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ORIGINAL ARTICLE

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

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Abstract The genus *Fragaria* (Rosaceae) contains 24 species, including hybrid species such as the garden strawberry (*Fragaria* × *ananassa* Duch.). Natural hybridization between *Fragaria* species has repeatedly been reported, and studies on the hybridization potential between *F.* × *ananassa* and its wild relatives have become increasingly important with the outlook for genetically modified garden strawberries. In Europe, a candidate species for hybridization with garden strawberries is the common woodland strawberry (*Fragaria vesca* L.). Although a previous field survey indicated that the potential for hybridization between *F. vesca* and *F.* × *ananassa* is low, it is not clear whether the lack of natural hybrids is caused by known pre- and postzygotic barriers, or whether hybrid plants lack the fitness to establish in natural *F. vesca* populations. We grew different *F. vesca* and *F. vesca* × *F.* × *ananassa* hybrid clones with and without competition in a greenhouse and assessed biomass production, clonal reproduction, and sexual reproduction of plants. While some hybrid clones exceeded *F. vesca* in biomass production, general clonal reproduction was much lower and delayed in hybrids. Furthermore, hybrids were sterile. These results demonstrate a mechanism by which the general lack of *F. vesca* × *F.* × *ananassa* hybrids in natural habitats can be explained, in addition to the known low hybridization potential between garden and woodland strawberries. We conclude that hybrids have a competitive disadvantage against co-occurring *F. vesca* plants due to inferior and delayed clonal reproduction, and that the potential for hybrid establishment under natural conditions is low.

Keywords Competition · Hybrid fitness · Hybridization · Transgenic plants · Clonal reproduction

Introduction

The octoploid garden strawberry (*Fragaria* × *ananassa* Duch.) 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; Schulze et al. 2011). Generally, *Fragaria* 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. Furthermore, *Fragaria* species can be crossed experimentally with species of the closely related genus *Potentilla* and yield viable, but highly sterile hybrid plants (Ellis 1962). Such intergeneric hybrids have been used by breeders to develop new strawberry cultivars (Mabberley 2002).

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 (*Fragaria chiloensis* Mill.) and the diploid woodland strawberry (*Fragaria vesca* L.) have been described (Bringhurst and Khan 1963; Bringhurst and Senanayake 1966). These hybrid populations were described as infertile but competing well with their co-occurring parental species due to superior stolon productivity (Bringhurst and Khan 1963). With

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prospects of genetically modified (GM) strawberry cultivars in the near future (Qin et al. 2008), studies on the hybridization potential between *F.* × *ananassa* and related wild species have become increasingly important. To date, two field surveys that addressed the potential of natural hybridization between *F.* × *ananassa* and wild relatives have been carried out. Substantial gene flow between octoploid *F.* × *ananassa* and one of its wild octoploid parental species, the Virginia strawberry (*Fragaria virginiana* Mill.), was found in the southeastern USA (Westman et al. 2004). However, no indications for gene flow between octoploid *F.* × *ananassa* and the common diploid *F. vesca* have been found in Central Europe (Schulze et al. 2011). The reasons for the absence of *F. vesca* × *F.* × *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.* × *ananassa*, resulting in low germination rates of hybrid seeds and generally sterile hybrid plants (Evans 1974; Mangelsdorf and East 1927; Marta et al. 2004; Schulze et al. 2011). 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.* × *ananassa* can yield viable and vigorous hybrids (Olbricht et al. 2006; Schulze et al. 2011). Furthermore, *F. vesca* and *F.* × *ananassa* share major pollinators, such as solitary bees, and their flowering times overlap (Schulze et al. in press). Pollen flow between *F.* × *ananassa* and *F. vesca* is therefore likely in areas where they grow in close vicinity. Regarding these findings, it is unclear whether the major obstacles for an establishment of natural *F. vesca* × *F.* × *ananassa* hybrids are pre- and post-zygotic barriers or whether the later developmental stages of hybrids are not fit enough to compete with co-occurring plants.

The aim of the present study was to assess the differences in growth parameters between *F. vesca* × *F.* × *ananassa* hybrids and *F. vesca* plants, which may affect plant fitness and thus could further explain the absence of *F. vesca* × *F.* × *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.

Methods

Experimental setup

We grew *F. vesca* × *F.* × *ananassa* hybrids and *F. vesca* plants in flower boxes (63 boxes) under the conditions shown below.

