were otherwise small and deformed. These two plants had specific *F.* × *ananassa* alleles at all seven loci and lacked any of the specific *F. vesca* alleles at the monomorphic and diallelic loci for *F. vesca*. This clearly identified them as feral *F.* × *ananassa* plants. Furthermore, the two individuals had more than two alleles at four loci, which indicated their polyploid status. None of the 368 *F. vesca* samples had an allele that was specific for *F.* × *ananassa* at any of the four loci that were either monomorphic or diallelic for *F. vesca* (Table 2). In fact, we only found alleles already known from the *F. vesca* reference populations for these four loci at all survey sites.

At the three loci that showed high variability, many new alleles were found for survey site samples. Two plants from site Forst and 12 plants from site Unterboezberg had allele fragment lengths that matched *F.* × *ananassa* alleles at the highly variable locus ARSFL 22 and were not present in reference populations. These overlapping alleles were cloned and sequenced (see below). Due to the absence of characteristic *F.* × *ananassa* alleles at all seven loci the remaining 354 plants were classified as genetically pure *F. vesca* plants. At all loci we never found more than two alleles per sampled *F. vesca* individual. The number of multilocus genotypes in survey site samples was estimated (Table 1). These results are based on some markers with high allele scoring error rates (see below) and are likely to overestimate genotype numbers. However, genotype numbers show that *F. vesca* is highly clonal and consists of a limited number of genets in survey site populations.

Ten per cent of *F. vesca* samples were re-amplified. No re-amplifications were made with markers ARSFL 27 and ARSFL 31 that were monomorphic for all samples from survey sites. The allele scoring error rate for EMFv 27 and EMFvi 136 was 0 %. The allele scoring error rate for loci that yielded stutter peaks in *F. vesca* were 20, 21 and 29 % for EMFvi 108, EMFvi 109 and ARSFL022, respectively, but re-scoring errors did not exceed a range of ± 1–3 bp.

TABLE 3. *Relative DNA contents of cell nuclei of* F. vesca *(*F. v.*) ×* F. ananassa *(*F. a.*) hybrids, their* F. vesca *mothers and ten* F. vesca *field samples*

| | Relative DNA content | s.d. |
|---|---|---|
| *F. v.* 1 | 0·354 | 0·012 |
| *F. v.* 2 | 0·361 | 0·009 |
| *F. v.* 3 | 0·351 | 0·018 |
| *F. v.* 4 | 0·362 | 0·003 |
| *F. v.* 5 | 0·342 | 0·019 |
| *F. v.* 6 | 0·352 | 0·001 |
| *F. v.* 1 × *F. a.* 'Calypso' | 0·692 | 0·013 |
| *F. v.* 2 × *F. a.* 'Calypso' | 0·666 | 0·007 |
| *F. v.* 3 × *F. a.* 'Calypso' | 0·672 | 0·008 |
| *F. v.* 4 × *F. a.* AN93.231.53 | 0·684 | 0·006 |
| *F. v.* 5 × *F. a.* AN93.231.53 | 0·678 | 0·002 |
| *F. v.* 6 × *F. a.* AN93.231.53 | 0·708 | 0·031 |
| *F. v.* field samples (10) | 0·357–0·395 | – |

Relative DNA contents were calculated with *F.* × *ananassa* 'Calypso' as standard. Samples of hybrids and their mother plants were measured three times and field samples once.

### Fragaria vesca *sampling at farm survey sites in 2010*

All *F. vesca* sites were revisited and a total of 3050 plants were inspected. Ten plants that had one or more conspicuous traits (i.e. unusually thick leaves, broad leaflets of rather obovate shape and extraordinary size of plants) were sampled. The size of cell nuclei of all sampled plants matched nuclei sizes of *F. vesca* reference plants (Table 3).

### *Cloning and sequencing of overlapping alleles*

Two plants from site Forst and 12 plants from site Unterboezberg that were sampled in 2007 and 2008 had allele fragment lengths that matched *F.* × *ananassa* alleles at the highly variable locus ARSFL 22. The allele sizes were 192 and 206 bp for plants from Forst and Unterboezberg, respectively, and were not present in reference populations. To ascertain whether overlapping alleles were derived from

TABLE 4. *Haplotypes of two* F. vesca *plants and* F. ananassa *'Hummi grande' that have alleles of overlapping length at microsatellite marker ARSFL 22*

| Position (bp) | 1 | 6 | 7 | 27 | 36 | 41 | 44 | 60 Indel | 61 Indel | 63 Indel | 69 | 86 | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *F. vesca* F.1 | A | C | C | T | A | A | T | TA | AA | – | T | C | 9 |
| *F. vesca* F.2 | . | . | . | C | . | . | . | . | . | . | . | . | 4 |
| *F. vesca* U.1 | . | T | . | . | . | . | . | . | . | . | . | . | 4 |
| *F. vesca* U.2 | . | . | . | N | . | . | A | . | . | . | . | . | 2 |
| *F.* × *ananassa* 1 | . | . | A | . | C | . | . | . | . | . | – | . | 2 |
| *F.* × *ananassa* 2 | G | T | . | . | C | . | . | . | – | . | – | T | 1 |
| *F.* × *ananassa* 3 | . | A | . | . | C | . | . | . | – | . | – | T | 2 |
| *F.* × *ananassa* 4 | . | . | . | . | C | T | . | . | . | . | . | . | 3 |
| *F.* × *ananassa* 5 | . | A | . | . | C | . | . | . | . | GGTC | – | . | 8 |
| *F.* × *ananassa* 6 | . | T | . | . | C | . | . | – | – | . | – | . | 5 |
| *F.* × *ananassa* 7 | . | A | . | . | C | . | . | – | – | . | – | . | 2 |
| *F.* × *ananassa* 8* | . | . | . | . | C | . | . | . | – | TGTC | . | . | – |

Haplotypes of the sequences flanking the microsatellite repeats were collapsed from 22, 12 and 37 sequences for individuals *F. vesca* F, *F. vesca* U and *F.* × *ananassa*, respectively. *Fragraraia vesca* F and *F. vesca* U were sampled at sites Forst and Unterboezberg, respectively. The microsatellite repeat sequence is flanked by positions 62 and 63. The total length of the flanking sequences is 103 bp.

*Consensus haplotype based on four single haplotypes that differed in one nucleotide from one another at position 6 or 7.

*F.* × *ananassa* or not, we cloned and sequenced PCR products of one plant each from sites Forst (*F. vesca* F) and Unterboezberg (*F. vesca* U) and of *F.* × *ananassa* 'Hummi grande' that contained both the 192 bp and the 206 bp allele. A total of 37, 22 and 12 sequences were obtained for *F.* × *ananassa* 'Hummi grande', *F. vesca* F and *F. vesca* U, respectively. Collapsing of haplotypes resulted in eight, two and two haplotypes for *F.* × *ananassa* 'Hummi grande', *F. vesca* F and *F. vesca* U, respectively (Table 4). In one case a group of four single *F.* × *ananassa* haplotypes that all differed in one nucleotide from one another at positions 6 or 7 were collapsed to a consensus haplotype. A single *F.* × *ananassa* haplotype was classified as a recombinant of *F.* × *ananassa* haplotypes 4 and 5 and therefore discarded. Microsatellite repeats were variable in sequences of cloned alleles, which made it impracticable to assign specific sequences to overlapping alleles based on sequence length. *Fragaria* × *ananassa* and *F. vesca* haplotypes were clearly different (Table 4).

### *Defined crosses between* F. vesca *and* F. ananassa

Fruits from hand pollinations yielded a total of 2999 and 2987 seeds for crosses of the type *F. vesca* × *F.* × *ananassa* 'Calypso' and *F. vesca* × *F.* × *ananassa* AN93.231.53, respectively. From these, 67 and 55 seedlings of crossings *F. vesca* × *F.* × *ananassa* 'Calypso' and *F. vesca* × *F.* × *ananassa* AN93.231.53, respectively, were raised. Molecular analysis of 25 randomly selected plants of each crossing type showed that seven of them had only alleles of the *F. vesca* mother at all seven loci and never had more than two alleles per locus. Five of them were collected from the same fruit. They were classified as pure *F. vesca* plants that resulted from accidental pollination with *F. vesca* pollen, e.g. by pollen shattering during emasculation. Of the remaining 43 plants, 42 had specific *F.* × *ananassa* alleles at all seven loci. One plant had *F.* × *ananassa* alleles at six of the seven loci. Forty plants had *F. vesca* alleles at all seven loci and three plants had *F. vesca* alleles at six loci. Furthermore, all plants had more than two alleles at three loci, which suggests a polyploid status; average allele numbers were 4 (range 3–6), 3 (range 2–4) and 4 (range 3–5) for microsatellite markers ARSFL 22, ARSFL 31 and EMFvi 136, respectively. Based upon these results all 43 plants were classified as true hybrids. All *F.* × *ananassa* alleles scored in hybrids matched exactly the known alleles in the parental line. It was therefore concluded that alleles are inherited in unchanged size by hybrids. Similarly, alleles of parental *F. vesca* lines were inherited unchanged, and variations of scored fragment length sizes for the variable loci yielding stutter peaks never exceeded $\pm$1–3 bp. Only in one plant was an allele scored that differed from all known alleles in parental lines.

By extrapolating the results from our sub-sample, we estimated the proportion of true hybrids among our 122 seedlings to be 86 %. This results in an average germination and survival rate of hybrids of 1·8 % until the seedling stage.

## DISCUSSION

No hybrids were found at any of the ten survey sites, although some of the oldest commercial strawberry farms in Switzerland were included in the survey.

Alleles for some *F. vesca* and *F.* × *ananassa* samples at sites Forst and Unterboezberg overlapped at the highly variable marker ARSFL 22. Overlapping alleles were cloned and sequenced, and it was found that *F. vesca* and *F.* × *ananassa* alleles were clearly different (Table 4). As the results of our analyses of hybrids from controlled crosses clearly showed that microsatellite marker analysis and flow cytometry both have high power to detect $F_1$ hybrid plants, we are confident that we did not sample any $F_1$ hybrids at our survey sites. The reliability of our method was furthermore confirmed by definite classification of two conspicuous plants from site Berneck as feral *F.* × *ananassa*.

The finding of two feral *F.* × *ananassa* plants raises the question about the potential of strawberries to become feral, which is of special interest regarding future GM cultivars. In central Europe, feral *F.* × *ananassa* do occasionally occur on and beside former strawberry fields or in the vicinity of

garden waste dumpsites (pers. comm. from breeders and farmers). Such plants probably establish from runners. Seedlings often germinate in agricultural fields, but seedling establishment beyond the favourable conditions of agricultural fields seems to be an unlikely event. At least we are not aware of any such reports. Although there is occasional establishment of feral *F.* × *ananassa*, the species appears neither in the FloraWeb database of the Federal Agency for Nature Conservation of Germany that lists about 500 local or country-wide established neophytes (Bundesamt für Naturschutz, 2010) nor in the inventory of alien species in Switzerland containing >300 plants (Wittenberg *et al.*, 2005). Unless transgenes will enhance the fitness of cultivars under non-agricultural conditions the occurrence of feral *F.* × *ananassa* would probably remain a sporadic event in our survey area. Nevertheless, there are geographical regions in which *F.* × *ananassa* escape is more likely, e.g. the mid-western and southern USA (Rosskopf, 1999).

We assume that our sample size was large enough to rule out a widespread occurrence of *F. vesca* × *F.* × *ananassa* hybrids in our restricted survey area. It seems that hybrid formation or hybrid establishment under natural conditions is a rather rare event. Differing ploidy levels of the two species are the major obstacles for establishment of hybrids (Darrow, 1966; Evans, 1974). Nevertheless, our sample size was not big enough to rule out completely the possibility of hybrid establishment under natural conditions. Vigorous hybrids between *F. vesca* and octoploid *Fragaria* species have been reported from experimental crossings and from field sites (Mangelsdorf and East, 1927; Yarnell, 1931*a*; Bringhurst and Khan, 1963; Olbricht *et al.*, 2006), and our own observations of *F. vesca* × *F.* × *ananassa* hybrids confirm these reports. We therefore warn against an uncritical use of our results for promoting the cultivation of transgenic strawberries.

It still is unclear to what extent pollen flow between the species occurs and whether pollinator behaviour contributes to hybridization in the field. Little is known about pollinators and pollinator overlap of *Fragaria* species and even less about the frequency of pollinators visiting two populations of differing *Fragaria* species during the same period. Another complication for assessing the probability of pollen flow from *F.* × *ananassa* to *F. vesca* is the self-compatible nature of the latter. *Fragaria vesca* seems to be a predominantly selfing species (Arulsekar and Bringhurst, 1981). This reduces the chances of interspecific outbreeding in comparison with dioecious species such as *F. chiloensis* that can hybridize naturally with *F. vesca* as reported in Bringhurst and Khan (1963).

Our results from germinating hybrid seeds showed, in good accordance with results from previous workers (Mangelsdorf and East, 1927; Yarnell, 1931*a*; Marta *et al.*, 2004; Ulrich *et al.*, 2007), that germination and survival rate of hybrids between *Fragaria* species with differing ploidy levels is low (approx. 1–2 %). As a comparison, the germination rate of *F. vesca* achenes collected at four different field sites on field soil in the greenhouse was 46 % after 8 weeks (unpubl. res.). This rate is 25 times higher than the germination rate of our hybrid seeds, although *F. vesca* seeds were exposed to less favourable germination conditions. Assuming that some pollen flow occurs, we do not know whether under natural conditions it is the relatively low germination rate of hybrid seeds that is a major obstacle for establishment of *F. vesca* × *F.* × *ananassa* hybrids or if natural selection selects against later developmental stages of hybrids, that are not fit enough to compete with co-occurring plants. Further experiments with pollinators as well as competition experiments between hybrids and *F. vesca* plants are underway and will clarify whether the small probability of hybridization between *F.* × *ananassa* and *F. vesca* can be explained by pollinator preferences for any of the species and/or hybrids lacking fitness.

## SUPPLEMENTARY DATA

Supplementary data are available online at ww.aob.oxfordjournals.org and consist of the following. Table S1: Allele numbers and ranges of allele lengths at seven microsatellite loci of major *F.* × *ananassa* cultivars grown at farm survey sites and reference samples of *F. moschata* and *F. viridis* accessions.

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# Chapter II

## Solitary bees - potential vectors for gene flow from cultivated to wild strawberries

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# Solitary bees - potential vectors for gene flow from cultivated to wild strawberries

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**Abstract**

The genus *Fragaria* (Rosaceae) contains 24 plant species, including hybrid species such as the octoploid garden strawberry (*F.* x *ananassa*). As natural hybridization between *Fragaria* species has repeatedly been reported, the potential future cultivation of genetically modified strawberries has made the study of hybridization potential between *F.* x *ananassa* and its wild relatives increasingly important. In Europe, *F.* x *ananassa* is the only octoploid species present, and the most likely candidate for hybridization is the common diploid woodland strawberry (*F. vesca*). To date, it is unknown whether pollinator spectra of the two species overlap and thus promote interspecific gene flow. We carried out a pollinator survey in northwestern Switzerland to identify major pollinators of *F.* vesca and *F.* x *ananassa*. This survey indicated that wild bees are the most important shared pollinators of *F.* x *ananassa* and *F. vesca*. Therefore, we studied flower choice behavior of the common wild bee *Osmia rufa* in a greenhouse experiment. *Osmia rufa* did not discriminate between *F.* x *ananassa* and *F. vesca* flowers. We conclude that wild bees are important shared pollinators of both *F.* x *ananassa* and *F. vesca* and are potential vectors for gene flow between cultivated and wild strawberries.

Keywords: *Fragaria x ananassa*, *Fragaria vesca*, hybridization, pollination, flower choice

**Introduction**

The genus *Fragaria* (Rosaceae) contains 24 herbaceous species, including well defined hybrids, that are found in large parts of the northern hemisphere and in South America (Hancock 1999; Staudt 2009). Different species show various ploidy levels that range from di- to octoploid. Natural hybridization between species of similar ploidy levels has repeatedly been reported (Hancock 1999; Staudt et al. 2003; Westman et al. 2004), and stable colonies of hybrids between octoploid *F. chiloensis* Mill. and diploid *F. vesca* L. have been described from coastal California (Bringhurst and Khan 1963; Bringhurst and Senanayake 1966). The cultivated garden strawberry (*F.* x *ananassa* Duch.) is a hybrid between the wild octoploid American species *F. chiloensis* and *F. virginiana* Mill. and is grown worldwide. With prospects of future genetically modified strawberry cultivars (Qin et al. 2008) knowledge about the hybridization potential between cultivated *F.* x *ananassa* and its wild relatives has become increasingly important in order to assess the risk of transgene escapes into wild populations. In Europe, cultivated *F. x ananassa* is the only octoploid species present, while the diploid woodland strawberry (*F. vesca*) is the most prevalent wild species (Hancock 1999) and therefore the most likely candidate for hybridization with *F.* x *ananassa*. However, it is unknown whether pollinator spectra of the two plant species overlap or whether common pollinators show a preference for a particular species, as flowers of *F. vesca* and *F.* x *ananassa* differ in size. Typically, *F.* x *ananassa* flowers have larger diameters and can reach twice or three times the size of *F. vesca* flowers. Pollinator behavior could thus limit chances for interspecific hybridization. Wild bees seem to be major pollinators of wild strawberry species. A study of pollinators of *F. virginiana*, an American strawberry species, showed that wild bees are likely the most important pollinators of this species (Ashman 2000). Furthermore, we found that solitary wild bees visited flowers of both *F.* x *ananassa* and *F. vesca* during a preliminary pollinator survey (see below). Wild bee species of the genus *Osmia* (Megachilidae) have received scientific and economic interest because populations can be easily managed in agroecosystems, and because *Osmia* species are good pollinators of fruit crops (Bosch et al. 2006; Cane 2005; Marquez et al. 1994; Matsumoto et al. 2009; Tepedino et al. 2007; Torchio 1976; Tuell et al. 2009; Vicens and Bosch 2000). Analyses of brood cell provisions have shown that polylectic solitary bees of the genus *Osmia* generally display high levels of flower constancy, but are also foraging on flowers of less frequent plant species during single forage bouts (Raw 1974; Rust 1990; Torchio 1976; Williams and Tepedino

2003). The behavior of these bees thus renders them potential vectors for interspecific cross-pollinations. Honey bees are recognized as major pollinators of *F.* x *ananassa* (Hancock 1999), but due to their foraging behavior, with scout bees recruiting pollen and nectar collecting workers to rewarding flower patches and strong floral constancy (Winston 1987), their potential as pollen vectors between *F.* x *ananassa* and *F. vesca* seems limited.
In this paper we present a preliminary survey of pollinators of *F. vesca* and *F.* x *ananassa* in northwestern Switzerland. Furthermore, we tested whether the wild red mason bee *Osmia rufa* L. differentiates between garden and woodland strawberries in a flower choice experiment to generally assess the potential of solitary wild bees for promoting gene flow between *F.* x *ananassa* and *F. vesca*. *Osmia rufa* is a widespread polylectic solitary bee species in Central Europe (Amiet et al. 2004; Westrich 1990). Flower discrimination of *O. rufa* between *F.* x *ananassa* and *F. vesca* would indicate that pollinator behavior is unlikely to promote hybridization between these species.

**Materials and Methods**

Survey of *F. vesca* and *F.* x *ananassa* pollinators

In May and June 2008 a survey of pollinators of *F. vesca* and *F.* x *ananassa* was carried out at five different sites in northwestern Switzerland where wild and cultivated strawberries co-occur. At each site and for every strawberry species we recorded insect flower visits during observation periods of 20 minutes in the morning (10-12 am), at midday (12-2 pm) and in the afternoon (2-4 pm). At two sites, observations were repeated resulting in 21 observation periods per *Fragaria* species. We identified insects to the levels of order or family, or classified them as honey bees or wild bees. The numbers of strawberry flowers in observation patches (ca. 4 $m^2$) varied substantially between cultivated strawberry fields and wild strawberry populations. Therefore, we corrected the number of insect visits for the number of observed flowers. We estimated functional pollen loads of the most frequent flower visitors. Insects were caught at study sites and killed in jars containing potassium cyanide. Pollen grains were dabbed off from the head, the legs and the body underside with small glycerine-gelatine cubes stained with fuchsin. Glycerine-gelatine cubes were transfered to microscope slides and pollen was counted under a microscope. In some cases pollen grains were so numerous that their number had to be estimated.

Flower choice experiments

A cuboidal mosquito net cage (L x W x H: 4 m x 2 m x 2 m; mesh size: 1 mm) was put up in a greenhouse. Holes of ≥ 8 cm depth and 7 mm diameter were drilled into hardwood boards to serve as nesting sites. We placed boards on a table of 1 m height that stood in the center of a long side wall of the cage (Fig. 1). Nesting site openings were facing south and were orientated perpendicularly to the cage wall. One hundred pupae of *O. rufa* (WAB-Mauerbienenzucht, Konstanz, Germany) were placed in front of the nesting sites on 03-Apr-2009. We put pieces of sponge in small plastic cups and soaked them with either water or sugar solution (1:1) to feed hatched individuals. Cups were refilled regularly. Furthermore, buckets with flowering boughs of *Salix* spp. and *Prunus* spp. as well as potted and flowering *Lathyrus vernus*, *Potentilla neumanniana* and *Lamium* spp. were placed in the cage to provide additional food sources. To mark bees, all hatched individuals were caught and put in a cold room at 5º C for 10 - 20 min until they were immobilized. We then marked individuals with a one or two color code of enamel paint on the thorax. For flower choice experiments the cage was cleared of all forage plants. Two different groups of potted plants (see below) were arranged on the floor of the cage in two blocks separated by a distance of 50 cm (Fig. 1). Interspaces between pots within blocks were 15 cm. Each block consisted of 21 plants, either *F. vesca* or *F.* x *ananassa*, with a total number of 25 flowers (Tab. 1). We conducted choice experiments with two different combinations of plants. One of the blocks always consisted of *F. vesca* plants, the other of either *F.* x *ananassa* cv. 'Calypso' or *F.* x *ananassa* AN93.231.53. We repeated choice experiments with each of the two plant combinations with block positions of plant groups switched. Altogether, we collected data from four different plant arrangements on four different days. Observations were started at 8.30 am when first bees started to fly, and lasted till between 10 am and 11.30 am depending on bee activity. Weather conditions on all experimental days were similar ranging from clear sky to slightly clouded sky (18°C - 23°C). We recorded bee behavior during forage bouts with a voice recorder. Records were kept of bee identity, start and stop of every flower visit, plant species visited and ressource collected during visits (nectar or pollen). We classified bee forage bouts as either short or long if less than five or five and more flowers, respectively, were visited. Male bees perpetually tried to copulate with females and disturbed most forage bouts of females sooner or later. Therefore, we caught as many males as possible prior to experiments and kept them separately. Thus, only few observations of males were made and male observations were not included in the data analysis. We observed only very few bees

collecting pollen from *Fragaria* flowers and therefore excluded these observations in the analysis. Thus, data analysed are based on female bees foraging for nectar.

Experimental plants

We grew all plants in a greenhouse in 1 l pots with common garden soil. *Fragaria vesca* plants were collected from forest edges at three different field sites in northwestern Switzerland (communities of Riehen, Liesberg and Dornach). *Fragaria* x *ananassa* cv. 'Calypso' and *F.* x *ananassa* AN93.231.53 plants were provided by Bruno Mezzetti (Marche Polytechnic University, Italy) and were propagated from runners in the greenhouse. We chose flowering plants of similar size for choice experiments.

Data analysis

We used R (R Development Core Team 2009) for all analyses.

