ANNALS OF
BOTANY
Founded 1887

Resource allocation trade-offs and the loss of chemical defences during apple domestication

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• **Background and Aims** Most crops have been dramatically altered from their wild ancestors with the primary goal of increasing harvestable yield. A long-held hypothesis is that increased allocation to yield has reduced plant investment in defence and resulted in crops that are highly susceptible to pests. However, clear demonstrations of these trade-offs have been elusive due to the many selective pressures that occur concurrently during crop domestication.
• **Methods** To provide a robust test of whether increased allocation to yield can alter plant investment in defence, this study examined fruit chemical defence traits and herbivore resistance across 52 wild and 56 domesticated genotypes of apples that vary >26-fold in fruit size. Ninety-six phenolic metabolites were quantified in apple skin, pulp and seeds, and resistance to the codling moth was assessed with a series of bioassays.
• **Key Results** The results show that wild apples have higher total phenolic concentrations and a higher diversity of metabolites than domesticated apples in skin, pulp and seeds. A negative phenotypic relationship between fruit size and phenolics indicates that this pattern is driven in part by allocation-based trade-offs between yield and defence. There were no clear differences in codling moth performance between wild and domesticated apples and no overall effects of total phenolic concentration on codling moth performance, but the results did show that codling moth resistance was increased in apples with higher phenolic diversity. The concentrations of a few individual compounds (primarily flavan-3-ols) also correlated with increased resistance, primarily driven by a reduction in pupal mass of female moths.
• **Conclusions** The negative phenotypic relationship between fruit size and phenolic content, observed across a large number of wild and domesticated genotypes, supports the hypothesis of yield–defence trade-offs in crops. However, the limited effects of phenolics on codling moth highlight the complexity of consequences that domestication has for plant–herbivore interactions. Continued studies of crop domestication can further our understanding of the multiple trade-offs involved in plant defence, while simultaneously leading to novel discoveries that can improve the sustainability of crop production.

Key words: Plant–herbivore interactions, domestication, plant defence, resource allocation trade-offs, yield–defence trade-offs, *Malus domestica* Borkh., *Malus pumila* Mill., *Malus sieversii* (Ldb.) Roem, codling moth, *Cydia pomonella*, phytochemical diversity.

INTRODUCTION

Crop domestication is among the most significant achievements of mankind and provides striking observable examples of evolution (Diamond, 2002; Larson *et al.*, 2014). Humans have imposed strong intentional and unintentional selective pressures on crops, leading to such complex changes in plant traits that modern cultivars are often unrecognizable as progeny of their wild ancestors. The suites of traits affected can be variable among crops, but common changes include alterations in plant secondary metabolites, alterations in fruit and seed morphology and alterations to branching patterns of vegetative parts (Meyer *et al.*, 2012). However, perhaps the most dramatic change, occurring in virtually all domesticated crops, is selection for gigantism of the harvested organs as a means to increase yield (Gepts, 2004; Meyer *et al.*, 2012). This intensive selective pressure for increased yield may also have unintended

consequences for other traits, especially if plants experience trade-offs between increasing edible biomass and other functions, such as the production of chemical defences against herbivores (Chaudhary, 2013; Chen *et al.*, 2015).

Theories of plant defence generally assume that chemical defences are physiologically costly – their production requires a diversion of carbon- and nitrogen-based resources that may otherwise be allocated to growth and reproduction (Stamp, 2003). Although these allocation-based trade-offs have often proved difficult to detect and their strength can be highly context dependent (Strauss *et al.*, 2002; Cipollini *et al.*, 2015), a large body of empirical evidence has amassed showing that the expression of defence traits is indeed costly (Koricheva, 2002). In the context of plant domestication, the existence of allocation-based trade-offs has led to the hypothesis that domestication may indirectly reduce plant defences due to increased allocation of plant resources to increased yield (Rosenthal and

Dirzo, 1997; Chaudhary, 2013; Chen *et al.*, 2015; Simpson *et al.*, 2017). This hypothesis has been supported by comparisons of modern cultivars with their wild ancestors in a variety of crops, including corn, olives, cranberries and sunflowers (Rosenthal and Dirzo, 1997; Massei and Hartley, 2000; Chen and Welter, 2003; Rodriguez-Saona *et al.*, 2011). However, despite these valuable case studies, broad conclusions remain elusive. Recent studies that examined patterns across multiple domestication events found that defences are not always lost during domestication: patterns are highly variable across crops (Turcotte *et al.*, 2014; Whitehead *et al.*, 2017) and dependent on the plant organ measured, with the strongest loss of defence observed in fruits and seeds (Whitehead *et al.*, 2017).

Why might defence losses during domestication be most pronounced in fruits? Fruits, due to their high nutrient content, typically have higher construction costs relative to vegetative organs and, furthermore, are photosynthetic sinks that require carbon and nutrient investment from the rest of the plant to complete their development (Herms and Mattson, 1992; Poorter, 1994). During selection for larger fruits, the increased costs experienced by plants are 2-fold: the high direct cost of fruit biomass and the cost of reduced efficiency due to a reduced ratio of leaves (the photosynthetic source) to fruits (the photosynthetic sinks). Thus, if allocation-based trade-offs are a primary driver of defence losses during domestication, we expect these losses to be greatest in fruits and seeds that have experienced strong direct selection for gigantism of these parts. Our recent meta-analysis (Whitehead *et al.*, 2017) did show that both herbivore resistance and plant defence traits such as secondary metabolites are reduced more strongly during domestication in fruits than in other plant organs. However, the limited availability of data on yields in different crop varieties and their wild relatives made it impossible to assess whether the loss of defences in fruits is the indirect result of growth–defence trade-offs or other mechanisms. In particular, there may be direct selective pressures during domestication against chemical defence traits because these same traits are also likely to reduce the palatability of fruits and other harvested organs for humans.

To provide an improved understanding of the mechanisms underlying defence losses during domestication, we need additional detailed studies within specific crops that can disentangle the impact of direct selection against plant defences (to increase palatability) from indirect selection against plant defences that occur as a result of selection for increased yield. Past studies examining the effects of selection for increased yield have typically focused on a very limited number of wild types and modern cultivars. Although high-yielding, domesticated varieties have been found to have lower defences (Lindig-Cisneros *et al.*, 2002; e.g. Rosenthal and Dirzo, 1997), many plant traits may change with domestication, and thus it is unclear whether resource allocation trade-offs are a key mechanism behind defence loss. Here, we take a novel approach by examining patterns in secondary metabolite production and herbivore resistance across a large number of wild types and domesticated varieties of a single crop species that vary considerably in yield. Our study system is the domesticated apple (*Malus domestica* Borkh), where the fruits of modern varieties can range in size from around 15 g (comparable with wild *Malus* fruits) to orders of magnitude larger, with the largest apple recorded reaching >1800 g (Guinness World Records, 2005).

Studies of the genetic basis of apple domestication have suggested that larger fruit size has been a key trait under selection during domestication, both in early stages of wild fruit utilization in Central Asia and in subsequent breeding efforts (Khan *et al.*, 2014; Duan *et al.*, 2017). If resource allocation trade-offs have shaped defence loss during domestication, we expect that there should be a negative relationship between fruit size and secondary metabolite production, both within wild apples and within domesticated apples.

