Evolution of tolerance in an invasive weed after reassociation with its specialist herbivore

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Abstract

The interaction between the European wild parsnip *Pastinaca sativa* and its coevolved florivore the parsnip webworm *Depressaria pastinacella*, established in North America for over 150 years, has resulted in evolution of local chemical phenotype matching. The recent invasion of New Zealand by webworms, exposing parsnips there to florivore selection for the first time, provided an opportunity to assess rates of adaptive response in a real-time experiment. We planted reciprocal common gardens in the USA and NZ with seeds from (1) US populations with a long history of webworm association; (2) NZ populations that had never been infested and (3) NZ populations infested for 3 years (since 2007) or 6 years (since 2004). We measured impacts of florivory on realized fitness, reproductive effort and pollination success and measured phenotypic changes in infested NZ populations relative to uninfested NZ populations to determine whether rapid adaptive evolution in response to florivory occurred. Irrespective of country of origin or location, webworms significantly reduced plant fitness. Webworms reduced pollination success in small plants but not in larger plants. Although defence chemistry remained unchanged, plants in infested populations were larger after 3–6 years of webworm florivory. As plant size is a strong predictor of realized fitness, evolution of large size as a component of florivore tolerance may occur more rapidly than evolution of enhanced chemical defence.

Introduction

Selection pressure exerted by herbivory is thought to have shaped a wide range of plant defence traits over macroevolutionary time (Futuyma & Agrawal, 2009). Herbivores may also cause rapid adaptive evolution in plants over contemporary timescales. However, documenting microevolutionary change is challenging given that response to selection can be influenced by other processes within the ecological community. Only a handful of studies have quantified real-time ecological and evolutionary change in plants in response to selection by herbivores. These studies have generally relied on synthetic plant populations and controlled access by

herbivores to these populations (Agrawal *et al.*, 2012; Züst *et al.*, 2012; Uesugi & Kessler, 2013). Recent common-garden herbivore exclusion studies have shown that, in the absence of herbivory, rapid adaptive evolution in defence and life-history traits can occur. For example, when protected from herbivory, *Oenothera biennis* evolved earlier flowering time, higher seed defences and greater competitive ability in four generations (Agrawal *et al.*, 2012). Competitive ability and allelopathic compound production increased in *Solidago altissima* genotypes experiencing 13 years of herbivore exclusion (Uesugi & Kessler, 2013). Studies documenting rapid responses to selection imposed by the introduction, rather than exclusion, of herbivores are fewer in number. One such study, Züst *et al.*, (2012), demonstrated rapid evolution of specific glucosinolate defences (within five generations) in *Arabidopsis thaliana* in the presence of different species of aphids (Züst *et al.*, 2012).

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Because evolution occurs in an ecological context, phenotypic changes predicted by herbivore removal experiments may not follow in real populations. To date, no studies have examined rapid evolution in natural populations in response to a single herbivore or the impact of rapid adaptive evolution on other ecological processes such as plant–pollinator interactions. The invasion by the European weed, wild parsnip, *Pastinaca sativa* L. (Apiaceae) and its subsequent reassociation with its coevolved specialist herbivore, the parsnip webworm, *Depressaria pastinacella* Duponchel (Lepidoptera: Oecophoridae), in both North America and New Zealand have provided an opportunity to quantify ecological and evolutionary responses to herbivory in real time. *Pastinaca sativa* has been established as an invasive weed in North America for at least 400 years. The presence of phototoxic furanocoumarins in all aboveground tissues of the plant renders it relatively free from herbivory (Berenbaum, 1981; Berenbaum & Zangerl, 1996), with the notable exception of the parsnip webworm, a coevolved European florivore that feeds on buds, flowers and fruit introduced into North America in 1869 (Bethune, 1869). Wild parsnips are also invasive in New Zealand (NZ) (with the earliest record of its presence dating back to 1867). However, NZ populations were free from significant florivory until 2004, when parsnip webworms were discovered in the South Island (Zangerl et al., 2008).

High levels of three furanocoumarins – bergapten, xanthotoxin and sphondin – are associated with resistance of parsnips to webworms in its North American and native European ranges (Berenbaum et al., 1986; Zangerl & Berenbaum, 1993). Webworm florivory exerts selection pressure on furanocoumarin content and composition (Berenbaum et al., 1986), and phenotype matching in the Midwestern USA is strongly suggestive of reciprocal selection between parsnips and webworms (Berenbaum & Zangerl, 1998; Zangerl & Berenbaum, 2003). Moreover, retrospective chemical analysis of North American and European herbarium specimens has shown that, prior to webworm introduction, wild furanocoumarin production in North American seeds was significantly lower relative to plants in Europe and to US plants after 1890 once webworms were present (Zangerl & Berenbaum, 2005).

In New Zealand, Zangerl et al. (2008) found that webworm florivory by 2006–2007 resulted in an average reduction in seed numbers of 75%, with some plants in many populations producing no viable seeds at all. NZ parsnips had lower levels of some furanocoumarins (imperatorin, isopimpinellin and bergapten) but much higher levels of others (xanthotoxin and sphondin) than US parsnips. NZ parsnips also differed in having lower octyl butyrate levels, a larval feeding deterrent and higher octyl acetate levels, a larval attractant (Carroll & Berenbaum, 2001), compared with US populations. Thus, parsnip webworms have had a significant negative impact on plant fitness and have exerted strong selection pressure on chemical traits in NZ parsnip populations. Given that phenotypic variation in chemical defence traits was high in these populations (Zangerl et al., 2008), that these traits are characterized by high additive genetic variance (Zangerl & Berenbaum, 2005) and that plant response to selection by florivores should be especially pronounced (Wise & Rausher, 2013), phenotypic changes in infested NZ wild parsnips in response to webworm selection should occur rapidly.

Selection imposed by webworm florivory also has the potential to alter interactions between wild parsnip and its pollinators. Florivorous insects can directly or indirectly disrupt pollination by reducing the visual and chemical attractiveness of the floral display (McCall & Irwin, 2006). Alternatively, selection on resistance traits may be balanced by pollinator selection on floral traits, especially if either the same traits mediate interactions with florivores and pollinators or if pollinator attractants and florivore defences are genetically correlated (e.g. Kessler et al., 2013). Parsnip plants are obligate outcrossers and rely on large calyptrate flies and syrphids for effective pollination (Jogesh et al., 2013). That parsnip plants in an uninfested NZ population emitted a floral bouquet that was more attractive to pollinators compared with a US population with a long history of infestation (Jogesh et al., 2013), suggests that floral traits may be under selection by both florivores and pollinators.

