

SPECIAL ISSUE ON INTRASPECIFIC VARIATION IN PLANT FUNCTIONAL TRAITS

Growing up aspen: ontogeny and trade-offs shape growth, defence and reproduction in a foundation species

Christopher T. Cole^{1,*}, Clay J. Morrow¹, Hilary L. Barker¹, Kennedy F. Rubert-Nason^{1,2}, Jennifer F. L. Riehl¹, Tobias G. Köllner³, Nathalie D. Lackus³ and Richard L. Lindroth¹

¹Department of Entomology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706 USA; ²Department of Natural and Behavioral Sciences, University of Maine at Ft. Kent, 23 University Drive, Fort Kent, ME 04743 USA and ³Max Planck Institute for Chemical Ecology, Department of Biochemistry, Hans-Knöll-Strasse 8, D-07745, Jena, Germany
*For correspondence. E-mail ctcole@wisc.edu

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- **Background and Aims** Intraspecific variation in foundation species of forest ecosystems can shape community and ecosystem properties, particularly when that variation has a genetic basis. Traits mediating interactions with other species are predicted by simple allocation models to follow ontogenetic patterns that are rarely studied in trees. The aim of this research was to identify the roles of genotype, ontogeny and genotypic trade-offs shaping growth, defence and reproduction in aspen.
- **Methods** We established a common garden replicating >500 aspen genets in Wisconsin, USA. Trees were measured through the juvenile period into the onset of reproduction, for growth, defence chemistry (phenolic glycosides and condensed tannins), nitrogen, extrafloral nectaries, leaf morphology (specific leaf area), flower production and foliar herbivory and disease. We also assayed the TOZ19 sex marker and heterozygosity at ten microsatellite loci.
- **Key Results** We found high levels of genotypic variation for all traits, and high heritabilities for both the traits and their ontogenetic trajectories. Ontogeny strongly shaped intraspecific variation, and trade-offs among growth, defence and reproduction supported some predictions while contradicting others. Both direct resistance (chemical defence) and indirect defence (extrafloral nectaries) declined during the juvenile stage, prior to the onset of reproduction. Reproduction was higher in trees that were larger, male and had higher individual heterozygosity. Growth was diminished by genotypic allocation to both direct and indirect defence as well as to reproduction, but we found no evidence of trade-offs between defence and reproduction.
- **Conclusions** Key traits affecting the ecological communities of aspen have high levels of genotypic variation and heritability, strong patterns of ontogeny and clear trade-offs among growth, defence and reproduction. The architecture of aspen's community genetics – its ontogeny, trade-offs and especially its great variability – is shaped by both its broad range and the diverse community of associates, and in turn further fosters that diversity.

Key words: Community genetics, heterozygosity, intraspecific trait variation, leaf economic spectrum, ontogeny, *Populus tremuloides*, salicinoids, tannins, trade-offs.

INTRODUCTION

Over the past 15 years, accumulating research has documented that the magnitude of intraspecific variation (ITV) in plant functional traits, and the consequences of that variation, can rival those of interspecific variation (Whitlock, 2014; Siefert *et al.*, 2015; Des Roches *et al.*, 2018; Koricheva and Hayes, 2018; Raffard *et al.*, 2019). ITV for functional traits is, fundamentally, the expression of differential genetic, environmental and developmental factors, and interactions thereof (i.e. phenotypic plasticity), that influence plant traits (Lindroth and St. Clair, 2013). Many of the consequences of ITV arise from ecological interactions mediated by growth, defence and reproductive traits (Moore *et al.*, 2014; Des Roches *et al.*, 2018). These consequences play out at multiple organizational and

spatial scales, from simple trophic interactions to community organization and ecosystem function (Whitham *et al.*, 2006; Des Roches *et al.*, 2018).

Genetic contributions to ITV and associated impacts on organismal interactions have long been a subject of interest in ecology (Fritz and Simms, 1992). More recently, interest has shifted to explore the consequences of genotypic ITV in plants for closely associated communities (e.g. insects, pathogens and soil microbes), revealing that such communities are heritable 'extended phenotypes' (Whitham *et al.* 2006). This research has fostered development of the field of community genetics, now more widely known as eco-evolutionary dynamics (Bailey *et al.*, 2009).

From an evolutionary perspective, key emergent questions include: How much of plant ITV is genotypic? How is ITV

shaped and constrained by heredity, ontogeny and trade-offs among traits?

In addition to genotypic sources of variation, plant ontogeny also shapes ITV. Most studies of the magnitude and consequences of ITV, however, are based on a single life stage (Barton and Boege, 2017). Plant traits, especially defences, can have marked ontogenetic trajectories, which in turn affect interactions with associated organisms, such as herbivorous insects (Boege et al., 2011). In general, predictions about the ontogeny of plant defence derive from our understanding of differential resource availability and susceptibility to herbivory at different life stages (Boege and Marquis, 2005; Barton and Boege, 2017; Ochoa-López et al., 2018). Seedlings, for example, might be expected to be highly defended (especially by maternally provided defence compounds). Defences produced by juvenile plants would start at low concentrations and gradually increase to the adult stage, as plants increase their access to resources. High levels of defence in adult plants would ultimately decline after most of their reproduction has been completed (Boege and Marquis, 2005). For woody plants, however, these predictions have rarely been tested and more rarely met, especially for chemical resistance traits (Barton and Koricheva, 2010). While ontogenetic trajectories themselves may be subject to selection, little work has explored their heritability.

Expression of ITV is likely to be shaped by trade-offs between growth and defence, particularly when those trade-offs have a genotypic basis. Yet despite the rich body of literature on trade-offs between growth and defence, relatively few studies have addressed genotypic costs of defence. Indeed, Hahn and Maron (2016) concluded that there is little evidence for genotypic trade-offs between growth and defence – a conclusion adumbrated by Cipollini and Walters (2014). Moore et al. (2014) even concluded that plant defence may be one of the most evolvable traits precisely because of the low costs associated with defence.

As plants mature into the adult stage, the onset of reproduction provides another source of ITV. Reproduction may have its own genotypic trade-offs with growth and defence – described by Obeso (2002) as a ‘triangle of competing functions’. Defence may exact a fitness cost through effects not only on growth, but also on reproduction (Ochoa-López et al., 2020). Similarly, while both growth and reproduction contribute to a plant’s fitness, the transition to reproduction might restrict future growth (Gadgil and Bossert, 1970), while prolonged growth may delay reproduction. In general, females are expected to have greater reproductive effort than males, leading to delayed reproduction and higher investment in defence (Lloyd and Webb, 1977; Delph, 1999).

