Natural hybridization is increasingly recognized as an important process for the ecology and evolution of natural plant populations and species. There is a great need to initiate more studies based on natural populations in order to elucidate the possible role of hybrids in nature. The reproductive success of early generation hybrids can make or break hybrid lineages and may determine the genetic structure of hybrid swarms or the potential for gene flow through future generations, but studies of hybrid reproductive success are lacking. Here we measured components of male and female reproductive success in *Senecio jacobaea* and *S. aquaticus* (Asteraceae) species and F$_1$ hybrids between these species under laboratory conditions, and we measured reproductive output from crosses producing F$_1$, F$_2$, and backcross (BC) generation hybrids. F$_1$ hybrids were readily produced, and on average, the success of crosses producing subsequent generations (F$_2$, BC) decreased (though remained substantial), but the success of crosses was highly dependent on the genotypes involved. Also, F$_1$ hybrids were bigger, produced more flowers, and therefore produced more pollen than parental plants. Finally, crosses between parents were asymmetrical, such that *S. aquaticus* produced more and larger F$_1$ seeds than did *S. jacobaea*.

**Key words:** Asteraceae, fertility, hybridization (hybridisation), male fitness, Netherlands, reproductive success, *Senecio aquaticus, Senecio jacobaea*

*American Journal of Botany* (2005) **92**: 1467-1473
INTRODUCTION

Already in 1859, Darwin recognized the potentially important role of hybridization in his *Origin of Species*, and since Darwin's time, many authors have touched upon the possible ecological and evolutionary consequences of gene flow between species (Stebbins, 1950; Lewis, 1966; Grant, 1981). Especially in the last decade, an increase in research into natural hybridization has extended our knowledge about the evolutionary significance of this process (Arnold, 1997; Rieseberg & Carney, 1998; Rieseberg *et al.*, 1999).

While many researchers have in the past considered that hybridization can reduce biological diversity by homogenizing divergent genomes (Mayr, 1942; Wilson, 1965), we now know that hybridization can increase biological diversity within populations, and also potentially within and between species. For instance, at the population level, Whitham *et al.* (1999) showed that hybrid zones in plants support a higher diversity of phytophagous insects than pure parental populations. With regards to diversity within and between species, hybridization may generate novel traits (Rieseberg *et al.*, 1999; Orians, 2000) or novel combinations of traits, which can be introgressed to parental species (Anderson, 1949; Arnold, 1997; Abbot *et al.*, 2003), or can allow hybrid lineages to adapt to new environments (e.g., sunflowers; Rieseberg *et al.*, 2003). Not only can hybridization facilitate diversification at the trait level, but it can also result in the evolution of new species (Rieseberg *et al.*, 2003), perhaps to the extent of contributing to adaptive radiations (Seehausen, 2004).

Although there is mounting evidence for the contribution of hybridization to the evolutionary history of plants in particular (Ellstrand *et al.*, 1996), the ecological fates of individual natural plant hybrid populations are varied, with some highly ephemeral populations (Barton & Hewitt, 1985) and some stable, or growing hybrid zones (see Arnold, 1997). The success of natural hybrids is dependent on a wide variety of factors, including the compatibility of parental genomes (e.g., Dobzhansky, 1933; Muller, 1942), vegetative performance according to abiotic conditions (e.g., Campbell & Waser, 2001; Kirk *et al.*, 2005b), and interactions with pollinators and herbivores (Campbell *et al.*, 1997; Fritz *et al.*, 1999).