In view of the genetic, chemical and ecological conditions prevailing in NZ, we hypothesized that the 6 years of reassociation of parsnips and parsnip webworms should have been sufficient to result in rapid evolution of resistance via increased chemical defences or by increased tolerance to florivores. We also hypothesized that rapid evolution in response to florivory has altered parsnip–pollinator interactions. To test these hypotheses, we planted reciprocal common gardens in the USA and in NZ, consisting of US parsnips with a long history of infestation as well as infested and uninfested NZ populations collected from 2004 to 2009 (2–6 years postinfestation).

Materials and methods

Life histories of the herbivore and plant in the USA and NZ

Wild parsnips are biennial and overwinter as rosettes in both countries. In the USA, after overwintering, parsnips bolt in early May, flower in June and set seed in mid-late July. In mid-April, prior to hostplant bolting, parsnip webworm adults oviposit on rosette leaves. After bolting, the largest inflorescence, the primary umbel, unfurls at the apex of the main shoot, followed by the secondary umbels that unfurl at the tips of

branches and tertiary umbels on the stalks of secondary umbels. Webworm larvae, upon hatching, move up to unopened buds and feed almost exclusively on floral tissues. When umbels open, the larvae continue to feed on flowers and developing fruit and subsequently burrow and pupate within the hollow parsnip stems. In NZ, parsnip rosettes bolt in late November, flower throughout December and set seed in mid-late January; however, in contrast with the USA, webworms oviposit in early December, after the plants have already bolted.

Reciprocal common gardens

Reciprocal common gardens were planted in the USA and in NZ. In the USA, the common garden was established at the University of Illinois Phillips Tract research area (N 40.13205, W 88.143826) (Champaign Co., IL, USA) in an area that has been tilled every other year for the last 21 years. In NZ, the garden was planted on agricultural land in Sawyers Bay, Otago, previously used to grow potatoes (S 45.821413, E 170.598428). We planted parsnips grown from seeds collected from nine NZ populations in 2006, 2007, 2008 and 2009 (Table 1). At the time of experimental set-up, four of these populations had been infested for at least 6 years (CRIMP, TOWNLEY, LUMBER and WARRINGTON), two were infested for 3 years (since 2007) (OCEAN and ROCK) and three population had no or minimal (< 1% damage) history of infestation (COTTAGE, BUSHY and HERBERT). Nearly 100% of all plants with 6 years of infestation were damaged (Table 1). Populations infested for 4 years were larger, but > 50% of all plants were damaged. No webworms were found on BUSHY or HERBERT from 2006 until 2009, and with fewer than 1% of plants experiencing damage, the COTTAGE population was considered uninfested for this study. In 2008, seeds were collected from five US populations in central Illinois (DEA, KAN, PIO, OP and RAN), all of which were known to have been infested with webworms for many years (Zangerl *et al.*, 2008).

Gardens were planted with seeds from all nine NZ populations and five US populations. At least 40 seeds from 20 maternal plants in each population (two maternal half-sibs per parent plant), for each seed collection year (2006–2009), were randomly planted in each garden (a total of $40 \times 4 \times 9$ NZ plants and 5×40 US plants). In the USA, seeds were germinated in the UIUC Entomology greenhouse in early March 2009 in plastic pots filled with $1 : 1 : 1$ peat : perlite : Drummer soil and grown at 24 °C at 16 h day length. The seedlings were transplanted to the garden in early July 2009. In NZ, however, with no access to a greenhouse nearby, we planted seeds directly into the ground in September, 2010. Individuals were planted 0.5 m apart in 21 long rows in the USA and 29 long rows in NZ, with rows 1 m apart in both locations. The parsnips were regularly watered and weeded to reduce mortality.

In the spring of the year following planting, we scored all plants that had overwintered successfully. In the 2 weeks between oviposition and egg hatch, one member of each maternal half-sib pair grown in each garden (chosen randomly) was sprayed with insecticide. Plants were sprayed with acephate (USA: Orthene, Ortho, Columbus, OH; NZ: Orthene WSG), a systemic organophosphate insecticide, to create an herbivore-free treatment (= spray treatment). Acephate was purchased as a soluble powder and dissolved in water as per the manufacturer instructions and sprayed at the base of the plant. As female flowers matured on secondary umbels, 10 flowers were collected for chemical analysis in a preweighed Eppendorf centrifuge tube containing a glass bead. The tubes were immediately placed over dry ice to prevent the loss of volatile components and subsequently stored at −20 or −80 °C in the laboratory. When all webworms had pupated, we estimated the percentage (to the nearest 10%) of florets in each umbel sustaining damage. We also recorded umbel diameter with vernier callipers for each primary (stem apex), secondary

Table 1 New Zealand wild parsnip population sizes and parsnip webworm infestation levels from 2006 to 2009. Infestation level was measured as the percentage of all plants in the population with webworms. Number of plants at each site indicated in brackets.

	Population	Lat./Long.	% of population infested			
			2006	2007	2008	2009
Infested for 6 years	CRIMP	S 45.89976, E 170.439749	100 (65)	97 (30)	100 (9)	78 (23)
	LUMBER	S 45.900044, E 170.443954	100 (96)	100 (44)	100 (45)	100 (28)
	TOWNLEY	S 45.893159, E 170.459071	100 (116)	100 (79)	100 (26)	100 (3)
	WARRINGTON	S 45.707985, E 170.589459	71 (31)	100 (34)	95 (20)	100 (27)
Infested for 3 years	OCEAN	S 45.699938, E 170.603256	0 (200)	60 (126)	20 (30)	20 (54)
	ROCK	S 45.658185, E 170.641987	0 (200)	76 (144)	100 (82)	67 (200)
Uninfested	COTTAGE	S 45.678391, E170.625991	0 (200)	0.05 (200)	4 (48)	1 (113)
	BUSHY	S 45.468408, E 170.776248	0 (200)	0 (200)	0 (200)	0 (200)
	HERBERT	S 45.234797, E 170.78011	0 (200)	0 (200)	0 (200)	1 (200)

(branch apex) and tertiary (secondary branch apex) umbel on the plant. The diameter is an approximate predictor of the number of potential seeds (determined empirically, number of seeds = 91.6 × umbel stalk diameter + 98.3, $n = 10$, $r^2 = 0.80$, $P = 0.0005$) (Zangerl *et al.*, 2008). Using the umbel diameter and the estimate of percentage of florets damaged, we calculated the total damage (number of seeds eaten = number of potential seeds × percentage of flowers damaged). Seeds from all plants were collected as they matured on stalks; after sifting to remove debris, seeds were weighed to obtain a measure of fitness (= realized fitness). After all seeds had been collected, the plants were cut at the base, dried in an oven at 60 °C for 4–5 days and weighed to measure total biomass (= plant size).