Further advances in our understanding of how plant ITV may govern associated ecological processes and eco-evolutionary dynamics require research that identifies the extent to which ITV has a genotypic basis, as well as the roles of ontogeny and genotypic trade-offs among growth, defence and reproduction in generating ITV. Most previous work exploring ontogeny and genotypic trade-offs shaping ITV has been based on common environments of herbaceous plant species, as the space and effort required for common gardens of woody plants – especially trees – makes comparable work extremely challenging.

Quaking aspen (*Populus tremuloides*) provides an ideal experimental system with which to explore genotypic and ontogenetic variation in plant ITV, and trade-offs among the key functions of growth, defence and reproduction. Aspen is a foundation species (Ellison et al., 2005) and the most widespread tree species in North America, with close relatives across temperate and boreal regions of the Northern Hemisphere (Rogers et al., 2020). Its large population size, broad range and wind dispersal of pollen and seeds from dioecious trees make it one of the most genetically diverse species known (Mittton and Grant, 1996; Callahan et al., 2013). Aspen serves as a host to >100 species of insect and vertebrate herbivores, as well as to numerous pathogens.

The extraordinary ecological and evolutionary success of aspen is partly a product of its defence chemistry, most notably phenolic glycosides (PGs, also called salicinoids) and condensed tannins (CTs) (Lindroth and St. Clair, 2013). Levels of PGs and CTs exhibit extraordinary genotypic variation in aspen (Barker et al., 2018; Cope et al., 2019). The two major classes of defence compounds exhibit negative genotypic correlations (trade-offs) with each other (Barker et al. 2018) and with tree growth (Osier and Lindroth, 2006; Cole et al., 2016; Kruger et al., 2020). These chemical defence traits also exhibit strong, and distinctly different, ontogenetic trends. PGs decline with age, whereas CTs remain roughly constant or increase with age (Donaldson et al., 2006b; Doak et al., 2007; Cope et al., 2019), as predicted above for ontogenetic trajectories of defence. The chemical defences of aspen are supplemented by extrafloral nectaries (EFNs) that occur at the base of leaf blades. Densities of EFNs decline with increasing tree age ((Doak et al., 2007; Wooley et al., 2007).

Intraspecific variation, particularly of defence chemistry, strongly influences the ecology of aspen and related *Populus* species. Variation in foliar chemistry governs feeding interactions with invertebrate and vertebrate herbivores (Lindroth and St. Clair, 2013), susceptibility to pathogens (Holeski et al., 2009), the structure and function of associated insect (Bangert et al., 2006; Robinson et al., 2012; Barker et al., 2018) and soil microbial communities (Schweitzer et al., 2004; Madritch and Lindroth, 2011), as well as litter decomposition and nutrient cycling (Madritch et al., 2006, 2009).

Here, we explore the roles of heritability, ontogeny and genotypic trade-offs in generating ITV in aspen. Our experimental system consisted of a large common garden of clonally replicated aspen trees, assayed for individual heterozygosity, and measured repeatedly over several years for traits associated with growth, defence and reproduction. This garden included almost 1800 trees representing >500 clonally replicated aspen genets, allowing us to quantify both genotypic and marker (microsatellite) variation, and to track changes through the juvenile phase into the onset of reproductive maturity. The unprecedented size, detail and duration of this study revealed and clarified patterns of ITV, notably heritability of growth, defence and reproductive traits, as well as ontogenetic patterns in those traits, their heritabilities and trade-offs among them. We first quantified genotypic variation in the traits, and then explored patterns among those traits. Based on the predictions above and prior work with aspen, we expected those patterns to be that (1) PGs would decrease while CTs would increase with age;

and (2) EFNs would decrease with age. For genotypic trade-offs, we predicted that (3) growth would decrease from allocation to defence or to reproduction, more so for females than for males; (4) defence would decrease from allocation to reproduction; and (5) reproduction would decrease from allocation to defence. Finally, we predicted that (6) levels of herbivory and disease would be negatively related to defence traits.

MATERIALS AND METHODS

Study population

We established the WisAsp common garden in 2010 from root cuttings of aspen genets collected along a latitudinal cline from northern to southern Wisconsin, USA. Detailed information about the site and garden design is provided by [Barker et al. \(2019\)](#). Representative trees in 2014 and 2018 are shown in [Fig. 1](#). To verify genet identifications, we assayed multilocus genotypes at ten microsatellite loci for at least one tree of every genet, as well as for all trees in any genet having phenotypic anomalies among its members (total = 644 trees). At the same time, we identified the sex of all genets using the TOZ19 marker ([Pakull et al., 2015](#)). These genetic markers enabled us to confidently assign all trees to their correct genet and identify the sex of pre-reproductive trees ([Supplementary data Table S1](#)). The microsatellite data also provided estimates of individual heterozygosity (H_{obs}), which has been found to affect growth and defence chemistry in aspen ([Cole et al., 2016](#)). Six hermaphroditic genets, which had the TOZ19 female sex marker, were excluded from the mixed-effects models that used sex as a covariate. The population analysed included 1788 trees representing 510 genets (mean = 3.51 trees per genet); genomic

data for these trees show no evidence of population structure ([Barker et al., 2019](#)).

Growth and morphology measures

We measured initial tree diameter in 2012 at 10 cm above the soil surface, and diameter at breast height (1.4 m) from 2015 through 2018. Two orthogonal measures were made with calipers until trees reached approx. 7.5 cm in diameter, after which we used diameter tapes.

For analysing leaf traits, including specific leaf area (SLA; $\text{cm}^2 \text{g}^{-1}$) as well as chemical traits, from each tree we collected four leaves (more if they were small) in each cardinal direction, taken haphazardly from one or more branches in each direction. Leaves were stored on wet ice, transported to the lab and scanned. For collections in July of 2014–2015 and June of 2016–2017, we scanned the leaves on a LiCor 3100 flat-bed scanner (LiCor, Lincoln, NE, USA) to obtain the area of each leaf. For August collections in 2016–2017, we used digital flat-bed scanners and analysed the images using WinFolia software (Regent Instruments, Chemin Sainte-Foy, Quebec, Canada). After scanning, leaves were freeze-dried, weighed and stored at -20°C .

