Hybrid vigor in the biological control agent, *Longitarsus jacobaeae*

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Abstract
Hybridization is an important evolutionary mechanism that can increase the fitness and adaptive potential of populations. A growing body of evidence supports its importance as a key factor contributing to rapid evolution in invasive species, but the effects of hybridization have rarely been assessed in intentionally introduced biological control agents. We investigated hybrids between a Swiss and an Italian population of the beetle, *Longitarsus jacobaeae*, a biological control agent of *Jacobaea vulgaris*, by reciprocally crossing individuals in the laboratory. Phenological traits of F1 and F2 hybrid lineages showed intermediate values relative to their parental populations, with some maternal influence. Fitness of the F2 generation, measured as lifetime fecundity, was higher than that of the Italian parent in one of the lineages and higher than that of either parent in the other hybrid lineage. The increased fecundity of hybrids may benefit tansy ragwort biological control by increasing the establishment success and facilitating a more rapid population buildup in the early generations. Even though the long-term consequences of hybridization in this and other systems are hard to predict, intentional hybridization may be a useful tool in biological control strategies as it would promote similar microevolutionary processes operating in numerous targeted invasive species.

Introduction
Hybridization (admixture) between individuals from genetically distinct populations or taxa can be an important mechanism during the adaptation of species to new environments (Stebbins 1959; Arnold 1997; Ellstrand and Schierenbeck 2000; Schierenbeck and Ellstrand 2009). It may facilitate rapid evolution by increasing genetic variation in admixed populations, which then may be able to respond better and faster to novel selection pressures (Stebbins 1959; Arnold 1997). Furthermore, recombination of genomes from different source populations creates an array of novel genotypes with novel trait combinations, a few of which may be better adapted to new environments than any parental genotypes (Stebbins 1959; Arnold 1997). In addition, hybridization can alleviate genetic load caused by inbreeding in isolated populations, resulting in an immediate increase in fitness (Ellstrand and Schierenbeck 2000).

Multiple introductions of propagules from genetically divergent native populations often result in admixture in the introduced range (Novak and Mack 2005), which has been hypothesized to contribute to rapid evolution and an increase in the invasive potential in exotic species (Ellstrand and Schierenbeck 2000). While numerous examples provide indirect evidence in support of this hypothesis (reviewed in Schierenbeck and Ellstrand 2009), Whitney et al. (2009) did not find a causal relationship between hybridization and invasiveness in vascular plants. Nonetheless, a few recent case studies involving a wetland grass (Lavergne and Molofsky 2007), a freshwater snail (Facon et al. 2008), an ornamental tree (Culley and Hardiman 2009), and a perennial plant species (Keller and Taylor 2010) were able to establish a direct connection between hybridization and the evolution of invasiveness.

Management of invasive species commonly entails the intentional introduction of host-specific natural enemies...
from the native range of exotic weeds or pests (classical biological control) (DeBach 1964). In the past, biological control agents were often collected from distinct populations in the native range to increase genetic diversity through admixture, and thus promote adaptation post-release that may increase the establishment success and control potential (DeBach 1964; Lewontin and Birch 1966; Legner 1972; Mackauer 1972; Messenger et al. 1976; Marshall et al. 1980; DeBach and Rosen 1991; Hopper et al. 1993). However, there has been no consensus whether to propagate and release agents from genetically distinct populations independently (Lewontin 1965; Force 1967; Whitten 1970) or to merge them pre- or immediately post-release (Messenger et al. 1976). Empirical evidence providing support for either recommendation is sparse (Roderick and Navajas 2003; Hufbauer and Roderick 2005), since the fitness of pure versus hybrid populations (Legner 1972; Mathenge et al. 2010) or the importance of post-release adaptation has only rarely been assessed in biological control agents (Hufbauer 2002; Phillips et al. 2008; Szücs et al. 2012). The only study that evaluated post-introduction adaptation in a biological control program, in which parasitoids (Microctonus hyperodae Loan) from genetically distinct populations were introduced sympatrically, found that adaptive evolution had occurred and resulted in improved control (Phillips et al. 2008).