Chemical analysis

Frozen flower tissues collected in 1.5 mL Eppendorf tubes were pulverized with a glass bead in a Wig-L Bug Amalgamator (Crescent Dental Manufacturing, Chicago, IL, USA) for 1 min. The pulverized tissue was refrozen, to reduce volatizing of compounds, and the frozen material was vortexed with 750 µL of 100% HPLC-grade ethyl acetate containing 0.0001% tridecane, an internal standard. The tubes were centrifuged at 9000× for 3 min, and the supernatant was pipetted into a 2-mL GC-vial. The pellets were dried at 50 °C for 48 h and weighed to estimate tissue dry weight. Chemical extracts obtained in this manner were analysed by gas chromatography with flame ionization detection and subjected to gas chromatography–mass spectrometry (GC-MS).

One microlitre per sample was injected in the GC-MS (Shimadzu QP2010 Plus, Columbia, MD, USA; SHRXI-5MS capillary column, 30 m × 2.5 mm × 0.25 u) in splitless mode for 1.5 min with an injection temperature of 250 °C and helium as the carrier. The initial oven temperature was set at 50 °C, held for 50 s, increased at a rate of 10 °C min^{-1} until 250 °C and held at 250 °C for 5 min. A full scan of ions from 40 to 300 m/z was recorded. Chemical peaks were identified by comparison to the NIST08 library and to standards when available. Amounts were quantified based on peak area integrations of total ion chromatograms. Areas were adjusted with the peak area of the internal standard (tridecane) and divided by the weight of the dried tissue to obtain a peak area per milligram, a measure that could then be compared across samples.

Statistical analyses

Impact of webworms on parsnip fitness
Our first analysis was structured to examine the impact of webworms on fitness and to compare susceptibility of US and NZ plants (genotypic effects) grown in different gardens (environmental effects). Realized fitness was the dependent variable and country of origin (USA, NZ), garden (2010, 2011) and spray treatment were included as fixed effects, population was a random effect, and plant size (stalk weight) was added as a covariate in a linear mixed-effects model. Realized fitness was estimated as log-transformed (to ensure equal variance) total seed weight. We used this model to determine the effects of (1) spray treatment (do webworms have an impact on fitness?), (2) country × spray (are NZ parsnips more susceptible to webworms than US parsnips?), (3) garden × spray (does the NZ environment favour webworm susceptibility?), (4) country × garden × spray (is there a genotype-by-environment interaction in webworm susceptibility?) (5) garden × country (is there a genotype-by-environment interaction in realized fitness?) and (6) stalk weight (does realized fitness change with plant size?).

To quantify impacts of webworm herbivory on the cost of seed production, we calculated plant reproductive effort as the ratio of seed weight to stalk weight. Reproductive effort measures the proportion of available resources (biomass) allocated to seed production and as such accounts for variation in realized fitness that may be due to resource availability and thus the relative cost of investing in seeds. Reproductive effort was fitted with a linear mixed model with country of origin, spray treatment and garden location as fixed effects, and population as a random effect.

Models with population as a random effect and random intercept performed significantly better, and therefore, random effects were included in all models. Likelihood ratio testing comparing AIC values between full and reduced models were used to determine the significance of main and interaction terms in each model. All statistical analyses were conducted in R version 2.14.2 (R Core Team, 2012), and linear-mixed effects models were executed using the package 'nlme' (Pinheiro *et al.*, 2013).

Impact of florivores on pollination success
We compared pollination success between (1) US and NZ parsnips; (2) sprayed vs. unsprayed plants; (3) the two different garden plots; and (4) plant size; to determine whether pollination success was influenced by the country of origin (genotype); florivory, the environment, or plant size, or interactions among these factors. Pollination success was estimated from the weight of fertilized seeds and the total number of available seeds (total potential seeds minus the number of damaged seeds). The number of fertilized seeds was estimated from weight of the fertilized seeds: number of seeds = 329.77 × (Seed weight) − 3.8599 ($n = 27$, $r^2 = 0.99$). The regression equation was obtained by weighing 2–200 seeds. Pollination success (number of fertilized seeds/total number of available seeds) was

analysed as a GLM with a quasibinomial error structure.

Influence of genotype and environment on fitness traits
We also compared plant size, total damage, proportional damage and plant chemistry between US and NZ parsnips grown in different gardens to determine whether these traits: (1) differed between parsnips depending on their country of origin (genotype); (2) differed between gardens (environment); and (3) were influenced by genotype-by-environment interaction. For plant size, log-transformed stalk weight was the dependent variable in a linear mixed model. Total damage was analysed as a generalized linear model (GLM) with a negative binomial model error structure and proportional damage as a GLM quasibinomial model. Plant size was included as a covariate. The drop1 () function in R was used to compare models. Negative binomial GLMs were implemented using the package 'MASS' (Venables & Ripley, 2002). Plants sprayed with insecticide (i.e. webworm-free) were not included in comparisons of damage across countries and gardens. We also examined the effect of spray treatment on plant size to ensure that the insecticide spray did not influence plant growth.

A subset of eight compounds – octyl butyrate, octyl acetate, xanthotoxin, bergapten, isopimpinellin, imperatorin, sphondin and myristicin – that were highly variable among individuals and that have been shown previously to influence webworms either behaviourally (Carroll *et al.,* 2000) or physiologically (Berenbaum & Zangerl, 1992) were analysed for comparisons of floral chemistry. We compared these eight floral compounds between US and NZ parsnips using a MANOVA with peak areas of compounds as dependent variables and with country of origin and garden as main effects. Individual compounds were analysed independently in univariate models to examine the influence of genotype, environment and genotype-by-environment interactions for each compound.