Chemistry and defence

Leaf chemistry analyses were conducted on finely pulverized powder obtained by ball-milling freeze-dried leaves in 20 mL plastic scintillation vials. Nitrogen (N) values were determined by near-infrared reflectance (NIR) spectroscopy of leaf powder, calibrated using reference values from combustion gas chromatography (Vario MACRO, Elementar, Hanau, Germany; or Flash EA 1112, Thermo Finnigan, Milan, Italy), as described by [Rubert-Nason et al. \(2013\)](#) and [Barker et al. \(2019\)](#). We assayed salicinoid PG concentrations from 25 mg of leaf powder aliquots extracted in methanol, separated by ultra-high-performance liquid chromatography (UHPLC), and quantified using negative electrospray ionization single quadrupole mass spectrometry (Acquity iClass UPLC/MS system, Waters, Milford, MA, USA), following [Rubert-Nason et al. \(2018\)](#). June 2017 PGs were quantified by HPCL-UV spectroscopy as described by [Boeckler et al. \(2013\)](#) ([Supplementary data Methods S1](#)), adjusted to match data from 99 samples also quantified by the UPLC/MS system. Total PG concentrations in 2016 and 2017 included salicortin and tremulacin, which are the primary PGs found in aspen, as well as salicin and tremuloidin, which occur in trace amounts. PG data in 2014 and 2015 come from NIR spectroscopy as for N, calibrated for salicortin and tremulacin quantified by UPLC/MS, as described by [Barker et al. \(2019\)](#). Analytical PG standards were purified from aspen foliage ([Rubert-Nason et al., 2018](#)) except for salicin, which was purchased from Sigma-Aldrich (St. Louis, MO, USA). Condensed tannin (CT) concentrations were measured colorimetrically from 25 mg leaf powder aliquots using the acid butanol method of [Porter et al. \(1986\)](#) and [Barker et al. \(2019\)](#). Nitrogen, PG and CT levels are reported as a percentage of dry mass. Because leaf chemistry and SLA values change roughly linearly between early June



FIG. 1. Typical aspen trees in the WisAsp common garden, in (A) 2014 and (B) 2017.

and late August, we averaged the late June and early August samples of 2016 and 2017 to more accurately correspond to the July samples collected in previous years. PG and CT values for 2018 were estimated by measuring concentrations in 107 trees; for the remainder of the trees, we adjusted their 2017 values by the same proportion by which the sampled sub-set changed. These estimated values are not used in the linear models described below, but are used in the ternary graph summarizing the three-way trade-off between growth, defence and reproduction over multiple years.

Extrafloral nectaries, which in aspen are located at the base of the lamina, were quantified from digital leaf scans by counting the number on the upper surface of each leaf. EFN density estimates represent the mean number of nectaries per leaf.

Leaf damage and disease

We quantified the percentage of leaf area lost to herbivores and disease from digital scans of late summer leaves collected in August of 2016 and 2017. On scans of upper leaf surfaces, when herbivory or disease had removed a portion of a leaf edge, we reconstructed the missing boundary based on the shape of the remaining leaf (Couture, 2011). Scans were analysed using the WinFolia software, calibrated to quantify healthy leaf surfaces as well as those removed, damaged by leaf scrapers or diseased.

Reproduction

Aspen flower buds are formed during the late summer, exclusively on twigs formed during that growing season. By late winter of the following year, they are distinctly larger than vegetative buds, and the flowers dehisce before leaves emerge. We quantified levels of reproduction in the early spring of the years 2017–2019 by counting the number of twigs that bore flower buds (or inflorescences) on each tree, identifying the sex of each tree by visual inspection of flowers.

Data analyses

We applied several data transformations to produce models whose residuals were distributed normally. Size (basal area) was square-root transformed and counts of flowering twigs were log-normal transformed. Measures of percentage leaf area lost to herbivory or disease were logit transformed. Finally, because numeric scales and distributions differed greatly among variables, we *z*-scaled tree variables used in the model (e.g. concentrations of defence compounds and leaf area measures) but not environment and experimental variables (e.g. year, age, block, etc.). Since these variables are *z*-scaled, model estimates for fixed effects provide appropriate measures for comparing effect sizes. All analyses were conducted in R v. 3.5.1 and 3.6.1 (R Core Team, 2018).

We calculated clonal repeatability measures (here referred to as broad-sense heritabilities, H^2) using variance components extracted from the linear, mixed-effect models of trade-offs described below. For these calculations, the genet and

environmental variables (block, year) were treated as random effects, and we excluded fixed-effect tree traits. Because H^2 represents the ratio of genet variance over total variance, and the model variance components include only random effects, if genetically influenced tree traits (such as sex, heterozygosity or PG concentration) were fixed effects, their impact would be omitted from the numerator and we would underestimate H^2 ; conversely, if environmental variables were represented as fixed effects they would be omitted from the denominator and we would overestimate H^2 (de Villemereuil *et al.*, 2018).

Ontogenetic trajectories were quantified as slopes of change over age. Because the concentrations of PGs and CTs changed in approximately negative exponential patterns, we log-transformed those concentrations before calculating the slopes. Heritabilities of ontogenetic parameters were calculated from models consisting only of random effects. We quantified the reliability of these estimates by calculating the s.e. values of 1000 bootstrapped iterations of each model, and report those s.e. values along with the H^2 values calculated from the data. We do not report H^2 for the probability of flowering model, as the residual variance component of a glmm model fit to a binomial distribution is undefined. We also report ontogenetic trajectories of heritabilities, showing changes in H^2 across tree age. Because most of the heritability trajectories show non-monotonic patterns of change, we did not summarize their slope parameters as was done for the overall H^2 values.

We constructed a compound ontogenetic trajectory of growth, defence and reproduction using (basal area increment)^{1/2}, (PG + CT concentrations), and log(number of flowering twigs). For each variable, at each age, the population mean for each scaled variable was expressed as the proportion of the sum of means.

We used linear mixed-effects models to quantify the relationships between growth, defence and reproduction variables, using lme4 v. 1.21 (Bates *et al.*, 2015). Genet was treated as a random effect (intercept) term. Annual measurements of (basal area)^{1/2} serve as a reliable proxy for growth because our models also included initial (2012) size, so the models analyse the change from initial size (Supplementary Data Methods S2). Because EFNs were not measured during 2015, we used a separate model to evaluate their effect on growth, which had 1 year less of data than the model evaluating the effects of PGs and CTs. Comparisons of size were made on square-root-transformed basal area data using Welch's two-sample *t*-test implemented in R.

For reproduction, since the study population of trees entered reproductive maturity asynchronously, annual counts of flowering twigs were both highly zero-inflated and overdispersed. Consequently, we used a two-step process when modelling reproduction as the dependent variable. We first modelled the probability of flowering among all trees with a generalized linear mixed logistic model of the binomial family, also using the lme4 package. We then analysed the level of flowering (number of flowering twigs, log transformed) using only those trees that were reproducing each year. We also calculated a reproductive effort index [REI = no. of flowering twigs / (basal area)^{1/2}] of size-specific flowering effort. The effect of REI on growth was evaluated for only 2017, as that was the

only year of data in which we had measurements for all of the relevant covariates.