Many recent papers examining the success of natural hybrids have focused on vegetative survival in natural environments (Burke *et al.*, 1998; Campbell & Waser, 2001; Johnston *et al.*, 2001; Kirk *et al.*, 2005b). Especially early hybrids are often found to be superior to parents in terms of growth, often surviving, growing, and competing better than parental species in all or a limited number of habitats, and such hybrids are frequently expected to have a high ecological or evolutionary impact. Yet, the evolutionary impact of such hybrids can be restricted if they are sterile or if gene flow from hybrids is restricted by genomic instability or genetic incompatibilities. Even hybrids that are superior to parents in vegetative phases may fail to reproduce, which can effectively enforce isolation of parental populations. Alternatively, hybrids could breed most successfully with one or other of the parental species, or with each other, which may result in unidirectional introgression or even in sympatric speciation. Measurements of reproductive fitness of hybrids
have been underrepresented in hybrid literature, likely because studies involving reproduction involve a longer time scale, especially for biennial and perennial plants.

Here, we measure the success of crosses that produce early generation hybrids (F1, F2, and BC) between *Senecio jacobaea* L. and *S. aquaticus* Hill (Senecioneae; Asteraceae). *Senecio* is a genus containing more than 1500 species with a worldwide distribution. Many *Senecio* species are known to hybridize in nature (e.g., Lowe & Abbott, 2000; Abbott & Lowe, 2004; Kirk et al., 2004), and hybridization may make a large contribution to the evolutionary history of the genus. Phylogenetic study of *Senecio* sect. *Jacobaea*, which contains *S. jacobaea*, *S. aquaticus*, and at least 23 of their closest relatives, has provided some evidence that hybridization may contribute to the evolutionary history of these species (Pelser et al., 2003).

*Senecio jacobaea* and *S. aquaticus* have been widely reported to produce hybrid swarms throughout Europe (Chater & Walters, 1976), with a confirmed population in the Zwanenwater nature reserve in The Netherlands (Kirk et al., 2004). The Zwanenwater hybrid population has been present for at least 25 yr (R van der Meijden, National Herbarium of the Netherlands, personal communication), and there is some evidence that hybridization there facilitates introgression of chemical traits from *S. aquaticus* into *S. jacobaea* individuals that are located at some distance from the hybrid zone (Kirk et al., 2004). Under laboratory conditions, hybridization between these species may lead to the generation of altered pyrrolizidine alkaloids (H Kirk, unpublished data) and unique expression of primary and secondary metabolites in hybrid individuals (Kirk et al., 2005a). Furthermore, studies of the Zwanenwater population have shown that F1 hybrids are superior in vegetative fitness to parental species across a variety of abiotic growth conditions (Kirk et al., 2005b). Combined, these results indicate that natural hybridization between *S. jacobaea* and *S. aquaticus* plays a potentially important ecological and/or evolutionary role.

The aims addressed by this study were (1) to test whether early generation hybrids can be easily formed in nature, (2) to make initial estimates of both male and female reproductive success of F1 hybrids and parental species, and (3) to evaluate whether there is asymmetry in the success of crosses.

**MATERIALS AND METHODS**

**Crossings**

Six *S. jacobaea* and six *S. aquaticus* plants were collected from the Zwanenwater Nature Reserve (The Netherlands) during May 2003 (see Kirk et al., 2005b, for a detailed description of the layout of the hybrid population and of collection zones). When plants were beginning to develop flowering stems, they were transferred to a greenhouse, matched in pairs between species, and crossed pairwise twice per week by rubbing flower heads together. Seeds, which represented F1 offspring, were harvested from both parents.
F₁ seeds and seeds from unrelated parental individuals were placed in tissue culture to produce clonal plantlets. Of nine F₁ genotypes included in the experiment, there were three sets of two full-sibs. Thus in total, six F₁ genotypes were completely unrelated to one another.