Half-sib comparisons to determine environmental effects
We compared eight floral compounds, size, realized fitness and damage between half-sibs grown in US and NZ gardens to test for trait differences attributable to environmental factors. Half-sibs were compared with a pairwise Wilcoxon's sign test.

Evolution of wild parsnips in NZ
To determine whether susceptibility to webworms decreased in plants associated with webworms over time, we compared NZ parsnips with different infestation histories (infested for 3–6 years and never infested) over the period 2006 to 2009. We analysed the data using a linear mixed-effects model with log-transformed fitness as the dependent variable, webworm infestation history, year (2006–2009) and spray treatment as main
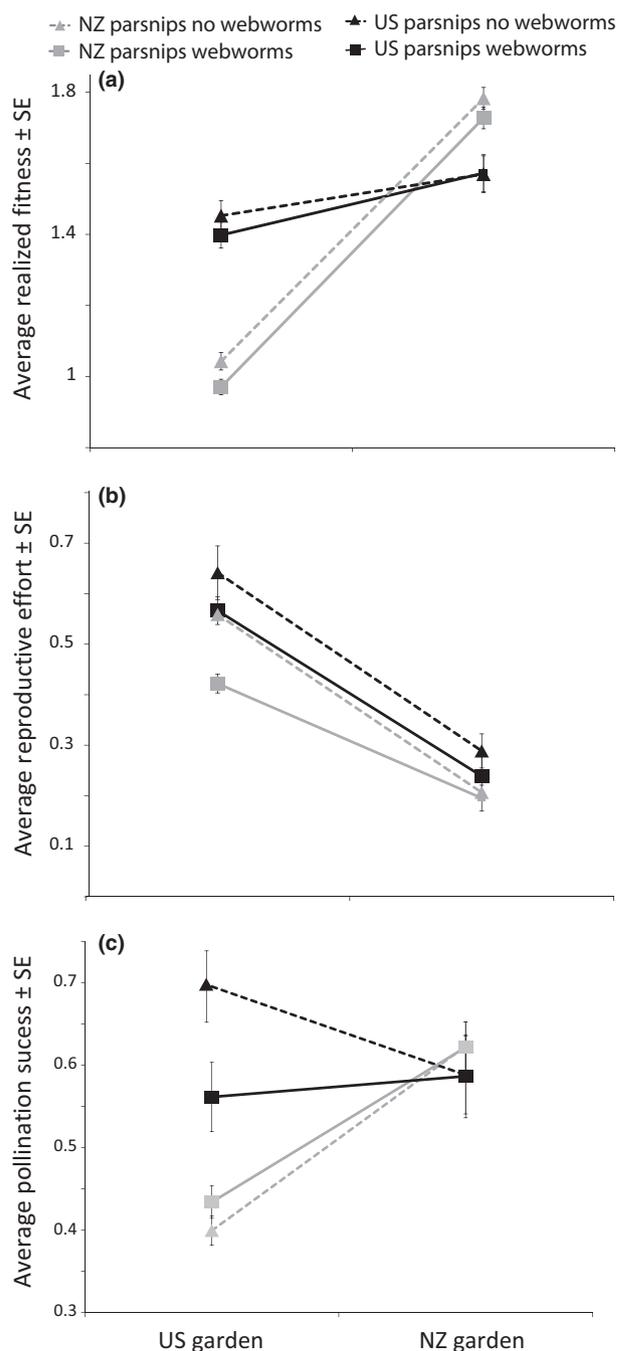
Fig. 1 Realized fitness (a), reproductive effort (b) and pollination success (c) for US and NZ parsnips, with and without webworms (spray treatment) grown in the US common garden and the NZ common garden. Realized fitness was measured as the total seed weight per plant (g), reproductive effort was measured as the ratio of seed weight (g) to stalk weight (g), and pollination success was measured as the proportion of fertilized seeds to the total number of potential seeds. Only parsnips sprayed with insecticide were analysed for pollination success. Effect of country × garden × spray on pollination success, scaled deviance = 7.23, $P = 0.007$.

Table 2 Models comparing phenotypic traits between countries and gardens with log-likelihood ratio (L) and P-values of main and interaction terms.

Model	Dependent variable	Stalk weight	Country	Garden	Country × garden	Spray	Spray × country	Spray × garden	Country × spray × garden
Model 1	Realized fitness	L = 166.03, P < 0.001*	L = 14.48, P < 0.001*	L = 113.22, P < 0.001*	L = 63.38, P < 0.001*	L = 7.57, P = 0.005*	L = 0.05, P = 0.81	L = 0.60, P = 0.43	L = 0.07, P = 0.78
Model 2	Reproductive effort	L = 40.67, P < 0.001*	L = 13.77, P = 0.001*	L = 204.22, P < 0.001*	L = 1.23, P = 0.26*	L = 13.21, P < 0.001*	L = 0.02, P = 0.87	L = 6.57, P = 0.01*	L = 1.20, P = 0.27
Model 3	Plant size	–	L = 1.83, P = 0.17	L = 1241.0, P < 0.001*	L = 179.40, P < 0.001*	L = 2.66, P = 0.10	–	–	–
Model 6	Total damage	L = 7.26, P = 0.007*	L = 1.54, P < 0.21	L = 31.06, P < 0.001*	L = 38.22, P < 0.001*	–	–	–	–
Model 7	Proportional damage	SD = 17.22, P < 0.001*	SD = 17.33, P < 0.001*	SD = 20.46, P < 0.001*	SD = 29.28, P < 0.001*	–	–	–	–
Model 8	Pollination success	SD = 8.06, P = 0.004*	SD = 18.38, P < 0.001*	SD = 11.74, P < 0.001*	SD = 11.32, P < 0.001*	SD = 0.06, P = 0.79	SD = 0.62, P = 0.74	SD = 0.04, P = 0.82	SD = 7.23, P = 0.007*

Difference in AICs in quasibinomial models is estimated with scaled deviance (SD) instead of L. Significant effects (*) are highlighted in bold.

effects, population as a random effect and stalk weight as a covariate.