We developed models using complete cases for which Aikike information criteria (AIC) levels could be compared, and then the initial overfit models were used on the complete data set, removing variables until only significant variables remained and residuals met assumptions of normality. Overall model strength was evaluated as a coefficient of determination based on likelihood ratios (denoted R^2) using the *rr2* package v. 1.0.2 (Ives, 2019), which we also used to evaluate the reduction in model strength when each covariate was omitted from the model. We used *lmerTest* v. 3.1.0 (Kuznetsova et al., 2017) to evaluate significance levels of model effects.

RESULTS

Levels of intraspecific trait variation

All traits measured showed high levels of genotypic variation (Fig. 2). Notably, while the range of variation for size increased over time, variation decreased for chemical defence (Fig. 2; individual PGs are shown in Supplementary Data Fig. S1). Final tree sizes (square root of basal area) differed 13-fold. Levels of PGs varied by 17- and 16-fold among genets in the first and last years of the study, respectively, whereas levels of CTs varied by 24- and 8-fold during the same period. Extrafloral nectaries varied by up to 78-fold among genets. The two measures of reproductive traits varied even more: in 2018 (the year of highest reproduction), the number of flowering twigs on reproducing trees varied 1300-fold; expressed as the REI, there was a 50-fold range of variation among genets. Leaf area lost to insect herbivores was low (mean = 2.3 %) during the 2 years surveyed, and the loss to disease was only slightly higher (mean = 3.8 %), but the range of variation among genets was large: 13-fold for herbivory and 43-fold for disease.

Heritabilities

Growth, defence and reproductive traits all had relatively high heritabilities, from $H^2 = 0.31$ for REI to 0.64 for EFNs. Heritabilities of disease and damage were lower, and the H^2 for disease was eight times higher than for insect damage (Fig. 3A).

These trait heritabilities changed over time, and variously so among traits (Fig. 3B–E). (Note that these ontogenetic trajectories of heritabilities are different from the heritabilities of ontogenetic trajectories in Table 1.) Ontogenetic trajectories of H^2 were most consistent for growth and reproduction. Values increased over time as the variance in traits became more determined by genotype, indicating that, within genets, ramets became more similar to one another (Fig. 3B). For other traits, H^2 increased but in a non-monotonic pattern (Fig. 3C–E). Interestingly, heritabilities for PGs and EFNs both tended to rise during the ages when their actual levels were declining, as described below. Of the defence traits (PG,

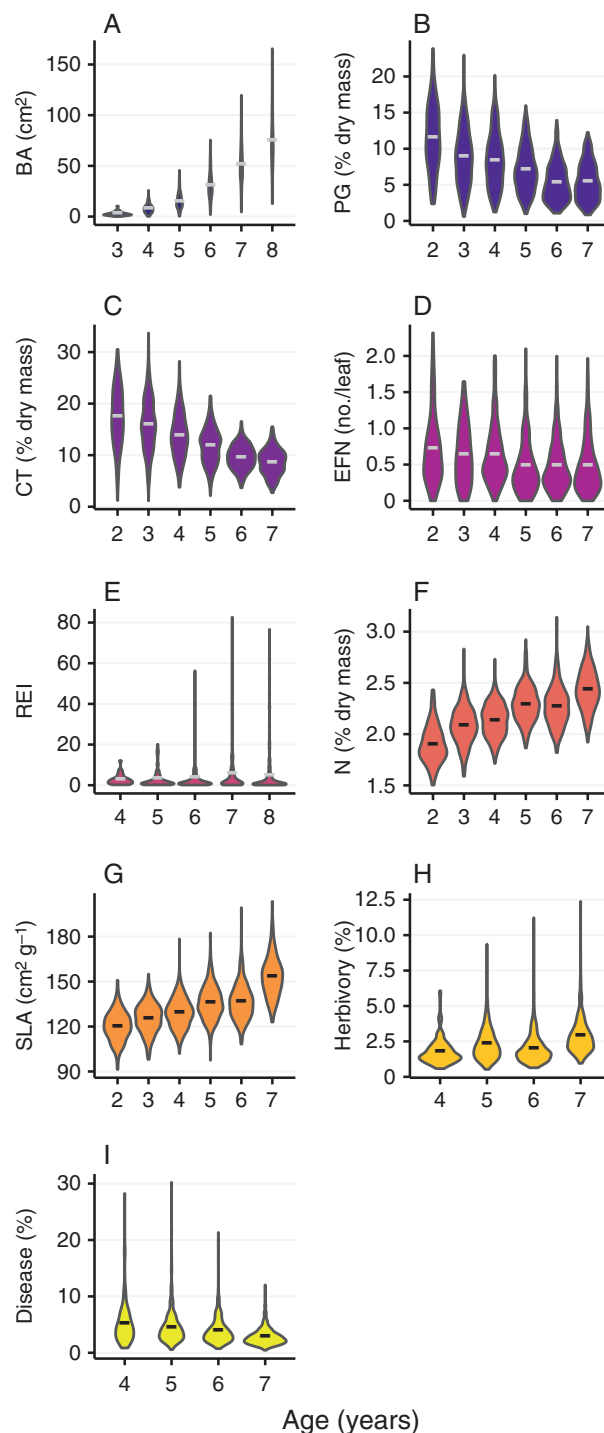


FIG. 2. Violin plots of ontogenetic trajectories for growth, defence, reproduction, leaf quality and damage traits over time in the WisAsp common garden. The vertical length of each violin represents the range of data, horizontal width represents the data density and bars within each violin indicate means. BA, basal area; PG, foliar phenolic glycosides; CT, foliar condensed tannins; EFN, extrafloral nectary density; REI, reproductive effort index [no. of flowering twigs/(basal area)^{1/2}]; N, foliar nitrogen; SLA, specific leaf area; Herbivory, leaf area consumed by herbivores; Disease, leaf area chlorotic, necrotic or lost to disease; Damage, sum of herbivory + disease.