The practice of collecting from and allowing hybridization between different populations of agents came under scrutiny as evidence for significant intraspecific differences in ecological traits, especially in host specificity, accumulated (Sands and Harley 1980; Messing and Aliniazee 1988; DeBach and Rosen 1991; Hopper et al. 1993; Hoffmann et al. 2002; Goldson et al. 2003; Mathenge et al. 2010). For example, two biotypes of Dactylopius opuntiae (Cockerell) were found to be specific to two different invasive Opuntia species (Hoffmann et al. 2002). Upon hybridization, the F1 generation was able to attack either host, while both host-specific and non-host-specific genotypes were produced in the F2 generation potentially reducing effectiveness of biological control in monocultures of either weed (Hoffmann et al. 2002). Hybridization between two biotypes of the hymenopteran parasitoid Microtusus aethiopoides (Loan), both specific to different invasive weevils, also produced progeny that was inferior biological control agents for both species (Goldson et al. 2003). These findings resulted in tighter regulations in New Zealand, setting a precedent, and allowing only the importation of one biotype of M. aethiopoides (Barratt et al. 2010).

As outlined above, the effects of hybridization may be positive or negative; moreover, these effects may depend on genetic and environmental factors, may change over time, and a combination of outcomes may be present in the same generation (Arnold and Hodges 1995; Erickson and Fenster 2006; Johansen-Morris and Latta 2006; Hwang et al. 2011). First generation hybrids may experience hybrid vigor (heterosis) because of heterozygote advantage (overdominance) or the masking of deleterious alleles (Lynch 1991; Edmands 2002). On the other hand, hybrid breakdown or outbreeding depression may result from underdominance (heterozygote disadvantage) or the disruption of coadapted gene complexes in the F2 or later generations and backcrosses (Dobzhansky 1950; Mayr 1963; Andersen et al. 2002). In addition, interaction between genes (epistasis) can affect fitness of hybrids both positively and negatively (Lynch 1991; Edmands 2002). The prevalence of the different outcomes is not well known, since the majority of studies measure fitness of F1 generation hybrids only (Edmands 2002), even though it is commonly recognized to be a weak predictor of fitness in the F2 and later generations when hybrid breakdown becomes more relevant through recombination (Edmands 1999). Long-term studies revealed that many (Erickson and Fenster 2006; Hwang et al. 2011) or at least a few hybrid genotypes (Johansen-Morris 2006) in a population can have superior fitness in later generations (but see Johnson et al. 2010).

With this study, we aim to document the effects of hybridization among different introduced populations of an established biological control agent in North America. An Italian biotype of the chrysomelid beetle Longitarsus jacobaeae (Waterhouse) has become widespread in the western US since its introduction in 1969, and provides successful biological control against the invasive tansy ragwort (Jacobaea vulgaris Gaertn., formerly Senecio jacobaeae L.) in most areas (McEvoy et al. 1991; Coombs et al. 2004). However, beetles collected from coastal Oregon have proved difficult to establish under more continental climates in eastern Oregon (Coombs et al. 1996) and in high-elevation alpine regions in Montana (Littlefield et al. 2008). Beetles collected from a high-elevation site on Mt. Hood, Oregon, managed to establish at high-elevation sites in Montana but seemed to have been restricted to moister areas (Littlefield et al. 2008). In hope of achieving quicker control and more widespread establishment, a cold adapted biotype of the beetle was introduced from Switzerland in 2002 (Littlefield et al. 2008). The host range of both biotypes is very narrow and similar, limited to a few species within the new genus Jacobaeae and the old genus Senecio s.l. (Frick 1970; K. P. Puliafico unpublished; Pelser et al. 2007), and thus, the potential for changes in their host specificity upon hybridization has not been considered.