In total, eight *S. jacobaea* genotypes, six *S. aquaticus* genotypes, and nine F₁ hybrid genotypes were used in the experiment. When enough clones were available, five clones from each genotype were transferred to 3-L pots filled with a 1:1 mixture of potting soil and dune sand. Three grams slow-release fertilizer (N:P:K = 15:11:13 + 2 MgO; Osmocote, Marysville, OH, USA) was added to the surface of pots. In total, 94 plants were used in the experiment (see Table 1 for details of the crossing scheme). Plants were grown in a climate cell for 4 months (light 16 h, temperature 20/15 °C, relative humidity 70%), after which they were transferred to a cooled climate cell (light 8 h, temperature 4 °C, relative humidity 70%) for 13 wk of vernalization. After vernalization, plants were transferred to two climate cells (light 16 h, temperature 20/15 °C, relative humidity 70%). Throughout the experiment, plants were watered several times weekly, so that the soil was never saturated or extremely dry. Flowering began after 4 weeks, with *S. aquaticus* generally beginning to flower several days before *S. jacobaea* and hybrid individuals. In another experiment (unpublished data), we noticed that *S. aquaticus* will rarely flower without vernalization, while *S. jacobaea* absolutely requires a cold period. Only two plants, both *S. jacobaea* individuals, failed to flower. After initiation of flowering, plants were given 30 mL Steiner solution (macronutrients: N, 167 mg/L; P, 32 mg/L; K, 282 mg/L; S, 11 mg/L; Ca, 180 mg/L; Mg, 49 mg/L) weekly for 7 weeks.

Plants were paired so that for each genotype, one clone was crossed with each of the other plant groups (i.e., each *S. aquaticus* genotype was crossed with at least one *S. jacobaea* genotype and one hybrid genotype). We also tried to self F₁ genotypes (as a negative control), by pairing two clones with each other, to ensure that there was no breakdown of self-incompatibility mechanisms or contamination by pollen from nearby plants. We made five crosses between *S. jacobaea* genotypes, as a positive control. A summary of the types of crossings made is presented in Table 1. Pairs of individuals were placed adjacently in the climate cell and were crossed by rubbing flower heads together, at least every 5 days. Each plant was reciprocally crossed with only one partner.

**Flower counting**

In some cases, flowers could not be crossed because of pollen limitation from the partner plant or because one partner finished flowering before the other. In these cases, unpollinated flowers were removed from the plant when fully developed, and recorded separately. Number of (pollinated) flowers were counted during seed harvesting, such that the contents of each flower head was harvested, and number of flower heads were recorded.

**Pollen estimation**

To estimate total investment in pollen by each plant, we sampled three flower heads for pollen production. Flowers were selected randomly during mid-flowering, such
that a substantial number of flowers were open, but the majority of flowers had not begun to senesce. Stigmas emerge sequentially from the outer edge to the center of the flower, pushing pollen from the anthers to the flower surface, and flowers were sampled when stigmas at the center were freshly emerged. In such flowers, all pollen produced by the flowers was present on the flower surface.

The three flowers were dipped and shaken into one plastic 20-mL vial containing 95% ethanol. A number of flowers were examined with a microscope to ensure that all pollen was transferred to the vial. Vials were refrigerated at 4 °C until pollen analysis.

To measure the mass of pollen produced by each plant, we siphoned 15 mL of ethanol from the surface of vials and allowed the rest of the ethanol to evaporate in a fume hood at room temperature of 2 days. We then added 200 μL of 70% ethanol in two samples to wash pollen out of the 20-mL vials and transferred the

Table 1 Crossing classes (cc) resulting from pairwise crosses involving *S. jacobaea* (Sj), *S. aquaticus* (Sa), and F₁ hybrids (H). Codes listed at top and left represent genotypes used. Filled squares indicate that a cross between intersecting genotypes was made, and different shades indicate different crossing classes. For example, black squares indicate F₂-producing crosses (H-H). Full-sibs included in the crossing scheme include H3-H4, H7-H8, and H13-H14.