Competition treatment

We planted either a *F. vesca* plant or a *F. vesca* × *F.* × *ananassa* hybrid centrally between two established flanking *F. vesca* plants (Fig. 1). As central *F. vesca* plants, we used three different clones that were replicated three times each (nine boxes). As central hybrid plants, we used three clones out of each of two different hybrid groups. Each hybrid clone was replicated three times (18 boxes). The two flanking *F. vesca* plants were two different clones and were identical in all boxes.

Control treatment

In the control treatment for central plants, we planted either a *F. vesca* plant or a *F. vesca* × *F.* × *ananassa* hybrid centrally without flanking plants (Fig. 1). *Fragaria vesca* clones (nine boxes) and hybrid clones (18 boxes) were the same as used in the competition treatment. In the control treatment for flanking plants, flanking *F. vesca* plants were grown without central plants (nine boxes). The two flanking *F. vesca* plants were two different clones and were identical in all 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). We added 3.2 kg of this substrate to 63 rectangular flower boxes ($L \times W \times H$: 36 × 14.5 × 12.5 cm) that were placed on individual saucers. On November 6, 2008, we selected runner plants 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, we washed roots of runners and cut them back to 6 cm length and similar density. We reduced the number of runner plant leaves 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 of central plants. We arranged boxes in three blocks on movable tables, with each block containing 21 boxes distributed on six tables. Positions of tables within a block were changed weekly in a regular rotation. In April of 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. We cut plants back to 2–3 leaves in March and June of 2009, to promote the development of even-sized plants within and among experimental boxes. On July 24, 2009, we planted either a *F. vesca* × *F.* × *ananassa* hybrid or a *F. vesca* plant in the center of 27 boxes that contained flanking plants (competition treatment) and in 27 empty boxes (control treatment) (Fig. 1). After transplanting of central plants, all plants were allowed to grow stolons and runner plants. For every plant, two square flower pots ($L \times W \times H$: 11 × 11 × 12 cm) filled with 480 ± 5 g attapulgite substrate (Oil Dri US special type II R, Damolin, Denmark) were placed beside the experimental boxes. We set a limit for flower pots per plant due to space limitations. If a plant formed runner plants, we

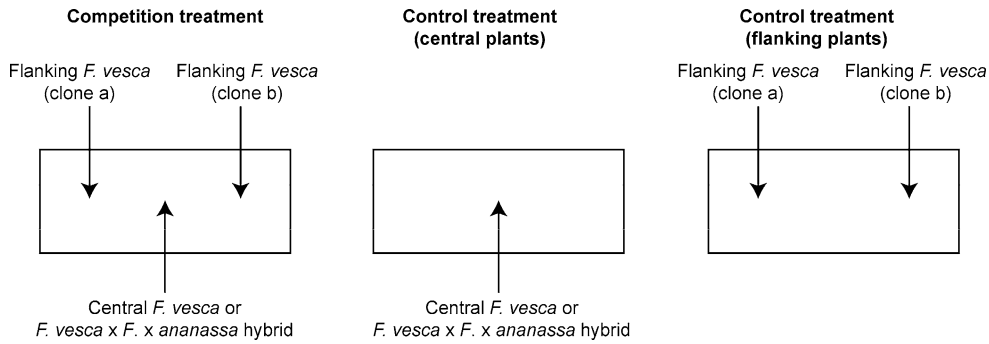

Fig. 1 Schematic top view on experimental flower boxes and plant positions in the competition and control treatments

fixed them in the flower pots with plastic hooks as soon as root tips became visible. We allowed for five runner plants per pot. If a plant had reached the maximal number of ten runner plants, newly formed stolons were regularly cut off. We counted runner plants produced by flanking and central plants on September 30, 2009, and on November 30, 2009. Growth of plants and runner plant production stagnated towards the end of November of 2009, and most leaves started to wither. We removed all runner plants grown in flower pots on December 3, 2009. In the spring of 2010, we placed pots with fresh substrate beside experimental boxes and treated newly formed runner plants of central and flanking plants as in the previous year. Between March 17, 2010 and September 29, 2010, we counted the number of ripe fruits and the runner plantlets produced by plants in biweekly intervals.

Withered inflorescences and fruits of plants were collected, dried at 80 °C for 48 h, and weighed and recorded as sexual reproductive biomass.

On September 29 and 30, 2010, we harvested above-ground biomass of flanking and central plants. We dried biomass at 80 °C for 48 h and weighed it. We harvested above-ground biomass of runner plants including interconnecting stolons separately. Root biomass was not harvested, as a separation of the intermingled roots of different individuals was not feasible for the competition treatment.

