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The effects of intraspecific hybridization on the host specificity of a weed biocontrol agent

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HIGHLIGHTS

- Hybridization might alter the host range of weed biocontrol agents.
- No-choice larval tests were conducted with parental and hybrid ragwort flea beetles.
- No change in host range of hybrids was found compared to parents.
- Hybridization did not improve performance on non-target species either.
- If host ranges are similar, hybridization likely poses minimal risk in biocontrol.

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ABSTRACT

Hybridization can alter the host-specificity and fitness of herbivorous insects when the hybridizing populations are adapted to different hosts. It is less clear what the effects of admixture may be when genetically distinct populations are crossed that have similar and narrow host ranges. We tested the effects of hybridization between an Italian and Swiss population of the ragwort flea beetle, *Longitarsus jacobaeae*, a biocontrol agent for tansy ragwort, *Jacobaea vulgaris,* in the USA. Development success, development time, and fecundity of parental and first- and second-generation hybrids were assessed on the primary host and ten closely related plant species in no-choice larval transfer experiments. Four of the non-target species supported limited development but none represented novel host use of hybrids compared to the parents. Development time showed maternal effects on one of the non-target species where offspring of crosses with Italian mother developed slower. Percent larval development was significantly greater for one replication of a non-target species, indicating a plant genotype effect. Overall, hybridization did not result in changes of the fundamental host range or improved performance of hybrids on non-target species even when some hybrid lineages exhibited hybrid vigor on the primary host. These findings underscore the reliability of currently-employed host-specificity testing procedures that identify the fundamental host range of potential agents. Our results also support the newly advocated approach to promote intraspecific hybridization in biocontrol agents when the parental populations have similar and narrow host ranges to increase genetic variation and create novel genotypes to facilitate adaptation and persistence in the new range.

1. Introduction

In classical biological control the safety of agents is of utmost importance to ensure that they will not damage populations of any non-target native species in the introduced range. Host specificity testing conducted prior to release has proven a very reliable method to assess the fundamental host range of weed biocontrol agents, that is, the range of species the agent is able to complete development on (Hinz et al., 2019, 2020; Schaffner, 2001; Suckling and Sforza, 2014). Only four cases of unpredicted direct non-target effects had been recorded of the

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457 weed biocontrol agent species released until 2008 (Hinz et al., 2019). Other cases of non-target attack involved non-target species that were not included in host specificity testing or were predictable based on results of host-testing (Hinz et al., 2019). In general, less than 1% of weed biological control agent introductions have resulted in any non-target effects (Hinz et al., 2019, 2020; Suckling and Sforza, 2014).

Despite the remarkable safety record of weed biological control agents, an emerging concern is the potential of species for rapid evolutionary change post-introduction that may include host shifts or host range expansion (Hufbauer and Roderick, 2005; Schaffner, 2001; Van Klinken and Edwards, 2002). Rapid evolution has been shown in a few weed biocontrol agents that have adapted to novel environmental conditions by changing cold tolerance or diapause requirements following release (Bean et al., 2012; McEvoy et al., 2012; Szűcs et al., 2012b, 2019b). To date there is no evidence for evolutionary change in the fundamental host range of weed biocontrol agents and only limited evidence for genetic change in the performance of agents on non-target species (Van Klinken and Edwards, 2002). However, we know that herbivorous insect species, particularly species that have relatively broad host ranges, can evolve rapidly to include new species in their diet or to increase performance on previously suboptimal hosts. For example, soapberry bugs (*Leptocoris tagalicus* Kirkaldy 1908) adapted in less than 50 years to exploit the introduced balloon vines *Cardiospermum halicacabum* L. and *C. grandiflorum* Sw. (Andres et al., 2013; Carroll et al., 2005). Experimental evolution studies in the laboratory using spider mites (*Tetranychus urticae* Koch), seed beetles (*Callosobruchus maculatus* F.) and flour beetles (*Tribolium castaneum* Herbst) have also shown that host use can evolve rapidly (Agrawal, 2000; Fellous et al., 2014; Fricke and Arnqvist, 2007; Magalhães et al., 2009; Messina and Durham, 2013; Messina et al., 2018, 2020; Szűcs et al., 2017).

One of the processes that is increasingly recognized as a likely agent of rapid evolutionary change is hybridization; either between species (interspecific) or between genetically distinct populations of the same species (intraspecific) (Dlugosch et al., 2015; Schierenbeck and Ellstrand, 2009; Stebbins, 1959). Hybridization can promote evolutionary change, for example by increasing genetic variation thereby facilitating adaptation to novel conditions or by creating novel genotypes that can exhibit traits that differ from parental populations (transgressive segregation) (Arnold, 1997; Rieseberg et al., 2007; Stebbins, 1959). Hybrids may also exhibit heterosis or hybrid vigor in early generations due to the masking of deleterious alleles or because of heterozygote advantage (overdominance) (Edmands, 2002; Lynch, 1991). In biological control, hybridization has been reported between imported biocontrol agents and native species (Davies et al., 2009; Havill et al., 2012; Naka et al., 2005; Toda et al., 2000; Yara et al., 2000), closely related species released against the same target (Bean et al., 2013; Bitume et al., 2017) and conspecifics (Hoffmann et al., 2002; Mathenge et al., 2010; Messing and Aliniazee, 1988; Szűcs et al., 2012a, 2019a), but the effects of such hybridization on host-specificity have rarely been investigated. In two cochineal insect species (*Dactylopius tomentosus* Lamark and *D. opuntiae* Cockerell) where host races are common, hybridization between biotypes adapted to different host species resulted in reduced host specificity of the first generation (F1) hybrids compared to parental biotypes in both cases (Hoffmann et al., 2002; Mathenge et al., 2010). Since the hosts of both biotypes were weeds targeted for biological control hybridization in these systems can be beneficial because the hybrids can attack both weed species where they occur in sympatry (Hoffmann et al., 2002; Mathenge et al., 2010). In the case of *D. opuntia* where crosses were followed beyond the first generation there were signs of a reversal to increased host specificity but only in some of the second generation (F2) individuals, suggesting that the longer-term impacts of hybridization on host range can be unpredictable (Hoffmann et al., 2002). Hybridization between three *Diorhabda* species used to control invasive saltcedars in the USA also revealed a shift in host preference towards either the target saltcedar or a non-target *Tamarix* species in some of the F2 crosses (Bitume et al., 2017). In this system, the different *Diorhabda* species used in the crosses had differing host preferences even prior to hybridization and were able to develop on the non-target *Tamarix aphylla* (L.) Karst (Bitume et al., 2017).

