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Hybridization affects life-history traits and host specificity in *Diorhabda* spp.

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ABSTRACT

Hybridization is an influential evolutionary process that has been viewed alternatively as an evolutionary dead-end or as an important creative evolutionary force. In colonizing species, such as introduced biological control agents, hybridization can offset losses in genetic variation due to population bottlenecks and genetic drift. Increased genetic variation associated with hybridization could benefit biological control programs, by increasing the chances of establishment success. However, hybridization also can lead to the emergence of transgressive phenotypes, potentially including changes in host use; an important consideration when assessing potential non-target impacts of planned agents. In a series of laboratory experiments, we investigated the effects of hybridization between three species of *Diorhabda* released to control invasive *Tamarix* (saltcedar), evaluating effects on development time, adult mass, and fecundity over two generations for all three cross types, and over a third generation for one cross. Depending on the cross, hybridization had either a positive or neutral impact on the measured traits. We evaluated preference for the target (saltcedar) relative to a non-target host *Tamarix aphylla* (athel), and found hybridization influenced preference in two of the three cross types, demonstrating the possibility for hybridization to shape host use. The overall effects of hybridization varied by cross, suggesting that the outcome of hybridization will be difficult to predict a priori.

1. Introduction

Hybridization is an influential evolutionary process that has been viewed alternatively as an evolutionary dead-end, because hybrids are often less fit than the parental species (Mayr, 1963; Dobzhansky, 1970) or as an important creative evolutionary force (Anderson and Stebbins, 1954; Ellstrand and Schierenbeck, 2000). On the detrimental side, hybrid breakdown, or outbreeding depression, can decrease performance of hybrid individuals across a suite of traits linked to fitness, such as development time, mortality, and fecundity (Burton et al., 1999; Edmands, 2002). On the positive side, hybridization can increase fitness relative to parents directly through heterozygote advantage (overdominance of beneficial traits) (Edmands, 2002; Hedrick and Garcia-Dorado, 2016; Lee et al., 2016) or by masking deleterious alleles (heterosis) and reducing inbreeding depression, and indirectly through increasing genetic variation and thus facilitating adaptive evolution (Fisher, 1930). Additionally, hybridization can facilitate the formation of novel genotypes, potentially producing 'transgressive' phenotypes that fall outside the range of either parent (Rieseberg et al., 1999). Alternatively, in some cases hybridization can have minimal effects, particularly when the genetic distance between parents is small (Mallet, 2005).

Hybridization between recently diverged taxa may be particularly beneficial in colonizing populations, which typically pass through strong bottlenecks in population size, losing genetic variation, and potentially becoming inbred (Ellstrand and Schierenbeck, 2000; Dlugosch and Parker, 2008; Rius and Darling, 2014; Laugier et al., 2016). In the planned release of specialized biological control agents, the goal is for the intentionally released population to establish and propagate (Seastedt, 2015), to feed on the target host (typically an invasive weed or insect), and not shift to use other, non-target hosts. Biological control programs have a fairly low success rate (< 50%), mostly due to lack of establishment of agents in their new environment (Van Driesche et al., 2010). As an evolutionary mechanism, hybridization might allow these establishing populations to better face adaptive challenges posed by a novel environment. There is some evidence that releasing different "strains" or ecotypes of biological control agents in an effort to increase genetic variation might improve establishment success (Hopper et al., 1993; Henry et al., 2010). New evidence suggests that increased genetic variation can be even more important

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than augmenting population size in promoting population growth (Frankham, 2015; Hufbauer et al., 2015; Frankham, 2016). Colonizing populations also experience novel environments in which transgressive phenotypes may, by chance, have higher fitness than parental phenotypes (Ellstrand and Schierenbeck, 2000). In addition to potential benefits for biological control, hybridization, by increasing variation and potentially altering phenotypes, warrants careful consideration of potential risks as well, particularly with respect to host use in the biological control of weeds. There is robust evidence that host range of specialized agents used in weed biological does not evolve readily, and that non-target effects that occasionally occur (e.g. (Pemberton, 2000) have largely been predicted from host-specificity testing prior to release (Van Klinken and Edwards, 2002).

To continue this robust record of predictably safe biological control, examining potential risks posed by hybridization is crucial. Only a few studies have examined hybridization in weed biological control agents (Hoffmann et al., 2002; Mathenge et al., 2010; Szűcs et al., 2012). Szűcs et al. (2012) found that hybridization improved performance in vital life-history traits, which could improve control of the target pest. In contrast, Hoffmann et al. (2002) and Mathenge et al. (2010) found that hybridization between two biotypes of *Dactylopius* could alter host specificity relative to parental lines. In the *Dactylopius* system, each biotype is specific to a single cactus species, and hybrids between them generally can feed on both hosts, and thus are suggested for release in areas with both hosts (Mathenge et al., 2010). Additional research along these lines will improve our understanding of the consequences of hybridization for biological control programs, including evaluating the degree to which it will be possible to draw general conclusions versus research being needed on a case-by-case basis.

To that end, we quantify the effects of hybridization between biological control agents in the genus *Diorhabda* that were released to control *Tamarix* (saltcedar, or tamarisk) in North America. Saltcedar in North America is comprised of a hybrid swarm of *Tamarix chinensis* and *T. ramosissima* (Gaskin and Schaal, 2002). It is an invasive weed that has colonized riparian habitats from Montana to Mexico (Gaskin and Schaal, 2002). In 2001, the USDA Animal and Plant Health Inspection Service (USDA APHIS) approved the release of the central Asian salt cedar leaf beetle, *D. elongata*, as a biological control agent for saltcedar (DeLoach et al., 2003). *Diorhabda elongata* was classified as a single wide-ranging species that specialized on saltcedar and comprised different subspecies and ecotypes. To match environmental conditions in North America, the saltcedar biological control program eventually utilized seven *Diorhabda* ecotypes with native ranges stretching from northern Africa to central Asia (Tracy and Robbins, 2009; Bean et al., 2013a). A recent taxonomic revision of the *Tamarix*-feeding *Diorhabda* has used morphological and biogeographical data to define this group as a complex comprising four species: *D. elongata*, *D. carinulata*, *D. carinata*, and *D. sublineata* (Tracy and Robbins, 2009). Genetic studies using amplified fragment length polymorphisms (AFLPs) revealed four major clades within this group that coincide with the four morphospecies (Bean et al., 2013b). There was also a fifth species, *D. meridionalis*, not currently used in the saltcedar biological control program. Currently, *D. carinulata* is the most widespread of the species in North America and covers large areas in Oregon, Idaho, Wyoming, Colorado, Utah, Nevada, northern Arizona, and northern New Mexico (Bean et al., 2013a). The other three species have all been released in Texas and have started spreading (Michels et al., 2013). Hybridization is possible between all four taxa, but hybrids between *D. carinulata* and each of the other three species produce few viable offspring. In contrast, egg viability of hybrids between *D. elongata*, *D. carinata*, and *D. sublineata* is comparable to that of the parents (Bean et al., 2013b). We crossed these three species reciprocally, and tracked performance over three generations to quantify the effects of hybridization. We measured several phenotypic traits linked to fitness, as well as preference for saltcedar relative to a non-target plant, *Tamarix aphylla* (athel hereafter) and performance (development time) on saltcedar and athel for both hybrid offspring and the parental species.