Plant material

As flanking plants, we used two different *F. vesca* genotypes collected at forest sites in Riehen and Dornach, Switzerland. We propagated runner plants of these *F. vesca* genotypes on garden soil. As central plants, we used *F. vesca* × *F.* × *ananassa* hybrids and *F. vesca* plants. Two groups of 24 and 19 hybrid plants stood at our disposal, which were germinated in a greenhouse in summer 2008. These hybrid groups originated from hand-crosses between *F. vesca* and *F.* × *ananassa* cv. Calypso and *F. vesca* and *F.* × *ananassa* AN93.231.53, respectively, and are hereafter called hybrid group 1 and hybrid group 2 (*F.* × *ananassa* varieties provided by B. Mezzetti, Marche Polytechnic University, Italy). *Fragaria* × *ananassa* cv. Calypso is a day-neutral variety whereas *F.* × *ananassa* AN93.231.53 is a short-day type, i.e., it initiates flower buds either under short-day conditions or when the temperature is less than 15 °C (Hancock 1999). Hybrid breeding and identification methods have been described elsewhere (Schulze et al. 2011). Hybrid identity of putative hybrids was confirmed by microsatellite analysis with a set of seven microsatellite markers. Of each hybrid group we selected three hybrids. In both hybrid groups there was large phenotypic variation with differences in size, leaf morphology, leaf color, and clonal reproduction. The hybrid clones selected for the experiment were among the most vigorous within their group and all of them reproduced clonally. We estimated ploidy levels of hybrid plants by flow cytometry following the methods of Schulze et al. (2011). 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 watered the flower boxes 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 of tap water. Control treatment boxes received 150 ml of tap water. Boxes had to be watered 2–4 times a week during the summer and once every week or every other week in the winter, depending on weather conditions. We did not water flower pots with runner plants at the same time, as they contained different numbers of plants. Pots were watered with 100 ml of tap water on top of the substrate whenever the substrate surface became dry.

We sprayed the plants repeatedly against infections of spider mites (Tetranychidae), and against white flies (*Trialeurodes vaporariorum*). On March 10, 2010, all of the boxes were fertilized with 150 ml of 1/4 strength Hoagland solution.

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 (2007), i.e., nonsignificant interactions and variables were stepwise excluded from the original maximal models. We specified treatment contrasts 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.

Total above-ground biomass

- Central plants, 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 analyzed 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.
- Central plants, competition treatment: 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.
- Flanking plants: we analyzed 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.

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.

Clonal reproduction and biomass allocation to 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 ten runners. Therefore, data were analyzed as proportional data, i.e., proportions of the maximal possible number of runners realized. 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 analyzed 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 analyzed 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.

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.* × *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.

Total above-ground biomass

Central plants, 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.

Central plants, 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 (Table 2a). 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).

Table 1 Relative DNA contents of cell nuclei of *F. vesca* × *F.* × *ananassa* hybrid clones and their *F. vesca* mother lines

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

Relative DNA contents were calculated with *F.* × *ananassa* cv. Calypso as standard. Samples were measured three times. *Fragaria vesca* mother 1.2 died before plants were sampled

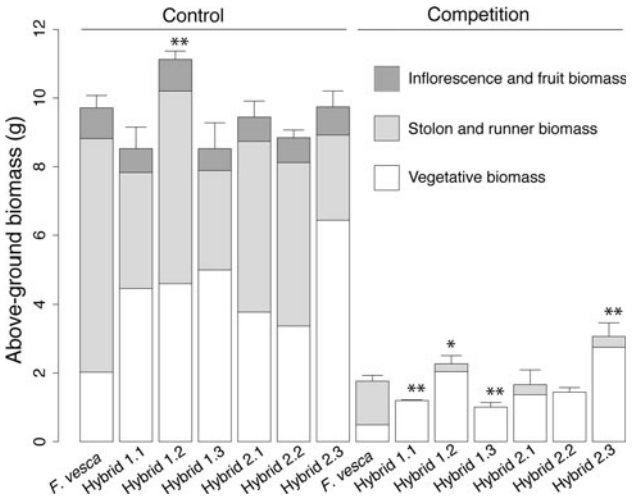

Fig. 2 Mean above-ground biomass of *F. vesca* plants and six *F. vesca* × *F.* × *ananassa* hybrid clones that were grown under a competition and a control treatment. Biomass of plants was calculated as the total of vegetative biomass, biomass of stolons and runner plants, and biomass of sexual reproductive 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

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). However, neither variable plant group nor variable plant type was significant.