Pollinator field survey:

We analysed pollinator visit data from the survey with a two-way Anova with plant species and pollinator group as independent factors. Only data of the six most important pollinator groups were analysed. We analysed pollen load data with a one-way Anova with pollinator group as independent factor. Pollen load data were logarithmically transformed to obtain normally distributed residuals. We used Tukey's test for multiple comparisons of factor levels.

Flower choice experiment:

Individual bees could sometimes be observed repeatedly, either on different days or on the same day. To test whether individuals showed a preference for *F. vesca* or *F.* x *ananassa* flowers we used a binomial test for equality of proportions on 24 repeatedly observed individuals. Depending on whether first visits of two different foraging bouts on the same day were made to the same plant species or not, individual bees were categorized as constant or non-constant. As there was no indication of species constancy of individual bees ($X^2 = 1.47$, n = 24, $P = 0.23$) repeated observations of individuals were left in the dataset for analyses and treated as independent observations.

We used generalized linear models to test effects of block position and plant species combination on the proportion of:

1. First visits of bees to either *F. vesca* or *F.* x *ananassa* flowers during individual forage bouts (using short and long forage bout data).
2. Total visits of bees to *F. vesca* or *F.* x *ananassa* flowers (using long forage bout data only).
We first tested whether block position had an effect on proportions of visits to *F. vesca* and *F.* x *ananassa*. Plant block position was not a significant factor (see Results section). We then tested whether proportions of visits to *F. vesca* and *F.* x *ananassa* differed significantly from 0.5 in any plant type combination. Models for first visits and total visits of bees were both overdispersed. Thus the error distribution was set to quasibinomial and dispersion parameters were estimated. Numbers of observations used for analyses are given in Table 1.

**Results**

Preliminary survey of *F.* x *ananassa* and *F. vesca* pollinators

Mean numbers of insect visits during observation periods of 20 min did not differ between *F. vesca* and *F.* x *ananassa* ($F_{1, 240} = 0.008$, $P = 0.93$). The number of visits of different pollinator groups to *F. vesca* and *F.* x *ananassa* were significantly different ($F_{5, 240} = 5.24$, $P < 0.01$). There was a significant interaction between factors plant species and pollinator group ($F_{5, 240} = 13.9$, $P < 0.01$). Wild bees and flies (Muscidae) were the most abundant insects that visited both, flowers of wild and cultivated strawberries (Fig. 2). The number of honey bee visits to cultivated strawberries was significantly higher than visit numbers of any other pollinator group, but the number of honey bee visits to wild strawberries was significantly lower than visit numbers of the most important wild strawberry pollinators (Fig. 2). Pollen loads differed significantly between the different pollinator groups ($F_{4, 24} = 6.82$, $P < 0.01$). Wild bees had significantly higher pollen loads than all other pollinator groups except honey bees (Fig. 2).

Flower choice experiments

Male bees were hatching before the females. We saw first female individuals on 09-Apr-2009 and copulating began on the same day. The bees accepted the hardwood boards as nesting and sleeping sites. We observed the first nesting site inspection of a female on 11-Apr-2009. Bees began to collect nectar shortly after hatching. Sealed nesting sites showed that the female bees were collecting pollen for nest provisions from forage plants. Although some bees collected pollen from forage plants, we only observed very few bees collecting pollen during the choice experiments.

First visit analysis:

Plant block position was not a significant factor ($X_1 = 0.98$, $P = 0.32$). Proportions of first visits to either *F. vesca*- or *F.* x *ananassa*-flowers did not differ significantly from 0.5 ($F_{2, 2} = 0.42$, $P = 0.71$). Mean proportions of first visits made to *F. vesca*-flowers were 55% (± 9% SE) and 57% (+ 9%, - 8% SE) for plant combinations *F. vesca*-*F.* x *ananassa* AN93.231.53 and *F. vesca*-*F.* x *ananassa* cv. 'Calypso', respectively.

Total visit analysis:

Plant block position was not a significant factor ($F_{1, 55} = 0.3$, $P = 0.58$). Proportions of total visits to either *F. vesca* or *F.* x *ananassa* did not differ significantly from 0.5 ($F_{2, 55} = 0.9$, $P = 0.41$). The proportions of total visits to either *F. vesca* or *F.* x *ananassa* reflected the proportions found for first visits to flowers. Mean proportions of total visits made to *F. vesca*-flowers were 54% (± 8% SE) and 57% (+ 6%, - 5% SE) for plant combinations *F. vesca*-*F.* x *ananassa* AN93.231.53 and *F. vesca*-*F.* x *ananassa* cv. 'Calypso', respectively. Bees switched between blocks in 65% of long forage bouts.

**Discussion**

The results of the survey of *Fragaria* pollinators suggest that wild bees are important and effective pollinators of wild *F. vesca* and cultivated *F.* x *ananassa* and justify the use of a wild bee species for the flower choice experiments. Moreover, our findings are in line with the results of Ashman (2000) who found that wild bees were the most important pollinators for another wild strawberry species, *F. virginiana*. Honey bees were important pollinators of *F.* x *ananassa* but were rarely observed on *F. vesca.*

The results of the flower choice experiments revealed no flower preference of *O. rufa* for either *F. vesca* or *F.* x *ananassa*. The differences in flower traits between the two species do not seem to influence pollinator behavior under the experimental conditions. However, our results are based on relatively young animals that did not seem to collect pollen from *Fragaria* plants. Animals collecting mainly pollen may behave differently. Furthermore, when cultivated and wild strawberries are present together in the field, cultivated strawberries usually outnumber by far wild strawberries. Larger floral displays of *F.* x *ananassa* may distract pollinators from *F. vesca* with increasing distance between plant groups, thereby reducing the probability of cross-pollinations. Thus, the results of our experiments rather apply for cultivated and wild strawberries growing in close vicinity. Nevertheless, the lack of discrimination of *O. rufa* bees between *F. vesca* and *F.* x *ananassa* flowers observed in our

experiments shows that wild bees could in principle act as vectors for gene flow from cultivated to wild strawberries and hence also for unwanted gene flow from transgenic cultivated to wild strawberries, a possible scenario in the near future. However, this finding contrasts with a previous survey conducted in Switzerland and southern Germany, in which no evidence for gene flow from *F.* x *ananassa* to *F. vesca* was found, and from which it was concluded that an establishment of *F. vesca* x *F.* x *ananassa* hybrids would be a rare event (Schulze et al. 2011). There are obvious reasons that limit or prevent hybridization: 1) The two species have different ploidy levels and germination and survival rates of hybrids are generally low, although the formation of vigorous hybrids is possible (Bringhurst and Senanayake 1966; Darrow 1966; Evans 1974; Noguchi et al. 2002; Schulze et al. 2011); 2) *Fragaria vesca* is self-fertile and predominantly a selfing species (Arulsekar and Bringhurst 1981), which also lowers chances for hybridization. In conclusion, there is inconsistent evidence for possible gene flow from cultivated to wild strawberries in Central Europe. On the one hand, data from field surveys suggest that hybridization between cultivated and wild strawberries seems to be at best a rare event, on the other hand indiscriminative pollinator behavior could still cause such unwanted gene flow. Further work to clarify these first findings is certainly indicated, particularly if the cultivation of transgenic strawberries should become a real option for agriculture, as must be expected for the near future.

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**Table 1.** Arrangement of plant combinations used for flower choice experiments and corresponding numbers of bee observations.

| Date | Block 1 | Block 2 | Plants, flowers (per block) | Nr. of bee observations used for analysis of: First visits, total visits |
|---|---|---|---|---|
| 17-Apr-2009 | *F.* x *ananassa* AN93.231.53 | *F. vesca* | 21, 25 | 30, 11 |
| 18-Apr-2009 | *F.* x *ananassa* cv. 'Calypso' | *F. vesca* | 21, 25 | 19, 16 |
| 20-Apr-2009 | *F. vesca* | *F.* x *ananassa* cv. 'Calypso' | 21, 25 | 27, 20 |
| 21-Apr-2009 | *F. vesca* | *F.* x *ananassa* AN93.231.53 | 21, 25 | 12, 10 |

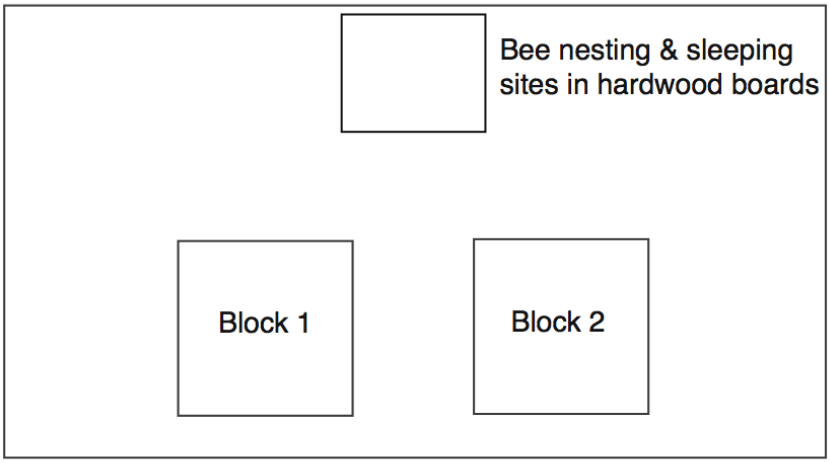


**Fig. 1.** Arrangement of bee (*Osmia rufa*) nesting and sleeping sites and plant blocks for flower choice experiments in a mosquito net cage. Dimensions of the cage were 4 m x 2 m x 2 m (L x W x H). Blocks were separated by 50 cm distance.

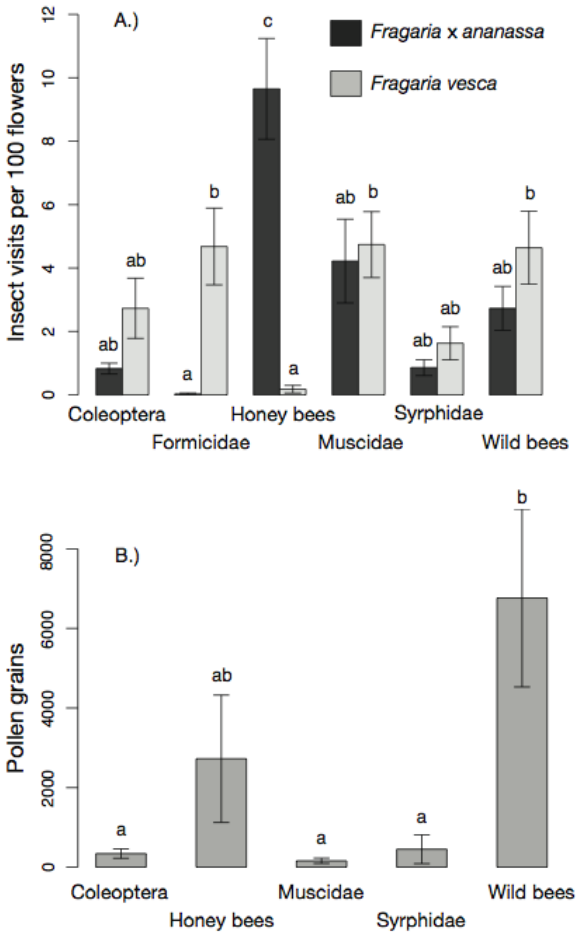


**Fig. 2** A.) Mean (± SE) insect visits to flowers of *F.* x *ananassa* and *F. vesca* during observation periods of 20 minutes. Mean insect visits were corrected for a number of 100 observed flowers. Data are pooled from 21 observation periods from 5 field sites for each plant species. Only the most abundant insect groups are presented. B.) Mean (± SE) pollen grain loads of the most frequent insect visitors, Coleoptera (n = 8), honey bees (n = 4), Muscidae (n = 6), Syrphidae (n = 4), wild bees (n = 7). Groups not sharing letters are significantly different as determined by Tukey's test.

# Chapter III

## Demography of woodland strawberries (*Fragaria vesca*): an indispensable basis for risk assessment of genetically modified strawberries

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# Demography of woodland strawberries (*Fragaria vesca*): an indispensable basis for risk assessment of genetically modified strawberries

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**Abstract**

The octoploid garden strawberry (*Fragaria* x *ananassa*) is a hybrid species derived from the wild American species *F. virginiana* and *F. chiloensis*, and is grown in all arable regions of the globe. Experimental hand-crosses between *F.* x *ananassa* and wild *Fragaria* species of different ploidy levels are feasible, but yield only small numbers of viable hybrid plants. However, such hybrids can be vigorous. With respect to the growing availability of genetically modified crop plants and the outlook for transgenic strawberries the study of natural hybridization between *F.* x *ananassa* and wild strawberry species has become increasingly important. In Central Europe, the diploid woodland strawberry (*F. vesca*) is the most likely candidate species for hybridization with *F.* x *ananassa*, as it is the most prevalent wild *Fragaria* species. To model possible consequences of establishment of *F. vesca* x *F.* x *ananassa* hybrids in *F. vesca* populations, detailed knowledge on the demography of natural *F. vesca* populations is an essential prerequisite. As a first step towards modelling of hybrid invasions into wild *F. vesca* populations we performed a demographic study on *F. vesca* populations at 12 sites in northwestern Switzerland from spring 2008 to spring 2010. The data were used to parameterize periodic stage-structured matrix population models. The population's finite rate of increase ($\lambda$) varied strongly between sites and between years, and we found differences in demographic parameters for plants growing in forest or forest edge habitats. Elasticity analyses and life table response experiments showed that changes in plant survival and clonal reproduction influenced population growth most strongly, whereas changes in seedling recruitment were insignificant for population growth, suggesting that the potential for clonal reproduction would also be an essential trait of hybrids, if they were to invade wild *F. vesca* populations.

Keywords: *Fragaria* x *ananassa*, Matrix population models, Gene flow, Transgenic plants, Clonal plants, Life table response experiments

**Introduction**

The octoploid garden strawberry (*Fragaria* x *ananassa* Duch.) is a high-value fruit crop that is widely grown in European agriculture. *Fragaria* x *ananassa* emerged from hybridization between the octoploid American species *F. chiloensis* (L.) Mill. and *F. virginiana* Mill., and has no related wild species of similar ploidy level in Europe. Therefore, unlike the situation in parts of the United States of America (Westman et al., 2004), gene flow from cultivated to wild strawberry species can not be expected to occur at a large scale in Europe. Nevertheless, it is known that experimental crosses between *Fragaria* species of different ploidy levels can yield vigorous hybrids (Mangelsdorf and East, 1927; Marta et al., 2004; Noguchi et al.; 2002, Olbricht et al., 2006; Yarnell, 1931a; Yarnell, 1931b; Schulze et al., 2011) and even stable natural populations of hybrids between octoploid *F. chiloensis* and diploid *F. vesca* L. have been reported (Bringhurst and Khan, 1963; Bringhurst and Senanayake, 1966). With respect to the growing availability of genetically modified (GM) crop plants and the outlook for GM strawberries (Qin et al., 2008), the study of natural hybridization and fitness of hybrids between cultivated and wild strawberry species in Europe has become increasingly important. The most likely candidate for hybridization is the diploid woodland strawberry (*F. vesca*), as it is the most prevalent wild *Fragaria* species in Europe (Hancock, 1999) and main flowering times of *F.* x *ananassa* and *F. vesca* can overlap during April, May and June in Central Europe. The range of *F. vesca* covers the British Isles and continental Europe with parts of Scandinavia and the Iberian Peninsula. Furthermore, it is the only *Fragaria* species that occurs naturally throughout the northern hemisphere.

To model possible consequences of a potential establishment of *F. vesca* x *F.* x *ananassa* hybrids in *F. vesca* populations, e.g. local extinction of natural *F. vesca* populations, detailed knowledge on the demography of natural *F. vesca* populations is an essential prerequisite. Demographical data can be used to parameterize stochastic matrix population models, which are a widely used tool in invasive species biology as well as in conservation biology to analyse population dynamics, e.g. to identify critical stages in the life cycle of plants and to quantify explosion or extinction risk (Caswell, 2001). As a first step towards modelling a potential invasion of *F.vesca* x *F.* x *ananassa* hybrids into wild *F. vesca* populations, we designed and performed a demographic study on natural *F. vesca* populations. Our goal was to estimate the spatial and temporal variation of vital rates from natural populations of *F. vesca*. As vital rates of

plants can be strongly influenced by changing environmental conditions (Colling and Matthies, 2006; Smith et al., 2005; Schleuning et al., 2008; Jongejans et al., 2008; Jurik, 1983; Chabot, 1978) a broad demographical study on *F. vesca* populations is also a prerequisite for future stochastic modelling of population dynamics, ultimately leading to a quantitative assessment of explosion and extinction risk.

To date the paper of Angevine (1983) is the only study that explicitly deals with the demography of *F. vesca* that we are aware of. Owing to this study's focus on the comparison of the demography of two *Fragaria* species, the number of surveyed populations was relatively small. However, Angevine (1983) detected large variations in demographic parameters between three *F. vesca* populations. To provide a good estimate of spatial variability in vital rates, we studied *F. vesca* populations at 12 sites in northwestern Switzerland over two years, from spring 2008 to spring 2010.

We analysed whether survival and sexual or clonal reproduction were dependent on plant biomass, and tested whether sexual and clonal reproduction are correlated. The demographic data were used to parameterize periodic stage-structured matrix population models. We calculated population's finite rate of increase ($\lambda$) and stable stage structures, and applied elasticity analyses and life table response experiments (LTRE) to identify life cycle components that contribute most to changes in $\lambda$. The aims of this study were to explore population dynamics of *F. vesca* and to provide a data set of vital rate estimates that can be used for future risk assessment and stochastic modeling of *F. vesca* x *F. x ananassa* hybrid invasions into wild *F. vesca* populations.

## Methods

### Study sites and plots

We selected twelve sites with *F. vesca* populations located either at relatively shaded forests or at forest edges (Table 1). The most obvious difference between these two habitats was the extent of solar irradiation. Within every population we established a permanent rectangular plot with an area ranging from 0.69 to 4 $m^2$ depending on plant density. A grid of 0.25 x 0.25 m squares was established within plots, and corners of plots were marked with wooden pegs and a piece of metal in case a plot had to be recovered with a metal detector. At every census a cord was spanned

along the outline of the plots and the inner grid subdivisions were determined with a measuring stick.

Meterological data

We obtained monthly precipitation data from four weather stations from the Swiss Federal Office of Meteorology and Climatology. The weather stations were distributed in and around the study area, i.e. in Arisdorf (47°30'53" N, 7°46'45" E), Binningen (47°32'28" N, 7°35'01" E), Laufen (47°25'05" N, 7°29'52" E) and Riehen (47°34'27" N, 7°37'26" E). Data were used to interpret the observed population dynamics on the landscape level.

Data collection

Data collection started in spring 2008 and either all or a selection of *F. vesca* plants present in the plots were marked with coloured wire below the oldest leaf. Initially, we marked between 54 to 91 plants per plot and mapped and numbered them on hand-drawn maps.

The first intraannual census was carried out at the end of April and a second and a third census were conducted four weeks (end of May) and eight weeks (end of June) later. The dates were chosen so as to cover the main flowering and fruiting period of *F. vesca*. A fourth intraannual data collection was carried out in the middle of September. Data collection from marked plants included measurement of (1) length of middle leaflet of live leaves (i.e. ≥ 50% of leaf area of green colour), (2) sexual reproduction, i.e. number of inflorescences, presence or absence of open flowers, number of developing and ripe fruits, number of fruits gone (empty calyx present) and (3) clonal reproduction, i.e number of stolons and numbers of nodes. Seasonal censuses were completed within less than 15 days for all 12 sites, depending on weather conditions.

In April, June and September, plants were measured and sexual and vegetative reproduction was recorded. In May, only sexual reproduction was recorded. At all censuses seedlings were searched and all seedlings were marked with coloured wire and mapped. We could find only very few seedlings and therefore planted seedlings into plots to estimate survival rates of seedlings. Ripe fruits were collected at each study site, cut in half and air dried on blotting paper. Seeds were collected from dried fruits and germinated on soil collected from study sites in a greenhouse. In the middle of August, 40 seedlings with one or two leaves were transplanted to

each plot. At each end of the plots, 20 seedlings were planted in two parallel rows separated by 20 cm and a distance of 1.5 cm between seedlings. Survival of seedlings was recorded at every subsequent census.

The lifetime of stolons is dependent on environmental conditions. Under unfavourable conditions connections between mother plant and nodes can die off within weeks, but they usually stay intact throughout the growing season and finally wither in winter. Therefore, clonal reproduction of plants can be assessed rather accurately by two censuses in summer and fall. To estimate survival and growth of nodes, 20 newly formed nodes, or as many as could be found if there were less, were marked and mapped at each plot in June and September.

In spring 2009 and spring 2010 we measured all plants present in plots to determine actual plant size structures with the exception of plots Eichmatt and Riehen.

Estimation of vegetative above-ground biomass

To estimate the vegetative above-ground biomass of plants non-destructively, we modeled the correlation of leaf lengths and dryweight of leaves. Inflorescences were not included in biomass estimation. Leaves of different sizes were collected from seven sites, dried at 80°C for 48 hours and weighed. We carried out a linear regression with leaf dryweight as the dependent variable and length of the middle leaflet of leaves as the independent variable. Data had to be logarithmically transformed to meet the assumption of normal distribution of errors.
The resulting equation was:

$\text{Log}(y) = 1.98\ (\pm 0.04\ \text{s.e.}) * \text{Log}(x) - 2.97\ (\pm 0.14\ \text{s.e.})\ \ (R^2 = 0.90, \text{df} = 277)$

where y = leaf dryweight (mg) and x = length of middle leaflet (mm).

Classification of life-cycle stages and calculation of transition matrices

In *F. vesca* as in many perennial plants, non–invasive age estimation based on above-ground morphological traits is not practicable. We therefore distinguished the following life-cycle stages of plants:

1. Seedlings with a pair of cotyledons and usually one or two leaves.
2. Nodes (clonal offspring) produced on above-ground stolons.
3. Small adult plants (based on estimated biomass).
4. Large adult plants (based on estimated biomass).

Size class borders of adults were chosen in such a way that vital rates of every class could be calculated for each of the twelve sites, i.e. that there were plants present in all classes at every site at every census.

Due to large differences in plant size structures between the 12 study sites (Fig. 1) it was not possible to use more than two size classes for adults. We calculated different size class borders between small and large adult plants for spring (76 mg), summer (150 mg) and fall (92 mg).

Transition probabilities from one life-cycle stage to another were calculated for each stage as the proportion of individuals remaining in that stage (stasis) or having changed to other stages (growth or retrogression) after a given time period (Fig. 2). By definition, seedlings and nodes could not change to another stage within the year of their formation. We did not include a seedling class in our population projection matrices because seedlings were very rare. Only 11 seedlings were found during the whole study, always in summer or fall. The effective sexual reproduction of adult plants within plots was rather calculated as the annual number of seedlings detected that survived and entered the small adult class in the following spring divided by the number of large adult plants (Fig. 2). Survival and transition rates of seedlings were calculated from the pooled data of natural and planted seedlings. As seedlings were rare and absent alltogether from many plots, the highest rate of effective sexual reproduction found was used for all sites. Clonal reproduction of adult plant classes was calculated as the mean number of nodes produced during each time period. We calculated separate matrices for parameters of (i) growth, stasis and retrogression (hereafter referred to as growth matrix, $B_G$), (ii) sexual reproduction ($B_S$) and (iii) clonal reproduction ($B_C$) of plants; summation of these matrices resulted in the population projection matrix B. For each year and site, vital rates were estimated for three

seasonal periods, i.e. 'spring – summer', 'summer – fall' and 'fall – spring' and for each period a seasonal population projection matrix was calculated. These matrices were combined to a periodic matrix model to describe the population dynamics over an annual cycle (Caswell, 2001):

$$A_{spring\ (t) - spring\ (t+1)} = B_{fall\ (t) - spring\ (t+1)}\ B_{summer\ (t) - fall\ (t)}\ B_{spring\ (t) - summer\ (t)}$$

Data analysis

All calculations and analyses were done in R (R Development Core Team, 2009).