Apple fruit pulp and skin are rich in phenylpropanoid-derived phenolic compounds (Escarpa and Gonzalez, 1998; Lee *et al.*, 2003; He and Liu, 2008), the production of which has demonstrated negative correlations with growth across many plant species (Koricheva, 2002). Loss of phenolic compounds would be likely to affect fruit defence against insect herbivores negatively, though there is surprisingly little information on the specific role of phenolics in defence against the numerous fruit-feeding insects that attack apples. A subset of the phenolics found in fruits are also found in apple leaves, where they can reduce feeding by generalist herbivores (Gutbrodt *et al.*, 2011), and many of the same phenolics have known direct defensive functions in other plant species (Hagerman and Butler, 1991; Appel, 1993; Treutter, 2006). Thus, in order to understand how changes in apple phenolic chemistry may affect herbivore resistance, we also need additional information on how apple phenolics impact insect pests. Here, we focus on the most important insect pest of apples worldwide, the codling moth (*Cydia pomonella*, Lepidoptera: Tortricidae), as a model herbivore feeding on apple fruits.

Our aim is to improve our understanding of the mechanisms underlying defence loss during crop domestication by examining variation in fruit phenolics and insect resistance across a large number of wild and domesticated apple genotypes that vary in fruit size. Specifically, we explored the following research questions. (Q1) Has selection for increased fruit size during apple domestication led to trade-offs with defence that reduce fruit phenolic production and herbivore resistance? To answer this question, we considered three hypotheses using a structural equation modelling framework (Fig. 1): (H1) domestication has reduced fruit defences directly (e.g. through direct selection for increased palatability for humans); (H2) domestication has reduced fruit defences indirectly through allocation-based trade-offs associated with increased fruit size; and (H3) domestication has reduced fruit defences through a combination of direct and indirect selective pressures. (Q2) Which individual phenolic compounds have changed during apple domestication? (Q3) Which specific aspects of phenolic chemistry are important for determining the preference and performance of the codling moth (*C. pomonella*), a major fruit-feeding apple pest, and are these traits similar for wild and domesticated apples?

MATERIALS AND METHODS

Study system and site

The domesticated apple is one of the most widespread and culturally important fruit crops in temperate regions. It was originally domesticated in the Tian Shan forests of Central Asia
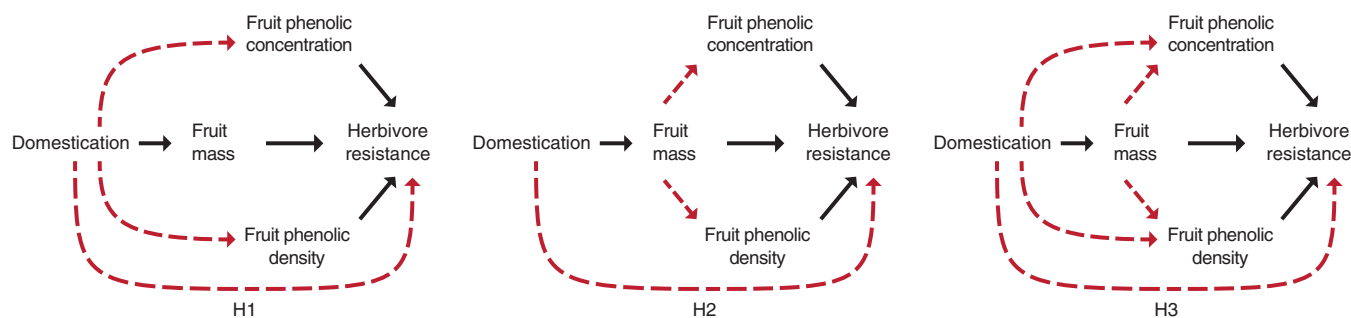
FIG. 1. Three alternative hypotheses for how domestication may influence chemical defences in apples. Domestication may directly reduce fruit phenolics, for example through selection for increased palatability (H1). Domestication may indirectly reduce fruit phenolics due to cost-based trade-offs between plant investment in fruit yield vs. defence (H2). Both mechanisms may occur simultaneously (H3).

and transported westward along the Silk Road trade routes. By 2000 BCE it was cultivated throughout Mesopotamia and Persia, and was later introduced to Europe and North Africa by the Greeks and Romans (Juniper and Mabberley, 2006; Cornille *et al.*, 2014). Multiple lines of genetic and morphological evidence have shown that the main progenitor is *Malus sieversii* (Ldb.) Roem (Cornille *et al.*, 2014; Duan *et al.*, 2017), and, in fact, genetic similarity between the two has prompted several authors to suggest that the two should be considered a single species, for which the more appropriate nomenclature of *M. pumila* could be adopted (Mabberley *et al.*, 2001; Juniper and Mabberley, 2006; Velasco *et al.*, 2010). However, the extent to which other wild species have contributed to the genome of the modern domesticated apple is still debated, and current evidence suggests that hybridization probably occurred along the Silk Roads with *M. orientalis* and *M. sylvestris* (Cornille *et al.*, 2014; Duan *et al.*, 2017). *Malus domestica* is still the name most widely used for the domesticated apple, and will be used here to distinguish from the wild progenitor, *M. sieversii.* In domesticated apples, each named variety is a unique genotype, propagated by grafting, typically onto dwarfing rootstocks that reduce tree height but have no clear effects on fruit size or quality (Al-Hinai and Roper, 2004).

The codling moth (*Cydia pomonella*) is one of the most economically important insect pests on apples worldwide (Beers *et al.*, 2003; Pajač *et al.*, 2011). In some areas, infestation rates can reach 95 % in unmanaged orchards (Peck and Merwin, 2010). Female moths lay eggs on fruit surfaces or nearby leaves, and the larvae hatch and burrow directly into fruits, where they feed internally for the remainder of their development (Beers *et al.*, 2003). These pests can be especially damaging for growers due to a low market tolerance (<1 %) for infested fruits (Beers *et al.*, 2003). Although most of the commonly grown commercial apple varieties are highly susceptible to codling moth, there is variation in resistance among wild and domesticated apples, with fruit damage rates varying from 0 to 78 % (Hogmire and Miller, 2005; Myers *et al.*, 2007).

All field research for this study was conducted in Geneva, New York at the United States Department of Agriculture (USDA) Plant Genetic Resources Unit collection of *Malus* germplasm. The collection includes trees of >2000 domesticated varieties and 200 genotypes of *M. sieversii* planted primarily on B9 dwarfing rootstock (NYSAES, 2011). All trees are managed with a regular programme of fungicide and insecticide. The 200 genotypes of *M. sieversii* represent a core

collection of genotypes obtained during USDA-sponsored field excursions to Kazakhstan conducted from 1989 to 1996 (Forsline *et al.*, 2003; Volk *et al.*, 2005). Seeds collected during the excursions were planted in 1997–1998 and evaluated using a combination of phenotypic traits and molecular analyses. A core collection was then selected from the seedling population to represent the large majority (estimated 90 %) of genetic diversity present (Volk *et al.*, 2005; Richards *et al.*, 2009) and grafted onto dwarfing rootstock in 2012.

Fruit collection

Fruits were collected from 56 trees of *M. domestica* (each a distinct genotype, i.e. named variety) and 52 trees of *M. sieversii* (each a distinct genotype) between 8 July and 21 July 2014. From each tree, we collected 15–20 individual fruits from various parts of the tree. One fruit per tree was haphazardly chosen for chemical analysis of phenolic metabolites. Although phenolic content is known to vary among individual fruits on a tree and among trees within genotypes (Escarpa and Gonzalez, 1998; Awad *et al.*, 2000), we sampled just one tree per genotype (all that is available for most genotypes in the collection) and just one fruit per tree. This allowed us to focus our available resources on extensive replication across genotypes. Variation across genotypes (rather than within) is the scale most important for our hypotheses regarding yield–defence trade-offs. Furthermore, past studies have found that variation in phenolic content and composition among genotypes is much larger than variation within trees or within genotypes (Escarpa and Gonzalez, 1998; van der Sluis *et al.*, 2001; Tsao *et al.*, 2003; Lata *et al.*, 2009; Thompson-Witrick *et al.*, 2014). However, two important caveats of our experimental design are that: (1) our data on fruit phenolics do not provide an accurate representation of the mean values for a genotype and should be interpreted with caution when characterizing specific apple varieties; and (2) we cannot fully disentangle phenotypic vs. genotypic correlations among traits (see the Discussion for more elaboration on this point).