2. Materials & methods

2.1. Organism

The beetles used in our experiments were from samples originally collected from saltcedar in Eurasia and North Africa. Descendants of these samples were used to establish laboratory populations maintained at the Palisade Insectary, Biological Pest Control Program, Colorado Department of Agriculture, Palisade, CO (CDA Palisade). Colonies were maintained on cuttings of saltcedar, including *T. ramosissima*, *T. chinensis*, and their hybrids (Gaskin and Schaal, 2002). In a study investigating 110 saltcedar plants from a wide range in the USA, Gaskin and Kazmer (2009) found that 83–87% of plants were some level of hybrid between the two species, with proportion *T. ramosissima* higher in the north, and proportion *T. chinensis* higher in the south, in a clear latitudinal gradient (Williams et al., 2014). While we expect various levels of hybridization to be present, we fed all beetles with cuttings from a restricted set of 3 saltcedar sampling sites on the 39th parallel, which would make the individual plants likely to be hybrid, and not parental (Williams et al., 2014, Fig. 1). Beetles were reared in 7.5-liter capacity plastic containers with mesh siding for ventilation in incubators under a light regime of 16:8 and 27 °C/16 °C. *Diorhabda carinata* ("C" hereafter) used in this study were originally collected in 2002 near Karshi (Qarshi), Uzbekistan (38.86 N, 65.72 E; elevation 350 m), and *Diorhabda sublineata* ("S" hereafter) originated near the town of Sfax, Tunisia (34.66 N, 10.67 E, elevation 10 m). Colonies of both of these species were maintained in the laboratory since their collection prior to our experiments. *Diorhabda elongata* ("E" hereafter) were collected from Sfakaki, Crete, Greece (35.83 N, 24.6 E, elevation 7 m) and in 2004 they were first released upstream of Esparto, CA along Cache Creek in the Capay Valley. Unlike the other two species, *D. elongata* were collected in 2015 from the field in the Capay Valley and used to start a laboratory colony. No other species were released into the Capay Valley nor have any been established within 150 miles. Therefore, it is reasonable to assume that there was no chance for hybridization before our experiments.

2.2. Crosses

To produce the first generation of hybrids, seven virgin females and seven males of each species were placed together into a plastic bucket with mesh siding (7.5 l) with saltcedar. Since male-female direction-

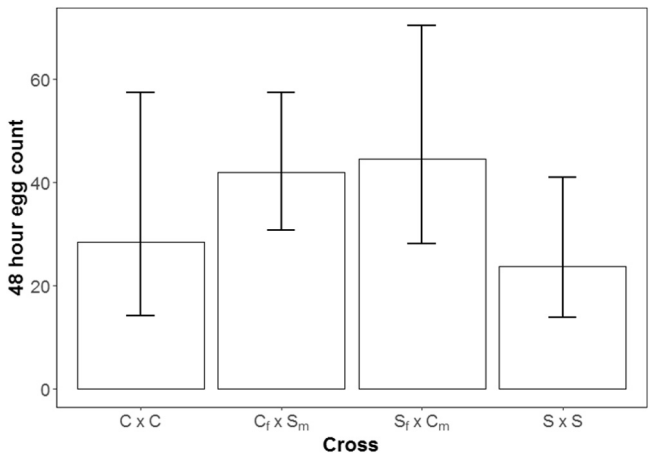

Fig. 1. Number of eggs produced in first 48 h by *D. carinata* (C × C), *D. sublineata* (S × S), and their hybrids in the second generation. Cross significantly affected egg production, with hybrids producing more than either parental species. Bars represent 95% confidence intervals.

ality can affect the fitness of hybrid offspring (Payseur and Rieseberg, 2016), we crossed each species reciprocally. We thus made the following hybrids: *D. carinulata* x *D. elongata* ($C_f \times E_m$, $E_f \times C_m$), *D. carinulata* x *D. sublineata* ($C_f \times S_m$, $S_f \times C_m$), *D. elongata* x *D. sublineata* ($E_f \times S_m$, $S_f \times E_m$), plus the parents ($C_f \times C_m$, $E_f \times E_m$, $S_f \times S_m$). To keep inbreeding depression to a minimum, we initiated two separate buckets for each of the parental lines so that density remained the same but so the parental generation had 14 families rather than 7 for the crosses. All adults were allowed to remain in the buckets for five days of egg-laying.

2.3. F_1 adult performance test

We counted the number of eggs produced over 48 h as an estimate of performance of first generation hybrids (Lewis et al., 2003). Buckets were checked daily for emergence of F_1 adults. On the day of emergence, adults were sexed and mating pairs were placed into a plastic container (0.4 L) with a paper towel lining the bottom and food. The containers were checked daily for eggs. The number of eggs produced was counted for 48 h after the first eggs were laid. After this time, F_1 adults were removed and killed by freezing.

2.4. F_2 larval performance test

We measured percent hatching of all eggs laid in the first 48 h, development time (in days), and adult mass (mg) attained by each F_2 larva. Upon emergence, the date was recorded as well as the number of eggs that successfully hatched. Counting eggs is challenging due to the three-dimensional nature of the egg clutches. Following (Bean et al., 2013b), to ensure accuracy we also counted the number of first instar larvae and compared this with the number of eggs. If the number of eggs was less than the number of larvae, we used the number of larvae as the total number of eggs produced. If the number of eggs was greater than the number of larvae, we conducted a recount of the clutch. Out of the hatched larvae from each mating pair, up to five were randomly chosen and allowed to develop individually.

Larvae were maintained in small plastic cups (0.4 L) and given fresh saltcedar with its stem in a water-filled 1.5 mL eppendorf tube each day. A paper-towel lined the bottom of each cup. When the larvae reached their last stage of development, 2 cm of sand was placed in each cup to provide conditions favorable for pupation. All larvae were maintained in incubators under a light regime of 16:8 (L:D) and 27 °C/16 °C, and rotated every other day to standardize environmental effects.