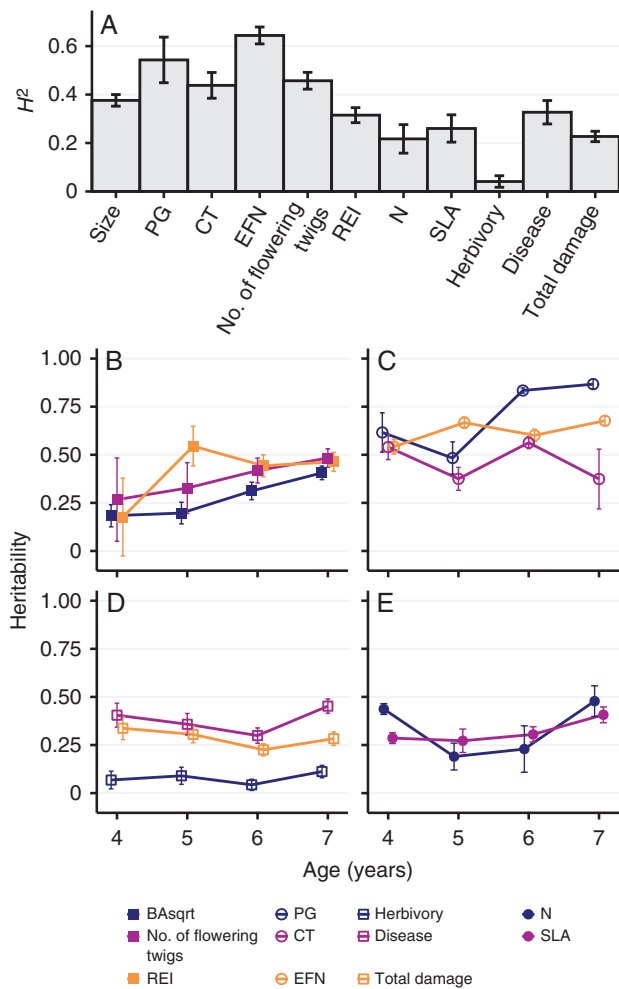


FIG. 3. (A) Broad-sense heritabilities (clonal repeatabilities, \pm s.e.) for growth, defence, reproduction, leaf and damage traits in aspen. (B–E) Ontogenetic trajectories (change with age) of broad-sense heritabilities. Abbreviations as in Fig. 2.

TABLE 1. Ontogenetic trajectories (slopes of increase or decrease) for defence and leaf traits, with s.e., broad-sense heritabilities (H^2) of the ontogenetic trajectories and s.e. of the H^2 values

Trait	Ontogenetic trajectory (slope)	s.e.	H^2 of ontogenetic trajectory	s.e. of H^2
PG	-0.19	0.003	0.39	0.027
CT	-0.19	0.003	0.24	0.028
EFN	-0.07	0.001	0.05	0.056
N	0.11	0.002	0.24	0.046
SLA	6.19	0.131	0.15	0.035

CT, condensed tannins (% dry mass); EFN, extrafloral nectary density; N, leaf nitrogen (% dry mass); PG, phenolic glycosides (% dry mass); SLA, specific leaf area ($\text{cm}^2 \text{g}^{-1}$).

CT and EFN), EFNs had the most consistent H^2 values, although the actual number of EFNs per leaf had the weakest ontogenetic trajectory of the defence traits examined (below). Heritabilities for herbivory remained low for all ages, while those for disease were consistently higher (Fig. 3D); neither showed a consistent trend over time.

Growth: ontogeny, trade-offs and sex

The exponential increase in tree size over time (Fig. 2A) sets the context for understanding all other ontogenetic changes; the trade-offs among growth, defence and reproduction; and the differences between sexes. Both chemical and indirect defence reduced growth: PG and CT concentrations as well as the EFN density all had negative relationships to growth (Table 2; Fig. 4A–C). Leaf N concentration was also associated with reduced growth, whereas SLA was associated with increased growth (Table 2; Fig. 4D). Reproduction also decreased growth: although growth and reproduction both increased with tree size, trees with a higher REI (number of flowering twigs per unit basal area) had reduced growth in the subsequent year (Table 2; Supplementary data Fig. S2). This growth reduction appeared to be more substantial for females than for males even though sex was not a significant factor in the mixed-effect models for growth. When analysing only the reproductive trees, initial (2012) size was slightly greater for females, but by 2018 there was no difference in size between the sexes (Supplementary data Table S2), suggesting that faster growth of males allowed them to become equal in size to females.

Defence: ontogeny, trade-offs and sex

Defence traits showed strong ontogenetic patterns, as well as trade-offs with growth and between defence types. PGs and CTs both declined by about 44 % during the juvenile years (Figs. 2B, C), with nearly identical ontogenetic trajectory slopes (Table 1). However, for PGs, this decline was affected by both size and age, and counterintuitively so (Table 2). PG content declined as trees grew larger but the modest age effect was positive, indicating that for trees of equal size, older trees had slightly higher PG levels. Moreover, while both classes of defence compounds declined as trees grew, there was a strong genotypic trade-off between PG and CT production that persisted across all age classes (Table 2; Fig. 5). EFNs also declined as trees grew larger (Tables 1, 2; Fig. 2D); densities dropped by 31 % from 2014 to 2017, but increased with SLA (Table 2; Supplementary data Fig. S3). EFN production had no effect on either CT or PG levels despite the strong trade-off between the two classes of chemical defences. Reproduction had no detectable effect on defence levels, nor did they differ by sex. PG levels declined with SLA, whereas CT levels declined with SLA, N and individual heterozygosity (Table 2; Supplementary data Figs S3 and S4).

Reproduction: ontogeny, trade-offs and sex

Flowering differed tremendously among the trees in the common garden: flowering levels varied from one to 1316 flowering twigs per tree, though only 469 trees (26.1 %) had produced ≥ 10 flowers. Probability of flowering, number of flowering twigs and the REI all varied widely (Fig. 2). This variation is reflected in lower overall R^2 values for the mixed-effect models of reproduction, compared with those for growth (Table 2), as the variation occurred both among and within

TABLE 2. Results from linear mixed-effects models for growth, defence, reproduction and damage to aspen trees.