The above laboratory studies show that host-specificity can be altered by hybridization in cases where the parental populations are adapted to different hosts. However, in these instances further testing of hybrids would likely be required under current regulations to ensure the safety of agents. What remains unclear, though, is whether hybridization could also change host-specificity of intraspecific crosses in cases where the parental populations have similar host ranges. Understanding how hybridization in these cases may alter the fundamental host range and/or the preference and performance of biocontrol agents on non-target species is important to ensure that current host-testing practices remain a good predictor of host range following introduction.

In this study, we assessed the effects of intraspecific hybridization between two phenotypically distinct and genetically diverged populations of the ragwort flea beetle (*Longitarsus jacobaeae* Waterhouse, Coleoptera: Chrysomelidae) (Szűcs et al., 2011). We tested whether hybridization would facilitate the inclusion of additional plant species in the diet or whether it would alter performance of hybrids on non-target species compared to the parental lineages. Two distinct populations, one from Italy and one from Switzerland, of the ragwort flea beetle were introduced to North America to control the invasive tansy ragwort (*Jacobaea vulgaris* Gaertn.) (Frick and Johnson, 1973; Littlefield et al., 2008). Both populations were tested separately for host specificity prior to release and were found to have very similar and narrow host ranges (De Clerck-Floate et al., 2010; Frick, 1970; Puliafico, 2003). Following introduction, hybridization between the Italian and Swiss populations occurred (Szűcs et al., 2019a, 2011). To test the effect of this hybridization on host specificity, we compared developmental success of larvae to adult, development time, and fecundity of parental populations with those of F1 and F2 hybrids on the target plant and on ten closely related species. We hypothesized that given the similar host ranges of parents, the fundamental host range of hybrids would not change compared to the parents. However, because previous studies showed hybrid vigor in this species (Szűcs et al., 2012a, 2019a), we expected that the hybrids could show increased fitness compared to either parent on non-target plants.

2. Materials and methods

2.1. Host range of Swiss and Italian L. jacobaeae

The Italian and Swiss populations of the ragwort flea beetle are able to feed and oviposit on a wide range of plants, but their larval host-range is limited to a few closely related species within the genera *Jacobaea*, *Senecio* sensu stricto, *Packera* and *Emilia* (De Clerck-Floate et al., 2010; Frick, 1970; Puliafico, 2003). The primary host of *L. jacobaeae* is tansy ragwort (*Jacobaea vulgaris* formerly *Senecio jacobaeae*: family Asteraceae, tribe Senecioneae). This target weed was recently reclassified into the genus *Jacobaea* from the genus *Senecio* sensu stricto (Pelser et al., 2002, 2007). Development to adulthood has been reported on three other species from the genus *Jacobaea* (*J. maritima, J. aquatica* syn. *S. aquaticus, J. erratica* syn. *S. erraticus*), on three species from the closely related *Packera* genus (*P. pseudaurea, P. paupercula, P. plattensis*), and on three species from the genus *Senecio* sensu stricto (*S. flaccidus* syn. *S. longilobus, S. vulgaris, S. erucifolius*) (De Clerck-Floate et al., 2010; Frick, 1970). In addition, one species in the genus *Emilia* was found to support larval development to adulthood (Frick 1970). This genus is now considered a sister genus or part of the genus *Packera*, and thus is closely related to *J. vulgaris* (Pelser et al., 2007). However, the development success on these non-target plants was lower than on the target weed.

2.2. Successful larval development of parental and hybrid lineages of *L. jacobaeae* on the primary and non-target hosts

To test the effects of intraspecific hybridization on host-specificity, we compared the performance of parental and hybrid lineages of *L. jacobaeae* on the primary host *J. vulgaris* and on 10 non-target plant species (Table 1). Six different lineages of ragwort flea beetles were used to infest the test plants: 1) Swiss parent, 2) Italian parent, 3) F1 hybrid where females were Swiss (Swiss F1), 4) F1 hybrid where females were Italian (Italian F1), 5) F2 hybrid where females were Swiss (Swiss F2), and 6) F2 hybrid where females were Italian (Italian F2). The number of replications for the different treatment combinations (11 plant species × 6 beetle lineages) is shown in Table 1. In general, there were 8 replicates for most treatment combinations, with more replicates for the primary host and *J. maritima,* the most closely related species, and fewer replicates for *S. eremophilus* because of difficulty growing plants (Table 1). In addition, the Italian F1 beetle lineage was only tested on *J. vulgaris* and *J. maritima* because there were not sufficient larvae available for this lineage to test it on all non-target species (Table 1).

The parental beetle lineages were collected either near Delémont, Switzerland or near Salem, OR (Italian) and were reared in a quarantine facility in Bozeman, MT. To create first generation reciprocal crosses, virgin Swiss or Italian females were paired with either Italian or Swiss males, respectively. The generation emerging from eggs laid by these crosses was considered F1. The subsequent generation produced by these F1 beetles constituted the second generation (F2) hybrids, thus no backcrossing took place to create F2 hybrid lineages. Beetles of parental and hybrid lineages were reared by keeping adults in clear plastic containers in groups of 20–50 beetles, which were fed tansy ragwort leaves inserted in moist foam blocks. Eggs were laid into the foam blocks during summer and fall and were then kept in a refrigerator at 2–6 °C over the winter. Eggs were removed from May to June. Upon eclosion, first instar larvae were transferred onto potted tansy ragwort plants. Infested plants were kept in cages, and adults emerging within 8–12 weeks were used to start a new generation of rearing.

The primary host and target weed, *J. vulgaris,* served as a positive control to quantify development of the beetles. The 10 test plants represented the genera *Jacobaea* (n = 1), *Senecio* sensu stricto (n = 6), and *Packera* (n = 3) (Table 1). Most of the species were used in previous host-specificity tests of either the Swiss or Italian population of the ragwort flea beetle as part of the approval process for their release in North America (De Clerck-Floate et al., 2010; Frick, 1970; Puliafico, 2003). Five of the test plants were previously found to support limited larval development to adulthood (Table 1). Nine of the test plant species plus the primary host were grown from seed starting in January 2016 in a greenhouse at 23Celsius temperature under 16 h light and 8 h dark conditions. The tenth test species (*P. plattensis*) was collected near Bozeman, MT in May 2016, transplanted into pots and kept in the same greenhouse as the other test plants.

Each potted plant (replication) was infested with 20 first instar ragwort flea beetle larvae between 18 May and 23 June 2016 as larvae for the different lineages became available. Larvae were transferred using a fine paint brush to the root crown and lower petioles of plants that were all in the rosette stage upon infestation. Each plant was covered individually with mesh cages prior to pupation (estimated at 4–5 weeks after larval transfer). Adult emergence was checked twice weekly until 12 Sept 2016. This was 11 weeks following the last larval transfer and would have allowed the emergence of all adults that completed their larval development.