2.5. F_2 adult preference test

We conducted a host preference test to determine if hybridization affected host preference for the non-target species, athel, presenting beetles with a choice between saltcedar and athel. Athel is an ornamental that is found at more southern latitudes in the US and is considered invasive in the southwestern U.S. (Gaskin and Shafroth, 2005). *Tamarix* hybrids of *T. ramosissima* and *T. chinensis* (saltcedar) are considered the preferred field host of *Diorhabda*. Previous host testing showed that the *D. elongata* can survive as well on athel as on saltcedar, will oviposit on either saltcedar or athel under laboratory no-choice conditions, and showed an inconsistent feeding preference for saltcedar under choice conditions (Milbrath and Deloach, 2006a,b). However, in choice conditions, *D. elongata* oviposited about 1/3 fewer eggs on athel than saltcedar (Milbrath and DeLoach, 2006b). In the field, some non-target attack on athel has occurred, but saltcedar is preferred (Moran et al., 2009). Further, the intrinsic rate of increase of beetle populations is reduced on athel due to smaller egg mass size and a delayed start to oviposition (Milbrath and DeLoach, 2006b).

Between 24 and 48 h after emergence, we sexed and weighed the F_2 adults. The beetles were placed in a plastic tub (3 L) with two eppendorf tubes containing equal amounts of either athel or saltcedar (average fresh weight of offered plant material 3.13 g ± 0.02 (mean ± SE)). The saltcedar used in the preference test was the same that had been used to rear the beetles throughout the experiment. The athel was sent from a population in Lake Mead National Park, Nevada. Each beetle was placed in the middle of the tub, with both plants placed equidistantly at 10 cm from the center. The beetle remained in the plastic tub for 24 h, at which time the amount of frass under each plant was weighed to the nearest 0.1 g (DeLoach et al., 2003).

2.6. F_3 larval performance test on two different hosts

We measured F_3 larval performance on athel and saltcedar. After the host-choice test, mating pairs were formed with F_2 adults from the same cross. All F_2 adults were given saltcedar foliage to feed on regardless of what they chose as their host in the adult preference test. They were placed in the same plastic dish as previously described and allowed to mate and oviposit. The date of first oviposition, the number of eggs laid in 48 h, and the percent hatching was recorded. Larvae from each mating pair were split and a maximum of five larvae were placed in a plastic dish with either athel or saltcedar. We measured development time to adult and adult mass.

2.7. Statistical analysis

Our interests centered on comparing the fitness of hybrids to their parental species. Thus, each analysis was done separately for each of the 7 pairs of parental species and their respective two hybrid crosses (male/female reciprocal). All statistical analysis was conducted using R version 3.3.2 (R_Core_Team, 2016). For the first generation, we analyzed differences in the total number of eggs produced between hybrids and parental species using a standard linear model. The number of eggs was log-transformed to meet the assumption of homogeneity of variance. Percent hatching was analyzed as a proportion of hatched eggs compared to the total number of eggs using a generalized linear model with quasibionmial error distribution. Cross was the only fixed effect for number of eggs produced and percent hatching in the first-generation analysis. For the second and third generations, we quantified the development time from egg to adult (days), adult mass (mg), and host choice. For development time and adult weight, we used linear mixed-effects models through the lme4 package (Bates et al., 2015) with cross, sex and their interaction as fixed effects, and family as a random effect. For host choice with a binary response (saltcedar or athel), we used a generalized linear mixed effects model with a binomial error distribution. For the third generation, we also included the random effect of cup nested within family for development time and adult weight.

3. Results

3.1. Egg count, percent hatching

Hybridization did not significantly affect the number of F_1 eggs laid in 48 h for any of the crosses (Tables 1–3). Cross had a marginally significant influence on percent of eggs that hatched with the E × E and $E_f \times S_m$ cross producing slightly fewer viable eggs than the other crosses ($F_{3,37} = 2.82$, $P = 0.052$, Table 2). In the F_2, only for the S × C cross was there a significant effect of cross on the number of eggs laid in 48 h, where hybrids produced significantly more eggs than either parental species ($F_{3,\ 39} = 2.97$, $P = 0.044$, Table 1, Fig. 1). Cross did not affect the percentage of eggs hatched for any crosses in the second generation (Tables 1–3).

3.2. Development time, adult mass

In the S × C crosses, females were larger (effect of sex: $\chi^2 = 12.98$, df = 1, $P < 0.001$, effect of cross: $\chi^2 = 16.39$, df = 3, $P < 0.001$)

Table 1
Trait means (95% CI) for each generation of the *D. sublineata* by *D. carinata* cross. Letters indicate significant differences between crosses.

Gen	Trait		C × C	$C_f \times S_m$	$S_f \times C_m$	S × S	F value	*P*
1	48 h egg count		25.53 (20.08, 32.46)[a]	21.54 (17.13, 27.08)[a]	30.14 (22.38, 40.59)[a]	20.25 (15.73, 26.08)[a]	$F_{3,\ 69} = 1.733$	0.1682
		N	20	22	13	18		
	Proportion hatching		91.4% (66.0, 99.0)[a]	90.9% (68.0, 98.6)[a]	91.8% (59.9, 99.6)[a]	94.3% (67.2, 99.8)[a]	$F_{3,\ 60} = 0.8425$	0.476
			17	20	12	15		
2	Dev time (days) (male)		43.6 (41.2, 45.9)[a]	38.2 (36.3, 40.1)[b]	39.6 (37.4, 41.9)[ab]	43.5 (41.0, 45.9)[a]	See Table 4 for statistical results	
		N	14	31	18	17		
	Dev time (days) (female)		44.1 (41.6, 46.7)[a]	36.2 (34.3, 38.2)[b]	38.7 (36.2, 41.3)[b]	39.8 (37.6, 41.9)[b]		
		N	11	22	12	13		
	Adult mass (mg) (male)		21.0 (19.3, 22.8)[ab]	22.6 (21.2, 24.0)[a]	19.4 (18.0, 21.0)[b]	18.3 (16.9, 19.8)[b]		
		N	14	31	18	17		
	Adult mass (mg) (female)		22.5 (18.5, 27.5)[ab]	25.8 (22.2, 29.9)[a]	20.6 (16.9, 25.1)[ab]	19.2 (16.2, 22.8)[b]		
		N	11	22	12	13		
	Preference for saltcedar		54.2% (34.6, 72.5)[a]	53.8% (40.3, 66.8)[a]	76.9% (57.2, 89.2)[a]	80.0% (62.1, 90.7)[a]		
		N	24	52	26	30		
	48 h egg count		25.0 (16.1, 38.8)[a]	41.7 (32.1, 54.1)[a]	45.6 (33.0, 63.1)[a]	27.0 (18.9, 38.7)[a]	**$F_{3,\ 39} = 2.9659$**	**0.0437**
		N	6	17	11	9		
	Proportion hatching		93.2% (26.5, 99.1)[a]	94.7% (67.7, 99.8)[a]	94.2% (50.0, 99.9)[a]	79.7% (29.7, 98.9)[a]	$F_{3,\ 26} = 1.3756$	0.2722
			3	15	7	5		
3	Dev time (days) saltcedar		31.9 (29.7, 34.3)[a]	34.6 (33.3, 35.9)[a]	35.3, (33.2, 37.6)[a]	32.4 (30.5, 34.3)[a]	See Table 5 for statistical results	
		N	9	38	9	12		
	Dev time (days) athel		34.1 (31.6, 36.9)[a]	37.0 (35.4, 38.6)[a]	37.7 (35.4, 40.2)[a]	34.6 (32.5, 36.9)[a]		
		N	3	24	11	7		
	Adult mass (mg) (male)		19.5 (15.9, 23.9)[a]	19.7 (17.7, 21.8)[a]	18.6 (15.8, 22.0)[a]	16.7 (14.1, 19.7)[a]		
		N	9	38	9	12		
	Adult mass (mg) (female)		22.5 (18.4, 27.6)[a]	22.8 (20.6, 25.3)[a]	21.6 (18.1, 25.6)[a]	19.3 (16.4, 22.7)[a]		
		N	3	24	11	7		
	Preference for saltcedar		69.0% (37.2, 89.3)[a]	61.8% (47.3, 74.5)[a]	50.2% (30.3, 75.7)[a]	71.4% (45.6, 88.1)[a]		
			12	62	20	19		