Models for survival and sexual and clonal reproduction of adult plants:

We checked whether survival and reproduction were size-dependent in *F. vesca* to show that a modeling approach based on a classification of plants according to size was reasonable. Furthermore, habitat (i.e. forest or forest edge) was included as a factor in our in models of survival and reproduction.

The influence of plant size and habitat on plant survival throughout the year and probability of sexual and clonal reproduction were tested with generalized linear mixed models. Models were calculated with either survival, sexual reproduction or clonal reproduction as dependent binomial variable, and estimated spring above-ground biomass (as independent continuous variable) and habitat as fixed effects. Because measurements from individuals within a site were not independent, we included site as a random effect.

We tested for correlation of clonal and sexual reproduction with generalized linear mixed models with the total yearly number of nodes produced as dependent variable and the total yearly number of fruits produced and habitat as fixed effects. Site was treated as a random effect. We hypothesized that there is a trade-off between the extent of clonal and sexual reproduction.

Seedling survival:

To analyse whether the survival of natural seedlings and planted seedlings was similar, we used a generalized linear model with the proportion of seedlings surviving from time of germination till the spring of the following year as dependent binomial variable and the seedling type (i.e. natural or planted seedlings) as independent factor.

Plant size distributions:

We tested for differences in plant sizes between sites and within-site differences between years with a two-way ANOVA using above-ground dry matter estimates as dependent variable and site and year as factors. We used data of the censuses in spring 2009 and spring 2010 when all plants present in plots were measured. Dry matter estimates were square root transformed prior to analyses.

Lambda, stable stage structure and elasticy analyses:

We calculated population growth rates $\lambda$ and stable stage structures for the periodic matrix models of all sites. Stable stage structures calculated for the periodic matrix model for 'spring 2009 – spring 2010' were compared to the observed stage structures found in spring 2009 using Keyfitz's $\Delta$. This standard measure quantifies the distance between stable and observed stage distributions with values ranging from 0 to 1, corresponding to maximum similarity and maximum difference, respectively (Caswell, 2001).

Furthermore, we applied sensitivity and elasticity analyses to the seasonal population projection matrices to identify life cycle components that contribute most to changes in $\lambda$. We followed the method for sensitivity analysis of periodic matrix models described in Caswell and Trevisan (1994). This method allows to analyse how $\lambda$ over the entire annual cycle responds to changes in the vital rates at each season within the cycle. Elasticities of $\lambda$ to changes in the entries of a seasonal population projection matrix B are given by

$$E_B = (1/\lambda)\, B \circ S_B \qquad (1)$$

where $E_B$ and $S_B$ are the elasticity and the sensitivity matrices of $\lambda$ with respect to matrix B, respectively, and $\circ$ denotes the Hadamard, or element-by-element, product. Some of the matrix elements of the seasonal population projection matrices were made up of different components, e.g. the retrogression of large adult plants to small adult plants and seedlings becoming adults in the 'fall-spring' matrices (Table S1). To calculate elasticities of the different components of a seasonal population projection matrix B, we decomposed the seasonal matrix into three matrices (i) $B_G$, containing the rates of stasis, growth and retrogression, (ii) $B_S$, containing the rates of sexual reproduction, and (iii) $B_C$, containing the rates of clonal reproduction. To attribute elasticities of $\lambda$ to elements of $B_G$, $B_S$ and $B_C$ we applied (1) to $B_G$, $B_S$ and $B_C$ separately.

Transition and elasticity matrices of $B_G$, $B_S$ and $B_C$ of all seasons and years are given as supplementary table (Table S1).

Life table response experiment (LTRE):

In a LTRE the effect of a treatment, e.g. an environmental factor, on population growth rate λ (or another statistic) is decomposed into contributions arising from the treatment effects on the different vital rates (Caswell, 2001). This decomposition reveals the vital rates most reponsible for the population level effect of the treatment retrospectively. Our models for survival, sexual reproduction and clonal reproduction showed that vital rates were influenced by habitat, i.e. forest or forest edge (see results section; Table 2). Therefore, we performed a LTRE with habitat as treatment factor. We analysed the effects of habitat on vital rates and how these effects contributed to differences in population growth rate λ. We calculated mean periodic transition matrices for forest sites ($M_F$) and forest edge sites ($M_{FE}$) for all periods in both years. As reference matrices we used the $M_F$-matrices. Matrices of differences for the different time periods were defined as

$M_{D(ij)} = M_{FE(ij)} - M_{F(ij)}$

where subscripts in parentheses denote the period (*i*) and the year (*j*). For the calculation of contribution matrices we worked out overall sensitivity matrices $M_{S(ij)}$ that were calculated from pooled data from all sites. A detailed description of sensitivity analysis of periodic matrix models is given in Caswell and Trevisan (1994).

Contribution matrices were then calculated as

$M_{C(ij)} = M_{D(ij)} \circ M_{S(ij)}$ (Caswell, 2001)

where ∘ denotes the Hadamard product. The sum of the entries of the three contribution matrices of one annual cycle should closely approximate the difference between annual population growth rates λ of the different treatments.

Matrix entries of the seasonal population projection matrices were never composites of the growth matrices and the clonal reproduction matrices. Therefore, calculation of contribution matrices was straightforward and their entries could be assigned to single vital rates. Although some matrix entries of the 'fall-spring' seasonal projection matrices were composites of sexual reproduction matrix entries and entries of the growth matrix (Table S1), sexual reproduction

matrix entries had no influence on the values of the difference matrix as they were chosen to be identical for all sites in our model (see section 'Classification of life-cycle stages and calculation of transition matrices').

**Results**

Meteorological data

In 2008 precipitation was higher than in 2009 in the study area (Fig. 3). Mean annual precipitation was 958 mm (± 54 mm s.e.) in 2008 and 856 mm (± 53 mm s.e.) in 2009. These annual differences may not seem large but differences were pronounced during periods that are important for growth and clonal reproduction of *F. vesca* plants, i.e. in April, August, September and October.

Models for survival and sexual and clonal reproduction of adult plants

Above-ground biomass had a highly significant influence on survival, sexual reproduction and clonal reproduction in both years (Table 2). However, survival did not differ between forest and forest edge habitats in 2008 and in 2009, although there was a significant interaction for biomass and habitat in 2009. The probability of clonal reproduction was significantly increased for plants growing at forest edges in 2008, but was not affected by habitat type in 2009, although there was a significant interaction for biomass and habitat. Furthermore, plants from forest edges had a significantly increased probability of sexual reproduction compared to plants growing in forests in 2009 but not in 2008. There was a high level of variation in the proportion of sexually and clonally reproducing plants between sites and between years (Fig. 4).

Total yearly number of produced nodes and total yearly number of produced fruits were positively correlated in 2008 ($t_{930} = 2.17$, $P = 0.02$) and 2009 ($t_{1098} = 5.08$, $P = < 0.01$), whereas habitat was not a significant factor in both years.

Survival of seedlings

Only 11 natural seedlings were found during the whole study, always in summer or fall, and 2 seedlings (i.e. 18%) survived their first winter. All six natural seedlings found in 2008 died within a year, and at the end of the study in spring 2010 one of the five natural seedlings that germinated in 2009 was still alive. The survival of the planted seedlings was even lower. Of the

960 seedlings that were planted only 7 (i.e. 0.7%) survived their first winter. None of the 480 seedlings planted in 2008 survived till spring 2010 and four of the 480 seedlings planted in 2009 were still alive in spring 2010. The proportion of natural seedlings that survived their first winter was significantly higher than the proportion of planted seedlings ($z_{1, 969} = -3.94$, $P < 0.01$).

Plant size distribution

By means of the above-ground dry matter formula we estimated size distributions of plants at the different sites (Fig. 1). Spring plant size differences between sites ($F = 38.2$, df = 9, $p < .01$), within-site differences between the two years ($F = 5.3$, df = 1, $p = .021$) and the interaction between factors site and year ($F = 9.4$, df = 9, $p < .01$) were significant.

Lambda, stable stage structure and elasticity analyses

Mean population growth rates λ for the two periodic matrix models 'spring 2008–spring 2009' and 'spring 2009–spring 2010' were 1.61 and 0.28, respectively, and had a large range with extreme mimimum and maximum values of 0.03 and 6.08 (Table 1). At all sites λ was smaller for the period 'spring 2009–spring 2010' and well below 1 with the exception of one site.

Keyfitz' Δ values for stable size distributions calculated for the periodic matrix model 'spring 2009–spring 2010' and the size distributions observed during the exhaustive census in spring 2009 were low, showing that actual size distributions were generally close to stable size distributions (Fig. 5).

To present an overview of the relative importance of plant survival and clonal and sexual reproduction, we summed up (1) elasticities of λ to changes in the growth matrices for adult plants (small and large) and for nodes separately, (2) elasticities of λ to changes in the clonal reproduction matrices of adult plants and (3) elasticities of λ to changes in the sexual reproduction matrices of adult plants (Table 3). In general, largest elasticities were found for survival of adult plants (mean for pooled data: 63%, range: 16-100%), but in a few cases elasticities were largest for survival of nodes (mean for pooled data: 34%, range: 2-84%). Elasticities of λ to changes in clonal reproduction were high (mean for pooled data: 22%, range: 1-44%), especially in 2008. However, elasticities of λ to changes in sexual reproduction were low

and never exceeded 1%. Complete elasticity matrices are given as supplementary table (Table S1).

Life table response experiments (LTRE)

For presentation, we added up transition matrix element differences and contributions for small plants, large plants and clonal offspring separately (Fig. 6); note that the sum of contributions of growth matrix entries yields the overall contribution of plant survival.

2008-2009:

Population growth rates λ for the period 'spring 2008-spring 2009' were 1.21 and 2.48 for mean periodic matrix models of forest and forest edge habitats, respectively. The largest differences between elements of seasonal projection matrices of forest and forest edge habitats were found for clonal reproduction rates in summer and fall (Fig. 6). Differences in clonal reproduction also contributed most to differences in λ. Contributions of clonal reproduction to differences in λ added up to 1.20. Differences in survival of adult plants were small but they yielded relatively high contributions to differences in λ that added up to 0.19. Adult plant survival was always higher at forest edges with the exception of large plants during the period 'spring 2008-summer 2008'. On the other hand, survival of clonal offspring was worse at forest edges and survival contributions to differences in λ added up to –0.06.

The difference in λ between forest and forest edge habitats was 1.27. The total of contributions of the three seasonal contribution matrices was 1.33.

2009-2010:

Contrary to the previous year, λ for the period 'spring 2009-spring 2010' was lower for forest edges (0.18) compared to forests (0.33). Clonal reproduction was very low at most sites and differences between seasonal projection matrices of forests and forest edges were small (Fig. 6). Similarly, contributions of clonal reproduction to differences in λ were small and added up to -0.005. Adult plant survival was mostly lower at forest edges. Generally, differences in adult plant survival were larger than in the previous year, but contributions to differences in λ were

relatively small, adding up to –0.11. Differences in survival of clonal offspring were similar to the previous year as were the contributions to differences in λ that added up to -0.04.

The difference in λ between forest and forest edge habitats was 0.15. The total of contributions of the three seasonal contribution matrices was 0.16.

**Discussion**

Above-ground biomass had a highly significant influence on the probability of survival, sexual reproduction and clonal reproduction in both years. These results justify the use of size classes for matrix population models of *F. vesca*.

Plants growing at forest edges had a significantly increased probability of clonal reproduction compared to plants growing in forests in 2008. Furthermore, plants from forest edges had a significantly increased probability of sexual reproduction compared to plants growing in forests in 2009. The most obvious difference between forest and forest edge habitats was the variation in light availability, which was most likely the major factor that caused differences in clonal and sexual reproduction. This conclusion is supported by other studies on *F. vesca.* For example, Chabot (1978) showed that with increasing light availability an increasing fraction of carbon was allocated to sexual and clonal reproductive structures in *F. vesca* in greenhouse experiments. In this study, variation in light availability, as compared to variation in temperature and nutrient supply, had the strongest effect on plant growth (Chabot, 1978). Furthermore, carbon was increasingly allocated to reproductive structures with increasing light availability in a field study of *F. vesca*, suggesting that photosynthetic carbon gain is likely to be the major factor limiting growth (Jurik, 1983).

We found a significant positive correlation between the yearly number of nodes and the yearly number of fruits produced by plants in both years, suggesting that there is generally no trade-off between clonal and sexual reproduction in *F. vesca* and that plants allocate resources to both sexual and clonal reproductive structures when required resources are available.

We used habitat as a factor in a LTRE and the results revealed that clonal reproduction was the vital rate contributing most to differences in λ between forest and forest edge habitats for the period ‘spring 2008-spring 2009’ (Fig. 6). Furthermore, lower survival rates for all plant stages at forest edges contributed to the lower λ of populations at forest edges for the period ‘spring 2009-

spring 2010'. It is noteworthy that during the period 'spring 2008-spring 2009' the overall survival of adult plants was generally higher at forest edges and contributed positively to the λ of forest edge populations. Our interpretation of these results in combination with the precipitation data for 2008 and 2009 is that higher exposition to solar irradiation positively influenced population growth at forest edge sites as long as water availability was sufficient (as in 2008), but caused lower plant survival at forest edges during relatively dry periods (as in 2009).

The range of values of λ for the different sites and years was large and the maximum and minimum values were rather extreme. But the site mean values over the two years were within the range of other studies on herbaceaous species (Silvertown et al., 1993) with the exception of the very low values for Eichmatt, Riehen and Schleifenberg (Table 1). At these sites plants within the plots faced increasing competition through plant succession or suffered from being covered for long periods by a thick layer of dead leaves. As a rule, elasticities of λ to changes in the survival of adult plants had the highest values (Table 3). But for some sites elasticities of λ to changes in the survival of nodes were highest when clonal reproduction was high, e.g. at sites Hochwald and Paradies for matrices 'summer 2008-fall 2008' and 'fall 2008-spring 2009'. Also for the pooled data matrix 'fall 2008-spring 2009', elasticity of λ to changes in the survival of nodes was highest. In contrast to clonal reproduction, elasticities of λ to changes in sexual reproduction were insignificant. Elasticities of λ to changes in sexual reproduction were calculated with the mean survival rates of planted and natural seedlings. Although survival of planted seedlings was lower than survival of natural seedlings, this difference did not influence the results of our elasticity analyses qualitatively, as elasticities of λ to changes in sexual reproduction were also well below 1% for sexual reproduction matrices calculated only with survival rates of natural seedlings (data not shown). Low elasticities of λ to changes in sexual reproduction of clonal plants are common (Silvertown et al., 1993). Our results suggest that sexual reproduction is of little importance for population growth within established *F. vesca* populations. The adaptation of the red fleshy fruits of *F. vesca* to endozoochorical dispersal seems obvious and mammals, birds and invertebrates have been described as seed dispersal mutualists of *Fragaria* spp. (Willson, 1993; Müller-Schneider, 1986). Therefore, one might expect that removal of the major part of fruits by animals could explain to some degree the observed low seedling recruitment within established *F. vesca* stands. However, we observed that

a large number of fruits was not consumed and remained withered attached to inflorescences. Also seed characteristics of *F. vesca* probably account to some extent for the low seedling recruitment. It has been proposed that in small seeded clonal plants seed production rather serves as a mechanism for dispersal to new sites than for plant reproduction within established populations, whereas clonal plants with larger seeds often have higher rates of seedling recruitment within stands of conspecific adults (Eriksson, 1997). *Fragaria vesca* can certainly be placed into the category of small seeded clonal plants (Eriksson, 1997).

The observed strong influence of clonal reproduction on population growth can be explained by the high survival rates of nodes, which were often similar to the rates of adult plants, and the fast growth of nodes. Nodes can reach the size of adult plants within the year of their creation, and we have even observed nodes that flowered during the year of their creation although this is not common. Low Keyfitz' $\Delta$ values for most sites indicate that as a consequence of the fast development of clonal offspring *F. vesca* populations reach their theoretical stable size distribution in a short time.

This study shows that reproduction and the survival of different life stages and their relative importance for population growth of *F. vesca* depends highly on spatio-temporal variation of environmental conditions. However, if the landscape level is considered, i.e. the pooled data from all sites, elasticities of $\lambda$ to changes in the vital rates of different years are relatively similar (Table 3). This shows that extreme conditions at the site scale can balance out at the landscape level and that the inclusion of spatial variability yields a more objective picture of the demography of a species. Retrospectively, we still find it difficult to define an ideal design to study the population dynamics of a highly mobile woodland understorey species such as *F. vesca* (or clonal plants with a similar life strategy, e.g. *Glechoma hederacea* (Hutchings and Price, 1999)). Due to its capacity to produce stolons that can reach a length of a few meters under favourable conditions, ramets of *F. vesca* can disperse in space very quickly. This can lead to the somewhat unsatisfying situation that plants that are surveyed within a marked plot do not perform well, e.g. due to competition, but that a few meters beside the plot *F. vesca* nodes from the previous season thrive and reproduce explosively in a newly occupied patch. Based on the data collected within plots we may conclude that *F. vesca* is going extinct at this site, but this conclusion would obviously be wrong. Generally, we found that plant succession quickly

displaced *F. vesca* from areas that are allowed to develop undisturbedly, yet *F. vesca* ramets could shift to more favourable patches via clonal growth. An approach to solve this problem would be to expand the study scale at the site level by increasing plot sizes and marking more newly produced plantlets or, as suggested by Crawley (1990), to set up randomly distributed empty quadrats at study sites where new recruitment could take place. In the case of *F. vesca*, increasing the plot size would probably be more appropriate. The recruitment of new plants within shorter time periods in actually randomly chosen empty quadrats would be an uncertain event for a plant like *F. vesca* that has low seedling recruitment and spreads patchily via clonal growth. Furthermore, information at the site level can only be increased at the expense of a reduction of total number of sites. It depends on the research goal whether precision at the site level or information on variation at the landscape level should be maximized. We conclude that it is best to maximize landscape level information if the goal is to study the general demography of a species that is expected to show high phenotypic plasticity in vital rates.

In conclusion, the present study contributes to the understanding of the population dynamics of *F. vesca* and plant species with similar life strategies, and forms a basis for a risk assessment of a potential future establishment of hybrids between *F. vesca* and GM *F.* x *ananassa*. Although demographic data on transgenic and non-transgenic *F. vesca* x *F.* x *ananassa* hybrids are still lacking, we can hypothesize on traits that would allow hybrids to compete successfully with *F. vesca* plants, based on our results of elasticity analyses and LTREs. The most relevant finding was the great importance of clonal reproduction for population growth of *F. vesca*. It seems likely that *F. vesca* x *F.* x *ananassa* hybrids would require clonal reproduction rates that are similar to *F. vesca* to compete successfully with the latter, as long as hybrids can not compensate any disadvantage of lower clonal reproduction rates in some other way. Transgenic effects that could augment the importance of sexual reproduction for spread and population growth of hybrids as compared to *F. vesca*, e.g. through increased fruit production or seed weight, seem less likely, because hybrids between diploid and octoploid *Fragaria* species are usually highly sterile (Bringhurst and Khan, 1963; Senanayake and Bringhurst, 1967; Olbricht et al., 2006). However, in addition to demographic data on *F. vesca*, data on *F. vesca* x *F.* x *ananassa* hybrids are needed to address the issue of hybrid fitness. Currently, competition experiments between hybrids and *F. vesca* are underway to allow for a comparison between growth parameters of hybrids and *F. vesca* plants.

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**Table 1.** Information on study sites, clonal and sexual reproduction and growth rates λ of natural *F. vesca* populations. Mean values are given with ± SD.

| | Adlerberg | Eichmatt | Gempen | Grammet | Holzen-berg | Hochwald | Paradies | Riehen | Schauen-burg | Schleifen-berg | Scharten | Ziefen | All sites |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Altitude (m a.s.l.) | 450 | 600 | 720 | 520 | 650 | 630 | 430 | 490 | 480 | 440 | 730 | 520 | - |
| Coordinates North/East | 47°30'24"/ 7°42'12" | 47°29'49"/ 7°39'10" | 47°28'43"/ 7°39'33" | 47°29'09"/ 7°46'02" | 47°25'12"/ 7°41'04" | 47°27'00"/ 7°37'27" | 47°30'17"/ 7°41'53" | 47°34'35"/ 7°40'49" | 47°29'50"/ 7°41'02" | 47°29'28"/ 7°44'10" | 47°28'38"/ 7°39'09" | 47°26'08"/ 7°42'22" | - |
| Plot area ($m^2$) | 1.13 | 1.88 | 2.00 | 1.75 | 0.75 | 1.13 | 1.50 | 4.00 | 1.75 | 0.69 | 0.88 | 1.69 | - |
| Site type | Forest | Forest | Forest | Forest | Forest edge | Forest edge | Forest edge | Forest edge | Forest | Forest | Forest edge | Forest | - |
| Mean clonal offspring 2008 | 0.27 ± 0.64 | 0.49 ± 0.91 | 1.89 ± 2.24 | 1.90 ± 2.12 | 3.82 ± 2.26 | 8.13 ± 6.78 | 8.57 ± 6.30 | 0.23 ± 0.73 | 3.00 ± 3.07 | 0.50 ± 0.92 | 2.58 ± 2.33 | 0.66 ± 1.07 | 2.47 ± 4.02 |
| Mean clonal offspring 2009 | 0.11 ± 0.34 | 0.06 ± 0.35 | 0.44 ± 1.12 | 1.39 ± 2.24 | 0.65 ± 1.28 | 1.16 ± 1.90 | 0.90 ± 1.47 | 0 | 3.86 ± 3.59 | 0.06 ± 0.25 | 0.80 ± 1.43 | 0.21 ± 0.66 | 0.83 ± 1.90 |
| Mean ripe fruits 2008 | 0.12 ± 0.47 | 0.48 ± 1.08 | 0.40 ± 1.09 | 0.26 ± 0.73 | 1.52 ± 1.98 | 0.05 ± 0.28 | 1.25 ± 2.89 | 0.51 ± 0.89 | 0.06 ± 0.29 | 0.08 ± 0.40 | 0.78 ± 1.34 | 0.15 ± 0.54 | 0.46 ± 1.28 |
| Mean ripe fruits 2009 | 0.06 ± 0.31 | 0.30 ± 0.88 | 0.02 ± 0.14 | 0.55 ± 0.84 | 1.12 ± 1.10 | 1.66 ± 1.81 | 2.16 ± 2.98 | 0.22 ± 0.50 | 0.25 ± 0.90 | 0.11 ± 0.32 | 1.27 ± 1.41 | 0.21 ± 0.58 | 0.66 ± 1.41 |
| Seedlings found 2008 | 1 | 2 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 6 |
| Seedlings found 2009* | 2 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| λ 2008-2009 | 1.13 | 0.41 | 1.65 | 1.10 | 1.89 | 4.92 | 6.08 | 0.37 | 3.04 | 0.64 | 1.85 | 1.19 | 1.61 |
| λ 2009-2010 | 0.64 | 0.09 | 0.46 | 0.25 | 0.40 | 0.37 | 0.17 | 0.04 | 2.34 | 0.03 | 0.35 | 0.43 | 0.28 |
| Mean λ | 0.85 | 0.25 | 1.06 | 0.68 | 1.14 | 2.68 | 3.14 | 0.21 | 2.74 | 0.33 | 1.11 | 0.81 | 0.94 |