Relative interaction index

The presence of flanking plants had a strong negative effect on final total above-ground biomass of central plants (Fig. 3). 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.

Clonal reproduction and biomass allocation to runner plants

Clonal reproduction

In 2009, only plants from the control treatment formed runner plants (Fig. 4a, b). Mean runner production of hybrid groups and hybrid clones was similar and sig-

Table 2 Maximal models for biomass of central *F.vesca* and hybrid plants (a), biomass of flanking *F. vesca* plants (b), and biomass allocation to clonal reproduction of *F. vesca* and hybrid plants (c)

	F	*df*	*p*
(a)			
ANOVA: biomass central plant ~ block * plant group (control treatment)			
No significant variables			
ANOVA: biomass central plant ~ block * plant type (control treatment)			
Plant type	2.83	6, 20	0.037
ANCOVA: log (biomass central plant) ~ block * plant group * biomass flanking plants (competition treatment)			
Block	3.37	2, 24	0.051
ANCOVA: biomass central plant ~ block * plant type * biomass flanking plants (competition treatment)			
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			
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			
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)			
Block	3.22	2, 24	0.059
Plant group	6.73	2, 22	0.005
GLM: biomass proportion ~ block * plant type (control treatment)			
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)			
Plant type	12.81	3, 14	< 0.001

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

nificantly lower than runner production of *F. vesca* plants (Fig. 4a, b; electronic supplementary material). At the end of the season, nearly all *F. vesca* plants achieved the maximum value of ten runner plants (mean number of runners = 9.9), whereas the mean number of runners per hybrid clone never exceeded 1.

On May 14, 2010, first *F. vesca* runner plants were observed in both treatments. In the control treatment, hybrid groups 1 and 2 produced significantly less runners than *F. vesca* plants after May 14, 2010 (Fig. 4c, 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. 4c, 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*. 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. 4d). 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, 2010, and May 28, 2010, respectively (Fig. 4e, f; electronic supplementary material). 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

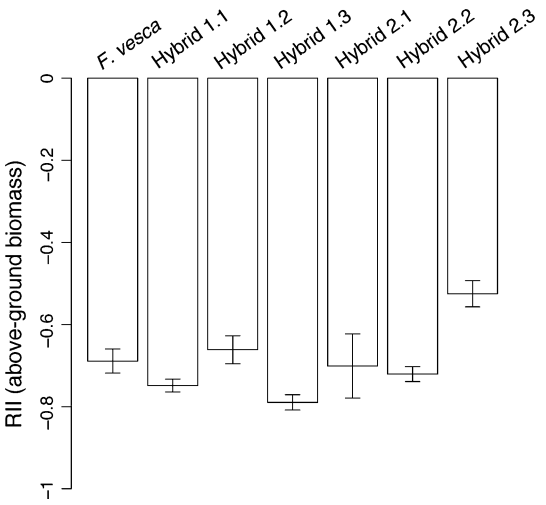

Fig. 3 Mean relative interaction index values (±SE) for *F. vesca* plants and *F. vesca* × *F.* × *ananassa* hybrid clones for total above-ground biomass ($n = 9$ for *F. vesca* and $n = 3$ for hybrid clones)

experiment without achieving the maximum possible number of runners. Furthermore, runner production of hybrid plants started 14 weeks after *F. vesca* and lagged behind.

Biomass allocation to runner plants

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 and 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 and 15 % in hybrid clones and 71 % in *F. vesca* plants.

Sexual reproduction

Fragaria vesca plants produced a mean of 9.8 ± 1.7 SE fruits in the control treatment. In the competition treatment, only one *F. vesca* plant formed a small inflorescence with three small fruits and mean *F. vesca* fruit production was 0.3 ± 0.3 SE. Most hybrid plants formed inflorescences in the control treatment (Fig. 2). However, they were sterile and all fruits remained rudimentary without any developed achenes. Hybrid plants did not form any inflorescences in the competition treatment.

Discussion

In this study, we made use of hybrid plants with vigorous vegetative growth and relatively good clonal reproduction. Even within this small subset of hybrids we found remarkable variability of growth parameters within hybrid groups and among hybrid clones. Growth of hybrid clones remained stable throughout the experiment and variability in growth parameters between hybrid clones did not seem to be related to late hybrid atrophy. Therefore, generalizations on growth characteristics and fitness of *F. vesca* × *F.* × *ananassa* hybrids should be made with care, and analysis of hybrid plants at the clone level is clearly more informative than analysis at the group level. In the following, we will therefore mainly discuss results of analyses carried out at 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. However, some hybrid clones exceeded *F. vesca* plants in total biomass production.