Bold terms indicate significance at $P < 0.05$.

and hybrids developed faster (effect of sex: $\chi^2 = 9.93$, df = 1, $P = 0.002$, effect of cross: $\chi^2 = 20.60$, df = 3, $P < 0.001$) (Tables 1 and 4, Fig. 2). For the S × E cross, there was a significant interaction between sex and cross in development time, in that males developed slower than females in the S × S line (interaction cross * sex: $\chi^2 = 8.90$, df = 3, $P = 0.031$, Tables 2 and 4). We also found a significant effect of sex and cross on adult mass in the S × E cross, with females being overall larger (effect of sex: $\chi^2 = 7.28$, df = 1, $P = 0.007$, effect of cross: $\chi^2 = 17.50$, df = 3, $P < 0.001$, Tables 2 and 4). There was no effect of sex or cross on development time or adult mass for the E × C cross, although overall, females tended to be larger.

For the third generation, we were only able to investigate the effects of hybridization for the S × C cross (*D. sublineata* x *D. carinata*) due to limitations in the availability of our host plants. Development time was significantly affected by host plant and by cross, with both parents and hybrids developing slower on athel (effect of cross: $\chi^2 = 9.74$, df = 4, $P = 0.029$; effect of plant: $\chi^2 = 10.22$, df = 1, $P = 0.001$, Tables 1 and 5, Fig. 3). While there was a trend for hybrids to develop more slowly than parents regardless of host plant, there was no significant decrease in development time in hybrids compared to parents (effect of cross: Hybrids develop more slowly than the parents regardless of host plant, yet contrasts between crosses were not significant. Adult weight was not affected by hybridization, however females were larger regardless of cross (effect of sex: $\chi^2 = 10.124$, df = 1, $P = 0.001$, Tables 1 and 5).

Table 2
Trait means (95% CI) for each generation of the *D. sublineata* by *D. elongata* cross. Letters indicate significant differences between crosses.

Gen	Trait		S × S	$S_f \times E_m$	$E_f \times S_m$	E × E	F value	*P*
1	48 h egg count		20.25 (15.45, 26.55)[a]	22.46 (16.12, 31.28)[a]	26.24 (18.84, 36.55)[a]	18.04 (10.16, 32.03)[a]		
		N	18	12	12	4	$F_{3,\ 42} = 0.4807$	0.5706
	Proportion hatching		94.3% (67.2, 99.8)[a]	90.6% (58.5, 99.3)[a]	87.62% (51.6, 98.9)[a]	87.8% (30.2, 99.9)[a]		
		N	15	12	10	4	**$F_{3,\ 37} = 2.8245$**	**0.05193**
2	Dev time (days) (male)		43.4 (40.8, 46.2)[a]	37.5 (34.7, 40.5)[b]	39.5 (35.5, 44.0)[ab]	42.2 (38.6, 46.3)[ab]	See Table 4 for statistical results	
		N	17	9	5	13		
	Dev time (days) (female)		39.8 (37.8, 42.0)[a]	38.0 (35.2, 41.0)[a]	42.4 (39.7, 45.3)[a]	43.3 (39.2, 47.7)[a]		
		N	13	5	10	3		
	Adult mass (mg) (male)		18.1 (16.2, 20.2)[a]	13.1 (11.5, 15.1)[b]	17.1 (14.1, 20.8)[ab]	17.0 (14.6, 19.9)[ab]		
		N	17	9	5	13		
	Adult mass (mg) (female)		19.1 (16.7, 21.9)[a]	15.6 (12.8, 19.0)[a]	21.5 (18.1, 25.5)[a]	18.8 (14.5, 24.3)[a]		
		N	13	5	10	3		
	Preference for *Tamarix* spp		80.0% (62.1, 90.7)[a]	38.5% (17.0, 65.6)[b]	50.0% (26.0, 74.0)[ab]	80.0 (53.0, 93.4)[ab]		
		N	30	13	14	15		
	48 h egg count		27.0 (17.7, 41.2)[a]	27.0 (14.4, 50.9)[a]	45.1 (21.8, 93.6)[a]	28.0 (11.4, 68.3)[a]	$F_{3,\ 14} = 0.622$	0.6124
		N	9	4	3	2		
	Proportion hatching		79.7% (29.7, 98.9)[a]	70.5% (14.2, 98.9)[a]	96.6% (18.1, 96.7)[a]	94.1% (4.3, 92.5)[a]	$F_{3,\ 7} = 0.9465$	0.4682
		N	5	3	2	1		

Bold terms indicate significance at $P < 0.05$.

Table 3
Trait means (95% CI) for each generation of the *D. elongata* by *D. carinata* cross. Letters indicate significant differences between crosses.