Fruits sampled for chemistry were divided into skin, pulp and seeds, and immediately frozen in liquid nitrogen in the field. They were then stored at –80 °C prior to freeze drying (see below). All other collected fruits were transported to the laboratory and washed three times in water prior to use in bioassay experiments with the codling moth (see below). For each fruit

collected, we measured fruit mass, width and height. All three variables were found to be strongly correlated with one another both within and across individuals ($r^2 > 0.85$, $P < 0.001$ for all relationships), and for the purposes of this study all analyses involving fruit size focused on the mean mass from all fruits sampled from an individual tree. At the same time as these collections, we also collected one small branch (including fruits and leaves) for use in oviposition preference bioassays (see below).

Codling moth resistance bioassays – larval survival and performance

Between 1 and 5 d following collection, 14–19 fruits from each tree of *M. sieversii* and *M. domestica* received a single neonate codling moth larva, transferred within 8 h of hatching with a fine bristle paintbrush. Each fruit was placed inside an individual plastic cup with a lid. All fruits were then placed inside a growth chamber set at 20 °C, 50 % relative humidity and a 12 h:12 h light:dark cycle. Five days after transferring the larvae, we checked each fruit to determine whether the neonate survived. It was clearly apparent when neonates had successfully entered the fruit due to a visible entry hole surrounded by frass. For fruits with surviving larvae, we returned the fruits to the growth chamber and allowed the larvae to complete their development. For fruits without surviving larvae, we added a second neonate, monitored survival and allowed surviving larvae to continue development as above (this allowed us to maximize the number of within tree replicates for larval survival and performance measures). Once the larvae pupated, we noted the sex (Peterson, 1965) and measured pupal mass as an indicator of performance. Male pupae are significantly smaller than female pupae and were therefore analysed separately (see below).

Codling moth resistance bioassays – adult oviposition preference

To assess oviposition preference, we used one small branch, including leaves and unripe fruits, cut from each tree in the study population. Each branch contained from one to four fruits (usually one or two), depending on the fruit size. We used branches with more fruits for genotypes with smaller average fruit size in order to control roughly for the amount of fruit surface area that moths had available for oviposition. Each branch was placed in a floral water tube and enclosed inside an upside-down 20 oz plastic drink cup with a dome-shaped smoothie lid. The floral water tube protruded from the straw opening of the lid and was placed in a test-tube rack. Racks were then left inside a growth chamber set at 20 °C, 50 % relative humidity and a 12 h:12 h light:dark cycle for a period of 24 h, beginning between 13.00 and 17.00 h.

On each branch we placed a single mated pair of adult codling moths for a period of 24 h. We only used moths in the trials that had successfully mated and laid eggs inside an emergence cup on the night prior to the start of the trial. For each trial, we recorded the number of eggs that moths laid over 24 h on the fruits, leaves and cup, and we used the proportion of eggs that were laid on the plant vs. the cup surface as our measure of oviposition preference.

Chemical analyses

All samples collected for chemical analysis were lyophilized and ground in 2 mL microcentrifuge tubes via a Retsch MM301 tissue grinder (Verder Scientific Inc., Newtown, PA, USA). Dried and ground samples were stored at room temperature in opaque boxes to prevent light exposure. Aliquots (approx. 50 mg) were transferred to new tubes and transcinnamic acid (17 µL 1000 ppm solution) was added as an internal standard. An extraction solution (600 µL) of 70 % methanol containing 2 % formic acid was added and tubes were tightly capped and placed on a vortex mixer for 30 min at 1500 rpm. Samples were then centrifuged for 10 min at 10 000 rcf and the supernatant was transferred to a clean tube. Two more extractions were conducted on the same plant material and the supernatants were combined and vortexed. The extracts were filtered through a 0.22 µm centrifuge tube filter (Costar Spin-X, Fisher Scientific) prior to analysis.

Phenolic compounds were analysed using an Agilent 1100 high-performance liquid chromatograph (HPLC) equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA) and an Inertsil ODS-3 column (5.0 µm particle size, 4.6 × 250 mm, GL Sciences Inc., Tokyo, Japan) preceded by an Inertsil ODS-3 guard column (5.0 µm, 4.0 × 10 mm). The flow rate was 1.0 mL min^{-1} and the injection volume was 5 µL. Two solvents were used in the gradient system for separation. Solvent A consisted of 10 % formic acid in water and Solvent B consisted of 10 % formic acid and 1.36 % water in acetonitrile. The gradient was as follows: 95 % A (0 min), 85 % A (25 min), 78 % A (42 min), 58 % A (49 min), 0 % A (55 min), 0 % A (60 min), 95 % A (62 min). Post-run time was 10 min. Simultaneous monitoring was performed at 280, 320, 365 and 525 nm. Peaks were identified wherever possible by comparison of retention time and UV spectra with authentic standards, and the concentrations of individual compounds were determined based on peak area and calibration curves from authentic compounds. Compounds for which there were no standards available were classified as dihydrochalcones, hydroxycinnamic acids, benzoic acids, flavonols, flavan-3-ols or anthocyanins based on UV spectra, and concentrations were estimated based on internal standard equivalents. Authentic standards for catechin, epicatechin, procyanidin B1, procyanidin B2, phloridzin, gallic acid, syringic acid, chlorogenic acid, gentisic acid, caffeic acid, *p*-coumaric acid, ferulic acid, quercetin, hyperin, isoquercitrin, quercitrin, rutin, reynoutrin and avicularin were obtained from Extrasynthese (Genay Cedex, France), Sigma-Aldrich (St. Louis, MO, USA) and AApin Chemicals (Abingdon, Oxon, UK).

The total phenolic concentration was determined in each sample by totalling the estimated concentration for each individual compound detected. Phenolic diversity was estimated using the Shannon diversity index, where the chemical diversity (H') is defined as: $H' = -\sum_{i=1}^{R} p_i \ln p_i$, where R is the total number of compounds present in a sample and p_i is the proportion of the total phenolic dry weight that is compound i. This

index accounts for both the number of different compounds present and their relative abundances.

Statistical analysis

To test alternative hypotheses for the mechanisms through which domestication may alter fruit chemical defence (Q1), we compared three structural equation models (SEMs) that differed in the causal factors influencing fruit chemistry (Fig. 1). SEMs provide a powerful framework to simultaneously test multiple complex relationships and to relate data to theory by allowing for latent variables that can represent theoretical constructs (e.g. plant defence or herbivore resistance) for which we have no direct measurement, but rather multiple measured indicators that reflect this underlying latent variable (Grace *et al.*, 2010). We modelled herbivore resistance as a latent variable, with oviposition preference, larval survival, male pupal mass and female pupal mass as its measured indicators. Fruit phenolic concentration and phenolic diversity were also modelled as latent variables, with the phenolics detected in sub-samples of skin, pulp and seeds as measured indicators. In the same model, we also tested how herbivore resistance was impacted by various other factors, including fruit size, phenolic concentration, phenolic diversity and other direct impacts of domestication. Prior to SEM analysis, all variables were re-scaled to similar orders of magnitude. The models were estimated by full information maximum likelihood (Enders and Bandalos, 2001).