All other genotypes are unrelated to one another.
pollen–ethanol mixture to 0.2-mL Eppendorf tubes. The pollen mixture was cen-
trifuged for 15 min at $9.7 \times g$, so that pollen formed pellets in the tips of tubes. The 
ethanol was siphoned from the surface of each pellet using a micropipette, and the 
remaining drops of ethanol were removed with the tip of a tissue. Pollen samples 
were dried in a Speed-vac (Savant) at medium temperature for 60 min. After drying, 
the solid pellet of pollen was removed from the Eppendorf tubes using the tip of a 
needle, and weighed on a microbalance (Sartorius, Goettingen, Germany).

**Seed counting and weighing**

Total material collected from seed heads of each plant included seed, pappus, and 
dried petals. Seed-head material from each plant was homogeneously mixed, and 
three subsamples, approximately equal in volume, were taken from each plant. Subsamples, ranging from 0.1 to 0.4 g, were weighed, as was the remainder (bulk) 
of the seed-head material.

Seed from subsamples were separated from chaff, and the number of seeds was 
separately counted from each subsample. We made an estimate of the total num-
ber of seeds produced by multiplying the number of seeds per gram in subsamples by the total bulk mass of seed-head material. We then divided by the number of pol-
linated flowers to get an estimate of number of seeds per flower. To estimate the 
total potential seed production per plant, we multiplied the number of seeds per 
flower by the total number of flowers (including both pollinated and unpollinated 
flowers). In most cases, the number of unpollinated flowers ranged from 10 to 30% 
of the total flowers, but in rare cases, approached 50% of total flowers. We realize 
that there are sometimes tradeoffs between seed and flower production (and we 
found that this is the case here; see Results, Table 2). In these experiments, hybrids 
were most affected by this bias because hybrids (which produced the largest num-
ber of flowers) were more likely to have a larger proportion of unpollinated flowers. 
However, hybrids produced few seeds anyway, so that the trade-off was small and 
had a relatively tiny effect on estimates of whole plant flower and seed production.

From each plant, three replicates of 10 seeds were weighed on a microbalance. The three measurements were averaged to yield one estimate of seed mass per 
plant (the variation between replicates was extremely low).

**Biomass measurement**

After plants finished flowering and began to die back, we harvested above- and 
belowground plant material and carefully washed the plant roots. Plant material 
was dried in an oven for 2 days at 80 °C and was weighed to the nearest 10 mg.

**Statistical analyses**

All statistical analyses were carried out in SPSS, version 11.0 (SPSS, Chicago, IL, USA). We analyzed total seed production with regards to crossing class. Crossing classes included crosses producing $F_1$S, $F_2$S, backcrosses to *S. jacobaea* and *S. aquaticus*, and negative and pos itive controls. We analyzed plant mass, seed mass, pollen produc-
tion, and flower production with regards to plant group (*S. jacobaea*, *S. aquaticus*, 
and $F_1$ hybrids), and genotypes within plant group.
To test which kinds of crosses produce the most seeds, we summed the total (potential) number of seeds produced by both maternal plants in each cross. We defined each cross as selfed (S), F2 producing (H x H), F1 producing (Sj x Sa), backcross to S. aquaticus (Sa x H) backcross to S. jacobaea (Sj x H) or cross between two S. jacobaea genotypes (Sj x Sj). We then applied a one-way ANOVA using cross type (fixed factor) to the log-transformed value of total seeds, and used a Tukey post hoc test (Zar, 1999) to identify differences between groups.

We used multiple regression analysis (separately for each plant group) to identify the factors that influence number of flowers produced. All clones from all genotypes were included for each plant group. We tested the independent variables plant biomass, mean seed mass, and number of seeds per flower (or alternatively, total number of seeds produced). Backward elimination and forward selection methods (Zar, 1999) yielded consistent models in all cases.

We used mixed model nested ANOVAs to test for plant group and genotype differences in plant biomass, pollen production, and number of seeds per flower, and seed mass. For analysis of seed mass, we removed selfed plants from the data set, and we tested for both maternal and paternal effects of plant group (S. jacobaea, S. aquaticus, and F1 hybrids). For analysis of seed production (number of seeds per flower), the analysis does not incorporate all the complexity in the experimental setup, because genotypes were used in multiple crosses and some F1 genotypes were related to one another. Because the number of genotypes used here was limited, the results should be interpreted with some caution. However, we feel that that this caveat does not affect the conclusions we draw from the data presented here.