Gen	Trait		E × E	$E_f \times C_m$	$C_f \times E_m$	C × C	F Value	*P*
1	48 h egg count		18.04 (9.93,32.77)[a]	18.90 (14.37, 24.85)[a]	18.36 (13.96, 24.14)[a]	25.53 (19.55, 33.35)[a]		
		N	4	19	19	20	$F_{3, 58} = 1.3855$	0.2837
	Proportion hatching		87.8% (35.1, 99.4)[a]	86.8% (58.1, 99.7)[a]	90.7% (61.9, 99.1)[a]	91.4% (66.0, 99.0)[a]		
		N	4	11	14	17	$F_{3, 42} = 0.7341$	0.5376
2	Dev time (days) (male)		42.4 (38.4, 46.3)[a]	39.5 (36.6, 42.4)[a]	42.5 (39.7, 45.4)[a]	43.6 (41.1, 46.3.4)[a]	See Table 4 for statistical results	
		N	13	12	18	14		
	Dev time (days) (female)		43.33 (38.57, 48.09)[a]	39.13 (36.55, 41.71)[a]	40.0 (36.3, 43.7)[a]	44.0 (41.02, 47.17)[a]		
		N	3	14	5	11		
	Adult mass (mg) (male)		17.1 (14.9, 19.6)[a]	18.8 (16.7, 21.0)[a]	20.5 (18.4, 22.9)[a]	21.0 (19.0, 23.3)[a]		
		N	13	12	18	14		
	Adult mass (mg) (female)		18.8 (13.7, 25.8)[a]	22.1 (18.7, 26.1)[a]	19.7 (15.4, 25.2)[a]	22.7 (18.7, 27.5)[a]		
		N	3	14	5	11		
	Preference for *Tamarix* spp.		81.5% (50.8, 94.9)[a]	45.6% (26.2, 66.4)[a]	54.3% (31.9, 75.1)[a]	54.1% (32.8, 74.0)[a]		
		N	15	26	22	24		
	48 h egg count		28.0 (15.0, 52.0)[a]	35.6 (27.9, 45.4)[a]	50.1 (33.8, 74.1)[a]	25.0 (17.5, 35.8)[a]	$F_{3, 22} = 2.6441$	0.07446
		N	2	13	5	6		
	Proportion hatching		94.1% (2.0, 97.2)[a]	74.6% (58.6, 99.9)[a]	93.0% (35.3, 99.3)[a]	93.2% (26.5, 99.1)[a]	$F_{3, 14} = 1.5797$	0.2387
		N	1	10	4	3		

Table 4
Results from generalized linear mixed-effects models for the second generation of hybridization for all crosses.

	S × E Cross			Random effects			
	Cross	Sex	Cross * sex	Family		Residual	
Trait	$\chi 2$, (df), *P*	$\chi 2$, (df), *P*	$\chi 2$, (df), *P*	Variance	Std dev	Variance	Std dev
Dev time	**10.82, (3), 0.013**	0.47, (1), 0.493	**8.90, (3), 0.031**	0.006174	0.07857	0.001771	0.04208
Adult mass	**17.50, (3), < 0.001**	**7.28, (1), 0.007**	2.56, (3), 0.464	0.01266	0.1125	0.02347	0.1532
Preference for saltcedar	**9.23, (3), 0.026**	0.98, (1), 0.322	0.99, (3), 0.804	0	0	0	0
C×E Cross							
Dev time	5.99, (3), 0.112	0.08 (1), 0.7837	5.79, (3), 0.122	0.008246	0.09081	0.001845	0.04295
Adult mass	5.68, (3), 0.128	3.47, (1), 0.0626	2.65, (3), 0.449	0.01058	0.1029	0.0348	0.1868
Preference for saltcedar	3.66, (3), 0.300	0.89, (1), 0.3464	3.69, (3), 0.297	0.6548	0.8092	0	0
S×C Cross							
Dev time	**20.60, (3), < 0.001**	**9.934, (1) 0.002**	5.82, (3), 0.120	9.787	3.128	4.205	2.051
Adult mass	**16.39, (3), < 0.001**	**12.982, (1), < 0.001**	2.73, (3), 0.435	0.02041	0.1429	0.02266	0.1505
Preference for saltcedar	**9.87, (3), 0.031**	0.3953, (1), 0.5295	1.89, (3), 0.596	0	0	0	0

Bold terms indicate significance at $P < 0.05$.

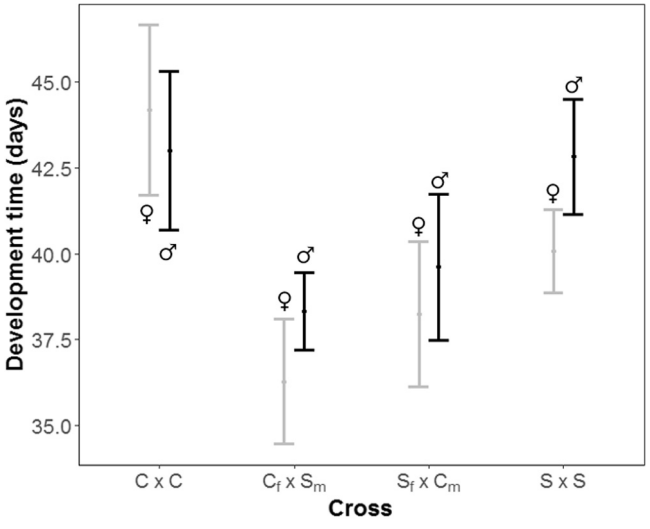

Fig. 2. Development time from hatching until adult for each sex of *D. carinata* (C × C), *D. sublineata* (S × S) and their hybrids in the second generation. Cross significantly affected development time, with hybrids developing faster than either parental species. Grey and black lines represent females and males, respectively. Bars represent 95% confidence intervals.

3.3. Host choice

We tested the host preference of individuals from all crosses in the second generation. Due to limitations in our host plant resources and, because of differences seen in the second generation, we also examined host preference for the S × C cross in the third generation. Sex did not affect host choice for any of the crosses in the second generation (Tables 1–3). Cross significantly affected host choice in the S × C cross (effect of cross: $\chi^2 = 9.87$, df = 3, $P = 0.031$) and S × E cross (effect of cross ($\chi^2 = 9.23$, df = 3, $P = 0.026$), whereas there was no difference in host preference between hybrids and their parents in the E × C cross (Tables 1–4, Fig. 4). There was no effect of hybridization on host preference in the S × C cross in the third generation (effect of cross: $\chi^2 = 1.163$, df = 3, $P = 0.7619$, Tables 1 and 5).

4. Discussion

In introduced species, the effects of hybridization can influence local adaption and determine the fate of colonization success and establishment (Rius and Darling, 2014). Introduced biological control agents undergo similar pressures as newly invading species, and understanding the mechanisms behind population growth and establishment are crucial to the implementation of successful biological control. In this study, we investigated the effects of hybridization on various life history traits and host preference for three different species

Table 5
Results from generalized linear mixed-effects models for the third generation of the *D. sublineata* by *D. carinata* cross.

Trait	S × C Cross				Random effects					
	Cross	Sex	Plant	Plant * Cross	Cup within family		Family		Residual	
	χ2, (df), *P*	χ2, (df), *P*	χ2, (df), *P*	χ2, (df), *P*	Variance	Std dev	Variance	Std dev	Variance	Std dev
Dev time	**9.74, (3), 0.030**	0.022, (1), 0.882	**10.22, (1), 0.001**	3.46, (3), 0.326	0.0027	0.052	0.0009	0.0303	0.00305	0.05531
Adult mass	4.16, (3), 0.244	**9.74, (1), 0.021**	1.39, (1), 0.239	2.67, (3), 0.449	0.02133	0.1461	0	0	0.04805	0.2192
Preference for saltcedar	1.16, (3), 0.762	0.12, (1), 0.728	1.00, (1), 0.317	0.30, (3), 0.960	0	0	0	0	0	0

Bold terms indicate significance at $P < 0.05$.