2.3. Development time and fecundity

To further investigate the performance of the different beetle lineages on the non-target plant species we measured development time (days between larvae transfer and first adult emergence) and the fecundity of adults emerging from host-specificity tests. Emerging adults were sexed, and single females were paired with one to two males, depending on availability. As the Swiss F2 lineage produced mostly females from the test plant, *S. flaccidus,* there were not enough males available to setup mating pairs from that test species. Thus, to measure fecundity of these females, they were paired with males of the same lineage that emerged from *J. vulgaris*. Adults were kept in clear plastic containers and fed with leaves of the test species from which they emerged. Floral foam blocks with inserted plant leaves provided oviposition substrate for females. Egg counts were made on a weekly basis for a three-month period.

2.4. Statistical analyses

To quantify the effect of beetle lineage on adult emergence, we used Bayesian hierarchical Poisson regression models with a log-link function. We constructed models for each of the test plants that had adults emerge from more than two replicates (*S. fremontii, S. flaccidus*, and *J. vulgaris*). We combined the two F1 crosses and the two F2 crosses because not all reciprocal crosses were tested on each host plant and emergence was very low in some of the crosses. The data from *S. fremontii* and *S. flaccidus* were highly zero-inflated, so adult emergence on those plants was modeled with a mixture of the Poisson and Bernoulli distributions, where the parameter of the Bernoulli distribution was the probability of a zero that is not accounted for by sampling variation alone. Significant differences between lineages were determined by the probability of direction (pd) for the difference between the

Table 1
Adult emergence of parental (Swiss and Italian), first (F1) and second (F2) generation hybrid *L. jacobaeae* lineages from the primary host, *J. vulgaris* and ten closely related non-target plant species. Cells with double rows indicate beetle lineage and plant species combinations with adult emergence. Numbers at the top are mean numbers of adults emerging and the standard deviation (SD). Note that for analyses the reciprocal crosses within the F1 and F2 lineages were combined but here non-model means and SD are shown for each lineage. The number of replications is shown in parentheses and in cells with single rows where no adults emerged. The last column indicates whether the plant species had been tested in the past to assess host range and the results of those tests. In parentheses, the number of parental *L. jacobaeae* that had emerged is shown from non-target plants.

	Beetle lineage						
Test species	Swiss	Italian	F1 Swiss	F2 Swiss	F1 Italian	F2 Italian	Prior host
Jacobaea vulgaris (syn. Senecio jacobaea)	5.8 ± 3.1 (14)	4.7 ± 2.8 (13)	10.4 ± 4.5 (10)	6.4 ± 2.8 (14)	5 ± 2.5 (4)	5.7 ± 1.9 (11)	Yes
Jacobaea maritima (syn. S. cineraria)	14	14	10	0.1 ± 0.3 (14)	5	0.1 ± 0.3 (10)	Yes (>10)
Senecio vulgaris	8	8	4	8	0	4	No
Senecio flaccidus (syn. S. longilobus)	0.4 ± 0.5 (8)	8	7	2.4 ± 5.5 (8)	0	0.2 ± 0.5 (5)	Yes (n = 1)
Senecio atratus	8	8	4	8	0	5	No
Packera plattensis (syn. S. plattensis)	8	8	4	8	0	4	No
Senecio riddellii	0.1 ± 0.4 (8)	8	6	8	0	5	Not tested
Senecio fremontii	3.4 ± 3.6 (8)	0.5 ± 1.1 (8)	0.9 ± 2.3 (7)	1.5 ± 2.0 (8)	0	3.3 ± 2.6 (4)	Larvae
Packera tridenticulata (syn. S. tridenticulata)	8	8	6	8	0	4	Yes (n = 3)
Packera pseudaurea (syn. S. pseudaureus)	8	8	6	8	0	4	No
Senecio eremophilus	5	5	0	5	0	0	Yes (n = 4)

means for all pairwise contrasts on each host plant. Probability of direction can be interpreted similarly to a frequentist p-value, where pd > 0.975 is similar to a p-value < 0.05.

To quantify the effect of beetle lineage and host plant on fecundity and development time, we used Bayesian hierarchical models. These data were not zero-inflated because they included only data from emerged adults. Due to uneven emergence across host plants and lineages, these factors were collapsed into one factor with either 11 levels (beetle lineage and test plant combinations) for fecundity or 13 levels for development time. Data were mean standardized before model fitting and inference was made on the standardized estimates. Mean fecundity or development time on each host plant was calculated by averaging the estimate for each lineage on that host plant. Probability of direction was used to determine significance of differences between means.

Three chains of the MCMC sampler were run for each model. Model fit was assessed with Bayesian p-values and convergence of chains was assessed with trace plots. Bayesian hierarchical models were fit using MCMC sampling in package rjags (Plummer 2018). All analyses were performed in R version 3.6.2 (R CoreTeam, 2018).

3. Results

3.1. Adult development

Besides the primary host, *J. vulgaris*, four non-target test plant species supported complete larval development; one *Jacobaea* and three *Senecio* species (Table 1). No adults emerged from any of the *Packera* species. On the primary host, *J. vulgaris*, the F1 hybrid lineage (mean: 8.5 adults (7.0, 10.1 95% HDPI–highest density posterior interval) had significantly higher emergence than F2 or parental lineages (F2: 6.1 adults (5.2, 7.1 95% HDPI); Swiss: 5.8 adults (4.7, 7.1 95% HDPI); Italian: 4.9, (3.7, 6.1 95% HDPI); probability of direction (pd) of the difference between F1 and each other lineage > 0.996). On average, 4.9–8.5 adults (24.5–42.5%) emerged for every 20 larvae transferred onto each *J. vulgaris* plant across the different beetle lineages (Fig. 1). Only 2 adults emerged across all beetle lineages and replications from the species most closely related to tansy ragwort, *J. maritima*; one each of the Swiss F2 and Italian F2 hybrid lineages (Table 1). Only a single Swiss adult emerged from *S. riddellii*. All the tested beetle lineages were able to develop on *S. fremontii* yielding on average 0.01–4.9 (0.07% – 24%) adults across the different beetle lineages (Fig. 1, Table 1). Swiss and F2 hybrids on *S. fremontii* had similar emergence (Swiss: 4.9 adults (1.3, 9.8 95% HDPI); F2: 3.9 adults (1.1, 8.1 95% HDPI)) and were significantly higher than Italian and F1 lineages (Italian: 0.04 adults; F1: 0.01 adults; pd of each pairwise contrast > 0.999). Swiss beetles and F2 hybrids were also able to develop on *S. flaccidus* yielding between 1 and 19 adults total across replications. This translated to 0.1% – 2.1% mean emergence rates per lineage (Fig. 1, Table 1). Mean emergence rates of the Swiss and F2 hybrids lineages, however, were not significantly different from each other or the other lineages (pd for all contrasts < 0.975) (Fig. 1).