We evaluated tolerance to herbivory as a result of size because plant size was very strongly correlated with fitness (see Results) and to determine whether parsnips from populations with a history of infestation become larger over time compared with uninfested populations. Log-transformed stalk weight was the dependent variable. We also compared total damage and pollination success in NZ plants. Damage was compared among unsprayed plants using a negative binomial model, whereas pollination success was compared in sprayed plants using a binomial model with proportion of seeds pollinated as a dependent variable.

To detect phenotypic changes in floral chemistry of infested populations from 2006 to 2009, we evaluated changes in floral chemistry in individual populations because the floral chemistry differed among populations. Zangerl *et al.* (2008) also found that the direction and complexity of selection differed among populations; these differences, along with low survivorship in four populations, limited our statistical power such that only five populations (HERBERT, COTTAGE, CRIMP, WARRINGTON and ROCK) could be analysed. With no *a priori* expectation for the direction of change, we used a multivariate distance approach to examine changes in all compounds simultaneously. The data were square-root-transformed, and a resemblance matrix was created with Bray–Curtis dissimilarities and used to conduct an analysis of similarity (ANOSIM) among plants from 2006, 2007, 2008 and 2009 in individual populations. ANOSIM tests for differences in rank dissimilarity between *a priori* defined groups compared with random groups. Much like an ANOVA, the ANOSIM statistic R compares the differences in ranks within years and between years for a population. The analysis was conducted in PRIMER 6 (Clarke & Warwick, 2001). Variation in octyl butyrate had a strong environmental component and was not included in this analysis.

Results

Of the 1238 parsnips planted in the US garden, 685 plants bolted, flowered and set seed. In NZ, 1603 parsnip seeds were sown and 626 plants bolted, flowered and set seed. Of the plants that bolted, some were lost to wind damage and some individuals could not be measured for certain parameters due to losses during processing.

Webworms reduce parsnip fitness

Irrespective of locality, both realized fitness and reproductive effort (the allocation of biomass to seed production) were higher in plants without webworms (Fig. 1a,

Table 3 Differences in damage, fitness, size and floral chemistry between maternal half-sibs grown in the US common garden and NZ common garden. Pairwise differences were estimated based on the Wilcoxon signed-rank test. Significant differences are denoted by *.

|	W+	W−	N	P	US garden	NZ garden
Damage (proportion)	359	1411	59	<0.001*	−	+
Size	9	1369	52	<0.001*	−	+
Fitness	33	495	32	<0.001*	−	+
Octyl butyrate	1	2484	70	<0.001*	−	+
Octyl acetate	1407	1078	70	0.33	=	=
Xanthotoxin	1226	1259	70	0.92	=	=
Bergapten	1211	1224	70	0.85	=	=
Sphondin	556	1040	56	0.05*	=	=
Myristicin	961	1454	69	0.14	=	=
Imperatorin	1656	759	69	<0.001*	+	−
Isopimpinellin	1855	630	70	<0.001*	+	−
Beta-Farnesene	1967	518	70	<0.001*	+	−

Table 2). We did not find a significant country × spray treatment interaction or a garden × spray treatment interaction, suggesting that the effect of webworm florivory on realized fitness does not differ with environment or genetic background (Table 2). However, insecticide treatment increased reproductive effort in the US garden but not in the NZ garden (Table 2, significant spray × garden effect), suggesting that environmental factors influence the effect of webworms on the cost of seed production (allocation of biomass to seeds) but not the effect of webworms on overall realized fitness.

Effect of webworms on pollination success is mediated by plant size

We did not find an overall significant effect of spray treatment on pollination success, but we did find a significant country × garden × spray interaction (Table 2). Webworms had a significant effect on pollination success of US parsnips in the US garden but not in the NZ garden (Fig. 1c); US parsnips without webworms had a higher proportion of flowers fertilized compared to sprayed parsnips in the US common garden. The effect of webworms on pollination success was mediated by plant size; webworms reduced pollination success in smaller plants but not in larger plants (interaction effect of plants size × spray treatment scaled deviance = 25.51, $P < 0.001$).

Genotype-by-environmental interactions influence parsnip fitness

Parsnips grown in NZ were on average 15 times larger than their half-sibs in the US garden. The substantial difference in size is likely a reflection of different abi-
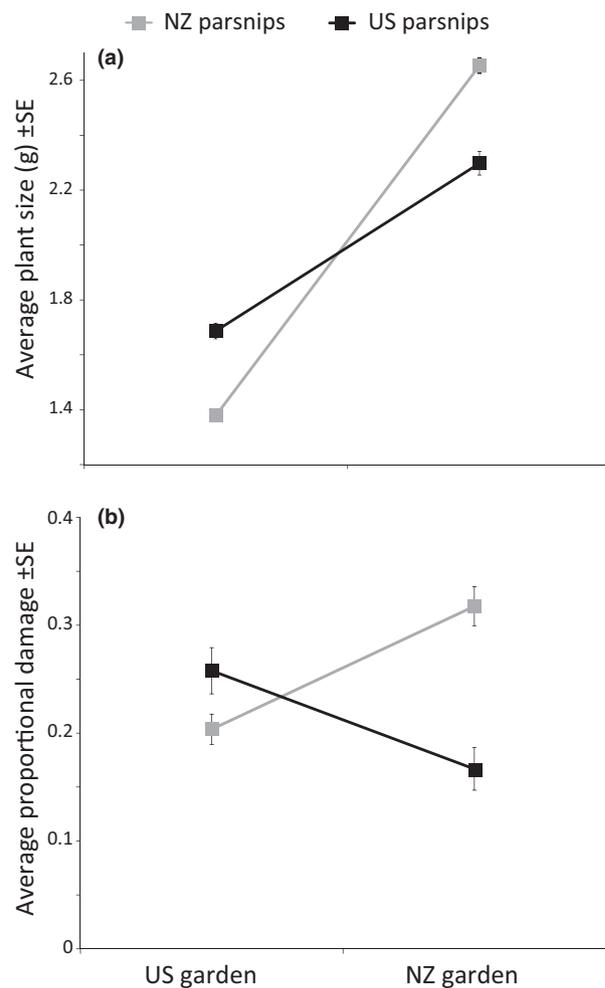
Fig. 2 Plant size (a) and proportional damage (b) for US and NZ parsnips grown in the US common garden and the NZ common garden. Damage was measured as the proportion of all florets that were consumed by webworms. Spray treatment had no effect on plant size ($F = 0.006$, $P = 0.93$), and only unsprayed plants were analysed for webworm damage.