As a complement to the SEM, we conducted a series of simple linear models (LMs) that tested individual hypothesized pathways in the model, i.e. comparing *M. domestica* and *M. sieversii* and examining the relationship between fruit size and chemistry. First, we compared total phenolics and phenolic diversity between *M. domestica* and *M. sieversii*, with analyses conducted separately for skin, pulp and seeds. Next, we compared different aspects of resistance between *M. domestica* and *M. sieversii*, including oviposition preference, survival, female pupal mass and male pupal mass. Finally, we used a series of LMs to assess the effects of fruit mass on total phenolic concentration and phenolic diversity in skin, pulp and seeds.

Next, to explore further which specific chemical traits or aspects of resistance change during domestication (Q2), we used a machine learning approach based on random forest models (Cutler *et al.*, 2007; Ranganathan and Borges, 2010). To identify compounds that can distinguish *M. domestica* from *M. sieversii*, we used random forest classification models conducted separately for apple skin, pulp and seeds. Variable selection was carried out using the Boruta algorithm, which identifies all relevant variables by comparing the importance of each variable with a set of 'shadow' variables created by randomly shuffling values of the original variables (Kursa and Rudnicki, 2010). These analyses gave us candidate sets of compounds that could differ in concentration between *M. sieversii* and *M. domestica*, and we further assessed the direction and significance of these differences using multivariate analysis of variance (MANOVA) models conducted across all selected compounds. To identify compounds that change with fruit size, we used random forest regression models, conducted separately for apple skin, pulp and seeds and with variable selection using the Boruta algorithm as above. For the selected set of candidate

compounds, we followed the random forest analysis with LMs, with fruit mass as the predictor and the concentration of the compound of interest as the response.

Finally, we used two approaches to assess which aspects of fruit chemistry are important in codling moth resistance (Q3). First, to test how total phenolic concentrations and diversity in different tissue types affected herbivore resistance, we used a model averaging approach. This began with a series of global general linearized models (GLMs) conducted separately for each resistance measure and including the following predictors: species (*M. domestica* or *M. sieversii*), total phenolics in skin, total phenolics in pulp, total phenolics in seeds, phenolic diversity in skin, phenolic diversity in pulp and phenolic diversity in seeds. We then analysed all possible sub-sets of the global models, eliminating models that contained any two variables that were correlated with $R^2 \geq 0.5$. From all possible sub-models, we retained a candidate set with ΔAICc <4 and used multimodel averaging based on Akaike weights to obtain final parameter estimates and significance levels for each variable. Secondly, to assess which individual phenolic compounds might be related to resistance, we used a series of random forest regression models, conducted separately for each species, tissue type (skin, pulp and seeds) and resistance measure. Variable selection was carried out with the Boruta algorithm and the random forests were followed by LMs for each selected candidate compound as above.

All statistical analyses were conducted in R, version 3.4.0 (R Development Core Team, 2017) using the packages 'lme4' (Bates *et al.*, 2017), 'lavaan' (Rosseel *et al.*, 2017), 'randomForest' (Breiman *et al.*, 2015), 'Boruta' (Kursa and Rudnicki, 2017) and 'MuMIn' (Bartoń, 2016).

RESULTS

Resource allocation trade-offs and fruit defence (Q1)

Results from the SEMs of the three hypothesized models (Fig. 1) indicated that the best model included both the direct effects of apple domestication and the indirect effects of selection for increased fruit size (H3). The next best model included the indirect effect of fruit size only (H2), but was much less supported (ΔAIC = 21.2), and the worst model included the direct effects of domestication only (H1, ΔAIC = 23.7). Our final model (Fig. 2) showed that apple domestication increased fruit size, and, as fruit size increased, both total phenolic concentration and phenolic diversity decreased. However, domestication also had a direct negative effect on both total phenolic concentration and phenolic diversity independent of the effect mediated by size. Herbivore resistance increased with increasing phenolic diversity; however, contrary to expectation, it decreased with increasing total phenolic concentration. Finally, the direct effects of apple domestication on herbivore resistance were unexpectedly positive, i.e. herbivores performed slightly better on wild apples than domesticated apples.

Results from the linear models largely reflected the results of the SEMs. Relative to *M. sieversii*, *M. domestica* had significantly lower phenolic concentration and phenolic diversity in skin (phenolic concentration, $F_{1,101} = 80.29$, $P < 0.0001$; phenolic diversity, $F_{1,101} = 19.06$, $P < 0.0001$), pulp (phenolic
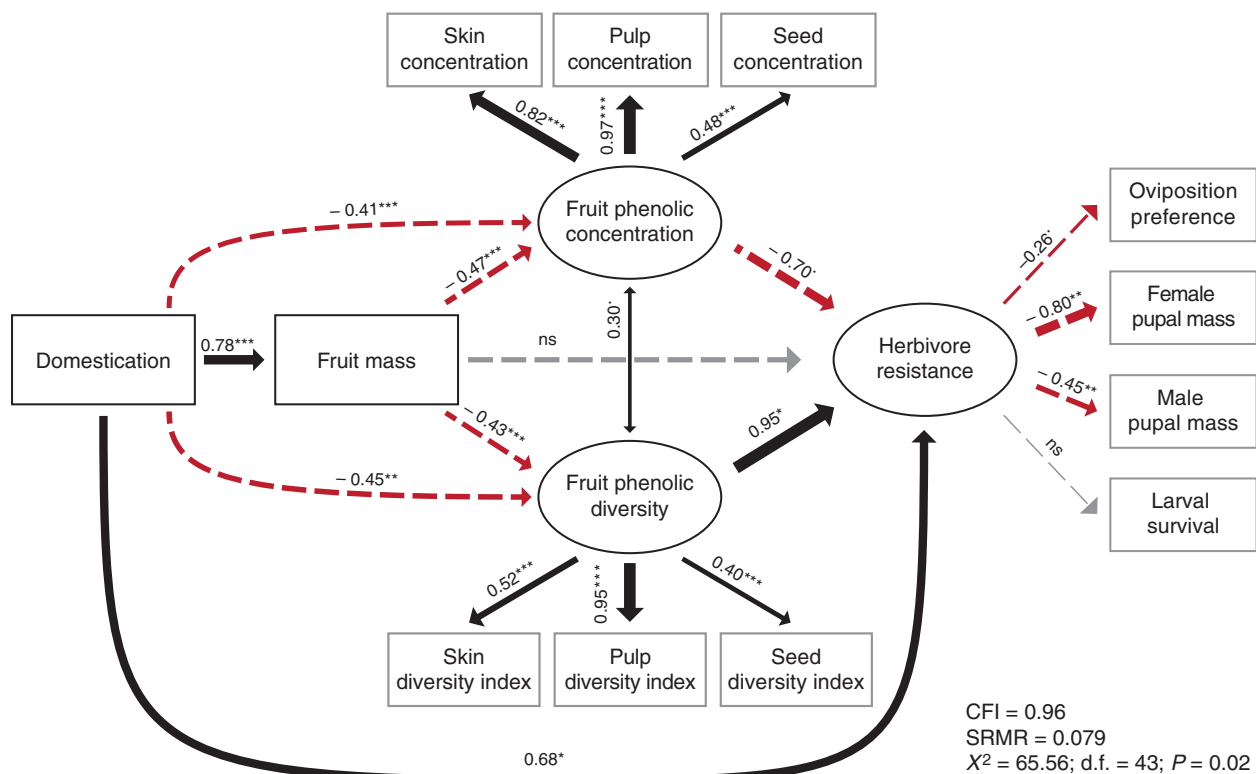
FIG. 2. Structural equation model exploring the relationships among apple domestication, phenolic production and resistance to codling moth, a major fruit-feeding herbivore pest. Phenolic diversity is estimated using the Shannon–Weiner diversity index (H'), where compounds are analogous to species and amounts are analogous to relative abundances. Continuous black arrows represent significant positive relationships, dashed red arrows represent significant negative relationships and grey arrows indicate non-significant relationships. Values above the lines are standardized path coefficients, analogous to relative regression weights, and the widths of the lines are proportional to the strength of the coefficients. Squares are observed variables, and ovals are latent variables (i.e. underlying or hypothetical variables not directly measured but inferred from observed variables). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ˙$P < 0.10$.