To compare the seed production of reciprocal crosses between groups, we used paired t tests on the number of seeds per flower, and on the potential seed production per plant.

RESULTS

Overall, there were large differences in potential seed production between different types of crosses (df = 5, F = 9.715, P < 0.001). We found that interspecific crosses (Sj...
× Sa) were most successful, producing significantly more seeds even than Sj × Sj crosses (Fig. 1). The trend showed that backcrosses (Sj × H and Sa × H) were about 1/3 as successful as interspecific crosses (Sj × Sa) and that F₂-producing crosses (H × H) were about four times less successful than backcrosses (Fig. 1). The variation within cross classes is very high though, because the success of individual crosses was highly dependent on interactions between genotypes. For example, one cross between two individual S. jacobaea genotypes produced no seeds, while these genotypes were completely fertile in other crosses. We found similar results in all other categories, such that some backcrosses produced more seed than the most productive interspecific crosses, which suggests that at least some backcrosses, and even some F₂ producing crosses, will be highly successful, even if the mean seed production in these crossing classes is low (i.e., Fig. 1).

Although biomass significantly differs between plant groups and according to genotype (Fig. 2A, Table 3), we found that plant biomass never influenced the number of flowers produced within plant groups. This result likely arose from a low amount of variation in biomass within plant groups, which was a result of standardized growth conditions. Plant biomass was thus excluded as a covariate in further ANOVA analyses. We also found that for all plant groups, total seeds per flower explained more variance in flower number and was more significant than total seeds produced, and we therefore discarded total seeds produced from the regression analyses. For S. jacobaea and hybrids, total flower number was dependent on seed mass, while total seeds per flower influenced total number of flowers produced by S. aquaticus and hybrids (Table 2). Overall, the amount of variance in flower number explained by the best model for S. jacobaea (adjusted $R^2 = 0.191$) was much lower than those for S. aquaticus (adjusted $R^2 = 0.468$) and hybrids (adjusted $R^2 = 0.538$), suggesting that flower number is more genetically controlled in S. jacobaea.
and less susceptible to plastic change (also see ANOVA results (Table 3), which show that flower number depends on genotype).

On average, we found that hybrids produced about 25% more flowers than parental species, followed by *S. aquaticus*, and then *S. jacobaea* (Fig. 2B, Table 3). The greater number of flowers produced by hybrids might be explained by the greater size of the plants (although we found that plant biomass never influenced flower number within plant groups, variation in biomass was greater between groups). Hybrids also likely produced more flowers because they produced fewer seeds per flower than parental species.

There was no significant effect of plant group on pollen production, but pollen production differed significantly according to genotype (Table 3). One *S. jacobaea* genotype (Sj3), for example, produced almost no pollen (consistently over five clones), and crosses with this genotype had to be removed from analyses involving seed production because pollen was extremely limiting in these crosses. The range in pollen production per flower was greater among *S. jacobaea* genotypes (0.18–2.48 mg/flower) than among *S. aquaticus* (0.56–1.88 mg/flower) or hybrids.