A recent study using molecular markers detected natural hybridization between the two biotypes in Montana, where both had been released in proximity (Szücs et al. 2011). Surprisingly, hybrid individuals constituted 47% of the sample at one location despite the different life histories of the individual Italian and Swiss biotypes that would only
were kept in an unlit greenhouse at 22 °C. On May 3, 2006, April 17, 2007, and June 2, 2008. Plants infested tansy ragwort plants with some surrounding soil at Salem, Oregon (44°N, 122°W), by digging up infested tansy ragwort plants with some surrounding soil on May 3, 2006, April 17, 2007, and June 2, 2008. Plants were kept in an unlit greenhouse at 22 ± 3°C day (14 h) and 12 ± 3°C night (8 h) temperatures at Moscow, Idaho, in plastic mesh covered cages (65 × 40 × 40 cm) until adult emergence. Swiss beetles were collected from a laboratory (2006 and 2007) and common garden (2008) rearing maintained at the University of Idaho, in Moscow, Idaho (46°44′N, 117°01′W), since 2004. Foundling beetles for the rearing originated from St. Imier (47°09′N, 6°59′E) and Mettembert (47°24′N, 7°20′E), Switzerland, and were imported to Montana in 2002, and from there to Idaho in 2004 (Littlefield et al. 2008). The laboratory rearing of Swiss beetles involved collection of eggs (4000–9000) from at least 100 females during July and August in 2005 and 2006. Eggs were kept in Petri dishes on moist filter papers at 2°C until the end of next April in both years. Eggs hatched within a week upon removal from the cold room, and larvae were transferred with a fine paint brush onto potted tansy ragwort plants between May 2 and 11, 2006 and May 2 and 14, 2007, respectively. Each plant was infested with about 50 larvae and kept in a greenhouse in cages until adult emergence. For the common garden rearing in 2008, at least 20 adults were released onto potted tansy ragwort plants, covered with a translucent mesh cage, in the fall and embedded in mulch during the winter. Each year, between 200 and 2000 adults emerged from either parental populations. All tansy ragwort plants used for rearing and experiments were grown from seeds collected at an infestation at Meadow Creek in Benewah County, Idaho (47°02′N, 116°44′W). The Italian and Swiss ancestry of the experimental populations was confirmed by molecular analyses (Szücs et al. 2011).

Two hybrid lines were established by crossing the Italian and Swiss populations to each other reciprocally (female Swiss × male Italian, FemSW; and female Italian × male Swiss, FemIT). Italian beetles, emerging between 22 and 25 June, and Swiss beetles, emerging between June 29 and July 1, 2006, were used for crossing. Fifty mating pairs were setup for each hybrid line on July 1, 2006 in transparent plastic cylinders (0.22 L volume), using freshly emerged adults collected twice daily from infested tansy ragwort plants from the rearing described above. One tansy ragwort leaf inserted in a moist cube of horticultural sponge served as food and as oviposition substrate. Cylinders, kept in a laboratory at room temperature under natural photoperiod, were checked twice weekly for eggs upon which fresh foliage and new horticultural cubes were provided. Egg collections continued until October 11, 2006 for the FemSW and until November 10, 2006 for the FemIT lines. The storage of eggs, transfer of larvae, and collection of adults followed the protocol described above for laboratory rearing of Swiss beetles. A common garden rearing of hybrids was also initiated using mating pairs from the cylinders following the methods described for the Swiss beetles. Eight potted tansy ragwort plants were infested each with two pairs of beetles for each hybrid line on October 11, 2006. This

Materials and methods

Study species

The ragwort flea beetle, *L. jacobaeae* (Coleoptera: Chrysomelidae), is a univoltine herbivore native to Eurasia (Shute 1975). The beetles show distinct phenologies in different geographic and climatic regions in the native range (reviewed by Windig 1991). We focus on the Italian and Swiss biotypes, which were introduced in the western US to control *Jacobaeae vulgaris*, and recently hybridized in nature in Montana (Szücs et al. 2011).