Dependent variable	Fixed effect	Effect size	s.e.	P value	R ²	Genet proportion of variance	R ² decrease w/o covariate
Growth							
Basal area	Age	0.638	0.005	<0.001	0.900	0.093	0.636
	SLA	0.057	0.004	<0.001			0.027
	PG	-0.029	0.006	<0.001			0.004
	N	-0.027	0.004	<0.001			0.003
	CT	-0.017	0.005	<0.001			0.002
	EFN	-0.012	0.005	0.023			0.870
Basal area increment	REI	-0.068	0.013	<0.001	0.930		0.083
	Defence						
PG	Size	-1.393	0.013	<0.001	0.758	0.460	0.037
	CT	-0.300	0.016	<0.001			0.093
	SLA	-0.180	0.024	<0.001			0.034
	Age	0.055	0.011	<0.001			0.006
CT	Size	-1.019	0.041	<0.001	0.580	0.180	0.102
	PG	-0.219	0.013	<0.001			0.059
	Age	-0.120	0.014	<0.001			0.014
	SLA	-0.086	0.010	<0.001			0.020
	N	-0.058	0.010	<0.001			0.024
	H _{obs}	-0.043	0.021	<0.001			0.001
Extrafloral nectaries	Size	-0.045	0.022	0.040	0.520	0.360	0.011
	SLA	0.040	0.014	0.004			0.004
	Reproduction						
Flowering probability	Sex = male	1.997	0.267	<0.001	0.418	0.190	0.030
	Size	1.919	0.137	<0.001			0.122
	PG	0.716	0.154	<0.001			0.013
	CT	0.663	0.165	<0.001			0.011
	N	0.269	0.090	<0.001			0.032
Flowering level	Size	0.531	0.042	<0.001	0.336	0.160	0.136
	Sex = male	0.232	0.096	<0.001			0.006
	SLA	-0.097	0.034	<0.001			0.024
	H _{obs}	0.085	0.043	0.049			0.004
Reproductive effort index	CT + PG	0.526	0.180	<0.001	0.240	0.140	0.020
	Size	0.219	0.050	<0.001			0.019
	Sex = male	0.179	0.085	0.037			0.004
	H _{obs}	0.097	0.038	0.012			0.006
	(CT + PG) × age	-0.077	0.030	0.011			0.007
	N	0.069	0.032	0.032			0.075
Damage							
Herbivory	CT	0.206	0.021	<0.001	0.165	0.017	0.033
	Size	0.178	0.025	<0.001			0.025
	Age	0.176	0.016	<0.001			0.033
	N	0.132	0.013	<0.001			0.092
Disease	N	-0.136	0.012	<0.001	0.315	0.100	0.077
	Size	-0.130	0.020	<0.001			0.021
	PG	-0.097	0.023	<0.001			0.010
	SLA	-0.073	0.013	<0.001			0.011
	EFN	0.046	0.014	0.001			0.003
	Size	-0.618	0.121	<0.001			0.174
Damage sum	CT	0.143	0.042	<0.001			0.008
	Age	0.136	0.028	<0.001			0.011
	PG	-0.121	0.037	0.001			0.008
	Size × age	0.106	0.022	<0.001			0.022
	SLA	-0.087	0.023	<0.001			0.006
	EFN	0.070	0.022	0.001			0.003

Genet was treated as a random effect; fixed effects are listed for each model, followed by effect size, s.e. and *P*-value. *R*² represents the overall model strength; genet proportion of variance is the proportion of decrease when genet is omitted from the model, and *R*² decrease is the proportion of decrease when each fixed effect is omitted from the model. Age, tree age (years); damage, sum of herbivory + disease (%); disease, leaf area chlorotic, necrotic or lost to disease (%); flowering level, number of flowering twigs per tree; flowering probability, probability of flowering; herbivory, leaf area consumed by herbivores (%); *H*_{obs}, observed heterozygosity at microsatellite loci; REI, reproductive effort index [no. of flowering twigs/(basal area)^{1/2}]; size, (basal area)^{1/2}, (cm). Other abbreviations as in Table 1.

genets. Nevertheless, some clear patterns emerged, particularly regarding differences between the sexes. Flowering levels were related strongly to the size and sex of the tree: being large

and male increased the probability of flowering, the level of flowering and the REI (Table 2). Although sizes of flowering and non-flowering trees completely overlapped, flowering trees

were both slightly larger and slightly older (Supplementary data Table S2). Additionally, flowering was positively related to individual heterozygosity (Table 2) and declined with SLA (Table 2; Supplementary data Fig. S5A, B).

We found no evidence that reproduction is reduced by allocations to defence; in fact, PG and CT levels were positively associated with the probability of flowering (Table 2). While the number of flowering twigs had no association with defence, the REI increased with the sum of PG and CT levels, an effect that declined with age (Table 2).

Across the 3 years for which we counted flowering twigs, 787 (43.7 %) of the trees entered reproductive maturity, with the greatest number flowering in 2018 (also the year with the highest number of flowering twigs per tree; Supplementary data Fig. S6A). Twice as many males flowered as females

(means: 340.3 trees, 159.0 genets vs. 172.3 trees, 80.3 genets) (Supplementary data Fig. S6B, C). This difference was a result of males flowering when younger and smaller (Table 1; Supplementary data Fig. S7) as well as having both a higher probability of flowering and producing 18 % more flowering twigs.

Herbivory, disease and other leaf traits

Herbivory and disease showed very different changes with tree size. Leaf area lost to insect herbivores increased with size and age (Supplementary data Fig. S8A), as well as with CT and N levels (Table 2; Supplementary data Fig. S9A, B). Disease, on the other hand, declined with size (Supplementary data Fig. S8B), as well as with PG levels, EFNs, N and SLA (Table 2; Supplementary data Fig. S10A–E). Other leaf traits also showed marked ontogenetic changes. Both leaf N and SLA increased over time (Fig. 2). Moreover, SLA increased with respect to N (Supplementary data Fig. S3).

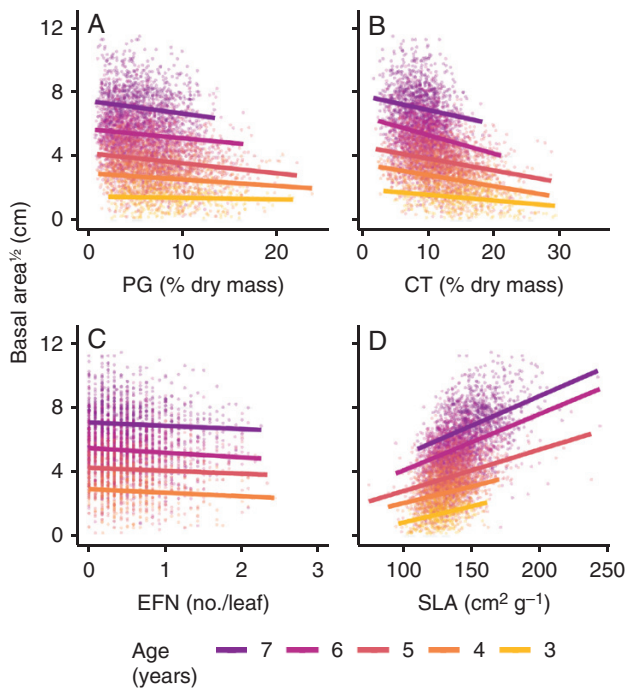


FIG. 4. Tree size in relation to defence traits and leaf morphology. Each point represents a genet mean for each age. Abbreviations as in Fig. 2.