3.2. Development time and fecundity

The development time of the different beetle lineages varied on the primary host. The Italian lineage took on average 63.2 days (62.8, 63.6 95% HDPI) to develop, which was significantly longer than that of other lineages (other lineage range: 62.3–62.8 days; pd of each pairwise contrast with Italian parent > 0.975) except the F2 Italian lineage (62.9 days (62.5, 63.3 95% HDPI), Italian vs. F2 Italian pd = 0.885). F2 Italian beetles also had significantly longer development time than F1 Swiss (62.3 days, (61.9, 62.6 95% HDPI); F2 Italian vs. F1 Swiss pd > 0.975). In general, Swiss beetles and those hybrids where the female was Swiss tended to develop faster than Italian beetles and hybrids where the female in the cross was Italian, though not all contrasts are significant (Italian vs. Swiss: pd = 0.988; F1 Italian vs. F1 Swiss: pd = 0.924; F2 Italian vs. F2 Swiss: pd = 0.922) (Fig. 2). Mean development times averaged across beetle lineages were overall longer on the two non-target plant species that yielded more than two adults than on the primary host (*J. vulgaris* vs. *S. fremontii* difference: 0.33 days, pd = 0.96; *J. vulgaris* vs. *S. flaccidus* difference: 1.73 days, pd = 1.0).

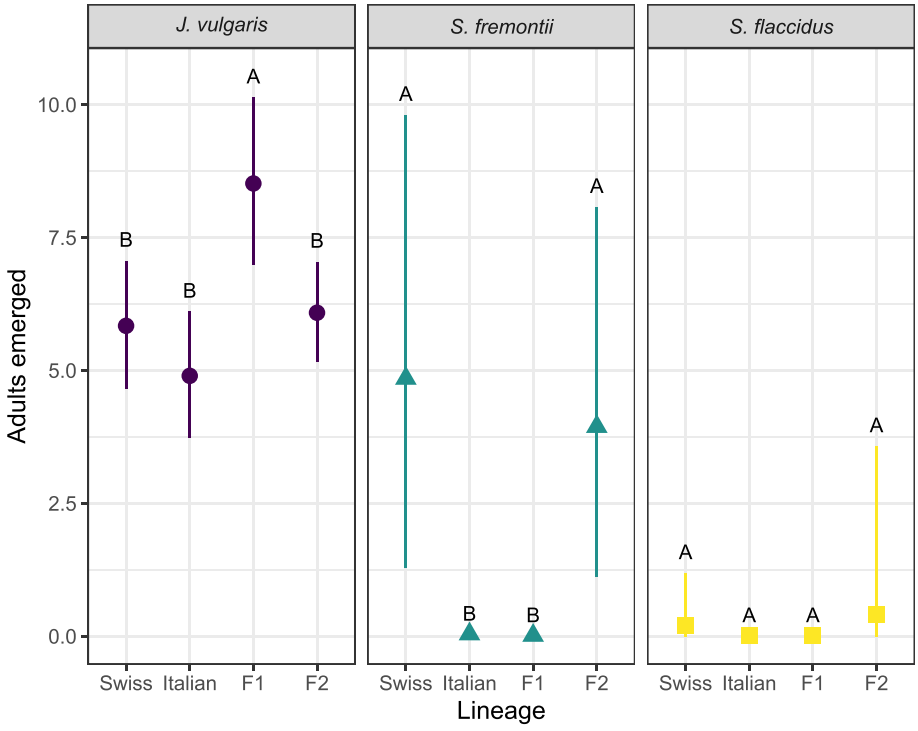

Fig. 1. The number of adults emerging of parental (Swiss and Italian) and first- and second-generation hybrid lineages (F1 and F2, respectively) from the primary host, *J. vulgaris* and two non-target species, *S. fremontii* and *S. flaccidus* following transfer of 20 larvae. Reciprocal F1 and F2 hybrids were merged for this analysis (see methods). Bars indicate 95% highest posterior density intervals around the mean. Letters indicate significant differences between beetle lineages.

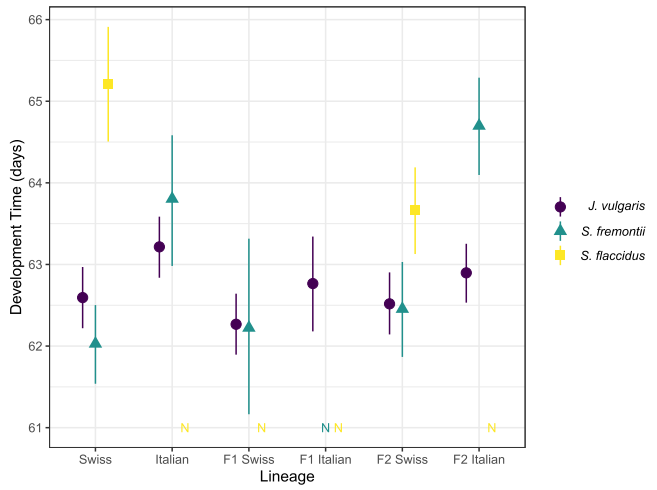

Fig. 2. Development time of parental (Swiss and Italian) and first- and second-generation hybrid beetle lineages (F1 Swiss, F1 Italian, F2 Swiss and F2 Italian) on the primary host, *J. vulgaris*, and on two non-target plant species, *S. flaccidus* and *S. fremontii*. Points are mean development times (days) and lines are 95% highest posterior density interval. The letter 'N' indicates beetle lineage and host plant combinations that did not yield development or were not tested (F1 Italian on *S. flaccidus* and *S. fremontii*).

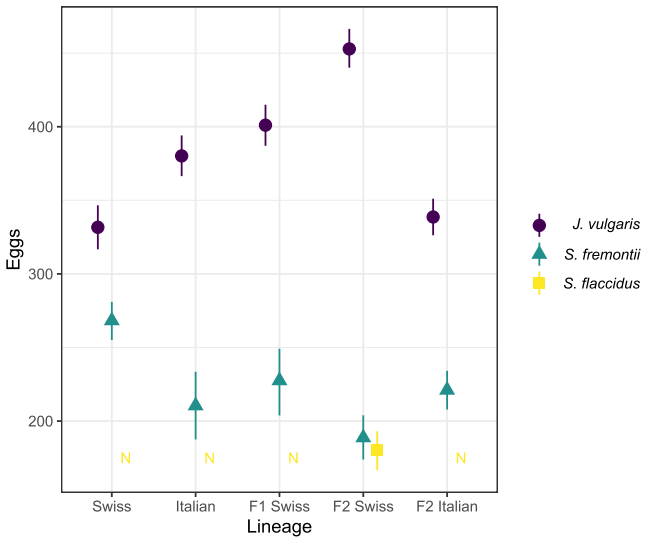

Fig. 3. Fecundity (number of eggs per female) of parental (Swiss and Italian) and first- and second-generation hybrid beetle lineages (F1 Swiss, F1 Italian, F2 Swiss and F2 Italian) on the primary host, *J. vulgaris*, and on two non-target plant species, *S. flaccidus* and *S. fremontii*. Points are mean number of eggs and lines are 95% highest posterior density interval. The letter 'N' indicates beetle lineage and host plant combinations where fecundity could not be measured due to lack of adult emergence.