The two biotypes are morphologically identical but show major differences in their phenologies (Frick 1971; Frick and Johnson 1973; Puliafico et al. 2008), which are likely to be adaptations to the prevailing climatic conditions in their respective areas of origin. Adults in Italy emerge in late spring to early summer, and after a brief feeding period enter a dormant stage throughout the hot and dry summer, called aestival diapause. They become active once temperatures cool and rain recommences in the fall. Most eggs are laid in the fall but adults are also able to overwinter and oviposit the following spring. Eggs laid around the root crowns of tansy ragwort rosettes hatch within 3 weeks. Larvae feed in the roots and petioles during fall, winter, and spring, developing through three larval instars (Frick and Johnson 1973). In Switzerland, where the summers are cooler and moister, adults emerge mid-summer and start oviposition within 2 weeks, which will continue until their death in late fall. Eggs diapause through the winter and only hatch the next spring (Puliafico et al. 2008). Thus, the life span, and egg and adult diapause characteristics differ markedly between the two biotypes.

Rearing and crossing

Italian *L. jacobaeae* were collected from a grazed meadow at Salem, Oregon (44°52′N, 122°57′W), by digging up infested tansy ragwort plants with some surrounding soil on May 3, 2006, April 17, 2007, and June 2, 2008. Plants were kept in an unlit greenhouse at 22 ± 3°C day (14 h) and 12 ± 3°C night (8 h) temperatures at Moscow, Idaho, and larvae were transferred with a fine paint brush onto potted tansy ragwort plants between May 2 and 11, 2006 and May 2 and 14, 2007, respectively. Each plant was infested with about 50 larvae and kept in a greenhouse in cages until adult emergence. For the common garden rearing in 2008, at least 20 adults were released onto potted tansy ragwort plants, covered with a translucent mesh cage, in the fall and embedded in mulch during the winter. Each year, between 200 and 2000 adults emerged from either parental populations. All tansy ragwort plants used for rearing and experiments were grown from seeds collected at an infestation at Meadow Creek in Benewah County, Idaho (47°02′N, 116°44′W). The Italian and Swiss ancestry of the experimental populations was confirmed by molecular analyses (Szücs et al. 2011).

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rearing served as a backup to our laboratory rearing and was used to start the F2 hybrid generation and to increase the number of adults available for experiments. F1 adults of the FemSW and the FemIT lines emerged from the common garden starting June 30 and July 1, 2007, respectively, were placed in 1.5 L volume plastic cylinders and allowed to mate randomly with all other adults from the same line (n = 74 for FemSW and n = 39 for FemOR). Mated beetles were used to start the F2 generation by infesting four potted tansy ragwort plants with five pairs of FemSW F1 hybrids on July 17, 2007 and an additional four plants with two pairs of FemSW F1 hybrids and two pairs of FemIT F1 hybrids each on August 27, 2007, yielding 776 FemSW and 295 FemIT F2 adults the following year. Emergence data from the common garden rearing were not used for any comparisons of F1 or F2 hybrid performance because, by the time of setup, the beetles were past their prime fecundity.

Aestival diapause

Aestival diapause characteristics of the parental populations and the F1 and F2 hybrid lines were assessed in an environmental chamber by measuring the time between adult emergence and first oviposition. Replications (n = 4 in 2007 and n = 5 in 2008) consisted of 5 pairs of newly emerged adults each, placed in 1.3 L volume plastic cylinders with tansy ragwort leaves inserted into a moist horticultural sponge. Cylinders were randomly placed in an environmental chamber and checked for eggs twice weekly. Upon each monitoring, foliage and horticultural cubes were replaced, and the cylinders re-randomized. Photoperiod in the environmental chamber was adjusted once a week to follow the natural cycle as experienced in Moscow, Idaho, while temperatures were left constant, at 25 ± 1°C and 15 ± 1°C (L:D). This setting ensured continuous daylight conditions for each population since all adults used for experiments emerged in Moscow. Moreover, the photoperiod regime in Moscow is close to that of the parental populations’ sites of origin (latitudes are within 2°). Photoperiod conditions are important because it is the most reliable cue for many insects for aestival diapause induction (Tauber et al. 1986; Danks 1987), including L. jacobaeae as demonstrated by Frick and Johnson (1973). The experiment started on 14 July (L: 15.5 h; D: 7.5 h) both in 2007 and in 2008, once a sufficient number of beetles emerged, and ended once at least 50 eggs were found.