concentration, $F_{1,103} = 128.80$, $P < 0.0001$; phenolic diversity, $F_{1,103} = 130.10$, $P < 0.0001$) and seed (phenolic concentration, $F_{1,92} = 27.71$, $P < 0.0001$; phenolic diversity, $F_{1,92} = 30.39$, $P < 0.0001$) (Fig. 3). However, there were no differences between *M. domestica* and *M. sieversii* in any aspect of resistance, including larval survival ($F_{1,104} = 0.99$, $P = 0.32$), female pupal mass ($F_{1,88} = 1.38$, $P = 0.24$), male pupal mass ($F_{1,80} = 0.02$, $P = 0.90$) or oviposition preference ($F_{1,90} = 0.03$, $P = 0.86$) (Fig. 4). Increasing fruit mass had a negative impact on both phenolic concentration and phenolic diversity, for both wild and domesticated apples, but the effects were primarily restricted to fruit pulp (see Fig. 5 for statistical results). There was a marginal negative effect ($P = 0.055$) of fruit mass on total phenolic concentration in skin in wild apples, but no other effects of mass on phenolic concentration or diversity in skin or seeds ($P > 0.20$ for all other analyses; results not shown).

Effects of domestication on individual phenolic compounds (Q2)

We detected a total of 56 phenolic compounds shared between *M. sieversii* and *M. domestica*, an additional 24 individual phenolic compounds unique to *M. sieversii* and 16 individual compounds unique to *M. domestica* (Supplementary data Table S1). In total, these included 25 hydroxycinnamic acids, 13 benzoic acids, 22 flavan-3-ols/procyanidins, 17 flavonols,

16 dihydrochalcones and three anthocyanins (Supplementary data Table S1; Fig. S1). Eighteen major compounds were identified using authentic standards, together accounting for 80 % of the total estimated phenolics in *M. domestica* and 71 % of the total in *M. sieversii* (Supplementary data Table S1). More compounds were detected in skin than in other tissues (27.4 compounds on average per sample vs. 14.5 and 14.2 in pulp and seeds, respectively), and two classes of compounds, flavonols and anthocyanins, were detected almost exclusively in skin (Supplementary data Table S1; Fig. S1).

The random forest classification models clearly distinguished between *M. domestica* and *M. sieversii* with very low out-of-bag estimates of error rate (5.77 % for skin samples, 4.76 % for pulp and 11.58 % for seeds; Supplementary data Fig. S2). Variable selection based on the Boruta algorithm detected 18 compounds in apple skin, 20 compounds in pulp and 14 compounds in seeds that could be distinguished between *M. domestica* and *M. sieversii* (Supplementary data Table S2). Almost all of these compounds were found in significantly higher concentrations in (and sometimes only in) *M. sieversii*, with the only exceptions being phloretin in apple skin and an unidentified benzoic acid in seeds, which were higher in *M. domestica* (Supplementary data Table S2). Compounds lost during domestication included a disproportionately large number of flavan-3-ols, as well as phenolic acids and dihydrochalcones, and these compounds were lost in apple skin, pulp and seeds (Supplementary data Tables S1 and

S2; Fig. S1). No compounds from the two groups of phenolics that occur almost exclusively in fruit skin, flavonols and anthocyanins, were selected by the random forest as important for distinguishing between *M. domestica* and *M. sieversii*, and, in contrast to other compound classes, the total concentrations of these two classes of compounds were higher in domesticated than in wild apples (Supplementary data Fig. S1).
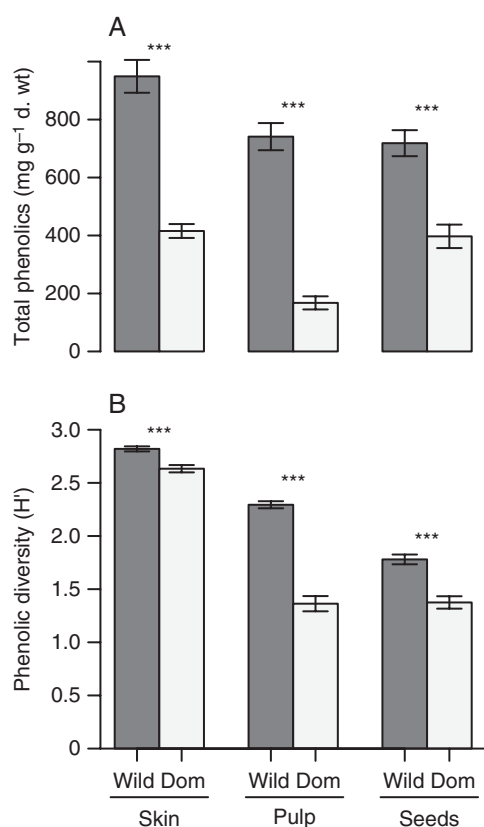

Fig. 3. Mean (± s.e.) total phenolic concentration (A) and phenolic diversity (B) in skin, pulp and seeds of wild and domesticated (Dom) apples. Phenolic diversity (*H′*) is estimated using the Shannon–Weiner diversity index, where compounds are analogous to species and amounts are analogous to relative abundances.

In our exploration of the relationship between fruit size and the concentrations of individual phenolic compounds, random forest regression models explained a very small amount of variance, with very low or negative pseudo-R^2 values (*M. sieversii* skin, $R^2 = 0.20$; pulp, $R^2 = -0.06$; seeds, $R^2 = -0.11$; *M. domestica* skin, $R^2 = 0.03$; pulp, $R^2 = 0.01$; seeds, $R^2 = 0.15$). Removing compounds that were rare (e.g. occurring in <5 samples) only slightly improved the model fit. Still, the Boruta algorithm selected a number of compounds that performed significantly better as predictors of fruit size than shadow attributes (Table 1). In domesticated apples, these compounds included three flavan-3-ols in fruit pulp (epicatechin, catechin and procyanidin B1) and an unidentified hydroxycinnamic acid in fruit skin, all of which were negatively correlated with fruit size ($P = 0.0001$, $P = 0.0016$, $P = 0.01$ and $P = 0.04$, respectively; Table 1), as well as one flavan-3-ol (procyanidin B2) that was marginally positively correlated with fruit size ($P = 0.09$; Table 1). In wild apples, the compounds selected by the Boruta algorithm included one flavan-3-ol (epicatechin), one unidentified dihydrochalcone and one unidentified hydroxycinnamic acid in pulp, all of which were negatively correlated with size ($P = 0.05$, $P < 0.0001$ and $P < 0.0001$, respectively). In skin, Boruta selected one unidentified hydroxycinnamic acid and four unidentified flavan-3-ols, all of which showed a negative trend with fruit size, but only one relationship with a flavan-3-ol was significant ($P = 0.002$) and one was marginally significant ($P = 0.09$; Table 1). In seeds we saw a different trend, with two unidentified benzoic acids selected by Boruta, both of which were positively correlated with fruit size ($P = 0.028$ and $P = 0.024$; Table 1).