The pattern of development time was similar on *S. fremontii* and their primary host. The Italian lineage and F2 Italian (Italian: 63.80 days (62.98, 64.58 95% HDPI), F2 Italian: 64.70 days (64.10, 65.29 95% HDPI)) had significantly longer development times than the Swiss, F1 Swiss, and F2 Swiss lineages (range: 62.03–62.45 days; pd of each pairwise contrast > 0.975). All lineages on *S. fremontii* had similar development times to the same lineage on the primary host (pd of each pairwise contrast less than 0.975), except for F2 Italian which took significantly longer to develop on *S. fremontii* (difference: 1.80 days; pd $= 1.0$) (Fig. 2). On *S. flaccidus* both the Swiss and F2 Swiss lineages that were able to develop took longer to complete development than the same lineage on the primary host (difference Swiss: 2.62 days, pd $= 1.0$; difference F2 Swiss: 1.15 days; pd $= 1.0$).

On the primary host, fecundity differed significantly among beetle lineages (pd of each pairwise contrast > 0.975), except for F2 Italian and Swiss (F2 Italian: 338.75 eggs; Swiss: 331.74 eggs; pd of difference $=$ 0.765), which both had significantly lower fecundity than all other lineages (Fig. 3). On *S. fremontii*, fecundity for each lineage was lower than the same lineage on the primary host (pd of all pairwise contrasts $=$ 1.0) (Fig. 3). For *S. flaccidus*, fecundity could only be measured for the F2 Swiss beetles as this was the only lineage where females emerged. Fecundity was significantly lower for F2 Swiss on *S. flaccidus* than for the same lineage on the primary host (difference: 272.89 eggs; pd $= 1.0$).

4. Discussion

We did not find evidence that intraspecific hybridization altered the fundamental host range, or the performance of *L. jacobaeae* hybrids compared to parental lineages on non-target plant species. There was only one instance when hybrid adults did, but parental lineages did not emerge from a non-target plant. One adult each emerged of F2 Swiss and F2 Italian hybrids and none for parental lineages from, *J. maritima*, which is the closest relative to the primary host, *J. vulgaris*. While these emergence rates are extremely low (0.36% and 0.5% of larvae transferred, respectively) and statistically insignificant, one might argue that even a single case of full development by hybrids should be cause for concern. However, in previous host-specificity tests Swiss beetles were able to develop on *J. maritima* (De Clerck-Floate et al., 2010) (Table 1), therefore some emergence was expected from this plant species based on parental host range and it does not signal use of a new host by hybrid beetles.

It is common to see development of a few adults during no-choice tests on plants that are not accepted as hosts in the field (Schaffner, 2001). No-choice tests, where only a single species, either the target or non-target plant species, are offered for oviposition or larval development (as done here) measure the fundamental host range of species, that is, the plant species that support full development of a herbivore (Schaffner, 2001; Sheppard et al., 2005). These tests are very conservative and are often criticized for overestimating possible non-target effects that may occur in the field where the full range of host-choice behavior can be exhibited (Schaffner, 2001; Sheppard et al., 2005). The list of species actually used in nature as hosts is just a small subset of species included in the fundamental host range and constitute the realized or ecological host range (Schaffner, 2001; Sheppard et al., 2005). Our results of very low rates of adult emergence from three of the non-target species, *J. maritima*, *S. flaccidus* and *S. riddellii*, and the low fecundity of emerging females indicate that (Fig. 3; Table 1), these plants are unlikely to be field hosts of either the parental or hybrid lineages of *L. jacobaeae*.

One of the non-target hosts, *S. fremontii* supported development consistently across all tested beetle lineages, but the hybrids did not have higher emergence rates or fecundity compared to the parental beetle lineages on this host. In contrast, on the primary host hybrids performed better than parents, with F1 hybrids showing the highest emergence rates and F2 hybrids laying the most eggs (Figs. 1, 3); a pattern also found in an earlier study (Szűcs et al., 2012a). These results suggest that hybrid vigor exhibited on the primary host does not necessarily translate into improved performance on a suboptimal host plant, or at least not in the first two generations of hybridization.

These findings are not entirely surprising given the diversity of responses intraspecific hybrid herbivores can show on novel or suboptimal host plants. For example, intraspecific hybrids of *T. castaneum* flour beetles performed better both on the natal (wheat) and a novel (corn) host compared to parental lineages over seven generations of an experimental evolution study (Szűcs et al., 2017). However, similar hybrids of the same flour beetle populations did not show hybrid vigor in the first generation compared to parental lineages when the natal host environment was manipulated to become more challenging by reducing

its nutrient content (Szűcs et al., 2014). In the seed beetle *C. maculatus* performance of hybrid and parental lines was similar initially on a novel host (lentil) with less than 5% survival of F1 larvae, but over five generations of selection the performance of hybrid lineages improved at a much higher rate than that of parental lines (Messina et al., 2020). The same *C. maculatus* hybrids also performed better on a different novel host (pea) after five generations of selection compared to parental populations (Messina et al., 2020). It should be noted though that the magnitude of improvement on the novel hosts in survival and growth rates varied depending on which geographically distinct populations were crossed, a pattern that is common and likely stems from initial differences in performance of parental populations on various novel hosts (Messina and Durham, 2013; Messina et al., 2018, 2020).