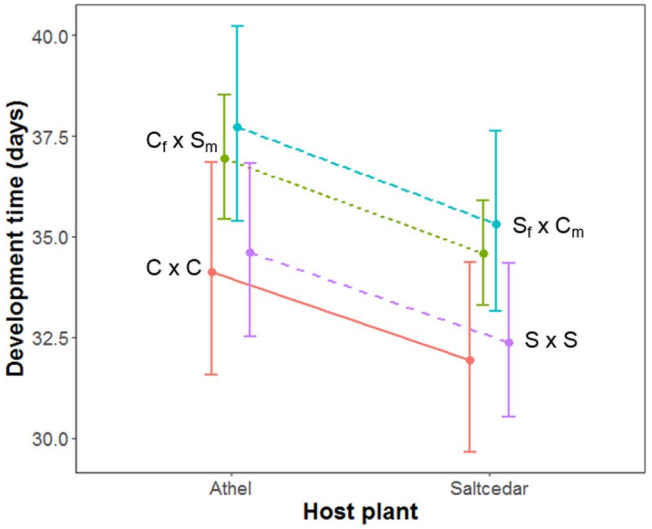

Fig. 3. Development time on athel (non-target) and saltcedar (target) for *D. carinata* (C × C), *D. sublineata* (S × S), and their hybrids after three generations of hybridization. $S_f \times C_m$ are long close dashes, $C_i \times S_m$ small dashes, S × S long spaced dashes, and C × C solid line. Host plant significantly affected development time with beetles developing slower on the non-target host.

of the biological control agent *Diorhabda*. We confirmed that all three species are reproductively compatible (Bean et al., 2013b), and found that reciprocal crosses produced viable offspring through at least two generations. Life history traits beyond the production of viable eggs were either unchanged or improved with hybridization when compared to the parental species. These results support the hypothesis that these species have not experienced reproductive isolation for long enough to allow the evolution of genetic incompatibilities.

Hybridization can have positive, neutral, or negative effects on fitness. These effects depend on the genetic distance between mixing populations and the interactions between genes and environment. Hybrid vigor is commonly seen in the first generation of admixture between genetically distinct populations, and is typically thought to be due to masking of deleterious alleles rather than overdominance (Szulkin et al., 2010), whereas hybrid breakdown is commonly seen in the second or later generations due to recombination of the parental genes, allowing for the possibility of deleterious allele combinations (heterozygote disadvantage) (Dobzhansky, 1950; Edmands, 2002). In our study, there was no difference between parents and their hybrid offspring in fecundity or percent hatching in the first generation in any cross. Previous molecular work done by Bean et al. (2013b) showed that while all four *Diorhabda* species separated into their own clades, the three species examined here were likely more closely related to each other than to the congeneric *D. carinulata*. It is possible that these

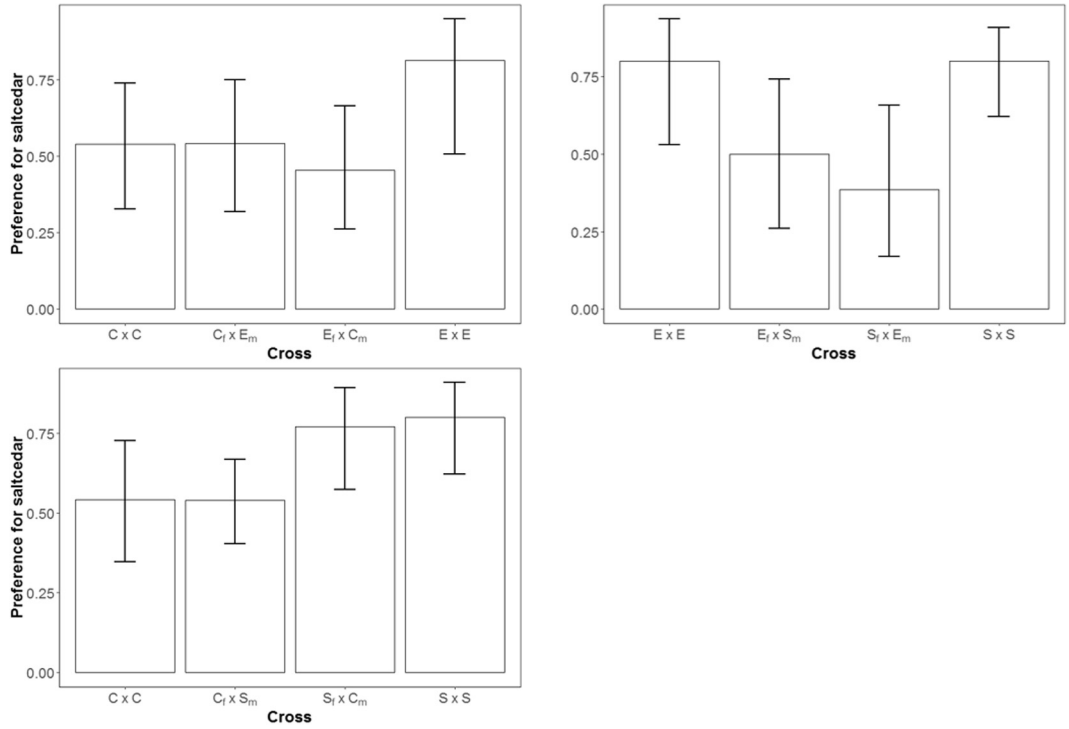

Fig. 4. Preference for saltcedar (target) over athel (non-target) across all parental species (*D. carinata* "C", *D. sublineata* "S", *D. elongata* "E") and their hybrids in the second generation of hybridization. Hybridization significantly affected host preference for the S × C and S × E crosses. Error bars represent 95% confidence intervals.

species are not genetically distinct enough to be detrimentally affected by hybridization. However, the beetles used in our study had been laboratory reared for varying amounts of time (at least ten generations), and may have lost heterozygosity via inbreeding or drift, which could reduce fitness. Thus, an alternative explanation is that positive effects of crossing, via masking of deleterious mutations, could have balanced out potentially negative effects of hybridization, leading to zero, or close to zero, net change in life history traits. Because the masking of deleterious mutations can persist for many generations (Frankham, 2016; Hedrick and Garcia-Dorado, 2016), further study investigating the effects of hybridization for more than three generations would allow us to disentangle the effects of masking deleterious alleles of potentially inbred parental species on the one hand from the effects of potential genetic incompatibilities between species on the other.