Lifetime fecundity and life span

Lifetime fecundity and life span of the parental populations and the F2 reciprocal hybrids were measured in the 2008/2009 season using the same environmental chamber, the same settings, and methods as described above. Ten replications were setup for each population in plastic cylinders of 0.22 L volume on July 14, 2008, each with one pair of newly emerged adults. Egg numbers and adult survival were monitored once a week until the last beetle died in May 26, 2009. Males were transferred between cylinders to ensure that females were not left without a mate in case their partner died. One replicate for the parental Swiss and two replicates for the FemIT populations each had to be excluded from statistical analyses because of reproductive impairment (only two deformed eggs laid) and accidental death of females, respectively.

Egg diapause, viability, and size

Egg diapause characteristics and viability of the Italian parental population (Salem, OR) and the F2 reciprocal hybrid lines were tested at three temperatures (2, 4, and 6°C). The Swiss parental population is not included because, by the time, we were able to start the experiment (October 2008), most Swiss females died or stopped laying eggs. However, for better statistical power, we included an additional Italian population, which originated from the Oregon coast, three kilometers north of Neskowin (45°07′N, 123°57′W) from Scherzinger road. Eggs, laid by females participating in the above-described fecundity experiment, were collected between 30 September and October 15, 2008. Four replications per population (Salem, Scherzinger, F2 FemSW, and F2 FemOR) were setup, each consisting of a Petri dish with 20 eggs on a filter paper for the 2°C, 38–60 eggs for the 4°C, and 37–56 eggs for the 6°C temperature treatments. Two separate walk-in climate rooms were used for the 2 ± 0.5°C and 4 ± 0.5°C and a refrigerator for the 6 ± 2°C temperature treatments. Egg hatch was monitored weekly until June 2009. A few Petri dishes (replicates) contracted an unknown fungus that killed all eggs within. Therefore, one Salem (Italian parent) replicate at 6°C, two Salem and one Scherzinger (other Italian) replicates at 4°C, and one Salem replicate at 2°C had to be excluded from analyses. Egg sizes (longest diameter) of both parental and F2 reciprocal hybrid populations (n = 100) were measured at 40× magnification under the microscope using an ocular ruler to the closest 0.033 mm during summer 2008.

Statistical analyses

Analysis of variance (ANOVA) was used to assess differences among populations in length of aestival diapause, lifetime fecundity, life span, egg size, as well as egg viability at the three experimental temperatures. The length of adult aestival diapause was compared among populations accounting for year effects. As there were no significant
population × year effects, noted the data were pooled for graphical presentation. Egg count data for the fecundity experiment were log-transformed, and percentages for egg viability were square-root transformed to meet assumptions of tests. The length of egg diapause was assessed by comparing egg hatch proportions of populations over time using a generalized linear model, assuming binomial distribution and a logit link. The model was formed as a split-plot in time repeated measures design with four populations (Salem, Scherzinger, F2 FemSW, and F2 FemIT) and five times (months 2, 3, 4, and 5). All statistical analyses were conducted in SAS 9.2 (SAS Institute, Cary, NC, USA) using PROC GLM for ANOVA and PROC GENMOD for maximum likelihood procedures.