otic conditions between the two gardens. Plant size was a very strong predictor of realized fitness with larger plants having proportionally higher fitness compared with smaller plants (Table 2). Total damage and pollination success were also strongly correlated with plant size (Table 2, significant effect of stalk weight). Significant genotype-by-environment effects and differences between half-sibs in both gardens suggests that many traits are phenotypically plastic (Table 3). US parsnips were larger and had higher realized fitness and greater pollination success in the US garden whereas NZ parsnips were more successful in the NZ garden (Figs 1 and 2; Table 2, significant country × garden interactions). US parsnips also sustained more damage in the US garden, whereas NZ parsnips
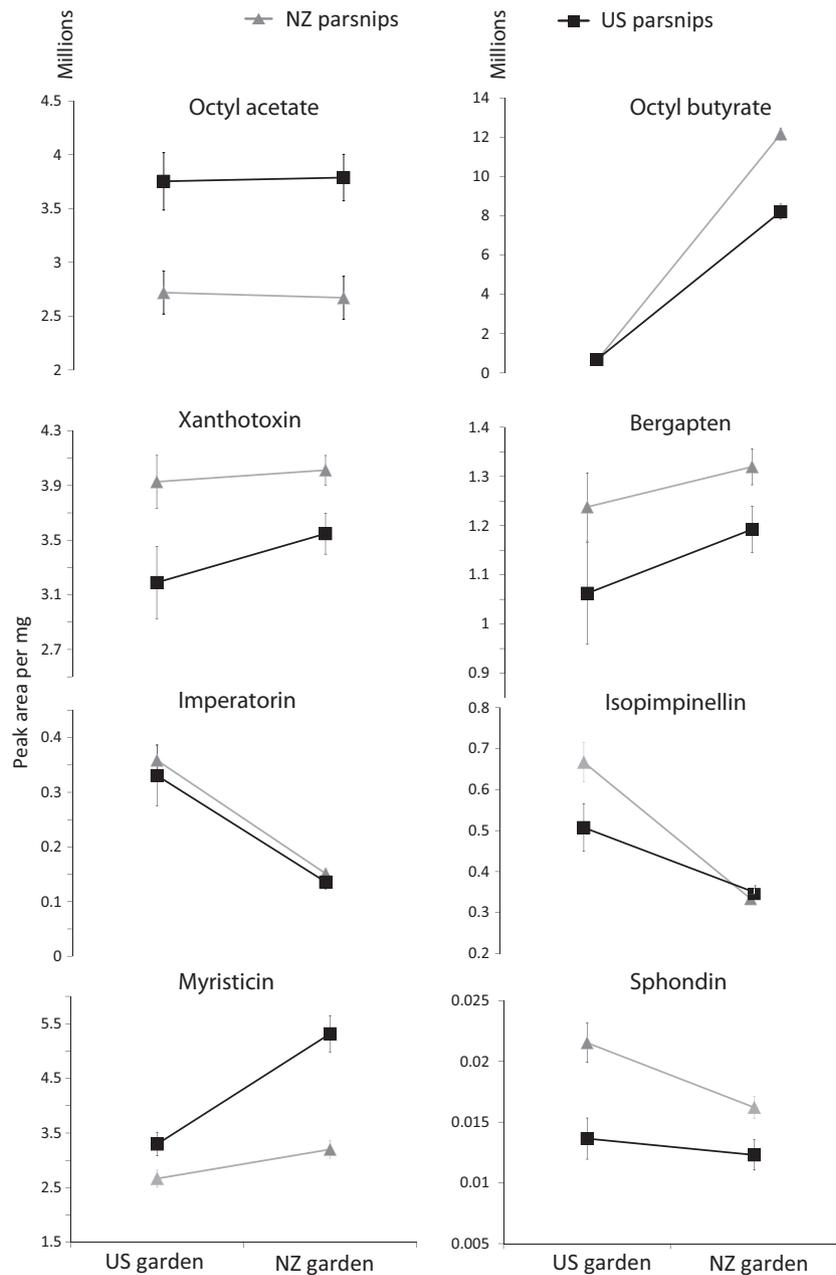
Fig. 3 Comparison of floral compounds between US and NZ parsnips grown in US and NZ gardens. Average peak areas per mg of compound are plotted in the x-axes. Error bars represent standard error values.

sustained more damage in the NZ garden (Fig. 2). Plants biomass did not differ based on spray treatment ($F = 0.006$, $P = 0.93$).

Genotype-by-environment interactions influence floral chemistry

Floral chemistry was strongly determined by genotype-by-environment interactions (country of origin × garden, Pillai's trace = 0.06, $P < 0.001$), indicating phenotypic plasticity in the expression of many floral compounds. Examination of individual compounds and comparison of half-sibs suggests that some compounds (bergapten, xanthotoxin and octyl acetate) are not environmentally variable, whereas others (imperatorin, isopimpinellin, sphondin, myristicin and octyl butyrate) are strongly environmentally determined (Fig. 3, Table 4). The phenotypic plasticity of octyl butyrate, isopimpinellin and imperatorin was partially driven by plant size; large plants produced higher amounts of octyl butyrate and lower amounts of isopimpinellin and imperatorin. (octyl butyrate, $\rho = 0.71$, $P < 0.001$, isopimpinellin, $\rho = -0.12$, $P = 0.003$ and imperatorin, $\rho = -0.12$, $P = 0.001$, $n = 739$).

Dependent variable	Country	Garden	Country × garden
Octyl butyrate	**L = 37.58, *P* < 0.001***	**L = 852.44, *P* < 0.001***	**L = 55.16, *P* < 0.001***
Octyl acetate	**L = 4.89, *P* = 0.026***	L = 0.11, *P* = 0.73	L < 0.01, *P* = 0.99
Xanthotoxin	**L = 8.35, *P* = 0.003***	L = 0.75, *P* = 0.38	L = 0.45, *P* = 0.50
Bergapten	**L = 2.89, *P* = 0.08***	L = 2.18, *P* = 0.13	L = 0.19, *P* = 0.65
Isopimpnellin	L = 1.16, *P* = 0.28	**L = 60.06, *P* < 0.001***	**L = 4.72, *P* = 0.02***
Imperatorin	L = 0.05, *P* = 0.81	**L = 55.16, *P* < 0.001***	L = 0.65, *P* = 0.41
Sphondin	L = 1.96, *P* = 0.16	**L = 25.16, *P* < 0.001***	L = 0.004, *P* = 0.94
Myristicin	**L = 10.05, *P* = 0.001***	**L = 26.30, *P* < 0.001***	**L = 8.91, *P* = 0.002***

Table 4 Models comparing chemical traits between countries and gardens with log-likelihood ratio (L) and *P*-values of main and interaction terms.