Development time of different beetle lineages on *S. fremontii* showed maternal effects in that the Italian parent and those crosses where the female was Italian took longer to complete development than lineages where the female was Swiss (Fig. 2). Maternal effects, stemming from the environment experienced and the genotype of the mother, are common in insect herbivores and are known to influence life history traits and to contribute to adaptation to novel hosts (Mousseau and Dingle, 1991; Mousseau and Fox, 1998; Newcombe et al., 2015; Van Asch et al., 2010). Life history and fitness traits of intraspecific hybrids are often intermediate between the parental lineages because many life history traits are governed by additive genetic variance, but maternal effects are common in early crosses where the phenotype of hybrids resembles more the maternal host (Carroll et al., 2001; Dingle et al., 1982; Mathenge et al., 2010; Messina and Slade, 1997). For example, F1 hybrids between two biotypes of *D. tomentosus* used for biological control of *Cylindropuntia* cacti showed maternal effects both in host specificity and in fitness traits (Mathenge et al., 2010). Maternal effects in egg diapause, egg size and adult aestivation of F1 and F2 hybrids had been detected in a prior study of *L. jacobaeae* on the primary host (Szűcs et al., 2012a), and our results show that maternal effects associated with life history traits can be present on suboptimal host plants.

On the non-target test plant *S. fremontii*, which supported beetle development, the emergence rates of parental and hybrid lineages were similar (Swiss to F2 and Italian to F1) (Fig. 1). In contrast, *S. flaccidus* presents a somewhat unique case because 20 F2 hybrid individuals of mixed sexes emerged from this non-target plant but only three F2 males of the Swiss parental line. This could be biologically important because in this case the emerging F2 adults, in theory, have the potential to produce a new generation but not the Swiss parental lineage. However, further investigation of the emerging F2 beetles revealed that they had very low fitness; only half of the emerging females laid any eggs and egg production was less than 5% compared to what the same lineage could produce on its primary host. What is notable for this test plant is that almost all F2 adults (16 of the 20) emerged from a single plant (replication). All the other plants produced either none or only one adult which indicates a strong genotype effect of the plant and not a genetic effect related to hybridization of the herbivore. It is well-established that plant genotype can affect host quality, resistance and tolerance to herbivory and even the population dynamics of herbivore populations (Cronin and Abrahamson, 1999, 2001; Strauss and Agrawal, 1999; Underwood and Rausher, 2000). It appears that one of the *S. flaccidus* plants used in experiments had reduced resistance or differed in some other trait(s) that affected plant-herbivore interactions. It is well known that plants in the *Senecio* and *Jacobaea* genera are defended by toxic pyrrolizidine alkaloids but *Longitarsus* species are adapted to these secondary chemicals and their performance is generally unaffected by variation in alkaloid profiles or concentrations (Dobler et al., 2000; Kirk et al., 2012; Vrieling et al., 1993). Regardless of the underlying mechanism that allowed development of beetles, the significant reduction in fecundity of emerging females indicates that *S. flaccidus* is a suboptimal host for *L. jacobaeae* that is unlikely to support viable beetle populations in the field on the long term.

The effects of intraspecific hybridization on host use and host range evolution of herbivorous insects depend on a multitude of factors including the origin of parental populations and their historical host use, the identity of closely related species overlapping in distribution with the primary host, maternal effects, the genotype of potential host plants and the hybrid class (Cronin and Abrahamson, 1999; Messina and Durham, 2013; Messina et al., 2018; Mousseau and Fox, 1998; Newcombe et al., 2015; Strauss and Agrawal, 1999). Experimental evolution studies show that hybridization can facilitate evolution and can lead to adaptation on marginal or novel host species, and that hybrid vigor can improve performance of early generation hybrids on both natal and novel hosts (Agrawal, 2000; Fellous et al., 2014; Fricke and Arnqvist, 2007; Magalhães et al., 2009; Messina and Durham, 2013; Messina et al., 2018, 2020; Szűcs et al., 2017). While experimental evolution studies are invaluable to elucidate mechanisms of evolutionary change, they have limited utility for assessing the potential risks intraspecific hybridization may pose in biological control agents under natural field conditions. Experimental evolution experiments usually start with hundreds or thousands of individuals and maintain strong selection pressures over multiple generations by restricting all individuals to the novel host and by inhibiting gene flow between individuals developing on the primary host and those emerging from a novel host. For example, many populations of *C. maculatus* are unable to survive on the novel lentil host and when adaptation of either parental or hybrid lines was achieved it required replicate starting populations of 2500 beetles each and many of those replicates still went extinct (Messina et al., 2018, 2020). The types of selection pressures that result in self-sustaining populations of hybrid or parental lineages on novel hosts in laboratory experiments would be unlikely to occur in nature in biological agent populations.

There are very few examples from natural populations when hybridization facilitated host range expansion to a novel host. In Australia, the native soapberry bug (*L. tagalicus*) expanded its realized host range to include the introduced balloon vine *Cardiospermum halicacabum* v. *halicacabum* in their diet (Andres et al., 2013). Andres et al. (2013) found that the soapberry bug populations attacking the annual *C. halicacabum* are hybrids between two subspecies of the soapberry bug with a novel phenotype that has long beaks and high beak and body length ratios. However, even this example does not represent the scenario we set out to test because the soapberry bug subspecies that crossed had been adapted to different hosts. We were interested in whether intraspecific hybridization between herbivores that are adapted to the same host would lead to host range expansion or better performance on non-target plants because this question has great relevance for the practice of biological control.

Intentional hybridization between genetically distinct populations of biological control agents has newly been advocated as an approach that could facilitate adaptation to changing environmental conditions, increase establishment success, persistence and possibly impact of biocontrol agents (Szűcs et al., 2019b). *Longitarsus jacobaeae* provides a good example for the positive effects of hybridization whereas hybrid beetles were shown to have higher densities and greater impact on larger *J. vulgaris* plants in the field (Szűcs et al., 2019a). However, the lack of data on how host-specificity may evolve in hybrids can hinder widespread adoption of this approach. Here we provide an example where intraspecific hybridization did not change the fundamental host range of an herbivorous insect, neither did it increase performance on non-target hosts. We also could not find any well-documented examples from the wider insect herbivore literature or from other biocontrol systems for host range evolution of intraspecific hybrids where the parents have similar host ranges. Long-term data on hundreds of biological control releases did not find evidence for evolution of the fundamental host range either (Van Klinken and Edwards, 2002). All of the above indicates that the likelihood of change in host-specificity that would result in population level negative impact on non-target species is likely negligible if weed biocontrol agent populations were to hybridize that are shown to have similar and narrow host ranges during host-specificity

testing.

5. Data statement

Upon acceptance data will be available in Mendeley Data.