Results

In the FemSW crosses, Swiss females began laying eggs on 20 August, while, in the FemIT crosses, the Italian females did not start oviposition until September 20, 2006. Forty-two percent of the mating pairs in the FemSW and 40% percent in the FemIT crosses did not lay any eggs or laid only one egg. Overwintering survival of eggs, defined as the proportion of all collected eggs that did not acquire mold during the winter and looked healthy at the time of removal from the cold room, was similar for the two hybrid lineages and for the Swiss parental population (95–97%). The proportion of eggs hatching was lowest for the FemIT line (12.6 ± 2.4%; mean ± SE), intermediate for the FemSW line (40.4 ± 2.7%), and highest for the Swiss parental population (63.8 ± 1.6%). Following transfer of F1 larvae to potted tansy ragwort plants, 33.5 ± 5.2% of larvae emerged as adults in the FemSW (n = 268), 33.5% in the FemIT lineages (n = 54), and 52.5 ± 2.8% in the Swiss parental population (n = 1050).

The length of aestival diapause differed significantly among the hybrid lines and the parental populations in both years (F1 in 2007; F3,12 = 21.93, P < 0.0001; F2 in 2008; F3,16 = 486.75, P < 0.0001), with the populations showing similar characteristics between years (population*year interaction: F3,21 = 1.49, P = 0.2474; year: F1,23 = 15.42, P = 0.0057; Fig. 1). Swiss females laid eggs about 2 weeks after emergence, while Italian females began to lay eggs more than 7 weeks after emergence. The two hybrid lines showed intermediate characteristics, relative to their parents, with the FemSW beetles starting oviposition approximately 3 weeks and the FemIT beetles within 4 weeks following emergence (Fig. 1).

There were significant differences among populations in fecundity (F3,34 = 7.96, P = 0.0004). Females of both F2 hybrid lines laid more eggs than the Italian parental population, and the FemSW hybrids laid more eggs than the Swiss parental population as well (Fig. 2). The life span of males and females was similar (P > 0.05 for all pairwise comparisons within populations) but differed significantly among populations (females: F3,33 = 5.91, P = 0.0024, males: F3,28 = 5.04, P = 0.0064; Fig. 3). In general, Swiss beetles died before the winter, while both hybrid lines and the parental Italian beetles survived until the following spring.

Egg viability (percentage hatched) did not differ significantly among the parental Italian (Salem), a second Italian (Scherzinger), and the two F2 hybrid populations at either of the tested temperatures (2°C: F3,11 = 1.65, P = 0.2355; 4°C: F3,9 = 2.29, P = 0.1475; 6°C: F3,11 = 3.21, P = 0.0658). Eggs from all study populations diapaused during the winter in the two lower temperature treatments (2 and 4°C) and only began hatching in mid-May 2009. Even though

![Figure 1](image1.jpg)  
**Figure 1** Pooled averages for 2007 (F1 generation) and 2008 (F2 generation) data comparing the length of aestival diapause (time between emergence and first oviposition) in days (mean ± 1SE) of the two parental (Swiss and Italian) and the two reciprocal hybrid (Swiss female = FemSW and Italian female = FemIT) *Longitarsus jacobaeae* populations. Different letters on top of bars indicate significant difference at 95% confidence.

![Figure 2](image2.jpg)  
**Figure 2** Lifetime fecundity (mean ± 1SE) of reciprocal F2 hybrids (Swiss female = FemSW and Italian female = FemIT) and their parental (Swiss and Italian) *Longitarsus jacobaeae* populations. Different letters on top of bars indicate significant difference at 95% confidence based on log-transformed data.
Figure 3 Longevity of reciprocal F2 hybrids (Swiss female = FemSW and Italian female = FemIT) and their parental (Italian and Swiss) *L. itarsus jacobaeae* populations in days (mean ± 1SE). Different letters on top of bars indicate significant difference at 95% confidence.

we did not use the Swiss parental population in this particular experiment, our regular rearing over the years showed that eggs of Swiss beetles also diapause during the winter when kept at 2°C. The length of egg diapause differed significantly among populations when kept at 6°C (population*time interaction: \( \chi^2 = 383.59, P < 0.0001 \)). Eggs laid by the two Italian populations had a very short diapause period with most viable eggs (93–97%) hatching within 2 months (by December 10, 2008). In contrast, by the same time, only 37% FemIT and 11% FemSW eggs hatched. FemIT eggs had a longer diapause with more than half (63%) of the eggs hatching within 4 months and all of them within 5 months (March 3, 2009). Eggs laid by FemSW beetles had the longest diapause with only 27% hatching within 4 months, 88% within 5 months, and all by the end of March (6 months).