* No new seedlings were found during the final census in spring 2010

**Table 2.** Results of generalized linear mixed effect models for survival, sexual reproduction and clonal reproduction of *F. vesca* in 2008 and 2009. For every model, the maximal model is given. * indicates inclusion of interactions between variables and factors. Site was included as a random effect in all models. Significant variables, factors and interactions are listed. For models with overdispersion test statistics were evaluated using Student's *t*-distribution.

| | Test statistic | df | *P* |
|---|---|---|---|
| Survival 08-09 ~ Biomass spring 08 * Habitat type | | | |
| Biomass | $t = 3.51$ | 928 | < 0.01 |
| Sexual reproduction 08 ~ Biomass spring 08 * Habitat type | | | |
| Biomass | $z = 7.42$ | 933 | < 0.01 |
| Clonal reproduction 08 ~ Biomass spring 08 * Habitat type | | | |
| Biomass | $t = 8.98$ | 933 | < 0.01 |
| Habitat type | $t = 2.99$ | 10 | < 0.01 |
| Biomass x Habitat type | $t = 2.22$ | 933 | 0.013 |
| Survival 09-10 ~ Biomass spring 09 * Habitat type | | | |
| Biomass | $t = 4.03$ | 1098 | < 0.01 |
| Habitat type | $t = 0.57$ | 10 | 0.29 |
| Biomass x Habitat type | $t = 2.55$ | 1098 | < 0.01 |
| Sexual reproduction 09 ~ Biomass spring 09 * Habitat type | | | |
| Biomass | $z = 9.60$ | 1089 | < 0.01 |
| Habitat type | $z = 2.54$ | 10 | 0.01 |
| Clonal reproduction 09 ~ Biomass spring 09 * Habitat type | | | |
| Biomass | $z = 10.98$ | 1089 | < 0.01 |
| Habitat type | z = 0.73 | 10 | 0.46 |
| Biomass x Habitat type | $z = 3.71$ | 1089 | < 0.01 |

**Table 3** Elasticities of λ of periodic matrix models of natural *F. vesca* populations for 2008-2009 and 2009-2010 to changes in survival of adult plants (small and large) and nodes, clonal reproduction of adult plants and sexual reproduction of adult plants. Elasticities are given as rounded percent values. Entries for sexual reproduction are not 0 but are very small. NA means that no observations were made.

| | | Spring 08-summer 08 | Summer 08-fall 08 | Fall 08-spring 09 | Spring 09-summer 09 | Summer 09-fall 09 | Fall 09-spring 10 |
|---|---|---|---|---|---|---|---|
| Adlerberg | Survival adults | 87 | 86 | 86 | 98 | 98 | 98 |
| | Survival nodes | . | 13 | 14 | . | 2 | 2 |
| | Clonal reproduction | 13 | 1 | . | 2 | NA | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Eichmatt | Survival adults | 78 | 68 | 68 | 94 | 91 | 90 |
| | Survival nodes | . | 22 | 32 | . | 6 | 9 |
| | Clonal reproduction | 22 | 10 | . | 6 | 3 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Gempen | Survival adults | 80 | 67 | 67 | 88 | 79 | 79 |
| | Survival nodes | . | 20 | 33 | . | 12 | 21 |
| | Clonal reproduction | 20 | 13 | . | 12 | 9 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Grammet | Survival adults | 85 | 77 | 77 | 73 | 50 | 49 |
| | Survival nodes | . | 15 | 23 | . | 27 | 50 |
| | Clonal reproduction | 15 | 8 | . | 27 | 23 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Holzerberg | Survival adults | 78 | 55 | 55 | 93 | 76 | 75 |
| | Survival nodes | . | 22 | 45 | . | 7 | 24 |
| | Clonal reproduction | 22 | 22 | . | 7 | 18 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Hochwald | Survival adults | 56 | 20 | 19 | 82 | 61 | 61 |
| | Survival nodes | . | 44 | 80 | . | 18 | 39 |
| | Clonal reproduction | 44 | 36 | . | 18 | 21 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Paradies | Survival adults | 56 | 16 | 16 | 92 | 88 | 88 |
| | Survival nodes | . | 44 | 84 | . | 8 | 12 |
| | Clonal reproduction | 44 | 40 | . | 8 | 4 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Riehen | Survival adults | 96 | 89 | 88 | 100 | 100 | 100 |
| | Survival nodes | . | 4 | 11 | . | NA | NA |
| | Clonal reproduction | 4 | 7 | . | NA | NA | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Schauenburg | Survival adults | 76 | 39 | 39 | 67 | 38 | 38 |
| | Survival nodes | . | 24 | 61 | . | 33 | 62 |
| | Clonal reproduction | 24 | 37 | . | 33 | 29 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Schleifenberg | Survival adults | 91 | 80 | 79 | 83 | 76 | 76 |
| | Survival nodes | . | 9 | 20 | . | 17 | 24 |
| | Clonal reproduction | 9 | 12 | . | 17 | 7 | . |
| | Sexual reproduction | . | . | . | . | . | . |
| Scharten | Survival adults | 82 | 54 | 54 | 85 | 81 | 81 |
| | Survival nodes | . | 18 | 46 | . | 15 | 19 |
| | Clonal reproduction | 18 | 28 | . | 16 | 4 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |
| Ziefen | Survival adults | 87 | 82 | 82 | 88 | 83 | 83 |
| | Survival nodes | . | 13 | 18 | . | 12 | 17 |
| | Clonal reproduction | 13 | 5 | . | 12 | 5 | . |
| | Sexual reproduction | . | . | 0 | . | . | 1 |
| All sites | Survival adults | 73 | 49 | 48 | 79 | 65 | 65 |
| | Survival nodes | . | 27 | 51 | . | 21 | 35 |
| | Clonal reproduction | 27 | 24 | . | 21 | 14 | . |
| | Sexual reproduction | . | . | 0 | . | . | 0 |

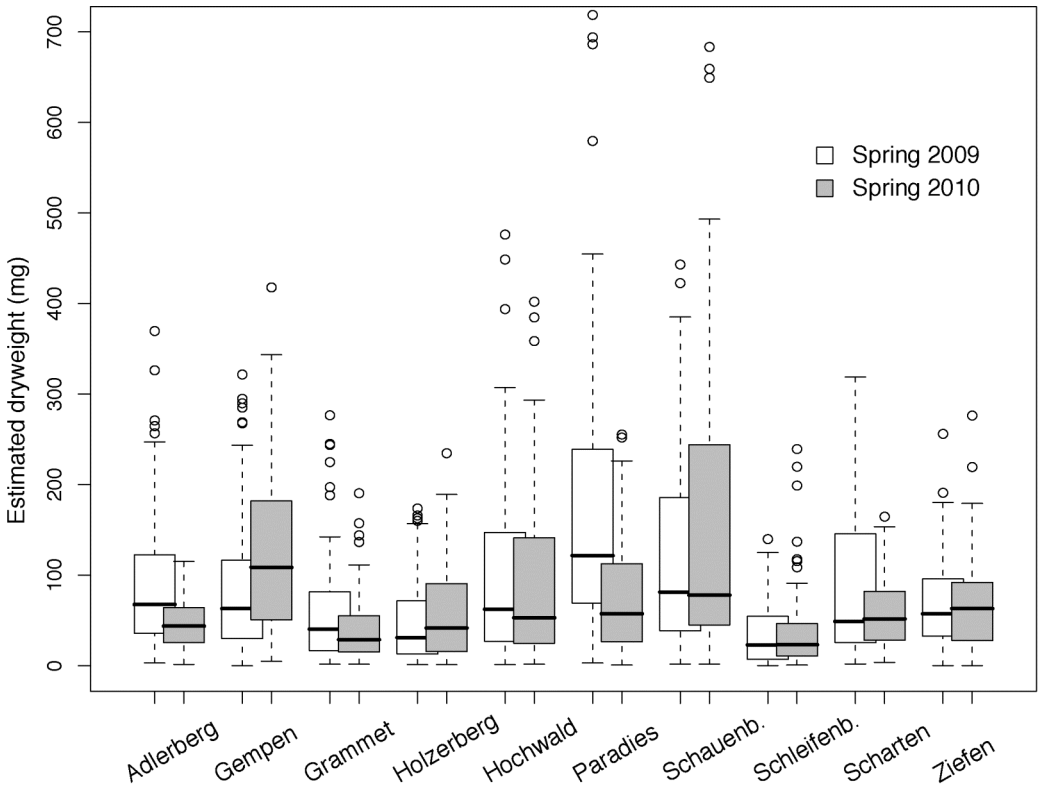


**Figure 1.** Box plots of estimated above-ground dryweights of *F. vesca* plants from ten Swiss study sites in spring 2009 and 2010.

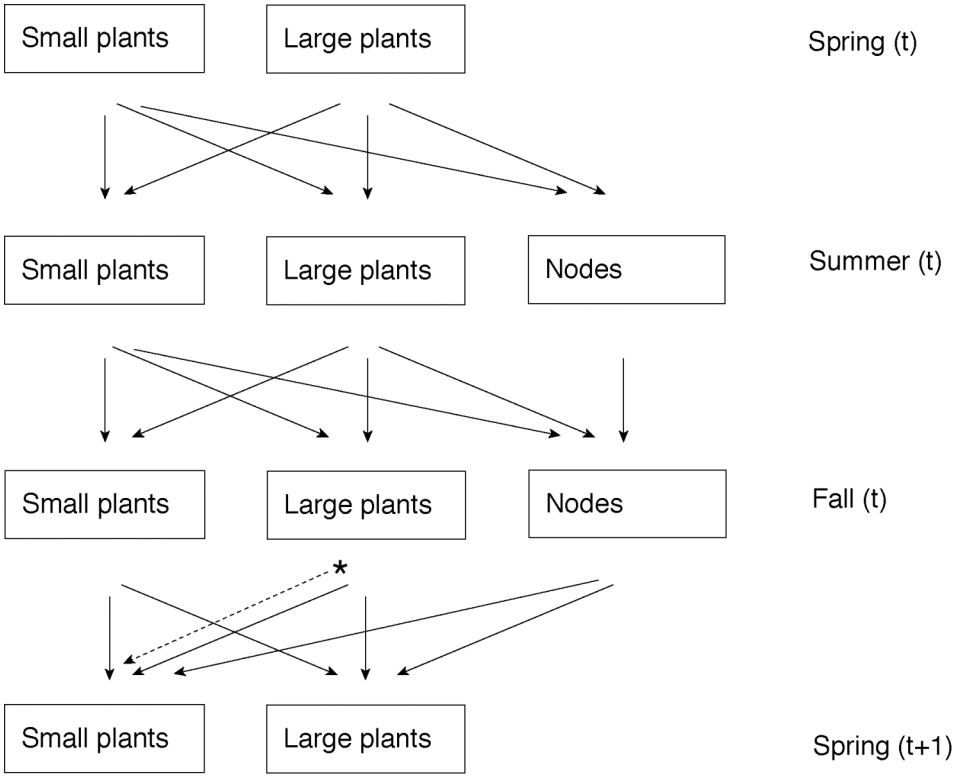


**Figure 2.** A seasonal life cycle graph for *F. vesca* depicting transitions as used in the periodic matrix models. Each horizontal row represents a season of the year. Small and large plants may remain in their size class or change class and may reproduce clonally from spring till summer and from summer till fall. Clonally formed nodes may grow to small or large plants from fall to spring. * For our models sexual reproduction was defined as the number of seedlings found within plots per year and per number of large plants present, that grow to small plants from fall to spring.

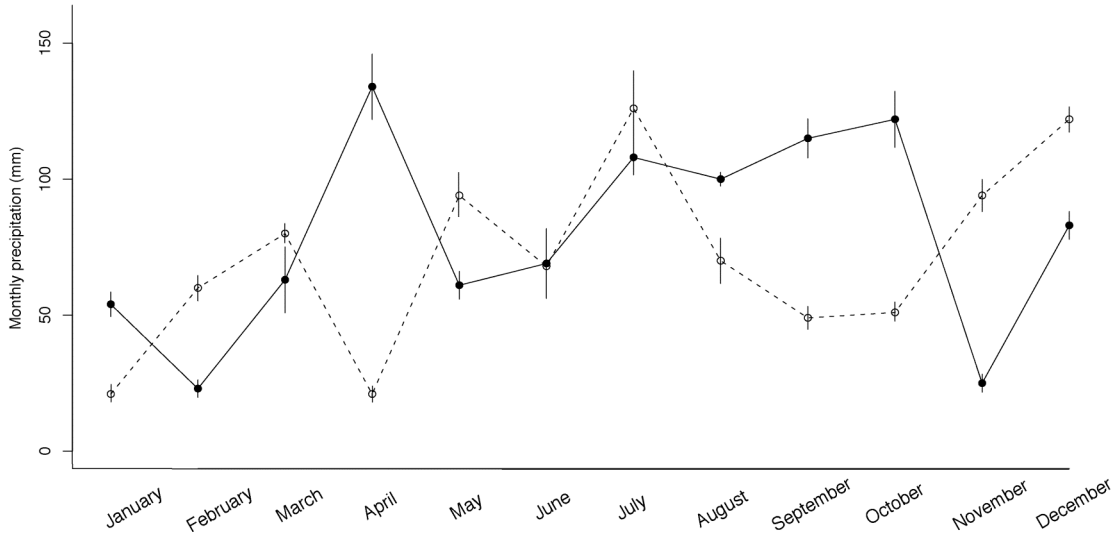


**Figure 3.** Mean monthly precipitations calculated from four weather stations in the study area in 2008 (solid line) and 2009 (dashed line). Error bars represent ± standard errors. Mean annual precipitation was 958 mm (± 54 mm s.e.) in 2008 and 856 mm (± 53 mm s.e.) in 2009.

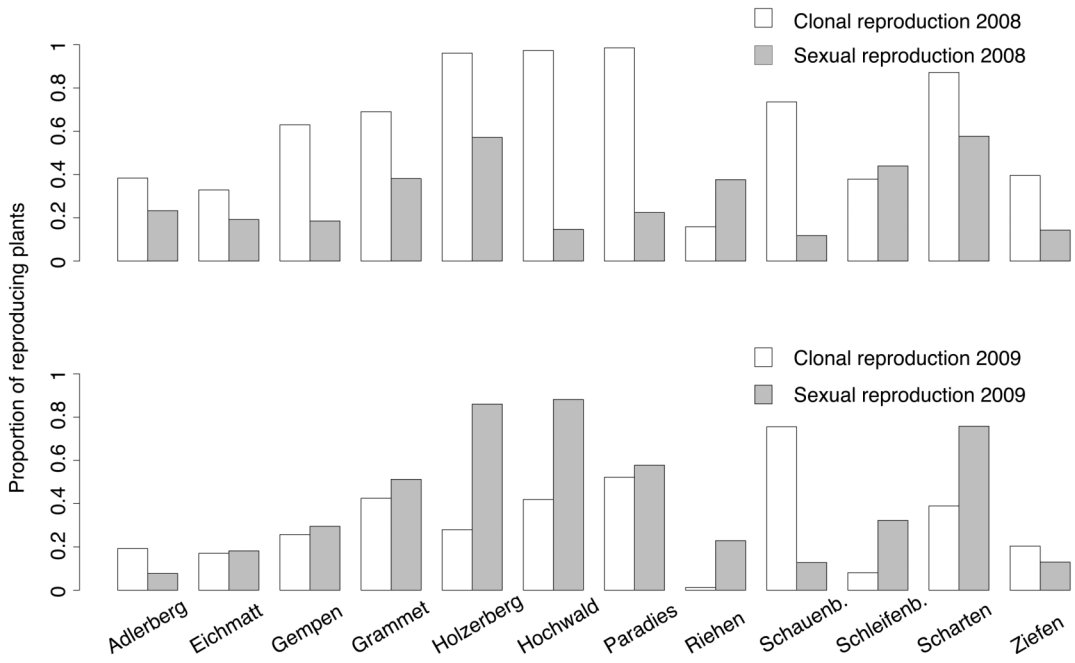


**Figure 4.** Proportions of sexually and clonally reproducing *F. vesca* plants at 12 Swiss study sites in 2008 and 2009.

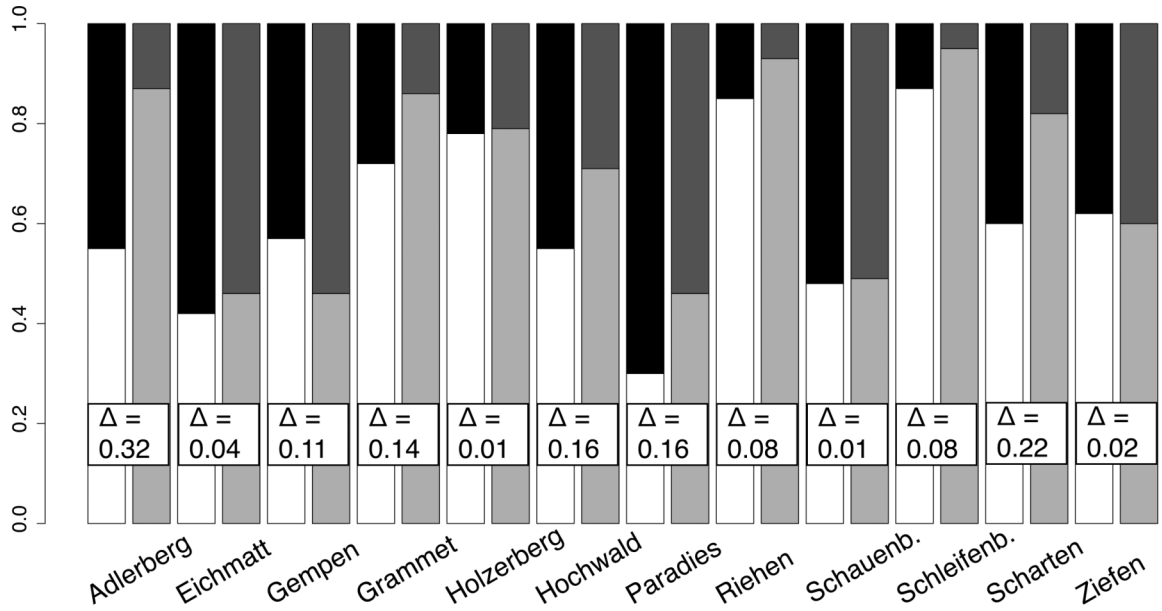


**Figure 5.** Observed *F. vesca* population size distributions (□ = small plants, ■ = large plants) in spring 2009 and stable population size distributions calculated from population projection matrices for the period 'spring 2009-spring 2010' (▒ = small plants, ▒ = large plants) at 12 Swiss study sites. Keyfitz's Δ values are shown for each site.

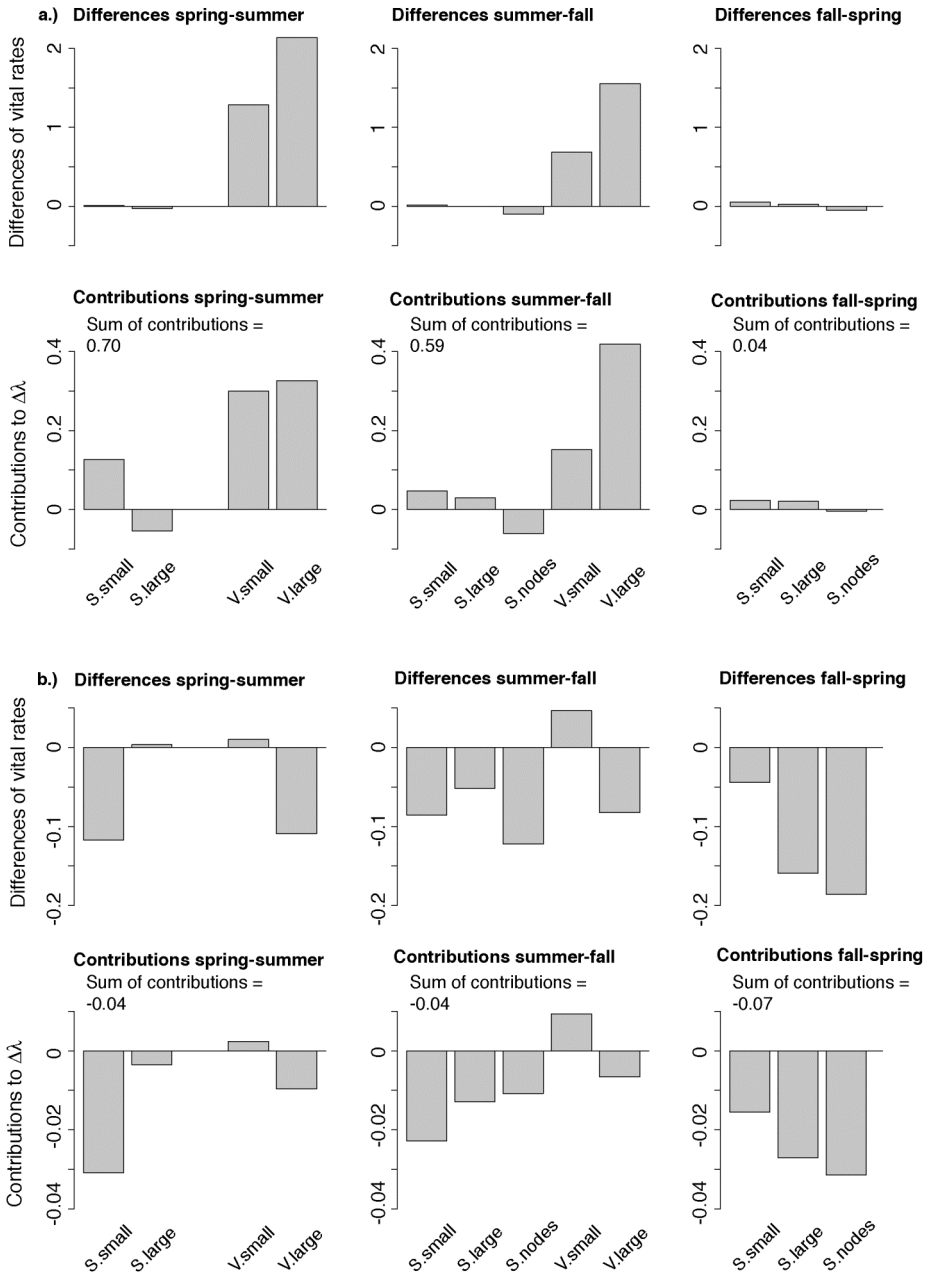


**Figure 6.** Differences of vital rates of *F. vesca* plant stages between the mean seasonal population projection matrices of forest and forest edge populations and contributions of differences in vital rates to differences in λ for the periods 'spring 2008-spring 2009' (a.) and 'spring 2009-spring 2010' (b.). Mean forest population projection matrices were used as reference matrices. Note that clonal reproduction started later in the year, therefore there were no clonal offspring survival rates for season 'spring-summer'. Note different axis-scales in a.) and b.). S.small = survival of small plants; S.large = survival of large plants; S.nodes = survival of clonal offspring; V.small = clonal reproduction of small plants; V.large = clonal reproduction of large plants.