### Table 3
Results of mixed model nested ANOVA tests to identify effects of plant groups (*S. jacobaea*, *S. aquaticus*, and F1 hybrids) and genotypes within plant groups [genotype (group)] on plant biomass, flower number, pollen production.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Factor</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>Plant group</td>
<td>2</td>
<td>1168</td>
<td>3.734</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>Genotype(group)</td>
<td>20</td>
<td>332.6</td>
<td>2.183</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>73</td>
<td>152.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flower number</td>
<td>Plant group</td>
<td>2</td>
<td>6.084*10^5</td>
<td>9.082</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Genotype(group)</td>
<td>20</td>
<td>7.109*10^4</td>
<td>2.126</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>73</td>
<td>3.344*10^4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pollen</td>
<td>Plant group</td>
<td>2</td>
<td>0.177</td>
<td>0.104</td>
<td>0.902</td>
</tr>
<tr>
<td></td>
<td>Genotype(group)</td>
<td>20</td>
<td>1.190</td>
<td>1.995</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>65</td>
<td>0.596</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figure 2* Total biomass, total flower production, pollen production, and seed mass of *S. aquaticus*, *S. jacobaea*, and F1 hybrids. Different letters indicate significant differences between groups (Tukey test, *P* < 0.05), NS indicates no significant differences, and error bars represent standard error.
(0.89–1.96 mg/flower). Hybrids produce on average more pollen over the whole plant than parental species, because the number of flowers was greater in hybrids than parents (Fig. 2B), while pollen production was equal (Fig. 2C).

To test whether seed production (seeds/flower head) was asymmetrical in reciprocal crosses (Fig. 3), we used a paired t test to test for pairwise differences. We found no reciprocal differences in *S. aquaticus* × hybrid crosses (*t* = 1.026, df = 9, *P* = 0.332), or *S. jacobaea* × hybrid crosses (*t* = 1.515, df = 9, *P* = 0.164), but *S. aquaticus* produced significantly more seeds than *S. jacobaea* in reciprocal crosses (*t* = 2.762, df = 8, *P* = 0.024). Production of F₁ seeds by *S. aquaticus* vs. *S. jacobaea* is also likely increased by the trend that *S. aquaticus* produces more flowers than *S. jacobaea* (Fig. 2A).

Regarding seed mass, we found that maternal species and genotype within maternal species were significant determining factors, with no paternal species or genotype effects, and no interaction between maternal and paternal species (Table 4). *Senecio aquaticus* mothers produced the largest seeds, followed by hybrids, and lastly *S. jacobaea* (Fig. 2D). We also found an extremely low level of seed production in some selfed-hybrid crosses. The mass of such putatively selfed seeds was lower than that of outcrossed seeds, which supports the conclusion that such seeds result from selfing, and not from pollen contamination by other genotypes.

**Table 4** Results of mixed model nested ANOVA tests to identify effects of maternal group (mother), paternal group (father), and genotypes within maternal and paternal groups on seed mass.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Factor</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed mass</td>
<td>Mother</td>
<td>2</td>
<td>8.069</td>
<td>10.420</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Genotype (mother)</td>
<td>20</td>
<td>0.854</td>
<td>3.257</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>2</td>
<td>0.330</td>
<td>1.461</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>Genotype (father)</td>
<td>20</td>
<td>0.219</td>
<td>0.835</td>
<td>0.656</td>
</tr>
<tr>
<td></td>
<td>Mother × father</td>
<td>3</td>
<td>0.134</td>
<td>0.511</td>
<td>0.679</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>23</td>
<td>0.262</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3** Seed production per flower in reciprocal crosses between F₁ hybrids (H) and *S. aquaticus* (A; *N* = 10), F₁ hybrids and *S. jacobaea* (J; *N* = 10), and *S. aquaticus* and *S. jacobaea* (*N* = 9). Error bars represent standard error. * Significant difference (*P* < 0.05, paired t test).
DISCUSSION

Clearly, initial crosses between *S. jacobaea* and *S. aquaticus* are highly successful, and result in the production of many seeds. Previous experiments have shown that F₁ seeds are viable, and seedlings grow heterotically (extremely vigorously) (Kirk *et al.*, 2005b). Furthermore, F₁ hybrids are fertile, and there is thus high potential for gene flow to continue to later generation hybrids after the initial crosses in nature. The lower mean success of crosses involving F₁s does not necessarily mean that gene flow in general is restricted after the F₁ stage, since some crosses remain highly productive, and on average even F₂ producing crosses yield more than 500 seeds. Genotype dependence of cross success has been found in other studies as well; for instance, high variability in the fertility of early generation hybrids was dependent on parental genotypes of *Microseris douglasii* and *M. bigelovii* (Bachmann & Hombergen, 1997). Also, other authors have shown that lost fertility in early generation hybrids is quickly regained in subsequent generations (e.g., Wang *et al.*, 2001).