Effects of fruit phenolics on herbivore resistance (Q3)

Overall, phenolics had very limited effects on codling moth resistance, but a few patterns did emerge. In our examination of multiple models for how phenolic concentration and diversity in skin, pulp and seeds affect larval survival, there were 20 candidate models with a ΔAIC <4, and the best model included species only (Supplementary data Table S3). Model averaging showed a marginally significant effect of species (Supplementary data Table S4), with the mean percentage
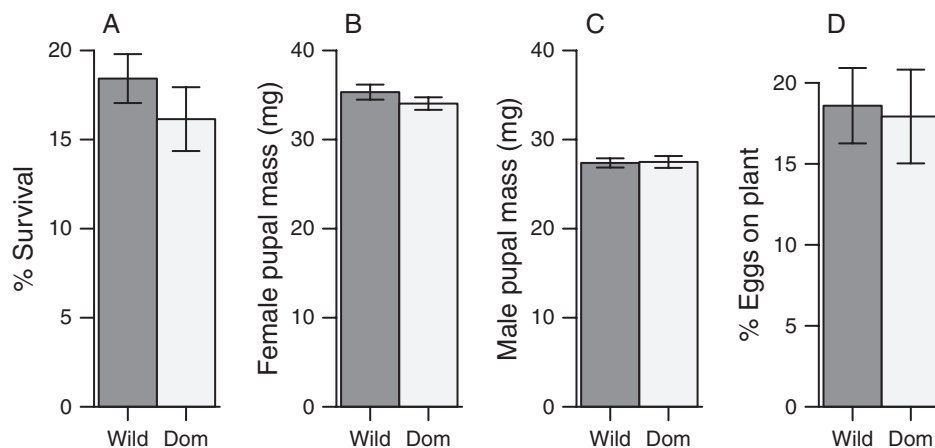

Fig. 4. Mean (± s.e.) percentage survival to pupation (A), female pupal mass (B) and oviposition preference (C) of codling moth (*Cydia pomonella*) on wild and domesticated (Dom) apples.
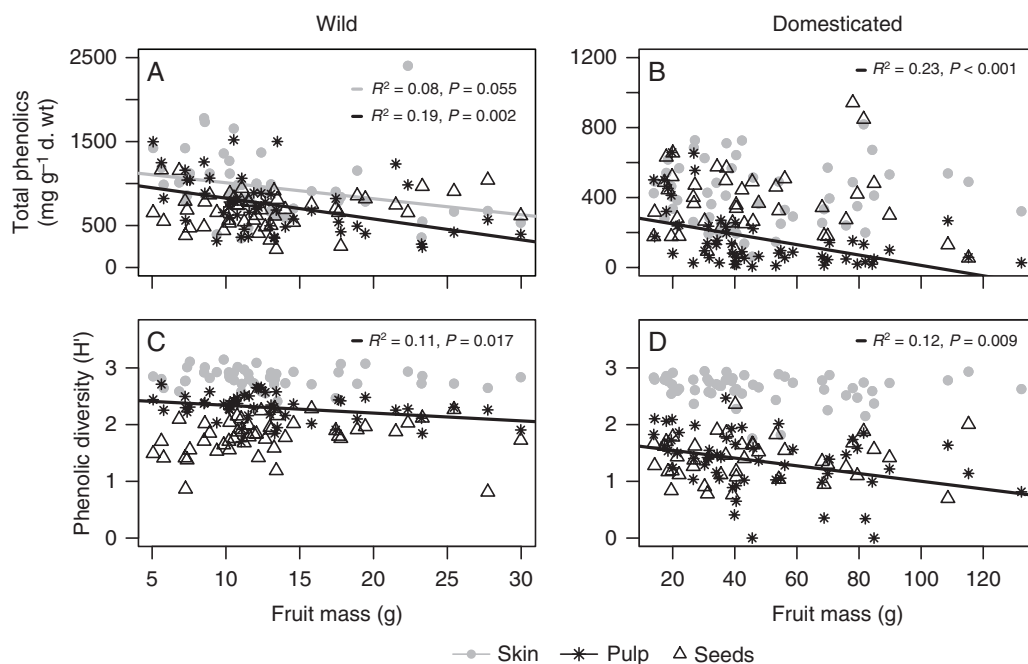
FIG. 5. Relationship between fruit mass and phenolics in wild and domesticated apples. In wild apples, phenolic concentration (A) and phenolic diversity (C) decreased with fruit size in both skin and pulp. In domesticated apples, phenolic concentration (B) and phenolic diversity (D) decreased with fruit size in pulp only. Phenolic diversity (H') is estimated using the Shannon–Weiner diversity index, where compounds are analogous to species and amounts are analogous to relative abundances.

TABLE 1: *List of individual phenolic compounds identified by random forest analyses that change with fruit size in* M. domestica *and* M. sieversii

Analysis	Compound ID[a]	Compound class[b]	RF variable importance[c]	Coefficient[d]	R^2	P-value
M. domestica						
Skin phenolics	BO	Hydroxycinnamic Acid	10.44	−0.76	0.08	0.046
	Procyanidin B2	Flavan-3-ol	8.62	2.74	0.05	0.094
Pulp phenolics	Epicatechin	Flavan-3-ol	8.7	−29.15	0.25	0.0001
	Catechin	Flavan-3-ol	7.49	−28.35	0.18	0.0016
	Procyanidin B1	Flavan-3-ol	4.62	−15.77	0.12	0.01
M. sieversii						
Skin phenolics	C	Flavan-3-ol	5.89	−22.87	0.19	0.0018
	H	Benzoic acid	5.64	−5.55	0.0011	0.81
	AA	Flavan-3-ol	5.36	−11.29	0.058	0.092
	G	Flavan-3-ol	4.68	−29.67	0.0043	0.65
	K	Flavan-3-ol	4.12	−126.5	0.028	0.25
Pulp phenolics	M	Hydroxycinnamic acid	9.64	−24.64	0.4	< 0.0001
	AD	Dihydrochalcone	6.1	−300.91	0.3	< 0.0001
	Epicatechin	Flavan-3-ol	4.52	−274.9	0.078	0.05
Seed phenolics	D	Benzoic acid	9.94	23.42	0.096	0.028
	BH	Benzoic acid	5.16	10.19	0.1	0.024

[a] Unknown compounds are denoted by letters.

[b] Compound classes for unknown compounds assessed based on characteristic UV-Vis spectra in HPLC analysis.

[c] Mean standardized variable importance (Z-score) assigned across all permuted random forest models.

[d] Regression coefficients and associated R^2 and P-values from regression analyses exploring the effects of size on the concentration of each compound selected as important in the random forest analysis.

survival slightly higher in wild compared with domesticated apples (Fig. 4), and no effects of phenolics. For female pupal mass, there were only six models with ΔAIC <4, and the top model included phenolic diversity in pulp, phenolic diversity in seeds, and species (Supplementary data Table S3). There was a

negative effect of pulp phenolic diversity on female pupal mass ($P = 0.006$; Fig. 6) (Supplementary data Table S4), a significant effect of species, again with slightly higher masses on wild apples ($P = 0.04$; Fig. 4), and a marginally positive effect of seed phenolic diversity on pupal mass ($P = 0.07$) (Supplementary

data Table S4). For male pupal mass, there were 16 models with ΔAIC <4, and the top model included species and total phenolics in skin (Supplementary data Table S3). However, model averaging indicated only a weak and marginally significant positive effect of total phenolics on pupal mass ($P = 0.07$) (Supplementary data Table S4). Finally, for oviposition, there were 22 models with ΔAIC <4 (Supplementary data Table S3), and model averaging revealed no significant effects of any predictor variables (Supplementary data Table S4).