**Table S1** Transition and elasticity matrices for the period 'spring 08-spring 10'. Elasticities are given as rounded percent values.

| Transition matrices spring 08-summer 08 | Ramet stasis & growth | | Clonal reproduction | |
|---|---|---|---|---|
| | Small plants | Large plants | Small plants | Large plants |
| **Adlerberg** | | | | |
| Nodes | 0 | 0 | 0 | 0.55 |
| Small plants | 0.89 | 0 | 0 | 0 |
| Large plants | 0.11 | 1 | 0 | 0 |
| **Eichmatt** | | | | |
| Nodes | 0 | 0 | 0 | 0.47 |
| Small plants | 0.48 | 0.04 | 0 | 0 |
| Large plants | 0.10 | 0.70 | 0 | 0 |
| **Gempen** | | | | |
| Nodes | 0 | 0 | 0 | 1.61 |
| Small plants | 0.84 | 0.05 | 0 | 0 |
| Large plants | 0.16 | 0.95 | 0 | 0 |
| **Grammet** | | | | |
| Nodes | 0 | 0 | 0.35 | 2.76 |
| Small plants | 0.48 | 0.03 | 0 | 0 |
| Large plants | 0.50 | 0.97 | 0 | 0 |
| **Holzerberg** | | | | |
| Nodes | 0 | 0 | 1.00 | 3.20 |
| Small plants | 0.83 | 0.28 | 0 | 0 |
| Large plants | 0.17 | 0.72 | 0 | 0 |
| **Hochwald** | | | | |
| Nodes | 0 | 0 | 2.62 | 6.18 |
| Small plants | 0.16 | 0.16 | 0 | 0 |
| Large plants | 0.78 | 0.84 | 0 | 0 |
| **Paradies** | | | | |
| Nodes | 0 | 0 | 2.64 | 5.68 |
| Small plants | 0 | 0.05 | 0 | 0 |
| Large plants | 1.00 | 0.95 | 0 | 0 |
| **Riehen** | | | | |
| Nodes | 0 | 0 | 0.05 | 0.15 |
| Small plants | 0.57 | 0.15 | 0 | 0 |
| Large plants | 0.17 | 0.54 | 0 | 0 |
| **Schauenburg** | | | | |
| Nodes | 0 | 0 | 0 | 1.74 |
| Small plants | 0.30 | 0.02 | 0 | 0 |
| Large plants | 0.07 | 0.98 | 0 | 0 |
| **Schleifenberg** | | | | |
| Nodes | 0 | 0 | 0.12 | 0.88 |
| Small plants | 0.78 | 0.50 | 0 | 0 |
| Large plants | 0.16 | 0.50 | 0 | 0 |
| **Scharten** | | | | |
| Nodes | 0 | 0 | 0.52 | 1.86 |
| Small plants | 0.57 | 0.40 | 0 | 0 |
| Large plants | 0.43 | 0.58 | 0 | 0 |
| **Ziefen** | | | | |
| Nodes | 0 | 0 | 0.11 | 0.93 |
| Small plants | 0.78 | 0.09 | 0 | 0 |
| Large plants | 0.20 | 0.91 | 0 | 0 |
| **All sites** | | | | |
| Nodes | 0 | 0 | 0.42 | 2.21 |
| Small plants | 0.59 | 0.13 | 0 | 0 |
| Large plants | 0.29 | 0.81 | 0 | 0 |

| Elasticity matrices spring 08-summer 08 | Ramet stasis & growth | | Clonal reproduction | |
|---|---|---|---|---|
| | Small plants | Large plants | Small plants | Large plants |
| **Adlerberg** | | | | |
| Nodes | 0 | 0 | 0 | 13 |
| Small plants | 39 | 0 | 0 | 0 |
| Large plants | 5 | 42 | 0 | 0 |
| **Eichmatt** | | | | |
| Nodes | 0 | 0 | 0 | 22 |
| Small plants | 12 | 1 | 0 | 0 |
| Large plants | 9 | 56 | 0 | 0 |
| **Gempen** | | | | |
| Nodes | 0 | 0 | 0 | 20 |
| Small plants | 27 | 1 | 0 | 0 |
| Large plants | 10 | 42 | 0 | 0 |
| **Grammet** | | | | |
| Nodes | 0 | 0 | 3 | 12 |
| Small plants | 24 | 1 | 0 | 0 |
| Large plants | 31 | 29 | 0 | 0 |
| **Holzerberg** | | | | |
| Nodes | 0 | 0 | 9 | 13 |
| Small plants | 40 | 6 | 0 | 0 |
| Large plants | 10 | 21 | 0 | 0 |
| **Hochwald** | | | | |
| Nodes | 0 | 0 | 10 | 34 |
| Small plants | 3 | 4 | 0 | 0 |
| Large plants | 19 | 30 | 0 | 0 |
| **Paradies** | | | | |
| Nodes | 0 | 0 | 9 | 35 |
| Small plants | 0 | 1 | 0 | 0 |
| Large plants | 20 | 36 | 0 | 0 |
| **Riehen** | | | | |
| Nodes | 0 | 0 | 2 | 2 |
| Small plants | 57 | 4 | 0 | 0 |
| Large plants | 19 | 15 | 0 | 0 |
| **Schauenburg** | | | | |
| Nodes | 0 | 0 | 0 | 24 |
| Small plants | 4 | 0 | 0 | 0 |
| Large plants | 21 | 50 | 0 | 0 |
| **Schleifenberg** | | | | |
| Nodes | 0 | 0 | 4 | 5 |
| Small plants | 59 | 7 | 0 | 0 |
| Large plants | 16 | 9 | 0 | 0 |
| **Scharten** | | | | |
| Nodes | 0 | 0 | 6 | 12 |
| Small plants | 21 | 8 | 0 | 0 |
| Large plants | 30 | 23 | 0 | 0 |
| **Ziefen** | | | | |
| Nodes | 0 | 0 | 2 | 12 |
| Small plants | 37 | 3 | 0 | 0 |
| Large plants | 10 | 36 | 0 | 0 |
| **All sites** | | | | |
| Nodes | 0 | 0 | 6 | 21 |
| Small plants | 18 | 3 | 0 | 0 |
| Large plants | 18 | 33 | 0 | 0 |

| Transition matrices summer 08-fall 08 | Ramet stasis & growth | | | Clonal reproduction | | |
|---|---|---|---|---|---|---|
| | Nodes | Small plants | Large plants | Nodes | Small plants | Large plants |
| **Adlerberg** | | | | | | |
| Nodes | 0.79 | 0 | 0 | 0 | 0 | 0.02 |
| Small plants | 0 | 0.95 | 0.62 | 0 | 0 | 0 |
| Large plants | 0 | 0.02 | 0.38 | 0 | 0 | 0 |
| **Eichmatt** | | | | | | |
| Nodes | 0.70 | 0 | 0 | 0 | 0 | 0.18 |
| Small plants | 0 | 0.40 | 0.13 | 0 | 0 | 0 |
| Large plants | 0 | 0.07 | 0.55 | 0 | 0 | 0 |
| **Gempen** | | | | | | |
| Nodes | 1.00 | 0 | 0 | 0 | 0 | 0.85 |
| Small plants | 0 | 0.63 | 0.08 | 0 | 0 | 0 |
| Large plants | 0 | 0.37 | 0.92 | 0 | 0 | 0 |
| **Grammet** | | | | | | |
| Nodes | 0.65 | 0 | 0 | 0 | 0.08 | 0.57 |
| Small plants | 0 | 0.92 | 0.59 | 0 | 0 | 0 |
| Large plants | 0 | 0.04 | 0.39 | 0 | 0 | 0 |
| **Holzerberg** | | | | | | |
| Nodes | 0.45 | 0 | 0 | 0 | 0.54 | 1.20 |
| Small plants | 0 | 0.75 | 0.55 | 0 | 0 | 0 |
| Large plants | 0 | 0.25 | 0.45 | 0 | 0 | 0 |
| **Hochwald** | | | | | | |
| Nodes | 0.90 | 0 | 0 | 0 | 2.43 | 3.87 |
| Small plants | 0 | 0.21 | 0.21 | 0 | 0 | 0 |
| Large plants | 0 | 0.64 | 0.76 | 0 | 0 | 0 |
| **Paradies** | | | | | | |
| Nodes | 0.81 | 0 | 0 | 0 | 0.33 | 3.53 |
| Small plants | 0 | 0 | 0.03 | 0 | 0 | 0 |
| Large plants | 0 | 1.00 | 0.97 | 0 | 0 | 0 |
| **Riehen** | | | | | | |
| Nodes | 0.50 | 0 | 0 | 0 | 0 | 0.22 |
| Small plants | 0 | 0.64 | 0.21 | 0 | 0 | 0 |
| Large plants | 0 | 0.08 | 0.44 | 0 | 0 | 0 |
| **Schauenburg** | | | | | | |
| Nodes | 0.86 | 0 | 0 | 0 | 0 | 1.61 |
| Small plants | 0 | 0.25 | 0.03 | 0 | 0 | 0 |
| Large plants | 0 | 0.75 | 0.97 | 0 | 0 | 0 |
| **Schleifenberg** | | | | | | |
| Nodes | 0.50 | 0 | 0 | 0 | 0.08 | 0.47 |
| Small plants | 0 | 0.48 | 0.32 | 0 | 0 | 0 |
| Large plants | 0 | 0.36 | 0.47 | 0 | 0 | 0 |
| **Scharten** | | | | | | |
| Nodes | 0.60 | 0 | 0 | 0 | 0.28 | 1.72 |
| Small plants | 0 | 0.47 | 0.02 | 0 | 0 | 0 |
| Large plants | 0 | 0.47 | 0.95 | 0 | 0 | 0 |
| **Ziefen** | | | | | | |
| Nodes | 0.76 | 0 | 0 | 0 | 0.05 | 0.19 |
| Small plants | 0 | 0.85 | 0.63 | 0 | 0 | 0 |
| Large plants | 0 | 0.13 | 0.35 | 0 | 0 | 0 |
| **All sites** | | | | | | |
| Nodes | 0.71 | 0 | 0 | 0 | 0.18 | 1.30 |
| Small plants | 0 | 0.63 | 0.27 | 0 | 0 | 0 |
| Large plants | 0 | 0.21 | 0.65 | 0 | 0 | 0 |

| Elasticity matrices summer 08-fall 08 | Ramet stasis & growth | | | Clonal reproduction | | |
|---|---|---|---|---|---|---|
| | Nodes | Small plants | Large plants | Nodes | Small plants | Large plants |
| **Adlerberg** | | | | | | |
| Nodes | 13 | 0 | 0 | 0 | 0 | 1 |
| Small plants | 0 | 38 | 27 | 0 | 0 | 0 |
| Large plants | 0 | 1 | 20 | 0 | 0 | 0 |
| **Eichmatt** | | | | | | |
| Nodes | 22 | 0 | 0 | 0 | 0 | 10 |
| Small plants | 0 | 9 | 4 | 0 | 0 | 0 |
| Large plants | 0 | 4 | 51 | 0 | 0 | 0 |
| **Gempen** | | | | | | |
| Nodes | 20 | 0 | 0 | 0 | 0 | 13 |
| Small plants | 0 | 13 | 2 | 0 | 0 | 0 |
| Large plants | 0 | 16 | 38 | 0 | 0 | 0 |
| **Grammet** | | | | | | |
| Nodes | 15 | 0 | 0 | 0 | 1 | 8 |
| Small plants | 0 | 23 | 29 | 0 | 0 | 0 |
| Large plants | 0 | 1 | 24 | 0 | 0 | 0 |
| **Holzerberg** | | | | | | |
| Nodes | 22 | 0 | 0 | 0 | 10 | 12 |
| Small plants | 0 | 26 | 10 | 0 | 0 | 0 |
| Large plants | 0 | 10 | 10 | 0 | 0 | 0 |
| **Hochwald** | | | | | | |
| Nodes | 44 | 0 | 0 | 0 | 4 | 32 |
| Small plants | 0 | 1 | 3 | 0 | 0 | 0 |
| Large plants | 0 | 2 | 14 | 0 | 0 | 0 |
| **Paradies** | | | | | | |
| Nodes | 44 | 0 | 0 | 0 | 0 | 40 |
| Small plants | 0 | 0 | 1 | 0 | 0 | 0 |
| Large plants | 0 | 1 | 15 | 0 | 0 | 0 |
| **Riehen** | | | | | | |
| Nodes | 4 | 0 | 0 | 0 | 0 | 7 |
| Small plants | 0 | 54 | 9 | 0 | 0 | 0 |
| Large plants | 0 | 7 | 19 | 0 | 0 | 0 |
| **Schauenburg** | | | | | | |
| Nodes | 24 | 0 | 0 | 0 | 0 | 37 |
| Small plants | 0 | 1 | 1 | 0 | 0 | 0 |
| Large plants | 0 | 4 | 33 | 0 | 0 | 0 |
| **Schleifenberg** | | | | | | |
| Nodes | 9 | 0 | 0 | 0 | 4 | 7 |
| Small plants | 0 | 31 | 6 | 0 | 0 | 0 |
| Large plants | 0 | 31 | 12 | 0 | 0 | 0 |
| **Scharten** | | | | | | |
| Nodes | 18 | 0 | 0 | 0 | 4 | 24 |
| Small plants | 0 | 10 | 0 | 0 | 0 | 0 |
| Large plants | 0 | 15 | 28 | 0 | 0 | 0 |
| **Ziefen** | | | | | | |
| Nodes | 13 | 0 | 0 | 0 | 1 | 4 |
| Small plants | 0 | 34 | 27 | 0 | 0 | 0 |
| Large plants | 0 | 5 | 16 | 0 | 0 | 0 |
| **All sites** | | | | | | |
| Nodes | 27 | 0 | 0 | 0 | 2 | 22 |
| Small plants | 0 | 12 | 6 | 0 | 0 | 0 |
| Large plants | 0 | 6 | 24 | 0 | 0 | 0 |

| Transition matrices fall 08-spring 09 | Ramet stasis & growth | | | Sexual reproduction | | |
|---|---|---|---|---|---|---|
| | Nodes | Small plants | Large plants | Nodes | Small plants | Large plants |
| **Adlerberg** | | | | | | |
| Small plants | 0.79 | 0.56 | 0 | 0 | 0 | 0.01 |
| Large plants | 0.08 | 0.40 | 0.84 | 0 | 0 | 0 |
| **Eichmatt** | | | | | | |
| Small plants | 0.61 | 0.40 | 0.02 | 0 | 0 | 0.01 |
| Large plants | 0.19 | 0.12 | 0.59 | 0 | 0 | 0 |
| **Gempen** | | | | | | |
| Small plants | 0.49 | 0.88 | 0.16 | 0 | 0 | 0.01 |
| Large plants | 0.12 | 0.06 | 0.84 | 0 | 0 | 0 |
| **Grammet** | | | | | | |
| Small plants | 0.26 | 0.58 | 0.19 | 0 | 0 | 0.01 |
| Large plants | 0 | 0.25 | 0.65 | 0 | 0 | 0 |
| **Holzerberg** | | | | | | |
| Small plants | 0.48 | 0.67 | 0.28 | 0 | 0 | 0.01 |
| Large plants | 0.10 | 0.31 | 0.72 | 0 | 0 | 0 |
| **Hochwald** | | | | | | |
| Small plants | 0.23 | 0.44 | 0.08 | 0 | 0 | 0.01 |
| Large plants | 0.28 | 0.44 | 0.88 | 0 | 0 | 0 |
| **Paradies** | | | | | | |
| Small plants | 0.28 | 0 | 0.06 | 0 | 0 | 0.01 |
| Large plants | 0.44 | 1.00 | 0.86 | 0 | 0 | 0 |
| **Riehen** | | | | | | |
| Small plants | 0.47 | 0.63 | 0.18 | 0 | 0 | 0.01 |
| Large plants | 0 | 0.04 | 0.43 | 0 | 0 | 0 |
| **Schauenburg** | | | | | | |
| Small plants | 0.47 | 0 | 0.03 | 0 | 0 | 0.01 |
| Large plants | 0.39 | 1.00 | 0.97 | 0 | 0 | 0 |
| **Schleifenberg** | | | | | | |
| Small plants | 0.40 | 0.56 | 0.50 | 0 | 0 | 0.01 |
| Large plants | 0.07 | 0.03 | 0.19 | 0 | 0 | 0 |
| **Scharten** | | | | | | |
| Small plants | 0.56 | 0.90 | 0.08 | 0 | 0 | 0.01 |
| Large plants | 0.03 | 0 | 0.88 | 0 | 0 | 0 |
| **Ziefen** | | | | | | |
| Small plants | 0.51 | 0.53 | 0.17 | 0 | 0 | 0.01 |
| Large plants | 0 | 0.46 | 0.79 | 0 | 0 | 0 |
| **All sites** | | | | | | |
| Small plants | 0.45 | 0.58 | 0.13 | 0 | 0 | 0.01 |
| Large plants | 0.15 | 0.24 | 0.74 | 0 | 0 | 0 |

| Elasticity matrices fall 08-spring 09 | Ramet stasis & growth | | | Sexual reproduction | | |
|---|---|---|---|---|---|---|
| | Nodes | Small plants | Large plants | Nodes | Small plants | Large plants |
| **Adlerberg** | | | | | | |
| Small plants | 12 | 32 | 0 | 0 | 0 | 0 |
| Large plants | 2 | 33 | 21 | 0 | 0 | 0 |
| **Eichmatt** | | | | | | |
| Small plants | 14 | 6 | 0 | 0 | 0 | 0 |
| Large plants | 18 | 7 | 54 | 0 | 0 | 0 |
| **Gempen** | | | | | | |
| Small plants | 20 | 12 | 4 | 0 | 0 | 0 |
| Large plants | 12 | 2 | 49 | 0 | 0 | 0 |
| **Grammet** | | | | | | |
| Small plants | 23 | 31 | 4 | 0 | 0 | 0 |
| Large plants | 0 | 21 | 21 | 0 | 0 | 0 |
| **Holzerberg** | | | | | | |
| Small plants | 34 | 21 | 4 | 0 | 0 | 0 |
| Large plants | 11 | 14 | 16 | 0 | 0 | 0 |
| **Hochwald** | | | | | | |
| Small plants | 29 | 1 | 1 | 0 | 0 | 0 |
| Large plants | 52 | 2 | 15 | 0 | 0 | 0 |
| **Paradies** | | | | | | |
| Small plants | 27 | 0 | 1 | 0 | 0 | 0 |
| Large plants | 56 | 1 | 15 | 0 | 0 | 0 |
| **Riehen** | | | | | | |
| Small plants | 11 | 60 | 7 | 0 | 0 | 0 |
| Large plants | 0 | 4 | 17 | 0 | 0 | 0 |
| **Schauenburg** | | | | | | |
| Small plants | 25 | 0 | 1 | 0 | 0 | 0 |
| Large plants | 36 | 2 | 36 | 0 | 0 | 0 |
| **Schleifenberg** | | | | | | |
| Small plants | 16 | 35 | 27 | 0 | 0 | 1 |
| Large plants | 4 | 2 | 15 | 0 | 0 | 0 |
| **Scharten** | | | | | | |
| Small plants | 43 | 11 | 3 | 0 | 0 | 0 |
| Large plants | 3 | 0 | 40 | 0 | 0 | 0 |
| **Ziefen** | | | | | | |
| Small plants | 18 | 28 | 3 | 0 | 0 | 0 |
| Large plants | 0 | 33 | 18 | 0 | 0 | 0 |
| **All sites** | | | | | | |
| Small plants | 30 | 10 | 2 | 0 | 0 | 0 |
| Large plants | 21 | 8 | 28 | 0 | 0 | 0 |

| Transition matrices spring 09-summer 09 | Ramet stasis & growth | | Clonal reproduction | |
|---|---|---|---|---|
| | Small plants | Large plants | Small plants | Large plants |
| **Adlerberg** | | | | |
| Nodes | 0 | 0 | 0 | 0.19 |
| Small plants | 0.83 | 0.57 | 0 | 0 |
| Large plants | 0 | 0.24 | 0 | 0 |
| **Eichmatt** | | | | |
| Nodes | 0 | 0 | 0 | 0.04 |
| Small plants | 0.38 | 0.07 | 0 | 0 |
| Large plants | 0 | 0.49 | 0 | 0 |
| **Gempen** | | | | |
| Nodes | 0.00 | 0.00 | 0 | 0.34 |
| Small plants | 0.61 | 0.10 | 0 | 0 |
| Large plants | 0.03 | 0.68 | 0 | 0 |
| **Grammet** | | | | |
| Nodes | 0 | 0 | 0.10 | 0.68 |
| Small plants | 0.37 | 0.06 | 0 | 0 |
| Large plants | 0.14 | 0.35 | 0 | 0 |
| **Holzerberg** | | | | |
| Nodes | 0 | 0 | 0 | 0.44 |
| Small plants | 0.67 | 0.40 | 0 | 0 |
| Large plants | 0.01 | 0.22 | 0 | 0 |
| **Hochwald** | | | | |
| Nodes | 0 | 0 | 0.13 | 0.39 |
| Small plants | 0.39 | 0.30 | 0 | 0 |
| Large plants | 0.07 | 0.46 | 0 | 0 |
| **Paradies** | | | | |
| Nodes | 0 | 0 | 0 | 0.72 |
| Small plants | 0.20 | 0.06 | 0 | 0 |
| Large plants | 0.06 | 0.66 | 0 | 0 |
| **Riehen** | | | | |
| Nodes | 0 | 0 | 0 | 0 |
| Small plants | 0.37 | 0.08 | 0 | 0 |
| Large plants | 0.01 | 0.24 | 0 | 0 |
| **Schauenburg** | | | | |
| Nodes | 0 | 0 | 0.03 | 2.57 |
| Small plants | 0.48 | 0.01 | 0 | 0 |
| Large plants | 0.24 | 0.90 | 0 | 0 |
| **Schleifenberg** | | | | |
| Nodes | 0 | 0 | 0.01 | 0.04 |
| Small plants | 0.31 | 0.11 | 0 | 0 |
| Large plants | 0.03 | 0.08 | 0 | 0 |
| **Scharten** | | | | |
| Nodes | 0 | 0 | 0.03 | 0.79 |
| Small plants | 0.57 | 0.19 | 0 | 0 |
| Large plants | 0.03 | 0.51 | 0 | 0 |
| **Ziefen** | | | | |
| Nodes | 0 | 0 | 0.01 | 0.19 |
| Small plants | 0.71 | 0.14 | 0 | 0 |
| Large plants | 0 | 0.56 | 0 | 0 |
| **All sites** | | | | |
| Nodes | 0 | 0 | 0.02 | 0.58 |
| Small plants | 0.49 | 0.16 | 0 | 0 |
| Large plants | 0.04 | 0.47 | 0 | 0 |

| Elasticity matrices spring 09-summer 09 | Ramet stasis & growth | | Clonal reproduction | |
|---|---|---|---|---|
| | Small plants | Large plants | Small plants | Large plants |
| **Adlerberg** | | | | |
| Nodes | 0 | 0 | 0 | 2 |
| Small plants | 88 | 9 | 0 | 0 |
| Large plants | 0 | 2 | 0 | 0 |
| **Eichmatt** | | | | |
| Nodes | 0 | 0 | 0 | 6 |
| Small plants | 30 | 6 | 0 | 0 |
| Large plants | 0 | 58 | 0 | 0 |
| **Gempen** | | | | |
| Nodes | 0 | 0 | 0 | 12 |
| Small plants | 24 | 5 | 0 | 0 |
| Large plants | 2 | 57 | 0 | 0 |
| **Grammet** | | | | |
| Nodes | 0 | 0 | 12 | 15 |
| Small plants | 33 | 1 | 0 | 0 |
| Large plants | 28 | 12 | 0 | 0 |
| **Holzerberg** | | | | |
| Nodes | 0 | 0 | 0 | 7 |
| Small plants | 75 | 12 | 0 | 0 |
| Large plants | 1 | 5 | 0 | 0 |
| **Hochwald** | | | | |
| Nodes | 0 | 0 | 8 | 10 |
| Small plants | 27 | 8 | 0 | 0 |
| Large plants | 12 | 35 | 0 | 0 |
| **Paradies** | | | | |
| Nodes | 0 | 0 | 0 | 8 |
| Small plants | 4 | 1 | 0 | 0 |
| Large plants | 6 | 81 | 0 | 0 |
| **Riehen** | | | | |
| Nodes | 0 | 0 | 0 | 0 |
| Small plants | 97 | 2 | 0 | 0 |
| Large plants | 1 | 1 | 0 | 0 |
| **Schauenburg** | | | | |
| Nodes | 0 | 0 | 0 | 33 |
| Small plants | 7 | 0 | 0 | 0 |
| Large plants | 12 | 48 | 0 | 0 |
| **Schleifenberg** | | | | |
| Nodes | 0 | 0 | 14 | 3 |
| Small plants | 80 | 1 | 0 | 0 |
| Large plants | 2 | 0 | 0 | 0 |
| **Scharten** | | | | |
| Nodes | 0 | 0 | 2 | 13 |
| Small plants | 62 | 5 | 0 | 0 |
| Large plants | 4 | 14 | 0 | 0 |
| **Ziefen** | | | | |
| Nodes | 0 | 0 | 1 | 11 |
| Small plants | 49 | 6 | 0 | 0 |
| Large plants | 0 | 32 | 0 | 0 |
| **All sites** | | | | |
| Nodes | 0 | 0 | 2 | 19 |
| Small plants | 40 | 5 | 0 | 0 |
| Large plants | 6 | 28 | 0 | 0 |