Our results show that some of our crosses benefited greatly from hybridization in fecundity and development time in the second generation, and thus we see no evidence of hybrid breakdown. S × C crosses produced 67% more eggs and developed approximately 7 days shorter than the parental species. The E × C cross exhibit the same trend, although this was only marginally significant. Other crosses showed no effect of hybridization, and none of our crosses suffered a fitness cost. In the S × C cross, where we could examine a third generation, we saw no effect of hybridization on fecundity, but we did see a trend that hybrids were developing slower on both host plants. For this analysis, our sample size was lower than for the previous generations, and so further work is necessary to determine if development time slowed because of hybridization.

Unanticipated host use in a released agent is one of the most concerning issues to scientists studying biological control (Van Klinken and Edwards, 2002; Brodeur, 2012; McEvoy et al., 2012). Our results show that host preference can indeed be affected by hybridization, and that the phenotype can vary depending on the maternal or paternal species. In the S × C crosses, host preference of the hybrid followed the preference of the maternal species, whereas in the S × E cross, hybrids showed no preference for either host plant where the parents both showed a strong preference for the target host. Host specificity depends upon a suite of traits, such as behavior, morphology, and life-history strategies and as such is highly constrained (Zwolfer and Harris, 1971; Giebink et al., 1984; Chang et al., 1987). Even so, in more generalist species than are typically used for biological control, host use has been shown to have a genetic basis, and can thus vary between individuals and populations (Singer and Parmesan, 1993; Funk, 1998). In our study, the inherited pattern for host use depended not only on the cross, but the preference of the maternal species. A growing body of literature suggests that for herbivorous insect species, mothers have been shown to influence host use (Amarillo-Suarez and Fox, 2006; Egan et al., 2011; Cahenzli and Erhardt, 2013). Egan et al. (2011) specifically demonstrated that host-use and performance are traits with sex-linked maternal influence. Consequently, the pattern of host specificity in hybrid crosses can be hard to predict since it will depend not only on the amount of genetic variation across a suite of traits, but also parental influence. We suggest that for new releases, results such as found here should be followed up at a larger scale (e.g. field cages with growing plants rather than cut plants) to evaluate their robustness under a more natural setting.

It is worth noting that the release of different ecotypes or species of weed biological control agents is the exception rather than the norm. In the case of *Diorhabda*, various ecotypes were released because the original populations of *D. carinulata* did not establish below the 38th parallel (Bean et al., 2012; Winston et al., 2014). At the time, the different ecotypes were not considered different species, and the potential effects of eventual hybridization were not considered (Tracy and Robbins, 2009). Furthermore, the non-target host in our experiments, athel, was previously known to be an acceptable, yet less preferred, host for *Diorhabda* beetles in the field (Moran et al., 2009). Future studies are needed to determine if hybridization translates to an increase in attack on athel in the field. Thus, our results, like those on the *Datylopius* system (Hoffmann et al., 2002; Mathenge et al., 2010) do not indicate that hybridization has led to an unpredicted host-shift, only that preference for previously identified hosts can shift with hybridization.

Using hybridization in biological control presents unique challenges. On one hand, increasing genetic variation via hybridization, could reduce genetic load, and facilitate adaptation and thus increase the probability of establishment and effective control (Hopper et al., 1993). On the other, the genetic admixture of previously isolated populations might give rise to new phenotypes that are less desirable, such as a change in host use (Hoffmann et al., 2002; Mathenge et al., 2010). Our results demonstrate that while some crosses benefit from hybridization in terms of development time and fecundity, shifts in host preference may also arise. We suggest that in programs considering introduction of genetically distinct populations of biological control agents, pre-release testing of the effects of admixture on host use and other life history traits be conducted.

Statement of authorship

EVB, DB, and RAH designed the experiments, EVB performed the experiments and analyzed the data. EVB wrote the first draft of the manuscript, and DB, AS, and RAH contributed substantially to revisions.

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References

Amarillo-Suarez, A.R., Fox, C.W., 2006. Population differences in host use by a seed-beetle: local adaptation, phenotypic plasticity and maternal effects. Oecologia 150, 247–258.
Anderson, E., Stebbins, G.L., 1954. Hybridization as an evolutionary stimulus. Evolution 8, 378–388.
Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.
Bean, D., Dudley, T., Hultine, K., 2013. Bring on the beetles!. In: Sher, A., Quigley, M.F. (Eds.), Tamarix: A Case Study of Ecological Change in the American West. Oxford University Press, Oxford.
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, 511–523.
Bean, D.W., Kazmer, D.J., Gardner, K., Thompson, D.C., Reynolds, B., Keller, J.C., et al., 2013. Molecular genetic and hybridization studies of Diorhabda spp. released for biological control of tamarix. Invasive Plant Sci. Manage. 6, 1–15.
Brodeur, J., 2012. Host specificity in biological control: insights from opportunistic pathogens. Evol. Appl. 5, 470–480.
Burton, R.S., Rawson, P.D., Edmands, S., 1999. Genetic architecture of physiological phenotypes: empirical evidence for coadapted gene complexes. Am. Zool. 39, 451–462.
Cahenzli, F., Erhardt, A., 2013. Transgenerational acclimatization in an herbivore – host plant relationship. Proc. R. Soc. B-Biol. Sci. 280, 8.
Chang, N.T., Lynch, R.E., Slansky, F.A., Wiseman, B.R., Habeck, D.H., 1987. Quantitative utilization of selected grasses by fall armyworm larvae. Entomol. Exp. Appl. 45,