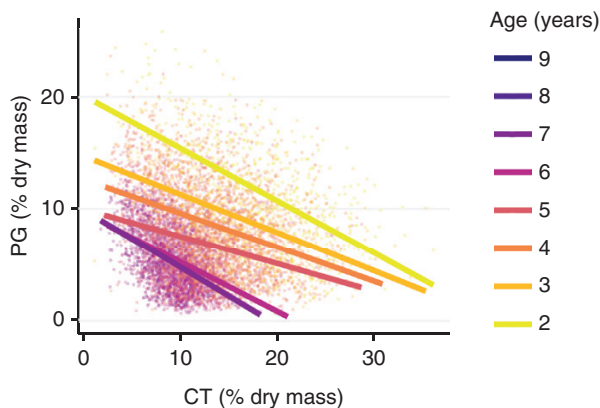


FIG. 5. Trade-off between production of phenolic glycosides and condensed tannins for trees of different ages. Each point represents a genet mean for each age.

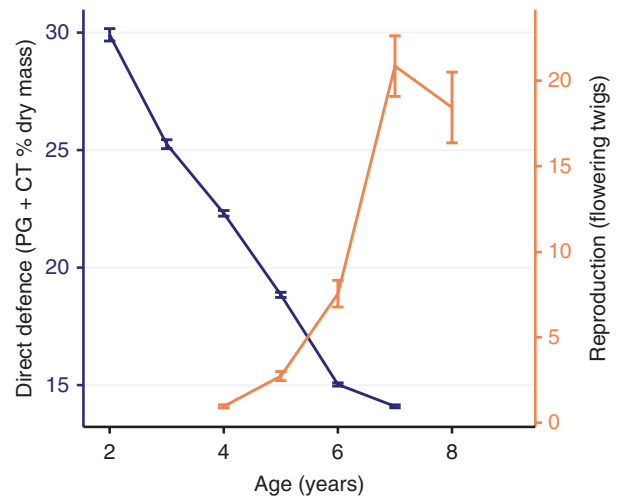


FIG. 6. Ontogenetic trajectories for direct defence and reproduction; population means vs. age.

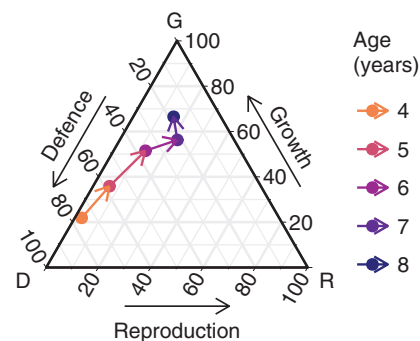


FIG. 7. Compound ontogenetic trajectory for aspen growth, defence and reproduction. Data are represented as a proportion of the range of each variable, based on population means.

Allocational shifts: compound ontogenetic trajectories

Age-specific changes produced compound ontogenetic trajectories for growth, defence and reproduction. Through the juvenile period into the onset of reproductive maturity, defence declined well before reproduction increased (Fig. 6). The compound ontogenetic trajectory for population means of relative allocation to these traits reflects the declining defence allocation as growth increases (Fig. 7). Reproduction increases with age as the population enters reproductive maturity, with the exception of a slight decrease in 2019 (Figs 6 and 7; Supplementary data Fig. S6).

DISCUSSION

ITV in aspen

Four principal conclusions emerge from this large-scale, multi-year study of clonally replicated aspen trees. First, high levels of genotypic ITV exist for all the traits studied, with high heritabilities for traits and their ontogenetic trajectories. Secondly, ontogenetic change acts as a major driver of growth, defence and reproductive ITV. Thirdly, accounting for ontogeny revealed trade-offs among growth, defence and reproduction. Finally, after accounting for ontogeny and genotypic trade-offs, high levels of residual variation remain among genets, with the potential to shape aspen community genetics.

The past 15 years have brought new understanding of the myriad consequences for community- and ecosystem-level processes resulting from ITV in plants. Increased primary productivity (Cook-Patton *et al.*, 2011), increased diversity of associated species (particularly arthropods) (Booth and Grime, 2003; Crutsinger *et al.*, 2006; Johnson *et al.*, 2006; Cook-Patton *et al.*, 2011; Crawford and Rudgers, 2013), reduced disease severity (Smithson and Lenne, 1996), altered multitrophic interactions (Bailey *et al.*, 2006), decreased colonization by invasives (Crutsinger *et al.*, 2008) and enhanced resistance to disturbance (Hughes and Stachowicz, 2004) have all been documented, although not universally (Fridley and Grime, 2010; Moreira and Mooney, 2013; Barton *et al.*, 2015; Abdala-Roberts *et al.*, 2015, 2016). Ecosystem processes such as litter decay and nutrient cycling are also affected by plant ITV (Madritch and Hunter, 2002; Schweitzer *et al.*, 2005; Madritch *et al.*, 2006).

Yet three limitations of this expanding body of work persist (Hughes *et al.*, 2008; Barton and Boege, 2017). Relatively few studies have (1) identified the mechanisms that determine community and ecosystem consequences; (2) focused on the genetic diversity of foundation species; or (3) explored the ontogeny of ITV that underlies community and ecosystem processes. For aspen, prior work has identified growth, defence and reproductive traits as the principal mechanisms linking this species to ecological associates and processes (Lindroth and St. Clair, 2013). Here we describe the genotypic diversity for these keystone traits in this foundation species, how that ITV changes with ontogeny and how it is shaped by trade-offs among the traits. The genetic and ontogenetic patterns identified give insight into the complex interplay among functional traits that shapes the expression of ITV and, by extension, mediates higher order ecological and evolutionary processes.

Heritability and ontogeny

Genotype was the most important factor shaping the high levels of ITV for defence and reproduction, and second only to age for growth (Table 2). Trait heritabilities were also high (Fig. 3), although H^2 values for reproductive measures were generally slightly lower than those for defence traits. Reproductive traits often have lower heritabilities than somatic traits, which is usually interpreted as the consequence of strong selection adjusting relevant allele frequencies to near mutation/selection equilibria (Lynch and Walsh, 1998). Alternatively, the lower heritability of life history traits may arise because they are affected by more genes than somatic traits (Price and Schluter, 1991).

After genotype, ontogenetic change had the largest effects on ITV (Table 2). The decline of PGs and EFNs (Fig. 2) matched our prediction. The decline in CTs, however, contradicted our prediction, based both on the expectation of increasing resources available for defence during the juvenile phase (Boege and Marquis, 2005; Barton and Koricheva, 2010) and on patterns of ontogenetic change documented in 0- to 2-year-old aspen trees (Donaldson *et al.*, 2006b). The ontogeny of CTs may also be shaped by their multifunctionality (Constabel *et al.*, 2014; Gourlay and Constabel, 2019) or because the growing trees had increased access to soil N, which lowers CT concentrations (Donaldson *et al.*, 2006a; Osier and Lindroth, 2006; Decker *et al.*, 2017).