| Transition matrices summer 09-fall 09 | Ramet stasis & growth | | | Clonal reproduction | | |
|---|---|---|---|---|---|---|
| | Nodes | Small plants | Large plants | Nodes | Small plants | Large plants |
| **Adlerberg** | | | | | | |
| Nodes | 0.50 | 0 | 0 | 0 | 0 | 0 |
| Small plants | 0 | 0.84 | 0.40 | 0 | 0 | 0 |
| Large plants | 0 | 0.05 | 0.16 | 0 | 0 | 0 |
| **Eichmatt** | | | | | | |
| Nodes | 0.64 | 0 | 0 | 0 | 0 | 0.02 |
| Small plants | 0 | 0.35 | 0.21 | 0 | 0 | 0 |
| Large plants | 0 | 0.01 | 0.25 | 0 | 0 | 0 |
| **Gempen** | | | | | | |
| Nodes | 0.71 | 0 | 0 | 0 | 0 | 0.26 |
| Small plants | 0 | 0.57 | 0.05 | 0 | 0 | 0 |
| Large plants | 0 | 0.07 | 0.66 | 0 | 0 | 0 |
| **Grammet** | | | | | | |
| Nodes | 0.65 | 0 | 0 | 0 | 0.03 | 0.53 |
| Small plants | 0 | 0.38 | 0.14 | 0 | 0 | 0 |
| Large plants | 0 | 0.08 | 0.33 | 0 | 0 | 0 |
| **Holzerberg** | | | | | | |
| Nodes | 0.50 | 0 | 0 | 0 | 0.17 | 0.35 |
| Small plants | 0 | 0.61 | 0.20 | 0 | 0 | 0 |
| Large plants | 0 | 0.14 | 0.18 | 0 | 0 | 0 |
| **Hochwald** | | | | | | |
| Nodes | 0.71 | 0 | 0 | 0 | 0.06 | 0.85 |
| Small plants | 0 | 0.27 | 0.08 | 0 | 0 | 0 |
| Large plants | 0 | 0.27 | 0.56 | 0 | 0 | 0 |
| **Paradies** | | | | | | |
| Nodes | 0.30 | 0 | 0 | 0 | 0.02 | 0.12 |
| Small plants | 0 | 0.11 | 0.24 | 0 | 0 | 0 |
| Large plants | 0 | 0.04 | 0.41 | 0 | 0 | 0 |
| **Riehen** | | | | | | |
| Nodes | 0.64 | 0 | 0 | 0 | 0 | 0 |
| Small plants | 0 | 0.35 | 0.05 | 0 | 0 | 0 |
| Large plants | 0 | 0.01 | 0.16 | 0 | 0 | 0 |
| **Schauenburg** | | | | | | |
| Nodes | 0.85 | 0 | 0 | 0 | 0 | 1.73 |
| Small plants | 0 | 0.17 | 0.04 | 0 | 0 | 0 |
| Large plants | 0 | 0.43 | 0.87 | 0 | 0 | 0 |
| **Schleifenberg** | | | | | | |
| Nodes | 0.64 | 0 | 0 | 0 | 0.01 | 0 |
| Small plants | 0 | 0.27 | 0.01 | 0 | 0 | 0 |
| Large plants | 0 | 0.03 | 0.09 | 0 | 0 | 0 |
| **Scharten** | | | | | | |
| Nodes | 0.70 | 0 | 0 | 0 | 0.01 | 0.18 |
| Small plants | 0 | 0.62 | 0.60 | 0 | 0 | 0 |
| Large plants | 0 | 0.01 | 0.01 | 0 | 0 | 0 |
| **Ziefen** | | | | | | |
| Nodes | 0.87 | 0 | 0 | 0 | 0 | 0.13 |
| Small plants | 0 | 0.40 | 0.02 | 0 | 0 | 0 |
| Large plants | 0 | 0.33 | 0.62 | 0 | 0 | 0 |
| **All sites** | | | | | | |
| Nodes | 0.64 | 0 | 0 | 0 | 0.03 | 0.38 |
| Small plants | 0 | 0.44 | 0.15 | 0 | 0 | 0 |
| Large plants | 0 | 0.10 | 0.38 | 0 | 0 | 0 |

| Elasticity matrices summer 09-fall 09 | Ramet stasis & growth | | | Clonal reproduction | | |
|---|---|---|---|---|---|---|
| | Nodes | Small plants | Large plants | Nodes | Small plants | Large plants |
| **Adlerberg** | | | | | | |
| Nodes | 2 | 0 | 0 | 0 | 0 | 0 |
| Small plants | 0 | 94 | 2 | 0 | 0 | 0 |
| Large plants | 0 | 3 | 0 | 0 | 0 | 0 |
| **Eichmatt** | | | | | | |
| Nodes | 6 | 0 | 0 | 0 | 0 | 3 |
| Small plants | 0 | 35 | 26 | 0 | 0 | 0 |
| Large plants | 0 | 1 | 28 | 0 | 0 | 0 |
| **Gempen** | | | | | | |
| Nodes | 12 | 0 | 0 | 0 | 0 | 9 |
| Small plants | 0 | 23 | 2 | 0 | 0 | 0 |
| Large plants | 0 | 5 | 48 | 0 | 0 | 0 |
| **Grammet** | | | | | | |
| Nodes | 27 | 0 | 0 | 0 | 3 | 21 |
| Small plants | 0 | 24 | 4 | 0 | 0 | 0 |
| Large plants | 0 | 7 | 14 | 0 | 0 | 0 |
| **Holzerberg** | | | | | | |
| Nodes | 7 | 0 | 0 | 0 | 15 | 3 |
| Small plants | 0 | 62 | 2 | 0 | 0 | 0 |
| Large plants | 0 | 10 | 1 | 0 | 0 | 0 |
| **Hochwald** | | | | | | |
| Nodes | 18 | 0 | 0 | 0 | 2 | 18 |
| Small plants | 0 | 7 | 1 | 0 | 0 | 0 |
| Large plants | 0 | 26 | 27 | 0 | 0 | 0 |
| **Paradies** | | | | | | |
| Nodes | 8 | 0 | 0 | 0 | 0 | 3 |
| Small plants | 0 | 2 | 15 | 0 | 0 | 0 |
| Large plants | 0 | 2 | 68 | 0 | 0 | 0 |
| **Riehen** | | | | | | |
| Nodes | 0 | 0 | 0 | 0 | 0 | 0 |
| Small plants | 0 | 97 | 1 | 0 | 0 | 0 |
| Large plants | 0 | 1 | 1 | 0 | 0 | 0 |
| **Schauenburg** | | | | | | |
| Nodes | 33 | 0 | 0 | 0 | 0 | 29 |
| Small plants | 0 | 1 | 0 | 0 | 0 | 0 |
| Large plants | 0 | 6 | 30 | 0 | 0 | 0 |
| **Schleifenberg** | | | | | | |
| Nodes | 17 | 0 | 0 | 0 | 7 | 0 |
| Small plants | 0 | 70 | 0 | 0 | 0 | 0 |
| Large plants | 0 | 4 | 1 | 0 | 0 | 0 |
| **Scharten** | | | | | | |
| Nodes | 15 | 0 | 0 | 0 | 1 | 3 |
| Small plants | 0 | 66 | 15 | 0 | 0 | 0 |
| Large plants | 0 | 0 | 0 | 0 | 0 | 0 |
| **Ziefen** | | | | | | |
| Nodes | 12 | 0 | 0 | 0 | 0 | 5 |
| Small plants | 0 | 24 | 0 | 0 | 0 | 0 |
| Large plants | 0 | 32 | 27 | 0 | 0 | 0 |
| **All sites** | | | | | | |
| Nodes | 21 | 0 | 0 | 0 | 2 | 11 |
| Small plants | 0 | 30 | 4 | 0 | 0 | 0 |
| Large plants | 0 | 12 | 19 | 0 | 0 | 0 |

| Transition matrices fall 09-spring 10 | Ramet stasis & growth | | | Sexual reproduction | | |
|---|---|---|---|---|---|---|
| | Nodes | Small plants | Large plants | Nodes | Small plants | Large plants |
| **Adlerberg** | | | | | | |
| Small plants | 0.86 | 0.78 | 0.10 | 0 | 0 | 0.01 |
| Large plants | 0 | 0.09 | 0.35 | 0 | 0 | 0 |
| **Eichmatt** | | | | | | |
| Small plants | 0.53 | 0.22 | 0.01 | 0 | 0 | 0.01 |
| Large plants | 0.08 | 0.20 | 0.28 | 0 | 0 | 0 |
| **Gempen** | | | | | | |
| Small plants | 0.47 | 0.40 | 0.06 | 0 | 0 | 0.01 |
| Large plants | 0.12 | 0.24 | 0.62 | 0 | 0 | 0 |
| **Grammet** | | | | | | |
| Small plants | 0.61 | 0.45 | 0.15 | 0 | 0 | 0.01 |
| Large plants | 0.03 | 0.04 | 0.27 | 0 | 0 | 0 |
| **Holzerberg** | | | | | | |
| Small plants | 0.50 | 0.57 | 0.09 | 0 | 0 | 0.01 |
| Large plants | 0.07 | 0.10 | 0.36 | 0 | 0 | 0 |
| **Hochwald** | | | | | | |
| Small plants | 0.60 | 0.38 | 0.13 | 0 | 0 | 0.01 |
| Large plants | 0.03 | 0 | 0.50 | 0 | 0 | 0 |
| **Paradies** | | | | | | |
| Small plants | 0.27 | 0.18 | 0.07 | 0 | 0 | 0.01 |
| Large plants | 0.04 | 0.15 | 0.43 | 0 | 0 | 0 |
| **Riehen** | | | | | | |
| Small plants | 0.53 | 0.31 | 0.03 | 0 | 0 | 0.01 |
| Large plants | 0.08 | 0.01 | 0.11 | 0 | 0 | 0 |
| **Schauenburg** | | | | | | |
| Small plants | 0.53 | 0.18 | 0.03 | 0 | 0 | 0.01 |
| Large plants | 0.30 | 0.24 | 0.88 | 0 | 0 | 0 |
| **Schleifenberg** | | | | | | |
| Small plants | 0.53 | 0.24 | 0.08 | 0 | 0 | 0.01 |
| Large plants | 0.08 | 0 | 0.04 | 0 | 0 | 0 |
| **Scharten** | | | | | | |
| Small plants | 0.49 | 0.56 | 0 | 0 | 0 | 0.01 |
| Large plants | 0.03 | 0.15 | 0.06 | 0 | 0 | 0 |
| **Ziefen** | | | | | | |
| Small plants | 0.85 | 0.56 | 0.19 | 0 | 0 | 0.01 |
| Large plants | 0 | 0.05 | 0.55 | 0 | 0 | 0 |
| **All sites** | | | | | | |
| Small plants | 0.53 | 0.42 | 0.08 | 0 | 0 | 0.01 |
| Large plants | 0.08 | 0.10 | 0.40 | 0 | 0 | 0 |

| Elasticity matrices fall 09-spring 10 | Ramet stasis & growth | | | Sexual reproduction | | |
|---|---|---|---|---|---|---|
| | Nodes | Small plants | Large plants | Nodes | Small plants | Large plants |
| **Adlerberg** | | | | | | |
| Small plants | 2 | 85 | 1 | 0 | 0 | 0 |
| Large plants | 0 | 10 | 2 | 0 | 0 | 0 |
| **Eichmatt** | | | | | | |
| Small plants | 7 | 21 | 1 | 0 | 0 | 0 |
| Large plants | 2 | 40 | 28 | 0 | 0 | 0 |
| **Gempen** | | | | | | |
| Small plants | 13 | 11 | 2 | 0 | 0 | 0 |
| Large plants | 8 | 15 | 51 | 0 | 0 | 0 |
| **Grammet** | | | | | | |
| Small plants | 45 | 23 | 4 | 0 | 0 | 0 |
| Large plants | 5 | 5 | 17 | 0 | 0 | 0 |
| **Holzerberg** | | | | | | |
| Small plants | 21 | 53 | 2 | 0 | 0 | 0 |
| Large plants | 3 | 11 | 9 | 0 | 0 | 0 |
| **Hochwald** | | | | | | |
| Small plants | 34 | 8 | 5 | 0 | 0 | 0 |
| Large plants | 4 | 0 | 49 | 0 | 0 | 0 |
| **Paradies** | | | | | | |
| Small plants | 5 | 2 | 1 | 0 | 0 | 0 |
| Large plants | 6 | 15 | 69 | 0 | 0 | 0 |
| **Riehen** | | | | | | |
| Small plants | 0 | 96 | 1 | 0 | 0 | 0 |
| Large plants | 0 | 2 | 1 | 0 | 0 | 0 |
| **Schauenburg** | | | | | | |
| Small plants | 19 | 0 | 0 | 0 | 0 | 0 |
| Large plants | 43 | 1 | 36 | 0 | 0 | 0 |
| **Schleifenberg** | | | | | | |
| Small plants | 21 | 71 | 3 | 0 | 0 | 0 |
| Large plants | 3 | 0 | 2 | 0 | 0 | 0 |
| **Scharten** | | | | | | |
| Small plants | 17 | 51 | 0 | 0 | 0 | 0 |
| Large plants | 2 | 29 | 0 | 0 | 0 | 0 |
| **Ziefen** | | | | | | |
| Small plants | 17 | 21 | 11 | 0 | 0 | 1 |
| Large plants | 0 | 3 | 47 | 0 | 0 | 0 |
| **All sites** | | | | | | |
| Small plants | 24 | 21 | 2 | 0 | 0 | 0 |
| Large plants | 10 | 13 | 29 | 0 | 0 | 0 |

# Chapter IV

## Reduced clonal reproduction indicates low potential for establishment of hybrids between wild and cultivated strawberries (*Fragaria vesca* x *F.* x *ananassa*)

Juerg Schulze, Andreas Erhardt and Peter Stoll

(to be submitted)

# Reduced clonal reproduction indicates low potential for establishment of hybrids between wild and cultivated strawberries (*Fragaria vesca* x *F.* x *ananassa*)

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**Abstract**

The genus *Fragaria* (Rosaceae) contains 24 plant species, including hybrid species such as the widely cultivated garden strawberry (*Fragaria* x *ananassa* Duch.). Natural hybridization between *Fragaria* species has repeatedly been reported, and studies on the hybridization potential between *F.* x *ananassa* and its wild relatives have become increasingly important with the outlook for future genetically modified strawberry cultivars. In Europe, the most likely candidate species for hybridization with *F.* x *ananassa* is the common diploid woodland strawberry (*F. vesca* L.). Although a previous field survey indicated that the potential for *F. vesca* x *F.* x *ananassa* hybrid formation and establishment is low, it is not clear whether the lack of natural hybrids is due to the known pre- and postzygotic barriers, or whether adult hybrid plants lack the fitness to establish and persist in natural *F. vesca* populations.

We grew different *F. vesca* and hybrid clones with and without competition in a greenhouse experiment to assess differences in growth parameters, i.e. biomass production, clonal reproduction and sexual reproduction of plants. While some hybrid clones exceeded *F. vesca* in biomass production, general clonal reproduction was considerably lower and delayed in hybrid clones and variability in clonal reproduction among hybrid clones was large. Furthermore, all hybrid plants were sterile.

We conclude that hybrid plants have a competitive disadvantage against co-occurring *F. vesca* plants due to inferior clonal reproduction and that the potential for hybrid establishment under natural conditions is low.

Keywords: Hybrid fitness, competition, hybridization, genetically modified plants

**Introduction**

The octoploid garden strawberry (*Fragaria* x *ananassa* Duch.), a worldwide grown fruit crop, belongs to the genus *Fragaria* (Rosaceae) that contains 24 herbaceous species, including well defined hybrids with various ploidy levels ranging from di- to octoploid (Staudt 2009). To date, numerous experimental crosses between different *Fragaria* species have been made to investigate the genetic compatibility of species and their phylogenetic relationship or to introduce novel traits into cultivars (Evans 1974; Mangelsdorf and East 1927; Marta et al. 2004; Noguchi et al. 2002; Olbricht et al. 2006; Schulze et al. 2011; Stegmeir et al. 2010; Yarnell 1931a; Yarnell 1931b). Generally, species with similar ploidy levels can be crossed successfully, whereas hybrids between species with different ploidy levels show high mortality and are highly sterile, but vigorous hybrids are possible. Natural hybridization between *Fragaria* species of similar ploidy levels has repeatedly been reported (Staudt 1989; Staudt *et al*. 2003; Westman *et al*. 2004). Furthermore, stable hybrid populations between the octoploid Chilean strawberry (*F. chiloensis* Mill.) and the diploid woodland strawberry (*F. vesca* L.) have been described (Bringhurst and Khan 1963; Bringhurst and Senanayake 1966). With prospects of genetically modified (GM) strawberry cultivars in the near future (Qin *et al*. 2008) studies on the hybridization potential between *F.* x *ananassa* and wild *Fragaria* species have become increasingly important. Two field surveys that addressed the potential of natural hybridization between *F.* x *ananassa* and wild relatives have been carried out to date. Westman et al. (2004) found substantial gene flow between octoploid *F.* x *ananassa* and one of its wild octoploid parental species, the Virginia strawberry (*F. virginiana* Mill.), in the southeastern USA. Schulze et al. (2011) found no indications for gene flow between octoploid *F.* x *ananassa* and the diploid *F. vesca* in Central Europe. The reasons for the absence of *F. vesca* x *F.* x *ananassa* hybrids in the field, as reported in the latter study, are not fully understood. There is limited genetic compatibility between *F. vesca* and *F.* x *ananassa*, and germination rates of hybrid seeds are low (Evans 1974; Marta et al. 2004). Moreover, *F. vesca* is self-fertile and a large portion of seeds may be selfed (Arulsekar and Bringhurst 1981). However, experimental hand-crosses between *F. vesca* and *F.* x *ananassa* can yield viable and very vigorous hybrids (Olbricht et al. 2006; Schulze et al. 2011). Furthermore, *F. vesca* and *F.* x *ananassa* share major pollinators, such as solitary bees, that do not discriminate between

flowers of the two species, and their flowering times overlap (submitted results). Pollen flow between *F.* x *ananassa* and *F. vesca* is therefore likely in areas where they grow in close vicinity. Regarding these findings it is however unclear whether the major obstacles for an establishment of natural *F. vesca* x *F.* x. *ananassa* hybrids are pre- and post-zygotic barriers (Evans 1974; Marta et al. 2004) or whether later developmental stages of hybrids are not fit enough to compete with co-occurring plants.

The goal of the present study was to assess differences in growth parameters between *F. vesca* x *F.* x *ananassa* hybrids and *F. vesca* plants which may affect plant fitness and thus could further explain the absence of *F. vesca* x *F.* x *ananassa* hybrids in the field (Schulze et al. 2011). We grew *F. vesca* and hybrid plants with and without competition in a greenhouse experiment and compared above-ground biomass, clonal reproduction and sexual reproduction of plants.

**Material & Methods**

Experimental setup:

To assess growth and reproduction of *F. vesca* x *F.* x. *ananassa* hybrids and *F. vesca* plants in the presence and absence of competition, plants were grown in flower boxes under the following conditions:

*Competition treatment*

- Either a *F. vesca* x *F.* x. *ananassa* hybrid or a *F. vesca* plant was planted centrally between two established flanking *F. vesca* plants (Fig. 1)

(Central *F. vesca* plants: Three different clones, each replicated three times (9 boxes)
Central hybrid plants: Two different hybrid groups, each with three different hybrid clones. Each clone was replicated three times (18 boxes)
Flanking *F. vesca* plants: Two different clones per box, with identical clones in all boxes)

*Control treatment*

- Either a *F. vesca* x *F.* x. *ananassa* hybrid or a *F. vesca* plant was planted centrally without flanking plants (Fig. 1)

(Central plants as described for the competition treatment (9 + 18 boxes))

- Two flanking *F. vesca* plants were grown alone without a central plant (Fig. 1)

(Flanking plants as described for the competition treatment (9 boxes))

Experimental workflow:

A loamy forest soil from a site in Riehen, Switzerland, was sieved through a 10 mm sieve and mixed with quartz sand (3:2). 3.2 kg of this substrate were added to 63 rectangular flower boxes (L x W x H: 36 x 14.5 x 12.5 cm) that were placed on individual saucers. On November 6$^{th}$, 2008, we selected runner plantlets of similar size of two different *F. vesca* genotypes. One runner plant of each *F. vesca* genotype was planted in the opposing ends of experimental flower boxes, 6 cm distant of the ends. Prior to planting roots of runners were washed with water to remove soil and were cut back to 6 cm length and similar density. The number of runner plant leaves was reduced to two if more were present. Altogether, we planted 72 runner plants in 36 experimental boxes. Twenty-seven boxes remained without plants for later control treatments. Boxes were arranged in three blocks on movable tables, each block containing 21 boxes distributed on 6 tables. Positions of tables within a block were changed weekly in a regular rotation. In April, 2009, some individuals began to form inflorescences and stolons. We regularly cut off developing inflorescences and stolons to promote an even resource allocation to vegetative biomass in the flanking plants. Plants were cut back to 2-3 leaves in March and June, 2009, to promote development of even sized plants within and among experimental boxes. On July 24$^{th}$, 2009, either a *F. vesca* x *F.* x *ananassa* hybrid or a *F. vesca* plant was planted in the center of 27 boxes that contained flanking plants (competition treatment) and in 27 empty boxes (control treatment) (Fig. 1). One central *F. vesca* plantlet died off after transplantation and was replaced 11 d after planting. After transplanting of central plants all plants were allowed to grow stolons and runner plants. For every plant, two square flower pots (L x W x H: 11 x 11 x 12 cm) filled with 480 ± 5 g Attapulgite substrate (Oil Dri US special type II R; Damolin, Denmark) were placed besides the experimental boxes. If a plant formed runner plants, these were fixed in the flower pots with plastic hooks as soon as root tips became visible. We allowed for five runner plants per pot. Pots were filled up with plants successively. If a plant had reached the maximal number of 10 runner plants, newly formed stolons were regularly cut off. A limit for runner plants had to be set due to space limitations. Runner plants produced by flanking and central plants were counted on September 30$^{th}$ and November 30$^{th}$, 2009. Growth of plants and runner plant production stagnated towards the end of November, 2009, and most leaves started to wither. All runner plants grown in flower pots were

removed on December 3rd, 2009. In spring 2010, pots with fresh substrate were placed besides experimental boxes and newly formed runner plants of central and flanking plants were treated as in the previous year. Between March 17th and September 29th, 2010, the number of ripe fruits and the runner plantlets produced by plants were counted in biweekly intervals. On September 29th and 30th, above-ground biomass of flanking and central plants was harvested and biomass was dried and weighed. Above-ground biomass of runner plants including interconnecting stolons was harvested separately. Root biomass was not harvested as a separation of the intermingled roots of different individuals was not feasible for the competition treatment.