The random forest regression models exploring the relationship between each aspect of herbivore resistance and the concentrations of individual phenolic compounds revealed few compounds that relate to resistance. All models explained a very low amount of the variance in resistance (estimated R^2 <3 % in all cases and most often negative; results not shown). The Boruta algorithm selected a very limited number of compounds that performed significantly better as predictors than shadow attributes (Supplementary data Table S5). The strongest relationships were in domesticated apple pulp, where epicatechin and two other unidentified flavan-3-ols showed a strong and highly significant negative relationship with female pupal mass ($P < 0.001$ for all relationships) (Supplementary data Table S5a). In apple skin, one unidentified flavan-3-ol was negatively correlated to male pupal mass ($P = 0.0055$), but another flavan-3-ol (procyanidin B2) was positively related to female pupal mass ($P = 0.0068$) (Supplementary data Table S5a). A few other compounds (nine in total in *M. domestica* and eight in total in *M. sieversii* across all tissue types and aspects of resistance) (Supplementary data Table S5) were identified by the Boruta variable selection algorithm; however, most of these were not significantly correlated with resistance in simple linear models and/or were found in only a very limited number of samples, making estimates of the relationship with resistance unreliable.

DISCUSSION

Although there is substantial evidence that domestication has resulted in lower levels of herbivore resistance in crops relative to their wild ancestors (Chaudhary, 2013; Chen *et al.*, 2015;
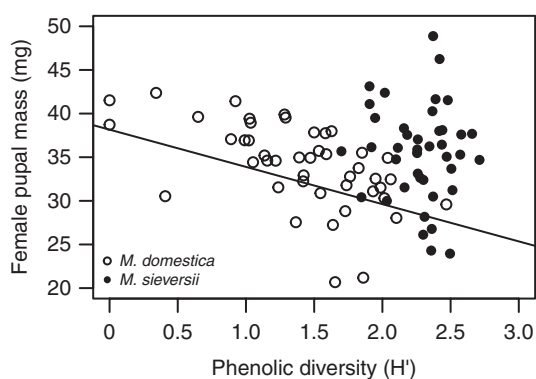

FIG. 6. Effects of phenolic diversity in apple fruit pulp on pupal mass of female codling moth. Phenolic diversity (*H′*) is estimated using the Shannon–Weiner diversity index, where compounds are analogous to species and amounts are analogous to relative abundances. The regression line shows the estimated relationship ($P = 0.006$) based on multimodel averaging across six models that included species, phenolic diversity in seeds and total phenolics in seeds as additional explanatory variables (see Supplementary data Tables S4 and S5).

Whitehead *et al.*, 2017), we still have a limited understanding of the underlying mechanisms that are driving these trends. We tested the hypothesis that higher yielding crops have fewer resources available to allocate to defence by examining variation in defensive chemistry and herbivore resistance across a large number of apple genotypes that vary >26-fold in fruit size. We found a clear negative relationship between fruit size and phenolic production (Figs 2 and 5), supporting the hypothesis that yield–defence trade-offs have occurred. However, these indirect selective pressures cannot entirely explain the large reduction in phenolics that has occurred during apple domestication (Fig. 3). Our results suggest that other mechanisms, such as direct selection for palatability, are also critical factors contributing to defence loss (Fig. 2). Phenolics have minimal effects on the codling moth, a major insect pest, but we did find that overall resistance was increased in fruits with higher phenolic diversity (Fig. 2), an effect primarily driven by negative effects of phenolic diversity on the pupal mass of female moths (Fig. 6). Several flavan-3-ols (e.g. epicatechin) are strongly reduced in the fruit pulp of larger apples (Table 1), and some of these same compounds are among the few compounds that appear to correlate with codling moth resistance (Supplementary data Table S5).

Trait correlations and evolutionary trade-offs

Our conclusion supporting the hypothesis of yield–defence trade-offs in apples is based primarily on a negative phenotypic relationship between fruit size and phenolics; however, it is important to consider that negative correlations between phenotypic traits do not always indicate an evolutionary trade-off (Agrawal *et al.*, 2010). There are two other scenarios that have been described in the evolutionary ecology literature to explain negative trait correlations in natural systems: (1) there may be developmental or physiological linkages between traits that are due to environmental factors, resulting in phenotypic (but not genotypic) correlations; or (2) the negative correlation may be adaptive, i.e. there are fitness benefits of not expressing the two traits simultaneously (Agrawal *et al.*, 2010). The first scenario would be typical for traits with low heritability that are shaped primarily by the environment and, in order to explain our results fully, would require extreme overlap in trait space for both fruit size and phenolics across the genotypes we sampled. There are multiple lines of evidence that we believe make this scenario implausible: (1) both fruit size (Srivastava *et al.*, 2012) and phenolic content (Verdu *et al.*, 2014) have high broad sense genetic heritability; (2) variation in fruit size within genotypes is much smaller than variation across genotypes [e.g. we found that variation in fruit size within genotypes explained only 7.6 % of total fruit size variation, while variation across genotypes explained 36.4 % and variation between species explained 56 % (Supplementary data 5)]; (3) although not directly measured in this study, variation in phenolics within genotypes is also much smaller than variation across genotypes, based on the numerous past studies that have shown large and highly significant differences in phenolic concentration and composition across varieties (e.g. Escarpa and Gonzalez, 1998; Tsao *et al.*, 2003; Lata *et al.*, 2009; Thompson-Witrick *et al.*, 2014); (4) apple domestication is known to have altered genes involved

in both fruit size and secondary metabolite production (Khan *et al.*, 2014; Duan *et al.*, 2017); and (5) all plants in our study were grown in a common field planting with the same management regime, minimizing the likelihood that environmental differences shaped the observed patterns. Still, it is important to note that both fruit size and phenolic composition are clearly shaped not only by genetics but also by epigenetics (Daccord *et al.*, 2017) and the biotic and abiotic environment (e.g. Bergh, 1990; Naor *et al.*, 2008; Petkovšek *et al.*, 2008, 2010; Gutbrodt *et al.*, 2012), and these factors may have contributed to our results. The second scenario, that fruit size and phenolic content might be negatively correlated due to an adaptive negative correlation, could theoretically apply in wild apples. For example, one could envisage a scenario where larger and smaller wild apples had different disperser assemblages with different fruit preferences. If large-bodied dispersers (e.g. deer) rejected apples with high phenolic content, but small-bodied dispersers (e.g. birds) ate them with impunity, fruits that were both large and high in phenolic content would be at a fitness disadvantage. However, this scenario is not probable in a domestication context where humans are the primary selective agent. Humans have most likely selected for fruits that are larger and fruits that are lower in phenolics, but with the negative selection on phenolics being largely independent of size. Thus, we conclude that the most parsimonious explanation for our results is that selection for increased fruit size has led to genetically based trade-offs that reduce fruit phenolic production, with numerous potential downstream consequences.

Variation across fruit tissues

Although apple domestication reduced phenolics in all fruit tissues, including skin, pulp and seeds (Fig. 3), the relative change was greatest for pulp, and pulp phenolics also were the primary group that changed with fruit size (Fig. 5). Pulp phenolics, because they affect fruit palatability and flavour, would theoretically be under the strongest direct selective pressure during domestication. However, this would not explain the negative relationship between pulp phenolics and fruit size (Fig. 5). Higher losses of phenolics per unit biomass in fruit pulp may occur because of the higher absolute investment required to defend pulp, which represents the large majority of fruit biomass, leading to stronger trade-offs between pulp phenolics and phenolic production as fruit size increases.