Egg sizes differed significantly among populations (\( F_{5,396} = 36.74, P < 0.0001 \)). Eggs from the Swiss biotype were significantly larger (0.797 ± 0.0046; mean ± 1 SE) than eggs of the FemSW (0.78 ± 0.0041) and the FemIT (0.78 ± 0.004) hybrid lines. Italian eggs (0.74 ± 0.0033) were smaller than the Swiss and the hybrids based on non-overlapping 95% confidence intervals.

Discussion

Our results provide evidence for increased vigor in the progeny of crosses between genetically and phenotypically divergent populations of the biological control agent *L. jacobaeae*. We found that both F2 hybrid lineages had a higher fitness (fecundity) than the Italian parent, and the FemSW hybrids also had a higher fitness than the Swiss parent. Overall, FemSW hybrids laid about 130% more, and FemIT hybrids about 50% more eggs than beetles from either parental population. The increased fitness was likely facilitated by the fact that both hybrid lineages inherited the longer life span of Italian parents and consequently were able to lay eggs over a longer period of time.

We found evidence for some level of prezygotic isolation upon pairing, which probably arises from phenological differences in the reproductive timing of the biotypes. Swiss beetles mate and lay eggs 2 weeks after emergence from mid-summer to fall, while Italian beetles aestivate during the summer and do not mate until fall. Reproductive timing of beetles used for reciprocal crossing was in agreement with the described life history characteristics of the biotypes (Frick 1971; Frick and Johnson 1973). Swiss females paired with Italian males were not able to lay eggs until late August, likely because Italian males aestivated and thus would not mate during the summer. In the reciprocal pairing, Italian females were not receptive for mating until September despite the presence of active Swiss males. Probably due to these behavioral differences, about 40% of the mating pairs in either reciprocal cross did not lay any eggs by mid-October. Egg viability of both F1 hybrid lineages was lower than that of the Swiss parent, which may be due to pre- or postzygotic isolation (Edmands 2002). However, this phenomenon seems to have been transient as egg hatch rates of F2 hybrids did not differ from those of the Italian parent at any of the three tested temperatures.

Most measured traits such as egg size, the length of adult aestivation, and egg diapause (at 6°C) showed intermediate values with some maternal influence for both hybrid lineages in the F1 or F2 generations when compared to the parental biotypes. These results are commonly found in crosses of insect biotypes and typically attributed to polygenic inheritance, some sex-linkage, and high additive genetic variance governing life history traits, which leads to intermediate expression of traits in the hybrids often with phenotypes more closely resembling either parent (e.g., Danilevskii 1965; Hoy 1975, 1978; Bradshaw and Lounibos 1977; Dingle et al. 1982; Tauber et al. 1986; Hard et al. 1993; but see Demont and Blankenhorn 2008). We believe that the detected maternal effects are most probably genetically based since F1 and F2 hybrid lines were reared under the same environmental condition.