Throughout the experiment old withered leaves of plants were collected, dried at 80°C for 48 h and weighed. Withered inflorescences and fruits of plants were collected, dried and weighed separately and recorded as sexual reproductive biomass.

Plant material:

As flanking plants we used two different *F. vesca* genotypes collected at forest sites in Riehen and Dornach, Switzerland. Runner plants of these *F. vesca* genotypes were propagated on garden soil. As central plants we used *F. vesca* x *F.* x. *ananassa* hybrids and *F. vesca* plants. Two groups of 24 and 19 hybrid plants stood at our disposal. These hybrid groups originated from hand-crosses between *F. vesca* and *F.* x *ananassa* cv. Calypso and *F. vesca* and *F.* x *ananassa* AN93.231.53, respectively (provided by B. Mezzetti, Marche Polytechnic University, Italy). *Fragaria* x *ananassa* cv. Calypso is a day-neutral variety whereas *F.* x *ananassa* AN93.231.53 is a short-day type, i.e. it initiates flower buds either under short-day conditions or when temperatures are less than 15°C (Hancock 1999). Hybrid breeding and identification methods have been described elsewhere (Schulze et al. 2011). We selected three hybrids of type *F. vesca* x *F.* x *ananassa* cv. Calypso (hereafter called hybrid group 1) and three hybrids of type *F. vesca* x *F.* x *ananassa* AN93.231.53 (hereafter called hybrid group 2). In both hybrid groups there was large phenotypic variation with differences in size, leaf morphology, leaf colour and clonal reproduction. Most hybrids formed inflorescences but none of them produced any well-developed fruits or fertile achenes. The hybrid clones selected for the experiment were amongst the most vigorous within their group and all of them reproduced clonally. Ploidy leves of

hybrid plants were estimated by flow cytometry (see below). Three different *F. vesca* clones were chosen as controls for the competition and the control treatment. These *F. vesca* clones were different from the *F. vesca* clones used as flanking plants in the competition treatment.

Watering, pesticide application and fertilization:

We did not want plants to have unlimited availability of water so that a minimal level of competition for water was reached. However, we were careful that plants did not experience severe drought stress and wilting. Boxes were watered all at the same time and water was always added to saucers. On each watering occasion, the competition treatment boxes and the boxes that contained only flanking plants received 250 ml tap water. Control treatment boxes received 150 ml tap water. Boxes had to be watered 2-4 times a week during summer and once every week or every other week in winter, depending on weather conditions. Flower pots with runners were not watered at the same time as they contained different numbers of plants. Pots were watered with 100 ml tap water on top of the substrate whenever the substrate surface became dry.

Plants were sprayed repeatedly with Vertimec (Syngenta Agro), Acarac (Syngenta Agro) and Spomill (Syngenta Agro) against infections of spider mites (Tetranychidae), and with Evisect (Syngenta Agro), Actara (Syngenta Agro), Plenum (Syngenta Agro) and Marshall (Syngenta Agro) against white flies (*Trialeurodes vaporariorum*) throughout the experiment. In both years some plants showed symptoms of a fungal infestation and were sprayed with Systhane C (Omya, Switzerland) and Topas vino (Syngenta Agro). On March 10$^{th}$, 2010, all boxes were fertilized with 150 ml of 1/4 strength Hoagland solution.

Flow cytometry:

DNA-contents of cell nuclei of the six hybrid clones, their *F. vesca* mother lines and *F.* x *ananassa* cv. Calypso were estimated by flow cytometry. Fresh young leaves of sampled plants were chopped together with leaves of *F.* x *ananassa* cv. Calypso as internal standard with a sharp razor blade in a Petri dish containing 0.8 ml nuclei isolation buffer (Galbraith et al. 1983) supplemented with 1% Polyvinylpyrrolidone K90. After 2 min. of incubation the solution was filtrated through a 50 µm CellTrics filter (Partec) and 1.6 ml of 4´,6-diamidino-2-phenylindole (DAPI) staining solution

(Cystain UV Precise P, Partec) was added. After 2 min. of staining fluorescence intensities of nuclei were measured with a CyFlow Ploidy Analyzer (Partec) equipped with a UV-LED of 365 nm emission wavelength. All measurements of reference samples were repeated three times.

The *F. vesca* mother line of hybrid clone 1.2 died before plants were sampled and could not be analysed.

Data analysis:

All analyses were done in R (R Development Core Team 2009).

Generalized linear models (GLM), analysis of variance (ANOVA) models and analysis of covariance (ANCOVA) models presented below were stepwise reduced as recommended by Crawley (Crawley 2007), i.e. nonsignificant interactions and variables were stepwise excluded from the original maximal models. Treatment contrasts were specified manually for all GLMs, ANOVAs and ANCOVAs. Hybrid plant groups or hybrid clones were always compared to *F. vesca* plants; hybrid groups or hybrid clones were not compared among themselves.

*Above-ground biomass, control treatment*

We did an ANOVA on final total above-ground biomass of the three plant groups, i.e. *F.vesca* plants, hybrid group 1 and hybrid group 2, with plant group and block as independent categorical variables. Total above-ground biomass was defined as the sum of vegetative biomass, biomass of inflorescences and fruits and the biomass of stolons and runner plants of each plant. Furthermore, we analysed the total above-ground biomass of *F. vesca* and the six different hybrid clones with an ANOVA, with plant type and block as independent categorical variables. The three *F. vesca* clones were treated as one group as there were no biomass differences between them according to ANOVA.

*Above-ground biomass, competition teatment*

We compared final above-ground biomass of the three plant groups, i.e. *F. vesca* group, hybrid group 1 and hybrid group 2, with an ANCOVA, with plant group and block as independent categorical variables and final above-ground biomass of the two flanking plants as independent continuous variable. Final above-ground biomass of *F.*

*vesca* and hybrid groups was logarithmically transformed to meet the assumption of normal distribution of errors. Furthermore, we compared the biomass of *F. vesca* plants with the biomass of the six different hybrid clones with an ANCOVA, with plant type and block as independent categorical variables and final above-ground biomass of the two flanking plants as independent continuous variable. The three *F. vesca* clones were treated as one group as there were no biomass differences between them according to ANCOVA.

*Number and biomass of runner plants*

Due to space limitations we had to set a limit to runner production of plants, i.e. each plant could produce up to 10 runners. Therefore, data were analysed as proportional data, i.e. proportions of the maximal possible number of runners realized. For many dates the variance of the numbers of runners produced for either *F. vesca* or the different hybrid groups or hybrid clones was 0. For the different dates, we compared the proportions of runners produced by *F. vesca* and hybrid groups and clones with post hoc pairwise comparisons of chi-square tests for equality of proportions (pairwise.prop.test in R). To correct for multiple comparisons the Bonferroni correction was used. Furthermore, we analysed the proportion of total above-ground biomass of central plants allocated to clonal reproduction, i.e. biomass of stolons and runner plants, with a GLM with logit transformed proportions. For the control treatment we used experimental block and either plant group or plant type as independent categorical variables. For the competition treatment biomass of flanking plants was added as an independent continuous variable. Three of the six hybrid clones did not produce any runners in the competition treatment. They could not be analysed with a GLM, as the variance of their runner numbers was 0. These clones were excluded from the analysis. Therefore, analysis with variable plant group was not carried out and we only calculated a GLM with variable plant type for the competition treatment.

GLMs of proportions of biomass allocated to clonal reproduction were overdispersed. Thus the error distribution was set to quasibinomial and dispersion parameters were estimated.

*Relative Interaction Index*

We calculated the Relative Interaction Index (RII) (Armas et al. 2004) for the total biomass of central plants. The RII is a measure for the relative interaction intensity in plants and has defined limits (-1, +1) with negative values indicating competition and positive values indicating facilitation between plants. RII was calculated as: $RII = (B_W - B_O)/(B_W + B_O)$, where $B_W$ is biomass produced under competition and $B_O$ is biomass produced in the control treatment.

For comparison of RII values ANOVA was not an appropriate method as there was non-constant variance and non-normality of errors. Therefore, we only show RII values to illustrate the effects of competition on biomass production of *F. vesca* and hybrid plants.

*Above-ground biomass of flanking plants*

We analysed the effect of central plants on the total biomass of the two flanking *F. vesca* plants with an ANCOVA. We carried out an ANCOVA with plant group and experimental block as independent categorical variables and the total biomass of the central plant as independent continuous variable. We then repeated the same analysis on the level of hybrid clones with the variable plant group replaced by variable plant type. Included in these analyses were the data of the control treatment of flanking plants grown without central plants. Above-ground biomass of flanking *F. vesca* plants was logarithmically transformed to meet the assumption of normal distribution of errors.

**Results**

DNA-contents of cell nuclei of all hybrid clones were similar and equaled rather precisely the mean of the cell nuclei DNA-contents of the *F. vesca* mother lines and *F.* x *ananassa* cv. Calypso plants (Table 1). This result in combination with the high sterility of all hybrid clones (see below), which is typical for odd-ploid plants, suggests that our hybrid clones all belong to a group of pentaploid hybrids. Biomasses of the three *F. vesca* clones used as central plants did not differ in either the control treatment ($F_{2,6} = 1.73$, $p = 0.26$) or the competition treatment ($F_{2,6} = 0.17$, $p = 0.85$).

Therefore, *F. vesca* plants were treated as one group in all comparisons with hybrid clones.

Above-ground biomass, control treatment:

No differences in biomass were found between the *F. vesca* group and hybrid groups 1 and 2. However, analysis of hybrid clones showed that hybrid clone 1.2 had significantly more biomass than *F. vesca* plants ($t_{10} = 3.15$, $p = 0.005$; Fig. 2). Experimental block did not influence biomass in the control treatment.

Above-ground biomass, competition treatment:

Plant group and biomass of flanking plants were not significant variables. Experimental block had a marginally significant effect on biomass in the ANCOVA of plant groups. However, in the ANCOVA of *F. vesca* plants and hybrid clones the plant type significantly influenced biomass (Table 2A). Hybrid 1.2 ($t_8 = 2.55$, $p = 0.020$) and hybrid 2.3 ($t_8 = 6.68$, $p < 0.001$) were significantly larger than *F. vesca* plants, whereas hybrid 1.1 ($t_8 = 2.92$, $p = 0.009$) and hybrid 1.3 ($t_8 = 4.00$, $p < 0.001$) were significantly smaller (Fig. 2). Furthermore, experimental block was a significant variable (Table 2A).

Production of runner plants:

There were large differences in runner production between *F. vesca* and hybrid plants.

In 2009, only plants from the control treatment formed runner plants (Fig. 3A,B). Mean runner production of hybrid groups and hybrid clones was similar and significantly lower than runner production of *F. vesca* plants (Fig. 3A,B; Table S1). At the end of the season, nearly all *F. vesca* plants achieved the maximum value of 10 runner plants (mean number of runners = 9.9), whereas the mean number of runners per hybrid clone never exceeded 1.

In 2010, first *F. vesca* runner plants were observed on May 14$^{th}$ in both treatments. In the control treatment, hybrid groups 1 and 2 produced significantly less runners than *F. vesca* plants after May 14$^{th}$ (Fig. 3C,D). However, analysis on the hybrid clone level showed that runner numbers of hybrids 1.2, 2.1 and 2.2 were not different from *F. vesca* towards the end of the experiment. Yet, these nonsignificant differences are caused by the fact that all *F. vesca* plants reached the upper limit set

for runner production already early during the experimental phase (Fig. 3C,D). Due to this limit it was not possible to find the plateau for runner production of *F. vesca* plants in the control treatment, and the more prolific hybrid clones reached similar runner numbers as *F. vesca*. Final mean runner plant numbers of the hybrid clones that did not differ from *F. vesca* at the end of the experiment were 10 (hybrid 1.2), 9 (hybrid 2.1) and 10 (hybrid 2.2). Mean runner plant numbers of the other hybrid clones were 6.7 (hybrid 1.1), 5.3 (hybrid 1.3) and 3.7 (hybrid 2.3). Runner production of hybrid clones that did not achieve the maximum runner number seemed to reach a plateau towards the end of the experiment (Fig. 3D). Furthermore, runner production of hybrid plants started 6 weeks later than *F. vesca* plants and lagged behind.
In the competition treatment, runner production of hybrid groups and hybrid clones was always significantly lower compared to *F. vesca* plants after May 14$^{th}$ and May 28$^{th}$, respectively (Fig. 3E,F; Table S1). Mean number of runners of hybrid clones never exceeded 1 and three hybrid clones did not form any runners at all. The mean number of runners of *F. vesca* was 8.9. Under competition, runner production of *F. vesca* and hybrid plants seemed to reach a plateau towards the end of the experiment without achieving the maximum possible number of runners. Furthermore, runner production of hybrid plants started 14 weeks after *F. vesca* and lagged behind.

The proportion of biomass allocated to clonal reproduction was significantly influenced by plant group and by plant type in the competition as well as in the control treatment (Table 2C). In the control treatment, hybrid group 1 ($t_{14} = -3.30$, $p = 0.003$) and hybrid group 2 ($t_{14} = -2.87$, $p = 0.009$) allocated significantly less biomass to clonal reproduction compared to the *F. vesca* group. On the hybrid clone level hybrid 1.1 ($t_8 = -2.62$, $p = 0.017$), hybrid 1.3 ($t_8 = -3.10$, $p = 0.006$) and hybrid 2.3 ($t_8 = -3.50$, $p = 0.003$) had a significantly lower biomass allocation to clonal reproduction. Mean proportions of total biomass allocated to clonal reproduction were between 24% - 54% in hybrid clones and 70% in *F. vesca* plants. Here too, proportions of biomass allocation at final harvest are biased due to the upper limit set for runner production. In the competition treatment, all three hybrid clones that actually reproduced clonally allocated significantly less biomass to clonal reproduction compared to the *F. vesca* plants, i.e. hybrid 1.2 ($t_7 = -3.37$, $p = 0.005$), hybrid 2.1 ($t_7 = -3.36$, $p = 0.005$) and hybrid 2.3 ($t_7 = -3.36$, $p = 0.005$). Mean

proportions of total biomass allocated to clonal reproduction were between 0% - 15% in hybrid clones and 71% in *F. vesca* plants.

Relative Interaction Index:

The presence of flanking plants had a strong negative effect on final above-ground biomass of central plants (Fig. 4). The distribution of RII values reflected the results of the ANCOVA for above-ground biomass with the hybrid clone values being similar to the *F. vesca* value and distributed around it.

Sexual reproduction:

*Fragaria vesca* plants produced a mean of 9.8 ± 1.7 SE fruits in the control treatment. Only one *F. vesca* plant formed a small inflorescence with three small fruits in the competition treatment and mean *F. vesca* fruit production was 0.3 ± 0.3 SE. Most hybrid plants formed inflorescences in the control treatment (Fig. 2), but we did not observe any developed achenes. Fruits remained rudimentary and showed at best a red and fleshy fruit part at the fruit basis. Hybrid plants did not form any inflorescences in the competition treatment.

Above-ground biomass of flanking plants:

Increasing biomass of the central plant had a significant negative effect on the biomass of flanking plants, and also experimental block was a significant variable (Table 2B). Neither variable plant group nor variable plant type were significant.

**Discussion**

We selected similar hybrid clones out of the best growing hybrid plants that stood at our disposal. All of them had vigorous vegetative growth and reproduced clonally. Even within this relatively small subset of hybrids we found remarkable variability of growth parameters within hybrid groups and among hybrid clones. Therefore, generalizations on growth characteristics and fitness of *F. vesca* x *F.* x. *ananassa* hybrids should be made with care, and analysis of hybrid plants on the clone level is clearly more informative than analysis on the group level. In the following, we will therefore mainly discuss results of analyses carried out on the hybrid clone level.

Mean total biomasses of hybrid clones were distributed around the mean biomass of *F. vesca* plants in both, the control and the competition treatment, and the relative ranks in total biomass production were in general the same in both treatments. Moreover, RII values were similar for *F. vesca* plants and hybrid clones. Therefore, we conclude that competition has a similar influence on total biomass production of *F. vesca* plants and hybrid clones.

While total biomass production was relatively similar among *F. vesca* plants and many hybrid clones in both treatments, there were stronger differences in the proportion of total biomass that was allocated to clonal reproduction. In the control treatment, data were somewhat biased due to the upper limit set for runner production which made it impossible to estimate the level of clonal reproduction that could be reached by *F. vesca* plants without restrictions. However, in the competition treatment plants did not reach the limit for runner production and biomass allocation to clonal reproduction was not biased. Under competition, all hybrids allocated less biomass to clonal reproduction with three hybrid clones not producing any clonal offspring at all. Furthermore, *F. vesca* plants produced far more runner plants than hybrid clones in the control treatment in 2009 and in the competition treatment in 2010. *Fragaria vesca* runner production in the control treatment in 2010 was significantly higher and earlier than runner production of all hybrid clones until the maximum possible number of runner plants was reached. Thereafter, some hybrid clones also approached or reached the maximum number of runner plants.

A striking difference between *F. vesca* plants and hybrids was the different timing of runner production. Clonal reproduction of hybrid plants lagged at least six weeks behind in the control treatment and even 14 weeks behind in the competition treatment in 2010. These results suggest that there is either a differential environmental trigger for clonal reproduction of *F. vesca* plants and hybrids or a differential biomass threshold that has to be reached before clonal reproduction starts or a combination of both factors. The low number or total absence of runners in hybrid plants and the increased delay in runner production under competition indicate that biomass plays an important role in clonal reproduction of hybrid plants.

Although hybrid plants formed inflorescences, sexual reproduction was not successful. Such high sterility was expected, as it is typical for odd-ploid *Fragaria*

hybrids (Bringhurst and Khan 1963; Folta and Davis 2006; Mangelsdorf and East 1927; Noguchi et al. 2002).

The presence of central plants decreased biomass production of flanking plants, however, there was no differential effect of *F. vesca* or hybrid plants.

In summary, *F. vesca* x *F.* x *ananassa* hybrids can exceed *F. vesca* plants in total biomass production, but biomass allocation to clonal reproduction and the number of runners produced is lower in hybrids. Furthermore, hybrid plants were sterile. How do these differences in growth parameters between hybrid clones and *F. vesca* plants affect plant fitness, and how representative are these results for *F. vesca* x *F.* x *ananassa* hybrids in general? Although hybrid plants did not reproduce sexually, high sterility is not necessarily a disadvantage for local competition. Bringhurst and Khan (1963) described wild occurrences of highly sterile pentaploid *F. chiloensis* x *F. vesca* hybrids that competed well with their co-occurring parental species due to superior stolon productivity. Furthermore, the results of demographic studies on *F. vesca* indicate that sexual reproduction seems to be insignificant for population growth within established *F. vesca* populations. *Fragaria vesca* seedlings were totally absent from study plots in a study from the USA (Angevine 1983). In an own demographic study, we also found only very few seedlings in established *F. vesca* populations in Switzerland (submitted results). In contrast to sexual reproduction, clonal reproduction seems to be more important for plant competitiveness. Our demographic study on twelve *F. vesca* populations showed, that population growth or maintenance is highly dependent on clonal reproduction of plants (submitted results). Furthermore, clonal reproduction results in a fast spread into unoccupied patches, which is crucial for persistence of genets, as *F. vesca* plants can be quickly overgrown by competitors if plant succession is not disturbed (submitted results). A 'dominance of clonal replication as a means of population maintenance' for *F. vesca* and *F. virginiana* has also been reported by Angevine (1983). Thus, clonal reproduction seems to be the dominant pathway for population maintenance and growth in *F. vesca*. As hybrid formation under natural conditions seems to be a very rare event (Schulze et al. 2011), fast and prolific clonal reproduction would be all the more important for a successful establishment of hybrid clones within a natural environment. The distinctly lower and delayed clonal reproduction of *F. vesca* x *F.* x *ananassa* hybrids in our experiment suggests a clear disadvantage of hybrids in a natural environment. Our results are

based on hybrids between two *F.* x *ananassa* varieties and a few *F. vesca* genotypes and represent therefore inevitably a limited genetic scope. However, Harbut et al. (2009) found similar differences in biomass allocation to runner plants between *F.* x *ananassa* cultivars and hybrids between *F.* x *ananassa* and synthetic octoploids of lower-ploidy *Fragaria* species (synthetic octoploid system (Evans 1982)). In this study, mean biomass allocation to runner plants of four cultivars was 15% whereas hybrids between *F.* x *ananassa* and synthetic octoploids allocated a mean of 46% to runner plants (Harbut et al. 2009). Furthermore, *F.* x *ananassa* cultivars are selected for fruit yield. In *F.* x *ananassa* and *F. vesca* plants each leaf carries an axilary bud, which can develop into an inflorescence or into a runner (Darrow 1966). Development into one of these structures is governed by environmental conditions and is genotype dependent, resulting in a direct trade-off between the formation of inflorescences and runners. Therefore, *F.* x *ananassa* cultivars usually produce lower stolon numbers than wild *Fragaria* species. For instance, a vigorous *F.* x *ananassa* plant produces about 10–15 stolons a year, whereas a clone of *F. virginiana* can produce two or three times that number (Hancock 1999).

In conclusion, the present results suggest that besides pre- and postzygotic barriers (Evans 1974; Marta et al. 2004) *F. vesca* x *F.* x *ananassa* hybrids would have a competitive disadvantage against co-occurring *F. vesca* plants due to inferior and delayed clonal reproduction. Taken together with a previous study on the hybridization potential of *F. vesca* and *F.* x *ananassa* (Schulze et al. 2011), we conclude that there is only low potential for hybrid establishment under natural conditions. As long as effects of transgenes can not compensate for the disadvantage of lower and delayed clonal reproduction rates, chances for transgene escape from transgenic *F.* x *ananassa* cultivars via *F. vesca* x *F.* x *ananassa* hybrids seem therefore also to be low.

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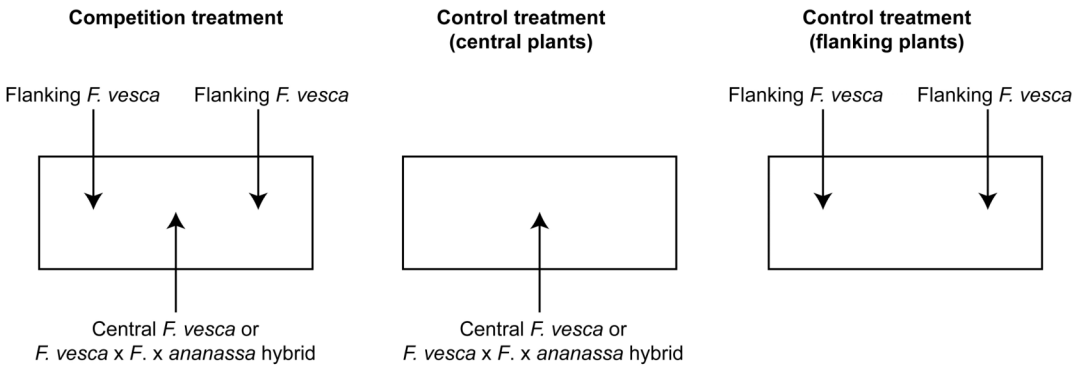


**Fig. 1.** Schematic top view on experimental flower boxes and plant positions in the competition and control treatments.