Other components of yield

An increase in fruit size is just one way in which greater yield can be achieved in apples or other crops. Domestication may select for increased growth rates, increased fruit load, more rapid development time or reduced interannual variability in fruit load (Evans, 1993). Fruit load has also been shown to impact phenolic production, with higher concentrations of phenolics in fruits from trees with low crop loads (Stopar *et al.*, 2002). Here, we focused on fruit size because it is likely to be the most important mechanism through which higher yields have historically been achieved in apples and other tree fruits. Wild apples are highly variable in fruit size (Cornille *et al.*, 2014), and fruit size not only increases the total potential yield per plant, but also greatly reduces the time and effort required for fruit harvest, storage or processing. Thus, fruit size has probably been a primary target for selection throughout the history of human–apple interactions (Cornille *et al.*, 2014; Duan *et al.*, 2017). Furthermore, changes in fruit size may trade-off more directly with other fruit traits (i.e. chemistry) than other mechanisms of increasing yield. A longer term study in apples examining the relative costs associated with various components of yield could provide valuable insight into the best ways to increase crop yield while minimizing costs in terms of reduced resistance.

Apple uses, harvest season and other selective pressures during domestication

Our study included a large number of domesticated apple varieties with origins in the USA, France, the UK, The Netherlands, Belgium, Switzerland and Israel. Most are heirloom varieties that are not produced commercially, and have a variety of uses including dessert apples, cooking apples and cider apples. Apple genotypes in our study that were traditionally preserved for use in hard ciders had higher concentrations and diversity of phenolics than eating (dessert or cooking) varieties, and on average were also smaller (Supplementary data 2). However, the negative relationships between fruit mass and pulp phenolics generally held both within cider varieties and within eating varieties, suggesting that any difference between cider and eating apples is not the major driver of our results. Apples in our study also varied widely in sugar content, and sweetness may also be an important trait that is under selection during domestication. Using data on sugar content from the USDA GRIN database (GRIN, 2018), we found a clear pattern of higher sugar content in domesticated (mean soluble solids = 27.8 ° Brix) compared with wild apples (mean soluble solids = 17.4 °Brix) (Supplementary data 3). However, there was no relationship between fruit size and sugar content (but see McClure *et al.*, 2018) and no relationship between total phenolics and sugar content (Supplementary data 3). These patterns suggest that apples may not experience similar trade-offs between fruit size and sugar production, and that selection for sweetness during domestication is not a major driver of our results. We also used data from the USDA GRIN database (GRIN, 2018) to assess whether differences in harvest season among apple genotypes might be an important driver of our results, but we found no relationships between harvest season and fruit size or between harvest season and fruit phenolics (Supplementary data 4).

Codling moth and apple domestication

Contrary to our predictions, there were minimal differences between wild and domesticated apples in herbivore resistance, with herbivores actually performing slightly better on wild apples. This may be due largely to our focus on codling moth, which is a specialist on apple (Beers *et al.*, 2003; Pajač *et al.*, 2011). The moth may be native to Central Asia and/or Europe, but the evolutionary history between codling moth and apple

is somewhat uncertain because, to our knowledge, there have been no worldwide studies of the population genetic structure of the codling moth. However, the origin of the apple in Central Asia (Cornille *et al.*, 2014) combined with evidence for a diverse assemblage of parasitoids that attack codling moth in that region (Kuhlmann and Mills, 1999) point to a long evolutionary history with wild apples in the region of origin followed by migration to Europe along with apples along the Silk Road. Thus, it is not surprising that codling moth is well adapted to the phenolics that occur in both wild and domesticated apples. Still, several compounds (particularly flavan-3-ols) did correlate with codling moth performance (Supplementary data Table S5), and these compounds could be explored further and used as potential breeding targets for improved natural resistance. Other chemical components, such as the volatiles used in codling moth host location (Bengtsson *et al.*, 2001; Vallat and Dorn, 2005), were not measured in this study but may also change during domestication (Rowen *et al.*, 2016) and explain variation in resistance among different genotypes.

Phenolic diversity and herbivore resistance

Although the concentrations of most phenolic compounds did not influence resistance (Fig. 2) (Supplementary data Table S5), overall phenolic diversity did have clear negative effects (Figs 2 and 6). Our non-targeted approach to phenolic analysis and inclusion of many rarely studied varieties revealed a much larger diversity of compounds than is typically reported in apples (Awad *et al.*, 2001; Lee *et al.*, 2003), and increasing evidence has suggested that mixtures of secondary metabolites can act synergistically and be more effective as a defence than expected based on additive interactions among compounds (Richards *et al.*, 2012; Whitehead and Bowers, 2014; Liu *et al.*, 2017). Our results support the hypothesis that phytochemical diversity *per se* may be a key resistance trait (Berenbaum and Zangerl, 1996), helping to explain the decades-old puzzle of why plants produce such an incredible diversity of secondary metabolites (Fraenkel, 1959). Here we focus on an index of phenolic diversity that provides only a snapshot of the total number of compounds and their relative abundances at one specific point in space and time, but we need more studies that specifically address which of the many dimensions of phytochemical diversity can influence herbivore resistance and ultimately plant fitness.

Broader ecological consequences of changes in phenolics

The changes in phenolics we saw with fruit size (Table 1) and in wild vs. domesticated apples (Supplementary data Table S2) probably have broad consequences for apple ecology. Although we did not detect strong effects of phenolics on resistance to codling moth, apple phenolics are likely to play a key role in defence against other insects (including generalists or non-adapted specialists) or pathogens. Therefore, we expect the reduction in phenolics during domestication to have important consequences for pest management. Phenolics also have diverse roles in plant ecology – they may protect against photodamage, reduce plant competition and influence microbial activity

and nutrient cycling (e.g. Close and Mcarthur, 2002; Lattanzio *et al.*, 2006; Meier and Bowman, 2008). Thus, changes in plant chemistry that occur during domestication may impact multiple aspects of tree physiology and health.

Conclusion

Understanding how plants allocate resources among different functions, including growth, reproduction and defence, is a key component of plant evolutionary ecology and has broad consequences for agricultural systems. Our study has taken an important step towards quantifying the trade-offs that may occur during domestication, providing evidence that plant allocation to chemical defences is reduced as a result of trade-offs between growth and defence. Furthermore, our detailed data set provides valuable information on the potential chemical mechanisms of insect resistance in one of the most valuable specialty fruit crops worldwide. Future work should continue to explore how the complex chemical changes we describe during domestication vary depending on ecological context and influence the multiple types of ecological interactions that can affect the yield and sustainability of our crop systems.

DATA ACCESSIBILITY

The full dataset and associated metadata are archived and publicly available online through the Dryad Digital Repository (DOI: 10.5061/dryad.dm33k0c).

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. S1: additional information on apple varieties and wild genotypes included in the study. S2: a comparison of phenolics in apples used for hard cider and fresh-eating. S3: fruit sugar content in wild and domesticated apples and relationship to phenolics. S4: fruit harvest season relationship to fruit size and phenolics. S5: fruit size variance within and across apple genotypes. Figure S1: a comparison of total concentrations of six major classes of phenolics across wild and domesticated apples. Figure S2: multidimensional scaling plot showing chemical similarity among individual samples of *M. domestica* and *M. sieversii* skin, pulp and seeds. Table S1: mean (s.e.) estimated phenolic concentration (mg g^{-1} d. wt) in skin, pulp and seeds of wild and domesticated apples. Table S2: list of compounds identified by a random forest analysis that distinguish *M. domestica* from *M. sieversii*. Table S3: Akaike's information criterion (AIC) scores and Akaike weights for top candidate models tested to describe the effects of phenolics on herbivore resistance. Table S4: estimated effects of phenolic concentration and diversity in different fruit tissues on various components of codling moth resistance, based on multimodel averaging across a candidate set of top models with ΔAICc <4. Table S5a: list of compounds identified by random forest analyses that are associated with various measures of codling moth resistance in *M. domestica*. Table S5b: list of phenolic compounds identified by a random forest analysis that are associated with various measures of codling moth resistance in *M. sieversii*.

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