CRediT authorship contribution statement

M. Szűcs: Conceptualization, Methodology, Investigation, Writing - original draft, Funding acquisition, Supervision. **E.I. Clark:** Formal analysis. **U. Schaffner:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **J.L. Littlefield:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **C. Hoover:** Investigation, Writing - review & editing. **R.A. Hufbauer:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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References

Agrawal, A.A., 2000. Host-range evolution: adaptation and trade-offs in fitness of mites on alternative hosts. Ecology 81 (2), 500–508.
Andres, J.A., Thampy, P.R., Mathieson, M.T., Loye, J., Zalucki, M.P., Dingle, H., Carroll, S.P., 2013. Hybridization and adaptation to introduced balloon vines in an Australian soapberry bug. Mol. Ecol. 22 (24), 6116–6130.
Arnold, M.L., 1997. Natural Hybridization And Evolution. Oxford University Press on Demand.
Bean, D.W., Dalin, P., Dudley, T.L., 2012. Evolution of critical day length for diapause induction enables range expansion of *Diorhabda carinulata*, a biological control agent against tamarisk (*Tamarix* spp.). Evol. Appl. 5 (5), 511–523.
Bean, D.W., Kazmer, D.J., Gardner, K., Thompson, D.C., Reynolds, B.(., Keller, J.C., Gaskin, J.F., 2013. Molecular genetic and hybridization studies of *Diorhabda* spp. released for biological control of *Tamarix*. Invasive Plant Sci. Manage. 6 (1), 1–15.
Bitume, E.V., Bean, D., Stahlke, A.R., Hufbauer, R.A., 2017. Hybridization affects life-history traits and host specificity in *Diorhabda* spp. Biol. Control 111, 45–52.
Carroll, S.P., Dingle, H., Famula, T.R., Fox, C.W., 2001. Genetic architecture of adaptive differentiation in evolving host races of the soapberry bug *Jadera haematoloma*. Microevolution Rate, Pattern, Process. Springer 257–272.
Carroll, S.P., Loye, J.E., Dingle, H., Mathieson, M., Famula, T.R., Zalucki, M.P., 2005. And the beak shall inherit – evolution in response to invasion. Ecol. Lett. 8 (9), 944–951.
Cronin, J.T., Abrahamson, W.G., 1999. Host-plant genotype and other herbivores influence goldenrod stem galler preference and performance. Oecologia 121 (3), 392–404.
Cronin, J.T., Abrahamson, W.G., 2001. Goldenrod stem galler preference and performance: effects of multiple herbivores and plant genotypes. Oecologia 127 (1), 87–96.
Davies, A.P., Takashino, K., Watanabe, M., Miura, K., 2009. Parental genetic traits in offspring from inter-specific crosses between introduced and indigenous *Diadegma* Foerster (Hymenoptera: Ichneumonidae): possible effects on conservation genetics. Appl. Entomol. Zool. 44 (4), 535–541.
De Clerck-Floate, R., Schaffner, U., Turner, S., 2010. Request for renewal of permit to field release the Swiss biotype of *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae) as a biological control agent for tansy ragwort, *Jacobaea vulgaris* (Compositae: Asteracea) in Canada.
Dingle, H., Blau, W.S., Brown, C.K., Hegmann, J.P., 1982. Population crosses and the genetic structure of milkweed bug life histories. In: Evolution and Genetics Of Life Histories. Springer, pp. 209–229.
Dlugosch, K.M., Anderson, S.R., Braasch, J., Cang, F.A., Gillette, H.D., 2015. The devil is in the details: genetic variation in introduced populations and its contributions to invasion. Mol. Ecol. 24 (9), 2095–2111.
Dobler, S., Haberer, W., Witte, L., Hartmann, T., 2000. Selective sequestration of pyrrolizidine alkaloids from diverse host plants by *Longitarsus* flea beetles. J. Chem. Ecol. 26, 1281–1298.
Edmands, S., 2002. Does parental divergence predict reproductive compatibility? Trends Ecol. Evol. 17 (11), 520–527.
Fellous, S., Angot, G., Orsucci, M., Migeon, A., Auger, P., Olivieri, I., Navajas, M., 2014. Combining experimental evolution and field population assays to study the evolution of host range breadth. J. Evol. Biol. 27 (5), 911–919.
Frick, K.E., 1970. *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae), a flea beetle for the biological control of tansy ragwort. 1. Host plant specificity studies. Ann. Entomol. Soc. Am. 63, 284–296.
Frick, K.E., Johnson, G.R., 1973. *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae), a flea beetle for the biological control of tansy ragwort. 4. Life history and adult aestivation of an Italian biotype. Ann. Entomol. Soc. Am. 66, 358–366.
Fricke, C., Arnqvist, G., 2007. Rapid adaptation to a novel host in a seed beetle (*Callosobruchus maculatus*): the role of sexual selection. Evolution 61, 440–454.
Havill, N.P., Davis, G., Mausel, D.L., Klein, J., McDonald, R., Jones, C., Fischer, M., Salom, S., Caccone, A., 2012. Hybridization between a native and introduced predator of Adelgidae: An unintended result of classical biological control. Biol. Control 63, 359–369.
Hinz, H.L., Winston, R.L., Schwarzländer, M., 2019. How safe is weed biological control? A global review of direct nontarget attack. Q. Rev. Biol. 94, 1–27.
Hinz, H.L., Winston, R.L., Schwarzländer, M., 2020. A global review of target impact and direct nontarget effects of classical weed biological control. Curr. Opin. Insect Sci. 38, 48–54.
Hoffmann, J.H., Impson, F.A.C., Volchansky, C.R., 2002. Biological control of cactus weeds: implications of hybridization between control agent biotypes. J. Appl. Ecol. 39, 900–908.
Hufbauer, R.A., Roderick, G.K., 2005. Microevolution in biological control: mechanisms, patterns, and processes. Biol. Control 35, 227–239.
Kirk, H., Vrieling, K., Pelser, P.B., Schaffner, U., 2012. Can plant resistance to specialist herbivores be explained by plant chemistry or resource use strategy? Oecologia 168, 1043–1055.
Littlefield, J.L., Markin, G.P., Puliafico, K.P., deMeij, A.E., 2008. The release and establishment of the tansy ragwort flea beetle in the Northern Rocky Mountains of Montana. In: Julien, M.H., Sforza, R., Bon, M.C., Evans, H.C., Hatcher, P.E., Hinz, H. L., Rector, B.G. (Eds.), Proceedings of the XII International Symposium on Biological Control of Weeds. CAB International, pp. 573–576.
Lynch, M., 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45, 622–629.
Magalhães, S., Blanchet, E., Egas, M., Olivieri, I., 2009. Are adaptation costs necessary to build up a local adaptation pattern? BMC Evol. Biol. 9, 182.
Mathenge, C.W., Holford, P., Hoffmann, J.H., Zimmermann, H.G., Spooner-Hart, R.N., Beattie, G.A.C., 2010. Hybridization between *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) biotypes and its effects on host specificity. Bull. Entomol. Res. 100, 331.
McEvoy, P.B., Higgs, K.M., Coombs, E.M., Karaçetin, E., Ann Starcevich, L., 2012. Evolving while invading: rapid adaptive evolution in juvenile development time for a biological control organism colonizing a high-elevation environment. Evol. Appl. 5, 524–536.
Messina, F.J., Durham, S.L., 2013. Adaptation to a novel host by a seed beetle (Coleoptera: Chrysomelidae: Bruchinae): effect of source population. Environ. Entomol. 42, 733–742.
Messina, F.J., Lish, A.M., Gompert, Z., 2018. Variable responses to novel hosts by populations of the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae). Environ. Entomol. 47, 1194–1202.
Messina, F.J., Lish, A.M., Springer, A., Gompert, Z., 2020. Colonization of marginal host plants by seed beetles (Coleoptera: Chrysomelidae): effects of geographic source and genetic admixture. Environ. Entomol. 49, 938–946.
Messina, F.J., Slade, A.F., 1997. Inheritance of host-plant choice in the seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). Ann. Entomol. Soc. Am. 90, 848–855.
Messing, R.H., Aliniazee, M., 1988. Hybridization and host suitability of two biotypes of *Trioxys pallidus* (Hymenoptera: Aphidiidae). Ann. Entomol. Soc. Am. 81, 6–9.
Mousseau, T.A., Dingle, H., 1991. Maternal effects in insect life histories. Annu. Rev. Entomol. 36, 511–534.
Mousseau, T.A., Fox, C.W., 1998. The adaptive significance of maternal effects. Trends Ecol. Evol. 13, 403–407.
Naka, H., Mitsunaga, T., Mochizuki, A., 2005. Laboratory hybridization between the introduced and the indigenous green lacewings (Neuroptera: Chrysopidae: Chrysoperla) in Japan. Environ. Entomol. 34, 727–731.
Newcombe, D., Moore, P.J., Moore, A.J., 2015. The role of maternal effects in adaptation to different diets. Biol. J. Linn. Soc. 114, 202–211.
Pelser, P.B., Gravendeel, B., van der Meijden, R., 2002. Tackling speciose genera: species composition and phylogenetic position of *Senecio* sect. *Jacobaea* (Asteraceae) based onplastid and nrDNA sequences. Am. J. Bot. 89, 929–939.