RII values were similar for *F. vesca* and hybrid clones and suggest that competition by *F. vesca* 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 impractical to estimate the final 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.

In addition to the differences in biomass allocation, *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

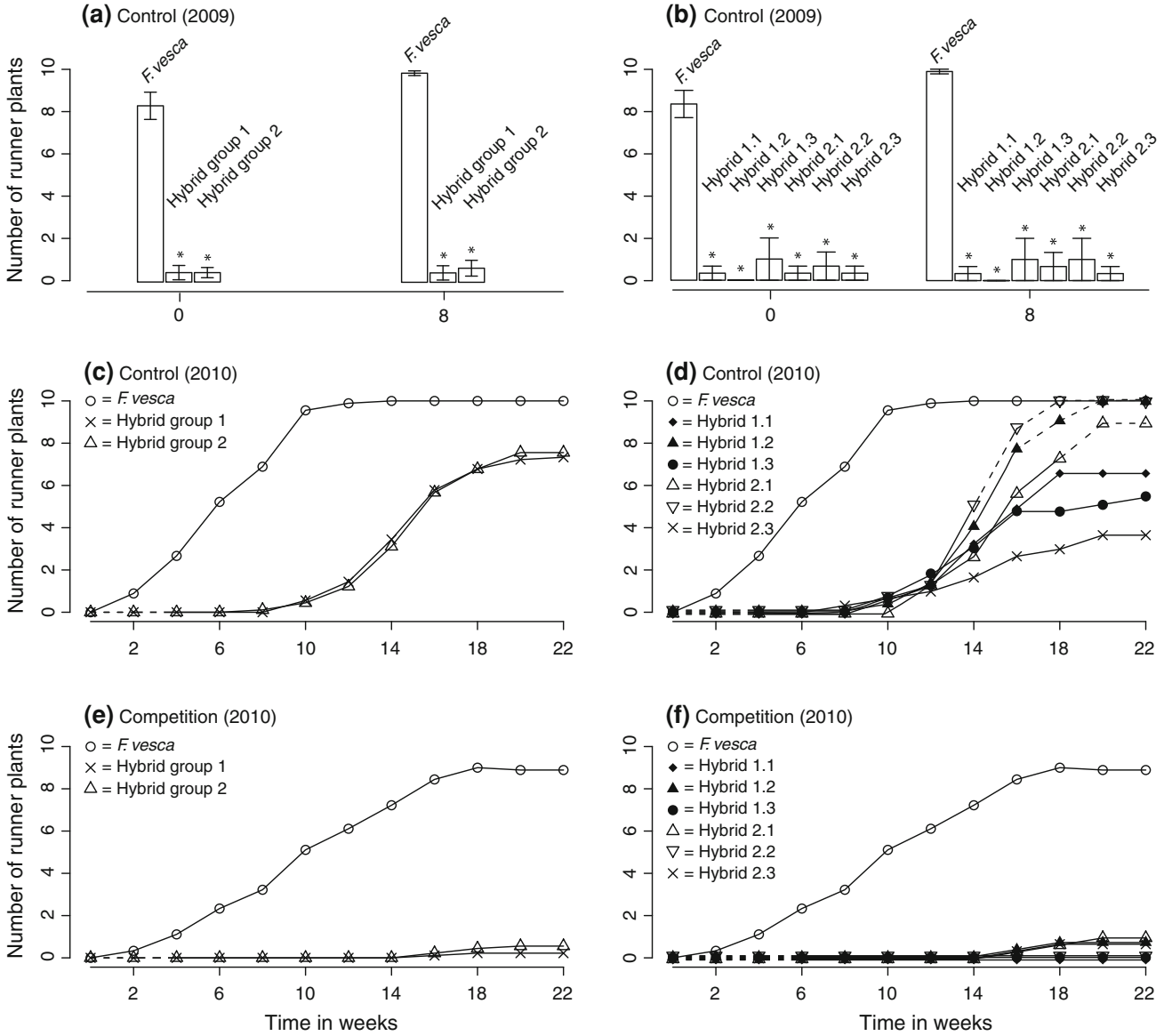

Fig. 4 Mean numbers of runner plants of *F. vesca* plants and two different *F. vesca* × *F.* × *ananassa* hybrid groups that are each made up of three different hybrid clones. Plants were grown under a competition or a control treatment. Data are presented for hybrid groups (**a**, **c**, **e**) and for hybrid clones (**b**, **d**, **f**). *Bars* marked with *asterisks* (**a**, **b**) and data points of hybrid plants that are connected with a *solid line* (**c**–**f**) differ significantly from *F. vesca* at the respective date ($p < 0.05$). Time period in 2009: September 30 to November 26; time period in 2010: April 30 to September 29. Plants from competition treatment did not form stolons in 2009. *Error bars* are ±SE ($n = 3$ for hybrid clones and $n = 9$ for *F. vesca*)

runner plants was reached. Thereafter, some hybrid clones also approached or reached the maximum number of runner plants.

The differences in biomass allocation to clonal reproduction and in numbers of runner plants between *F. vesca* and hybrid plants may result from the different timing of clonal reproduction. Clonal reproduction of hybrid plants lagged at least 6 weeks behind in the control treatment and even 14 weeks behind in the competition treatment in 2010. In *F.* × *ananassa* and *F. vesca* plants, each leaf carries an axillary bud, which can develop into an inflorescence, a stolon, or a new crown branch (Darrow 1966); development of the reproductive structures is governed by temperature and photoperiod in *F. vesca* (Chabot 1978; Heide and Sonsteby 2007; Sonsteby and Heide 2008) and *F.* × *ananassa* (Hancock 1999; Sonsteby and Nes 1998). Different requirements for induction of clonal reproduction may explain the observed differences in timing.

Furthermore, it has been observed that the proportion of biomass allocated to reproductive biomass is reduced with decreasing total biomass in *F. vesca* (Chabot 1978). The total absence of inflorescences and stolons in some of the hybrid clones in the competition treatment indicates that biomass allocation patterns are also size-dependent in hybrids. Differences in size-dependent allocation

patterns of *F. vesca* and hybrid plants may explain the increased time lag in clonal reproduction of hybrid plants in the competition treatment.