29–35.
DeLoach, C.J., Lewis, P.A., Herr, J.C., Carruthers, R.I., Tracy, J.L., Johnson, J., 2003. Host specificity of the leaf beetle, Diorhabda elongata deserticola (Coleoptera: Chrysomelidae) from Asia, a biological control agent for saltcedars (Tamarix: Tamaricaceae) in the Western United States. Biol. Control 27, 117–147.
Dlugosch, K.M., Parker, I.M., 2008. Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. Ecol. Lett. 11, 701–709.
Dobzhansky, T., 1950. Genetics of natural populations. 19. Origin of heterosis through natural selection in populations of drosophila-pseudoobscura. Genetics 35, 288–302.
Dobzhansky, T., 1970. Genetics of the Evolutionary Process. Columbia University Press, New York.
Edmands, S., 2002. Does parental divergence predict reproductive compatibility? Trends Ecol. Evol. 17, 520–527.
Egan, S.P., Janson, E.M., Brown, C.G., Funk, D.J., 2011. Postmating isolation and genetically variable host use in ecologically divergent host forms of Neochlamisus bebbianae leaf beetles. J. Evol. Biol. 24, 2217–2229.
Ellstrand, N.C., Schierenbeck, K.A., 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc. Natl. Acad. Sci. U.S.A. 97, 7043–7050.
Fisher, R.A., 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford, England.
Frankham, R., 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Mol. Ecol. 24, 2610–2618.
Frankham, R., 2016. Genetic rescue benefits persist to at least the F3 generation, based on a meta-analysis. Biol. Conserv. 195, 33–36.
Funk, D.J., 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in Neochlamisus bebbianae leaf beetles. Evolution 52, 1744–1759.
Gaskin, J.F., Kazmer, D.J., 2009. Introgression between invasive saltcedars (Tamarix chinensis and T-ramosissima) in the USA. Biol. Invasions 11, 1121–1130.
Gaskin, J.F., Schaal, B.A., 2002. Hybrid Tamarix widespread in U.S. invasion and undetected in native Asian range. Proc. Natl. Acad. Sci. U.S.A. 99, 11256–11259.
Gaskin, J.F., Shafroth, P.B., 2005. Hybridization of tamarix ramosissima and T. chinensis (Saltcedars) with T. aphylla (Athel) (Tamaricaceae) in the Southwestern USA determined from DNA sequence data. Madroño 52, 1–10.
Giebink, B.L., Scriber, J.M., Wedberg, J.L., 1984. Biology and phenology of the Hop-vine borer, Hydraecia-immanis Guenee, and detection of the potato stem borer, Hydraecia-micacea (Esper) (Lepidoptera, Noctuidae), in Wisconsin. Environ. Entomol. 13, 1216–1224.
Hedrick, P.W., Garcia-Dorado, A., 2016. Understanding inbreeding depression, purging, and genetic rescue. Trends Ecol. Evol. 31, 940–952.
Henry, L.M., May, N., Acheampong, S., Gillespie, D.R., Roitberg, B.D., 2010. Host-adapted parasitoids in biological control: does source matter? Ecol. Appl. 20, 242–250.
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.
Hopper, K.R., Roush, R.T., Powell, W., 1993. Management of genetics of biological-control introductions. Annu. Rev. Entomol. 38, 27–51.
Hufbauer, R.A., Szucs, M., Kasyon, E., Youngberg, C., Koontz, M.J., Richards, C., et al., 2015. Three types of rescue can avert extinction in a changing environment. Proc. Natl. Acad. Sci. U.S.A. 112, 10557–10562.
Laugier, G.J.M., Le Moguedec, G., Su, W., Tayeh, A., Soldati, L., Serrate, B., et al., 2016. Reduced population size can induce quick evolution of inbreeding depression in the invasive ladybird Harmonia axyridis. Biol. Invasions 18, 2871–2881.
Lee, Y.W., Fishman, L., Kelly, J.K., Willis, J.H., 2016. A segregating inversion generates fitness variation in yellow monkeyflower (Mimulus guttatus). Genetics 202, 1473.
Lewis, P.A., DeLoach, C.J., Knutson, A.E., Tracy, J.L., Robbins, T.O., 2003. Biology of Diorhabda elongata deserticola (Coleoptera: Chrysomelidae), an Asian leaf beetle for biological control of saltcedars (Tamarix spp.) in the United States. Biol. Control 27, 101–116.
Mallet, J., 2005. Hybridization as an invasion of the genome. Trends Ecol. Evol. 20, 229–237.
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, 331–338.
Mayr, E., 1963. Animal Species and Evolution. Harvard University Press, London.
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.
Michels, G.J., Royer, T.A., Jones, E.N., Lange, R.A., Bynum, E.D., Ruthven, D.C., et al., 2013. New establishment and county records for Diorhabda spp. (Coleoptera: Chrysomelidae) and Coniatus splendidulus (Coleoptera: Curculionidae) in the Texas Panhandle and Western Oklahoma. Southwestern Entomol. 38, 173–181.
Milbrath, L.R., Deloach, C.J., 2006. Acceptability and suitability of Athel, Tamarix aphylla, to the leaf beetle Diorhabda elongata (Coleoptera: Chrysomelidae), a biological control agent of Saltcedar (Tamarix spp.). Environ. Entomol. 35, 1379–1389.
Milbrath, L.R., DeLoach, C.J., 2006. Host specificity of different populations of the leaf beetle Diorhabda elongata (Coleoptera: Chrysomelidae), a biological control agent of saltcedar (Tamarix spp.). Biol. Control 36, 32–48.
Moran, P.J., DeLoach, C.J., Dudley, T.L., Sanabria, J., 2009. Open field host selection and behavior by tamarisk beetles (Diorhabda spp.) (Coleoptera: Chrysomelidae) in biological control of exotic saltcedars (Tamarix spp.) and risks to non-target athel (T. aphylla) and native Frankenia spp. Biol. Control 50, 243–261.
Payseur, B.A., Rieseberg, L.H., 2016. A genomic perspective on hybridization and speciation. Mol. Ecol. 25, 2337–2360.
Pemberton, R.W., 2000. Predictable risk to native plants in weed biological control. Oecologia 125, 489–494.
R_Core_Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rieseberg, L.H., Archer, M.A., Wayne, R.K., 1999. Transgressive segregation, adaptation and speciation. Heredity 83, 363–372.
Rius, M., Darling, J.A., 2014. How important is intraspecific genetic admixture to the success of colonising populations? Trends Ecol. Evol. 29, 233–242.
Seastedt, T.R., 2015. Biological control of invasive plant species: a reassessment for the Anthropocene. New Phytol. 205, 490–502.
Singer, M.C., Parmesan, C., 1993. Sources of variations in patterns of plant insect association. Nature 361, 251–253.
Szűcs, M., Eigenbrode, S.D., Schwarzländer, M., Schaffner, U., 2012. Hybrid vigor in the biological control agent, Longitarsus jacobaeae. Evol. Appl. 5, 489–497.
Szulkin, M., Bierne, N., David, P., 2010. Heterozygosity-fitness correlations: a time for reappraisal. Evolution 64, 1202–1217.
Tracy, J.L., Robbins, T.O., 2009. Taxonomic revision and biogeography of the Tamarix-feeding Diorhabda elongata (Brulle, 1832) species group (Coleoptera: Chrysomelidae: Galerucinae: Galerucini) and analysis of their potential in biological control of Tamarisk. Zootaxa 1–152.
Van Driesche, R.G., Carruthers, R.I., Center, T., Hoddle, M.S., Hough-Goldstein, J., Morin, L., et al., 2010. Classical biological control for the protection of natural ecosystems. Biol. Control 54, S2–S33.
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.
Williams, W.I., Friedman, J.M., Gaskin, J.F., Norton, A.P., 2014. Hybridization of an invasive shrub affects tolerance and resistance to defoliation by a biological control agent. Evol. Appl. 7, 381–393.
Winston, R.L., Schwarzländer, M., Hinz, H.L., Day, M.D., Cock, M.J.W., Julien, M.H., 2014. Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds. USDA Forest Service, Forest Health Technology Enterprise Team Morgantown, West Virginia, pp. 838.
Zwolfer, H., Harris, P., 1971. Host specificity determination of insects for biological control of weeds. Annu. Rev. Entomol. 16, 159.