It is not surprising that on average, seed production in F₂-producing crosses and backcrosses is lower than that of initial crosses, since backcrossing and intercrossing leads to segregation of genes, which can have many negative genetic consequences. Inability to produce embryos in hybrids (or embryo abortion) may result from, among other factors, chromosome rearrangements during meiosis in F₁s (Grant, 1981), the breakup of co-adapted gene complexes (Dobzhansky, 1933; Muller, 1942), or incompatibility of nuclear genes with a new cytoplasmic environment (Cruzan & Arnold, 1999). Other authors have reported similar findings; Song *et al.* (2004) for example, reported that reproductive success of F₁ hybrids between *Oryza rufipogon* and *O. sativa* was lower than that of parents, while vegetative success was higher.

Male function seems to be quite robust in hybrid individuals here. While we did not explicitly test pollen viability, our results showed that it is unlikely that pollen is significantly less viable in F₁ hybrids than in parents, because seed production was equal in both hybrids and parents in reciprocal crosses. Reduced reproductive success in parent-hybrid crosses most likely resulted from genomic incompatibilities. The greater size of hybrids in general and the significantly higher number of flowers in hybrids might also lead to increased attractiveness to pollinators. Other studies have shown that frequency of pollinator visitation is often correlated with size of plants and number of flowers (Klinkhamer & de Jong, 1990). This hypothesis should be tested in the field, though, since there is significant morphological variation in flower structure between parents, and also between hybrids and parents (H Kirk, unpublished data), which may also have a strong impact on plant–pollinator interactions.

Based on the results of this experiment, we might predict that hybrids are more likely to arise from *S. aquaticus* mothers, since *S. aquaticus* produce more seeds in reciprocal crosses with *S. jacobaea*. Also, seeds produced by *S. aquaticus* mothers are larger than those from *S. jacobaea* mothers, and we might expect that offspring from *S. aquaticus* might have a fitness advantage in early life stages. Yet, advantages to hybrids derived from *S. aquaticus* mothers may be balanced out by later vegeta-
tive growth advantages for $F_1$ hybrids arising from $S. jacobaea$ mothers (Kirk et al., 2005b). However, Kirk et al. (2005b) controlled for seed effects by using small, equally sized clonal plants. It would be interesting to study whether selection in the natural hybrid zone favors one maternal species over the other, since reproductively, $S. aquaticus$ mothers are most fit, while $S. jacobaea$ mothers produce the most fit offspring with regards to vegetative growth.

These results lead to many fascinating questions about the ecology of the natural hybrid population from which the parental plants were derived. How do pollinators affect the success of interspecific hybrids in nature (e.g., Campbell et al., 1997)? Can pollen competition influence the success of conspecific pollination, as found in other hybridizing species (e.g., Brassica, Pertl et al., 2002; Ipomopsis, Campbell et al., 2003)? Also, what is the evolutionary impact of hybridization within the genus Senecio? This last question may be of ultimate importance for explaining both the species and phenotypic diversity found within the genus.

Acknowledgements

The authors thank Kevin van den Hof, Henk Nell, Mieke Piekaar, Hans de Heiden, and Karin van der Veen for technical assistance. Also thanks to Mike Arnold and one anonymous reviewer for useful comments on the manuscript. Heather Kirk thanks the Natural Sciences and Engineering Council of Canada (NSERC) for financial support of her research.

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Reproductive fitness of hybrids