Our results are based on hybrids between two *F.* × *ananassa* varieties and a few *F. vesca* genotypes and therefore represent a limited genetic scope. However, similar differences in biomass allocation to runner plants between *F.* × *ananassa* cultivars and hybrids between *F.* × *ananassa* and synthetic octoploids of lower-ploidy *Fragaria* species have been found (Harbut et al. 2009); nine different hybrids partitioned a mean of 46 % dry-weight to runners, compared with a mean of 15 % in four different cultivars. These findings suggest that plants with a higher proportion of wild *Fragaria* genome tend to allocate more biomass to clonal reproduction than *F.* × *ananassa* cultivars.

In summary, *F. vesca* × *F.* × *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 *F. vesca* plants and hybrid clones affect plant fitness? 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* × *F. vesca* hybrids that competed well with their co-occurring parental species due to superior stolon productivity. Furthermore, in population studies on *F. vesca*, seedlings were either totally absent from study plots (Angevine 1983; Jurik 1985) or very rare (Schulze et al. 2012) and sexual reproduction may only be of small significance for population growth within established populations. On the other hand, clonal growth may secure local persistence of genets in various ways. Clonal growth allows for exploitation of spatially distributed resources and for sharing of unevenly distributed resources among connected ramets (Alpert 1996, 1999; Roiloa and Retuerto 2006; Stuefer et al. 1994, 1996). Furthermore, clonal fragmentation can be regarded as a "risk-spreading strategy", that minimizes the extinction risk of genets in a variable environment by spreading the risk of mortality to different ramets (Eriksson and Jerling 1990). Effective clonal reproduction would seem to be most important for the persistence of highly sterile genets. Therefore, the distinctly lower and delayed clonal reproduction of *F. vesca* × *F.* × *ananassa* hybrids in our experiment suggests a disadvantage for the long-term persistence of sterile hybrids in a natural environment.

In conclusion, the present results suggest that besides pre- and postzygotic barriers (Evans 1974; Marta et al. 2004), *F. vesca* × *F.* × *ananassa* hybrids would have a competitive disadvantage against co-occurring *F. vesca* plants due to inferior and delayed clonal reproduction. Based on these findings and on a study investigating the hybridization potential of *F. vesca* and *F.* × *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.* × *ananassa* cultivars via *F. vesca* × *F.* × *ananassa* hybrids seem therefore also to be low.

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