Our assessment of hybrid vigor in the F2 generation suggests that increased fitness may characterize future hybrid generations of *L. jacobaeae* as well; however, outbreeding depression due to the disruption of coadapted gene complexes may only become apparent in later generations or may only manifest under field conditions. High hybrid fitness in the early generations can contribute to biological control success by facilitating a more rapid population buildup during the initial years post-release. The higher fecundity of hybrids relative to parental biotypes may explain the high proportion of hybrid individuals (47%)
found in one of the study sites in Montana (Szűcs et al. 2011). Alternatively, the high proportion of hybrids at that field site may reflect higher availability and activity of hybrids at the time of sampling in mid-October. By that time, most Swiss beetles may have already died, but hybrids may still be widespread and active because of their differing life history and their increased life span compared to the Swiss biotype. The two explanations are not mutually exclusive, and thus, both processes may be responsible for high hybrid abundance at the field site in Montana. Even though sexual recombination can erode heterosis over time, the fitness of early generation hybrids is still important in predicting the long-term consequences of hybridization (Johansen-Morris and Latta 2006; Johnson et al. 2010). Since early generation hybrids with high fitness will produce numerous offspring with novel genotypes and trait combinations, chances increase that some of the recombinants are better adapted to the novel environment than either of their parental populations and may therefore be favored by selection (Johansen-Morris and Latta 2006). The combined release of both biotypes could probably provide the best long-term control for new tansy ragwort infestations east of the Cascades and for existing infestations in habitats with climatic conditions intermediate of those found in Italy and Switzerland, or at infestations at which previously either biotype failed to establish (for example, in eastern Oregon). However, release of the Swiss biotype in western coastal regions of the USA, where the Italian biotype provides successful control of tansy ragwort, may result in disruption of ecological adaptations of Italian beetles (outbreeding depression), which may have negative effects on their control potential. Since the host specificities of both biotypes were tested and found to be very similar and narrow (Frick 1970; K. P. Puliafico unpublished; U. Schaffner unpublished data), there is little reason to believe that this trait would change upon hybridization and compromise the environmental safety of the agent. In addition, our study system provides unique opportunities to test some relevant hypotheses, for example, how hybrid vigor documented in the laboratory would manifest under natural conditions, whether it would facilitate more rapid population buildup, whether populations would better be able to adapt to novel environments, or whether it would lead to a net increase in control success.

Rapid evolutionary changes often accompany biological invasions either by exotic species adapting quickly to novel environments, where hybridization can play a key role, or by recipient communities adapting to invaders (reviewed by Whitney and Gabler 2008). Biological control practices should take into account the evolving and hybrid nature of most target species, and design releases in ways that enable and promote the same microevolutionary processes operating in invasive populations. For example, pre-release hybridization of select biological control agent biotypes could be a viable option in cases when biological control agents could not be collected from similar environments in the native range than the target area in the introduced range, and this mismatch may impair agent establishment and control potential. Post-release hybridization of biological control agents may be warranted in places where a given biotype of an agent failed to establish or where successful control could not be achieved over several years despite establishment. Biological control agents with hybrid ancestry may also better be able to follow and control target species whose colonization of new habitats may also be facilitated by hybridization (Rieseberg et al. 2007). Theoretically, outbreeding depression can be a concern for later generation hybrids, but empirical evidence supporting it is scarce (Edmands 2007). Lexer et al. (2003) found that of 80 studies on hybrid fitness, in only two cases, were hybrids consistently less fit than parental genotypes. Other studies reporting on the long-term consequences of hybridization often find that either a few (Johansen-Morris and Latta 2006) or most hybrid genotypes have higher fitness in later generations (Erickson and Fenster 2006; Hwang et al. 2011), indicating that outbreeding depression may be a temporary phenomenon (Templeton 1986). Still, the possibility of outbreeding depression should be taken seriously (Edmands 2007), and intentional hybridization of biological control agents may be restricted to isolated populations to avoid spread of hybrids with novel ecological traits into well-connected areas where single biotypes provide successful control and where hybridization may cause disruption of local adaptation. The recent safety practices in biological control, which recommend (require) host specificity testing of each different biotype of an agent (Barratt et al. 2010) could single out those species in which biotypes are adapted to different hosts. Further tests on those species could assess the effects of hybridization, thereby avoiding possible negative outcomes as observed by Hoffmann et al. (2002) for D. opuntiae. However, we are not aware of any cases where specificity of hybrids between two agent biotypes, both specific to the same host, would change upon hybridization.

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