Significant effects (*) are highlighted in bold.

Large size evolves in infested NZ parsnip populations

Reduced realized fitness as a result of webworm florivory was consistent in parsnip populations with no infestation history from 2006 to 2009. However, parsnip populations with 3 and 6 years of webworm infestation showed a fitness loss due to florivory in the years 2006 and 2007 but a smaller effect of webworms in 2008 and 2009, suggesting the evolution of tolerance to herbivory although with small sample sizes and high year-to-year variation, this interaction did not prove to be statistically significant (year × infestation history × spray treatment L = 3.26, d.f. = 1, *P* = 0.35). Parsnips from infested populations were significantly larger after 3–6 years of infestation compared with parsnips from uninfested populations in 2009 (Fig. 4; year × infestation history L = 10.34, d.f. = 1, *P* = 0.01).
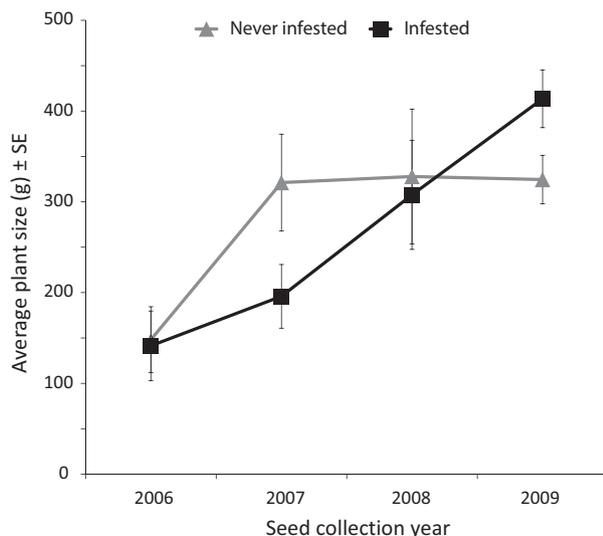
Fig. 4 Plant size of NZ parsnips from populations with a 3–6 year history of webworm infestation (black) compared with populations with no history of infestation (grey) collected from 2006 to 2009. Effect of infestation history × year on plant size L = 10.34, d.f. = 1, *P* = 0.01.

Parsnips with a history of infestation did not have lower total damage or higher pollination success in 2009 compared with parsnips with no infestation history (total damage L = 4.53, d.f. = 1, *P* = 0.20, proportional damage L = 4.78, d.f. = 1, *P* = 0.18 and pollination success SD = 1.62, d.f. = 1, *P* = 0.65).

No evidence for the evolution of increased chemical defences

We found no evidence of change in overall floral chemistry in all NZ populations from 2006 to 2009 (ANOSIM tests CRIMP R = −0.27, *P* = 0.79; WARRINGTON global R = 0.033, *P* = 0.25; ROCK R = 0.056, *P* = 0.015; COTTAGE R = 0.006, *P* = 0.35; and HERBERT R = −0.003, significance 0.50).

Discussion

Our results demonstrate that 6 years of reassociation between wild parsnip and its specialized herbivore has resulted in the evolution of increased plant size in New Zealand. Plant fitness was strongly dependent on plant size and size also mediated the effect of florivores on pollination success. Even though we found no evidence for increased chemical defences, large plants have much higher pollination success and realized fitness in spite of higher webworm damage and size is likely under strong selection after 3–6 years of intense florivory.

Plants have evolved a diversity of defence mechanisms against herbivory, including resistance (antibiosis), repellency (antixenosis) and tolerance. Tolerance is the ability of a plant to sustain damage without any demonstrable reductions in fitness (Mauricio *et al.*, 1997; Strauss & Agrawal, 1999) and it often measured as compensation for losses to herbivores via enhanced growth (Núñez-Farfán *et al.*, 2007). Recent studies suggest that plants may invest in mixed defence strategies (resistance vs. tolerance) under different ecological conditions (Agrawal, 2011; Turley *et al.*, 2013b). For example, Carmona & Fornoni (2013) found that, in *Datura stramonium*, resistance and tolerance act as complementary defences against two different folivores, which, when present together, select for intermediate levels of

the two strategies. Mixed defence strategies may also evolve under different abiotic conditions; tolerance to herbivory is greater in the absence of competition and under high resource availability (Mutikainen & Walls, 1995; Wise & Abrahamson, 2008). In wild parsnips, populations can evolve enhanced resistance via increased production of furanocoumarins (Berenbaum *et al.*, 1986; Zangerl & Berenbaum, 2005). In this study, however, we found no evidence of change in overall floral chemistry in any of the NZ populations from 2006 to 2009, so that rapid evolution of chemically mediated resistance to webworms did not appear to occur over the short duration of infestation in NZ.

Tolerance to webworms has not previously been demonstrated to exist in *P. sativa*. Because the number of inflorescences is predetermined prior to bolting, compensation growth in response to floral tissue loss cannot occur in this species, but increasing overall size appears to mitigate the effects of webworm florivory, suggesting that growing larger may be an alternate or additional strategy for *P. sativa*. The difference in size between parsnips with and without a history of infestation may become even more evident when environmental factors that can influence plant size, such as competition and resource availability, are controlled, as in a common garden setting. Previous work has shown that traits associated with plant size (e.g. height or biomass) can have high heritability and can respond rapidly to selection; for example, Johnson *et al.* (2009) found that biomass has high heritability and is under positive selection by herbivores in *Oenothera biennis*. Herbivore selection on life-history traits might be more common than previously assumed (Carmona *et al.*, 2011). In a longer-term exclusion study, after protection from rabbit herbivory for 26 years (Turley *et al.*, 2013a), *Rumex acetosella* in garden plots demonstrated reduced growth rate with no concomitant change in chemical defence (oxalate content) or competitive ability. Evolution of increased size has been documented in other invasive species (Siemann & Rogers, 2001; Prentis *et al.*, 2008; and Graebner *et al.*, 2012); however, in none of these systems has larger size in response to florivory been examined.