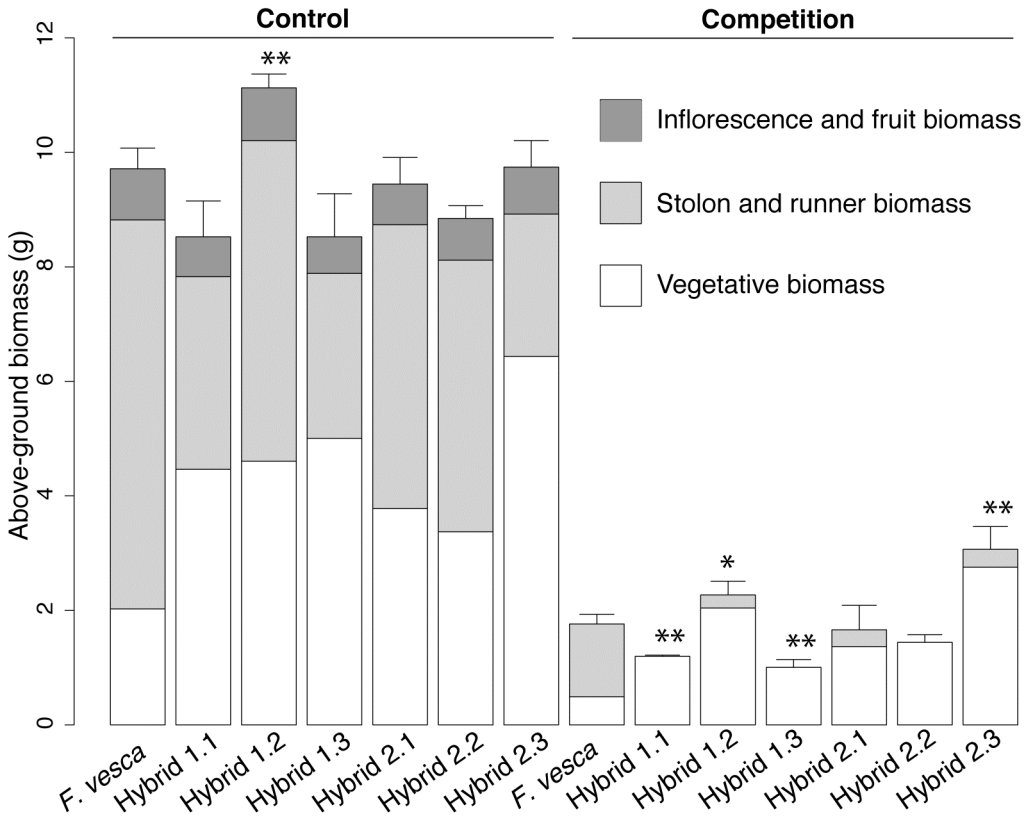


**Fig. 2.** Mean above-ground biomass of *F. vesca* plants and six *F. vesca* x *F.* x *ananassa* hybrid clones that were grown under a competition and a control treatment. Biomass of plants was calculated as the total of vegetative biomass, biomass of stolons and runner plants and biomass of sexual reproductive structures. Asterisks denote hybrid clone biomasses that are significantly different from *F. vesca* biomass within treatment (* $p < 0.05$; ** $p < 0.01$). Error bars are + SE; n = 9 for *F. vesca* and n = 3 for hybrid clones per treatment.

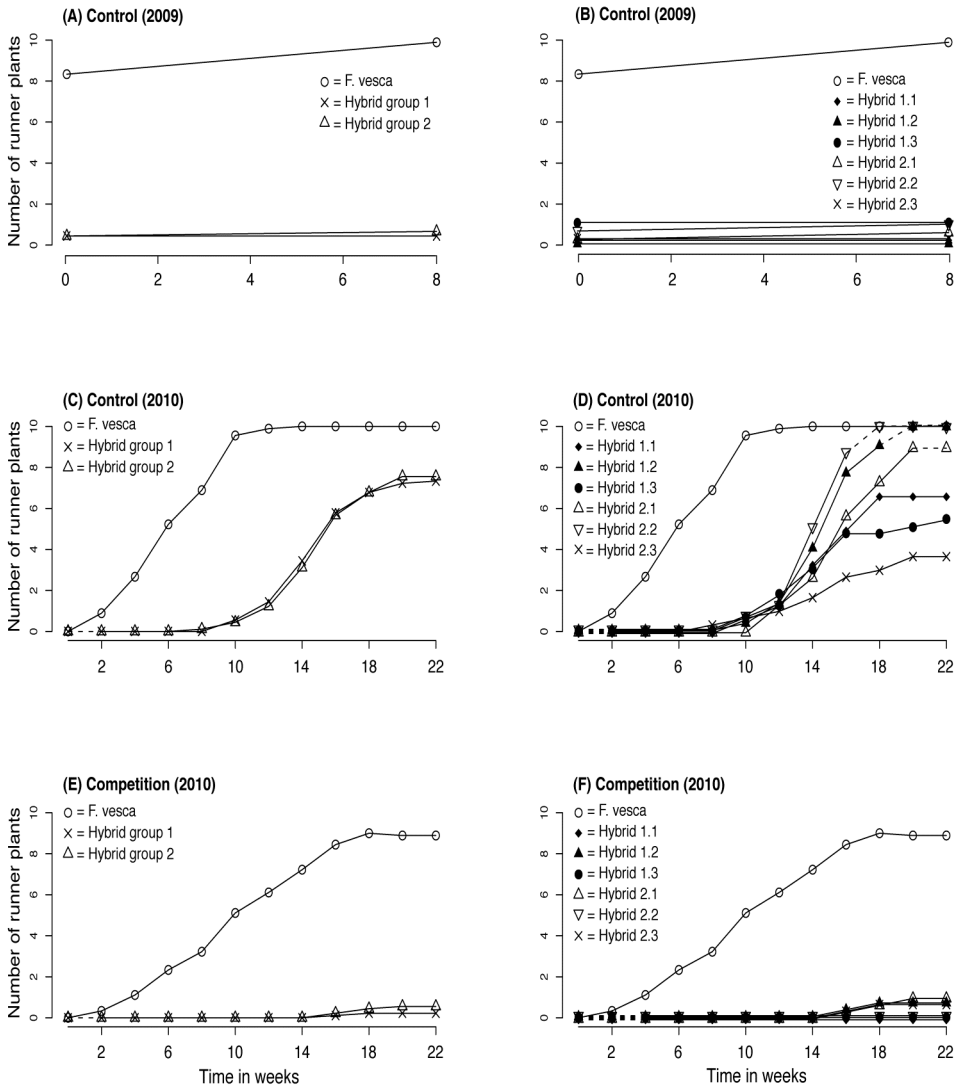


**Fig. 3.** Mean numbers of runner plants of *F. vesca* plants and two different *F. vesca* x *F.* x *ananassa* hybrid groups that are each made up of three different hybrid clones. Plants were grown under a competition or a control treatment. Data are presented for hybrid groups ((A), (C) and (E)) and for hybrid clones ((B), (D) and (F)). Data points of hybrid plants that are connected with a dashed line do not differ significantly from *F. vesca* at the respective date ($p > 0.05$). Time period in 2009: September 30$^{th}$ – November 26$^{th}$; time period in 2010: April 30$^{th}$ - September 29$^{th}$. Plants from competition treatment did not form stolons in 2009. N = 3 for hybrid clones and n = 9 for *F. vesca*.

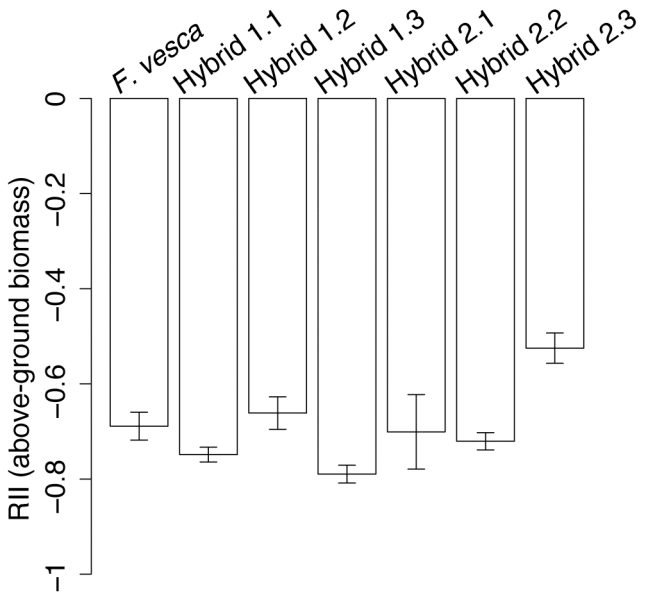


**Fig. 4.** Mean Relative Interaction Index values (± SE) for *F. vesca* plants and *F. vesca* x *F.* x *ananassa* hybrid clones for total above-ground biomass. N = 9 for *F. vesca* and n = 3 for hybrid clones.

**Table 1.** Relative DNA-contents of cell nuclei of *F. vesca* x *F.* x *ananassa* hybrid clones and their *F. vesca* mother lines. Relative DNA-contents were calculated with *F.* x *ananassa* cv. Calypso as standard. Samples were measured three times. *Fragaria vesca* mother 1.2 died before plants were sampled.

| Plant | Relative DNA-content | SD |
|---|---|---|
| *F. vesca* mother 1.1 | 0.361 | 0.009 |
| *F. vesca* mother 1.2 | NA | NA |
| *F. vesca* mother 1.3 | 0.354 | 0.012 |
| *F. vesca* mother 2.1 | 0.352 | 0.001 |
| *F. vesca* mother 2.2 | 0.362 | 0.003 |
| *F. vesca* mother 2.3 | 0.342 | 0.019 |
| Hybrid 1.1 | 0.666 | 0.007 |
| Hybrid 1.2 | 0.659 | 0.003 |
| Hybrid 1.3 | 0.692 | 0.013 |
| Hybrid 2.1 | 0.708 | 0.031 |
| Hybrid 2.2 | 0.684 | 0.006 |
| Hybrid 2.3 | 0.678 | 0.002 |

**Table 2.** Maximal models for (A) biomass of central *F.vesca* and hybrid plants, (B) biomass of flanking *F. vesca* plants and (C) biomass allocation to clonal reproduction of *F. vesca* and hybrid plants. Models were calculated either with hybrid plants pooled as hybrid groups (plant group) or on the level of hybrid clones (plant type). The maximal models were stepwise reduced by exclusion of nonsignificant interactions and variables. Only significant or marginally significant variables and significant interactions of the minimal adequate models are presented. Note that dependent variables were logarithmically transformed in some models to obtain normally distributed residuals.

**(A)**

*ANOVA: Biomass central plant ~ Block * Plant group* (control treatment)

No significant variables

*ANOVA: Biomass central plant ~ Block * Plant type* (control treatment)

| | *F* | df | *p* |
|---|---|---|---|
| Plant type | 2.83 | 6, 20 | 0.037 |

*ANCOVA: Log (Biomass central plant) ~ Block * Plant group * Biomass flanking plants* (competition treatment)

| | *F* | df | *p* |
|---|---|---|---|
| Block | 3.37 | 2, 24 | 0.051 |

*ANCOVA: Biomass central plant ~ Block * Plant type * Biomass flanking plants* (competition treatment)

| | *F* | df | *p* |
|---|---|---|---|
| Block | 9.64 | 2, 18 | 0.001 |
| Plant type | 11.72 | 6, 18 | <0.001 |

**(B)**

*ANCOVA: Log (Biomass flanking plants) ~ Block * Biomass central plant * Plant group*

| | *F* | df | *p* |
|---|---|---|---|
| Block | 6.89 | 2, 32 | 0.003 |
| Biomass central plant | 21.75 | 1, 32 | <0.001 |

*ANCOVA: Log (Biomass flanking plants) ~ Block * Biomass central plant * Plant type*

| | *F* | df | *p* |
|---|---|---|---|
| Block | 6.89 | 2, 32 | 0.003 |
| Biomass central plant | 21.75 | 1, 32 | <0.001 |

**(C)**

*GLM: Biomass proportion ~ Block * Plant group* (control treatment)

| | *F* | df | *p* |
|---|---|---|---|
| Block | 3.22 | 2, 24 | 0.059 |
| Plant group | 6.73 | 2, 22 | 0.005 |

*GLM: Biomass proportion ~ Block * Plant type* (control treatment)

| | *F* | df | *p* |
|---|---|---|---|
| Block | 3.46 | 2, 24 | 0.053 |
| Plant type | 3.64 | 6, 18 | 0.015 |

*GLM: Biomass proportion ~ Block * Biomass flanking plants * Plant type* (competition treatment)

| | *F* | df | *p* |
|---|---|---|---|
| Plant type | 12.81 | 3, 14 | <0.001 |

**General discussion**

The thesis aim (1), the assessment of the hybridization potential between *F.* x *ananassa* and *F. vesca*, was adressed by a hybrid survey which was designed to detect past and present hybridization processes under natural conditions (Chapter I). Furthermore, *F.* x *ananassa* and *F. vesca* plants were hybridized experimentally (Chapter I). No hybrids were found in the hybrid survey, although some of the oldest commercial Swiss strawberry farms were included. I assume that the sample size used in this study was large enough to rule out a widespread occurrence of *F. vesca* x *F.* x *ananassa* hybrids in the survey area. It seems that hybrid formation or hybrid establishment is a rather rare event. In the experimental hand-crosses the mean germination and survival rate of hybrids until the seedling stage was very low (1.8%) and showed that there are significant genetic incompatibilities between the two species (Chapter I). However, experimental hybridizations yielded some very vigorous hybrids with prolific clonal reproduction and vigorous hybrids between wild diploid and octoploid *Fragaria* species have been reported from field sites (Bringhurst and Khan 1963). Therefore, the possibility of hybrid establishment under natural conditions can not be ruled out completely.

The results of the flower choice experiment presented in Chapter II did not indicate that the behaviour of solitary bees, which are an important pollinator group for both *F. vesca* and *F.* x *ananassa*, would obstruct gene flow from cultivated to wild strawberries. However, the flower choice experiments were carried out with equal numbers of *F. vesca* and *F.* x *ananassa* flowers, whereas in the field cultivated strawberries usually outnumber *F. vesca* plants. Therefore, larger floral displays of *F.* x *ananassa* may distract pollinators from *F. vesca* with increasing distance between plant groups in the field. Thus, my results rather apply for cultivated and wild strawberries growing in close vicinity.

In conclusion, Chapters I and II give inconsistent evidence for possible gene flow from cultivated to wild strawberries in Switzerland. On the one hand, data from field surveys suggest that hybridization between cultivated and wild strawberries seems to be at best a rare event, on the other hand indiscriminative pollinator behaviour could still cause such unwanted gene flow.

In regard of these results it was unclear whether the major obstacles for establishment of natural *F. vesca* x *F.* x *ananassa* hybrids are pre- and post-zygotic barriers (Evans 1974; Marta et al. 2004) or whether later developmental stages of hybrids are not fit enough to compete with co-occurring plants.

The thesis aim (2), assessment of the fitness of hybrid plants and the potential effects of hybridization on natural *F. vesca* populations, was adressed with a demographic study on *F. vesca* to estimate the importance of different growth parameters for population growth and maintenance (Chapter III). In addition, growth of *F. vesca* and hybrid plants was compared directly in a competition experiment (Chapter IV).

The demographic study on *F. vesca* showed that growth parameters of plants can change dramatically between years within populations (Chapter III). Furthermore, it could be demonstrated that clonal reproduction is of significant importance for growth and maintenance of *F. vesca* populations. This can be explained by the high survival rates of clonal offspring, which were often similar to the survival rates of adult plants. However, seedlings were rarely found in study plots and sexual reproduction seems to be insignificant for population growth within established *F. vesca* populations. Such differences in the importance of sexual and clonal reproduction for population growth are well documented for other clonal plant species (Silvertown et al. 1993). Furthermore, it has been proposed that in small seeded clonal plants, such as *F. vesca*, sexual reproduction serves mainly as a mechanism for long-distance dispersal (Eriksson 1997). Regarding the results of the demographic study of *F. vesca*, it seems likely that *F. vesca* x *F.* x *ananassa* hybrids would require clonal reproduction rates that are similar to *F. vesca* to compete successfully with the latter, as long as hybrids can not compensate any disadvantage of lower clonal reproduction rates in some other way (Chapter III).

To directly compare growth characteristics of *F. vesca* x *F.* x *ananassa* hybrids and *F. vesca* plants I grew plants under a competition and a control treatment in a greenhouse (Chapter IV). The most vigorous hybrid clones that originated from experimental hand-crosses (Chapter I) were used in this experiment. I found that *F. vesca* x *F.* x *ananassa* hybrids can exceed *F. vesca* plants in total biomass production, i.e. vegetative biomass, biomass of sexual reproductive structures and biomass of clonal offspring. However, the number of clonal offspring and the biomass proportion that was

allocated to clonal reproduction was significantly lower in all hybrid clones under competition and significantly lower in most hybrid clones in the control treatment. Furthermore, there was a remarkable difference in the timing of clonal reproduction between *F. vesca* and hybrid plants with clonal reproduction starting six and 14 weeks later in hybrid plants in the control and the competition treatment, respectively. These results are in line with other findings that showed lower clonal reproduction of *F.* x *ananassa* cultivars in comparisons with hybrids between *F.* x *ananassa* and synthetic octoploids of wild *Fragaria* species (Harbut et al. 2009). The general lower potential for clonal reproduction in *F.* x *ananassa* cultivars or hybrids that contain parts of a *F.* x *ananassa* genome can be explained insofar as *F.* x *ananassa* cultivars are selected for fruit yield, a trait that stands in direct competition with clonal reproduction (Chapter IV). In addition, none of the hybrid clones produced normally developed fruits or fertile achenes. Sexual sterility is not necessarily a disadvantage for local competition as long as plants are able to maintain population growth by clonal reproduction (Angevine 1983; Bringhurst and Khan 1963). However, the distinct lower and delayed clonal reproduction of *F. vesca* x *F.* x *ananassa* hybrids seems to be a clear disadvantage in a natural environment. In summary, the results of Chapter IV suggest that besides the known pre- and postzygotic barriers (Evans 1974; Marta et al. 2004) *F. vesca* x *F.* x *ananassa* hybrids would have a competitive disadvantage against co-occurring *F. vesca* plants due to inferior and delayed clonal reproduction.

In regard of the differences in clonal reproduction parameters between *F. vesca* and hybrid plants, the lack of hybrids at farm survey sites and the low experimental hybrid germination rates, I conclude that there is low potential for hybrid establishment under natural conditions. Therefore, it seems that chances for transgene escape from transgenic *F.* x *ananassa* cultivars via *F. vesca* x *F.* x *ananassa* hybrids are also low as long as effects of transgenes can not compensate for any disadvantage of lower and delayed clonal reproduction rates of hybrid plants.

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**Summary**

Hybridization is a widespread phenomenon in many plant and animal species complexes and generally refers to crosses between individuals from different taxa. Plant scientists have studied hybridization to understand relatedness and evolution within particular plant groups and natural hybridization has been acknowledged as an important evolutionary process that can lead to new evolutionary lineages.

The introduction of genetically modified (GM) economic plants has raised questions about the potential for transgene escape from GM plants into populations of wild or weedy relatives via hybridization. To date, numerous studies have shown the potential of GM economic plants to hybridize with wild species. In the genus *Fragaria* (Rosaceae), the potential for hybridization between different species has been demonstrated repeatedly. The genus *Fragaria* contains 24 strawberry species, including well-defined hybrid species such as the garden strawberry (*F.* x *ananassa* Duch.), which is cultivated worldwide. Although future commercialisation of GM garden strawberries is very likely, there is limited knowledge about the potential for hybridization between garden strawberries and wild relatives under natural conditions. The goal of my thesis was to assess the hybridization potential between cultivated garden strawberries and wild relatives in Switzerland, and thus to provide a basis for estimating the risks of a potential future cultivation of transgenic garden strawberries. In Switzerland, the most likely wild candidate species for hybridization with cultivated *F.* x *ananassa* seems to be the common woodland strawberry (*F. vesca* L.). The main research aims of this thesis were:

(1) Assessment of the hybridization potential between *F.* x *ananassa* and *F. vesca*

(2) Assessment of fitness of hybrid plants and the potential effects of hybridization on natural *F. vesca* populations

To detect past and present natural hybridization between *F.* x *ananassa* and *F. vesca*, a hybrid survey was conducted in the surroundings of farms in Switzerland and southern Germany, where garden strawberries have been cultivated for at least ten years and wild *F. vesca* plants occur in the close vicinity (Chapter I). Samples of wild *F. vesca* plants were analysed with microsatellite markers and ploidy levels of plants were estimated by flow cytometry to identify putative hybrids. Furthermore, *F.* x *ananassa* and *F. vesca* plants were hybridized experimentally to assess the hybridization potential under

controlled conditions (Chapter I). No hybrid plants were detected in the field. Experimental hand-crosses yielded some vigorous *F. vesca* x *F.* x *ananassa* hybrid plants but germination and survival rates of hybrids were generally very low.

Solitary bees are important and effective pollinators that visit both *F.* x *ananassa* and *F. vesca* plants in the field. To assess whether natural hybridization between *F.* x *ananassa* and *F. vesca* is promoted by the behaviour of pollinators I studied the flower choice behaviour of the red mason bee (*Osmia rufa* L.) in a greenhouse experiment (Chapter II). Blocks of *F.* x *ananassa* and *F. vesca* plants were presented to bees and flower visits and flower handling of individual insects were recorded. Solitary bees did not show a preference for either *F.* x *ananassa* or *F. vesca*. The results indicate that the behaviour of solitary bees does not obstruct gene flow from cultivated to wild strawberries.

As a basis for the assessment of fitness of hybrid plants and the potential effects of hybridization on natural *F. vesca* populations, the demography of wild *F. vesca* populations was studied (Chapter III). Demographic data were used to parameterise matrix population models, and the importance of different growth parameters for population growth was assessed using prospective (elasticity analyses) and retrospective (life table response experiments) matrix analysis methods. It could be shown that clonal reproduction is of great importance for growth and maintenance of *F. vesca* populations, whereas sexual reproduction seems to be insignificant for population growth within established *F. vesca* populations.

Furthermore, growth characteristics of *F. vesca* x *F.* x *ananassa* hybrids and *F. vesca* plants were directly compared. Different hybrid and *F. vesca* clones were grown under a competition and a control treatment in a greenhouse experiment. I found that hybrids can exceed *F. vesca* plants in total biomass production, i.e. vegetative biomass, biomass of sexual reproductive structures and biomass of clonal offspring. However, the number of clonal offspring and the biomass proportion that was allocated to clonal reproduction was significantly lower in all hybrid clones under competition and significantly lower for most hybrids in the control treatment. Furthermore, there was a remarkable difference in the timing of clonal reproduction between *F. vesca* and hybrid plants with clonal reproduction starting later in hybrids. In summary, the results of Chapter IV suggest that

*F. vesca* x *F.* x *ananassa* hybrids have a competitive disadvantage against co-occurring *F. vesca* plants due to inferior and delayed clonal reproduction.

In conclusion, the lack of hybrids at farm survey sites, the low experimental hybrid germination and survival rates and the differences in clonal reproduction parameters between *F. vesca* and hybrid plants indicate that there is low potential for hybrid establishment under natural conditions. Therefore, it seems that chances for transgene escape from transgenic *F.* x *ananassa* cultivars via *F. vesca* x *F.* x *ananassa* hybrids are also low as long as transgene effects can not compensate for any disadvantage of lower and delayed clonal reproduction rates of hybrid plants.

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