Pelser, P.B., Nordenstam, B., Kadereit, J.W., Watson, L.E., 2007. An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of *Senecio* L. Taxon 56, 1077–1104.
Puliafico, K.P., 2003. Molecular taxonomy, Bionomics And Host Specificity of *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae): The Swiss Population Revisited. Montana State University-Bozeman, College of Agriculture.
Rieseberg, L.H., Kim, S.-C., Randell, R.A., Whitney, K.D., Gross, B.L., Lexer, C., Clay, K., 2007. Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129, 149–165.
Schaffner, U., 2001. Host range testing of insects for biological weed control: how can it be better interpreted? Data on the host range of biocontrol candidates are particularly relevant in assessing potential detrimental effects to nontarget organisms. Bioscience 51, 951–959.
Schierenbeck, K.A., Ellstrand, N.C., 2009. Hybridization and the evolution of invasiveness in plants and other organisms. Biol. Invasions 11, 1093–1105.
Sheppard, A., Van Klinken, R., Heard, T., 2005. Scientific advances in the analysis of direct risks of weed biological control agents to nontarget plants. Biol. Control 35, 215–226.
Stebbins, G.L., 1959. The role of hybridization in evolution. Proc. Am. Philos. Soc. 103, 231–251.
Strauss, S.Y., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecol. Evol. 14, 179–185.
Suckling, D.M., Sforza, R.F.H., 2014. What magnitude are observed non-target impacts from weed biocontrol? PLoS ONE 9, e84847.
Szűcs, M., Eigenbrode, S.D., Schwarzländer, M., Schaffner, U., 2012a. Hybrid vigor in the biological control agent, *Longitarsus jacobaeae*. Evol. Appl. 5, 489–497.
Szűcs, M., Melbourne, B.A., Tuff, T., Hufbauer, R.A., 2014. The roles of demography and genetics in the early stages of colonization. Proc. Royal Soc. B: Biol. Sci. 281, 20141073.
Szűcs, M., Melbourne, B.A., Tuff, T., Weiss-Lehman, C., Hufbauer, R.A., 2017. Genetic and demographic founder effects have long-term fitness consequences for colonising populations. Ecol. Lett. 20, 436–444.
Szűcs, M., Salerno, P.E., Teller, B.J., Schaffner, U., Littlefield, J.L., Hufbauer, R.A., 2019a. The effects of agent hybridization on the efficacy of biological control of tansy ragwort at high elevations. Evol. Appl. 12, 470–481.
Szűcs, M., Schaffner, U., Price, W.J., Schwarzländer, M., 2012b. Post-introduction evolution in the biological control agent *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae). Evol. Appl. 5, 858–868.
Szűcs, M., Schwarzländer, M., Gaskin, J.F., 2011. Reevaluating establishment and potential hybridization of different biotypes of the biological control agent *Longitarsus jacobaeae* using molecular tools. Biol. Control 58, 44–52.
Szűcs, M., Vercken, E., Bitume, E.V., Hufbauer, R.A., 2019b. The implications of rapid eco-evolutionary processes for biological control-a review. Entomol. Exp. Appl. 167, 598–615.
Team, R.C., 2018. R: A Language And Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Toda, S., Miyazaki, M., Osakabe, M., Komazaki, S., 2000. Occurrence and hybridization of two parasitoid wasps, *Torymus sinensis* Kamijo and *T. beneficus* Yasumatsu et Kamijo (Hymenoptera: Torymidae) in the Oki islands. Appl. Entomol. Zool. 35, 151–154.
Underwood, N., Rausher, M.D., 2000. The effects of host-plant genotype on herbivore population dynamics. Ecology 81, 1565–1576.
Van Asch, M., Julkunen-Tiito, R., Visser, M.E., 2010. Maternal effects in an insect herbivore as a mechanism to adapt to host plant phenology. Funct. Ecol. 24, 1103–1109.
Van Klinken, R.D., Edwards, O.R., 2002. Is host-specificity of weed biological control agents likely to evolve rapidly following establishment? Ecol. Lett. 5, 590–596.
Vrieling, K., de Vos, H., van Wijk, C.A., 1993. Genetic analysis of the concentrations of pyrrolizidine alkaloids in *Senecio jacobaea*. Phytochemistry 32, 1141–1144.
Yara, K., Yano, E., Sasawaki, T., Shiga, M., 2000. Detection of hybrids between introduced *Torymus sinensis* and native *T. beneficus* (Hymenoptera: Torymidae) in central Japan, using malic enzyme. Appl. Entomol. Zool. 35, 201–206.