Evolution of increased size in infested NZ populations may have occurred either by a change in genotype frequency or through the transgenerational inheritance of large size (maternal effects). Recent studies suggest plants can respond to herbivory experienced in the previous generation; for example, herbivory by *Pieris rapae*, the cabbage white butterfly, increased wild radish, *Raphanus raphanistrum*, seed mass and growth in the subsequent generation (Agrawal, 2001, 2002). Transgenerational increase in size may be based on an increase in maternal allocation of resources to seeds or via epigenetic changes in the plant in response to herbivory. With this experimental design, we did not specifically examine maternal effects on plant traits and

cannot determine the mechanism of evolution; both differential survival and transgenerational inheritance can change the distribution of plant traits in populations in response to herbivory by diverse mechanisms (Herman & Sultan, 2011; Rasmann *et al.*, 2012).

In addition to having an effect on wild parsnip survivorship and reproductive effort, the presence of webworms had a significant impact on pollination success in this study. Florivores directly influence plant fitness through the consumption of reproductive parts, but they can also indirectly influence fitness via a reduction in pollination visitation and efficacy. Florivore damage can change flower morphology, visual appearance (Lohman *et al.*, 1996) and volatile emissions to render flowers less attractive to pollinators (Mothershead & Marquis, 2000; Zangerl & Berenbaum, 2009; Cardel & Koptur, 2010; Botto-Mahan *et al.*, 2011). Damaged parsnip flowers emit high levels of octanol, a component of webworm frass, and have lower seed set compared with undamaged flowers and the presence of octanol itself can reduce pollination success (Zangerl & Berenbaum, 2009). In this study, webworms reduced pollination success in small plants but not in larger plants irrespective of where they originated and where they grew. Large plants emitted substantially more octyl butyrate, a putative pollinator attractant (Jogesh *et al.*, 2013), and had higher pollination success. An increase in size may also increase the strength of the visual and volatile signal, attracting more pollinators; umbel size in other species has been linked to pollinator visitation rates (Thomson, 1988; Danderson & Molano-Flores, 2010). Thus, effects of webworm damage in large plants may be moderated by enhanced pollination success. At least in the USA, seed set in wild parsnip is strongly pollinator-limited; inadequate pollination is the likely cause of parthenocarpic seed production in this species (Zangerl *et al.*, 1991).

We found strong evidence for local adaptation of US genotypes to the US garden and NZ genotypes to the NZ garden; US parsnips were larger with higher realized fitness and pollination success in the US garden, whereas NZ parsnips were larger with higher realized fitness and pollination success in the NZ garden. Higher fitness (both actual and realized) in the 'home country' appears to be driven by size, a phenotypically plastic trait, which may be driven by genetics or epigenetics (Holeski *et al.*, 2012; Des Marais *et al.*, 2013). Size is a strong indicator of resource acquisition and individual differences in resource acquisition determine the ability of a plant to invest resources so as to increase fitness (Robinson & Beckerman, 2013). That fitness is strongly determined by resource acquisition, and that resource acquisition is genetically and environmental driven, suggests that plant size can be under herbivore selection, but the evolutionary trajectories may differ with abiotic conditions. Despite the fact that furanocoumarins are strongly associated with parsnip resistance to

webworms in both Europe, where the interaction is native, and North America, where it is invasive, and despite the fact that Zangerl *et al.* (2008) found that several furanocoumarin traits were under selection by webworm florivory in New Zealand, we did not find any evidence to suggest increased chemical resistance to herbivory has evolved in NZ populations with a 3- to 6-year history of infestation compared with uninfested populations. Response to selection for increased chemical resistance may be constrained by genetic correlations between chemical traits and life-history traits (Johnson *et al.*, 2009) or by negative genetic correlations between chemical resistance traits (Berenbaum *et al.*, 1986). Octyl butyrate levels in our study were positively associated with size, whereas imperatorin and isopimpinellin levels were negatively associated with size. Our common garden experiment with half-sib parsnips grown in two different environments (USA and NZ) also showed that some of these chemical traits are phenotypically plastic, which has not been previously documented for chemical constituents of wild parsnip reproductive structures. Half-sibs grown in different gardens had strikingly different levels of octyl butyrate, isopimpinellin and imperatorin, and we found a significant genotype-by-environment interaction for these chemicals. Even though these compounds may be under selection, chemical phenotype is likely to be strongly influenced by the environment. Plasticity of resistance traits may be especially important if the costs of production are high (Coley *et al.*, 1985; Hare *et al.*, 2003), as they are for furanocoumarins (Zangerl & Berenbaum, 1997; Zangerl *et al.*, 1997). That small plants produced higher levels of the defensive compounds imperatorin and isopimpinellin, suggests that deterring florivory is more important for small plants; larger plants may have more resources to allocate to flower production and to pollinator attraction to offset losses due to florivory.

Evolution in invasive species has been documented with increasing frequency. Many evolutionary changes are associated with the colonization of a new range or escape from natural enemies (Maron *et al.,* 2004; Prentis *et al.,* 2008; Joshi & Tielbörger, 2012), but these responses are likely short-lived as plants form new associations with native species or reunite with coevolved enemies that also colonize the new range (Siemann *et al.,* 2006; Lu & Ding, 2012). Few studies have quantified responses in invasive plants to reassociation with their coevolved specialist herbivores even though this reassociation is the basis of classical biological control (e.g. Rapo *et al.,* 2010). Wild parsnips in New Zealand have responded remarkably rapidly to strong selection by webworms and this response is even more notable in view of the fact that wild parsnip is a biennial. Whether the evolutionary response is of tolerance or chemically mediated resistance is of less economic consequence than is the fact that coevolved species can evolve rapidly after reassociation. This rapid adaptation and post-establishment evolution may underlie some of the failures in initial weed biocontrol efforts and the long-time lags in some of the successful efforts (McFadyen, 2000; Muller Scharer *et al.*, 2004; Atwood & Meyerson, 2011).

Acknowledgments

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