The heritabilities of these ontogenetic trajectories of chemical resistance were substantial (Table 1). Relatively few studies have reported heritabilities of ontogenetic changes. Maherali *et al.* (2009) found lower heritabilities for ontogenetic changes in physiological traits in *Avena*, which also differed between wet and dry environments. Ochoa-López *et al.* (2018) found significant H^2 values for ontogenetic increases in trichome density of shadehouse-grown *Turnera velutina* shrubs, though not for other defence traits. Also, studying a smaller number of aspen genets than we used, Cope *et al.* (2019) found higher H^2 values for PG and CT ontogenies in trees of ages similar to those reported here.

Heritabilities themselves can show ontogenetic change, though few studies have examined H^2 values as plant traits change with ontogeny. Ochoa-López *et al.* (2018) reported that the H^2 values for trichome density of *T. velutina* increased from the seedling to reproductive stages. We found that H^2 values generally rose for reproductive traits and for CT and EFN defence traits (Fig. 3C) even as the average values of those traits declined.

Genotypic trade-offs

Trade-offs play central roles in the generation and maintenance of ITV, and the community- and ecosystem-level consequences thereof (Hunter, 2016; Agrawal, 2019). Genotypic trade-offs, in particular, reveal how combinations of traits are shaped by natural selection (Reznick, 1985; Agrawal, 2019). For example, biosynthetic constraints are likely to influence the joint evolution of chemically related defence compounds. PGs and CTs derive from a common cinnamic acid precursor, and we found strong genotypic trade-offs between PG and CT

concentrations at all ages. This pattern contrasts with results of a meta-analysis by [Koricheva et al. \(2004\)](#), who found no overall trade-off between chemical defences belonging to different groups of biosynthetically related compounds, such as PGs and CTs. Genotypic trade-offs can also indicate selective forces on different functions, but surprisingly few studies have explored genotypic trade-offs between growth and defence in woody plants. [Sampedro et al. \(2011\)](#) found a significant decrease in growth from genotypic allocation to defence in *Pinus pinaster*, which was alleviated by phosphorus fertilization. In contrast, [Villari et al. \(2014\)](#) found no growth decrease from constitutive chemical defence in *P. sylvestris*, and a positive relationship between growth and terpenoid defences. To complicate matters, [Strauss et al. \(2002\)](#) noted that assessing growth–defence correlations can be complicated by inbreeding levels, which can affect growth and/or defence ([Mopper et al., 1991](#); [Carr and Eubanks, 2002](#)). [Strauss et al. \(2002\)](#) suggested that work assessing genotypic trade-offs between growth and defence should account for inbreeding by measuring individual heterozygosity (H_{obs}), which declines to zero as inbreeding increases. This concern has received little attention, but our earlier work ([Cole et al., 2016](#)) found that aspen growth increases with H_{obs} and decreases with higher CTs. In the present study, we found that growth was reduced by defence and, indirectly, by reproduction, matching our third prediction. Furthermore, while H_{obs} (evaluated at a smaller number of loci than in the former study) was negatively related to CT levels and positively related to reproduction, it exhibited no discernible relationship in the aspen growth–defence trade-off reported here ([Table 2](#)).

Unlike the clear trade-off between growth and defence, we found no direct trade-offs between defence and reproduction, contrary to our predictions. This result contrasts with the finding of [Ochoa-López et al. \(2020\)](#) that defence investments [hydrogen cyanide (HCN) in seedlings and trichomes in juveniles] in *T. velutina* exact a fitness cost in the form of reduced seed production. Defence may, however, indirectly reduce reproduction in aspen, since it reduces growth that is positively related to reproduction.

In addition to resistance, defence against herbivores can take the form of tolerance or escape. The ontogenetic decline in resistance (PGs and CTs) and indirect defence (EFNs) in growing aspen may reflect a shift from defence through resistance to defence through tolerance (and, ultimately, through escape, as trees grow out of reach of most mammalian herbivores). Indeed, [Stevens et al. \(2007, 2008\)](#) found that aspen tolerance to defoliation increases with stem biomass. This shift from resistance to tolerance represents a form of ontogenetic trade-off, which occurs along with the genotypic trade-off between resistance and growth. The increase in tolerance is similar to that observed in radishes by [Boege et al. \(2007\)](#), but contrasts with the tolerance decrease found in *Turnera* by [Ochoa-López et al. \(2015\)](#), as defence from HCN in the seedling stage is supplanted by trichomes and EFNs in juvenile and adult plants.

A major development in plant ecology that reflects insights from evolutionary trade-offs among functional traits is the concept of the leaf economic spectrum (LES; [Wright et al., 2004](#); [Reich, 2014](#)). The LES contrasts leaves of fast-growing, resource-acquisitive, poorly defended plants with those of slow-growing, resource-conservative, well defended plants.

Resource-acquisitive species produce leaves with high concentrations of N, low levels of defence and high SLA, whereas resource-conservative species do the opposite ([Agrawal and Hastings, 2019](#)). This spectrum of trait variation has shown wide applicability at higher taxonomic levels ([Reich, 2014](#); [Anderegg et al., 2018](#)).

Despite the large and growing literature on ITV, few studies have explored intraspecific relationships among traits from an LES perspective. Also, as noted by [Agrawal and Hastings \(2019\)](#), virtually no work has related genotypic ITV in leaf defence traits to the LES. In a small-scale study (15 full-sibling families) with *Asclepias*, he reported a positive genotypic relationship between foliar N and SLA, but no significant relationships of N or SLA with trichome, latex or cardenolide defences ([Agrawal and Hastings, 2019](#)). Our study, however, revealed numerous patterns of ITV that are consistent with the LES paradigm: aspen genotypes exhibited a positive relationship between leaf N concentration and SLA ([Supplementary data Fig S2D](#)), negative relationships between defence (PGs, CTs and EFNs) and SLA ([Supplementary data Fig. S2A, B](#)), and negative relationships between resistance (PGs and CTs) and N ([Supplementary data Fig. S4](#)). These results suggest that the LES may provide a useful framework for exploring and explaining relationships among functional traits in future ITV research.

Residual ITV: the architecture of aspen community genetics

The mechanisms by which genotypic variation shapes ecological communities are increasingly well documented. Among woody plants, this is especially true for the Salicaceae. For example, genotypic variation arising from hybridization between the western (USA) cottonwoods *Populus angustifolia* and *P. fremontii* largely determines CT levels, which in turn affect palatability to mammalian herbivores, litter decomposition and nutrient cycling ([Schweitzer et al., 2008](#)). Genotypic variation in the traits (size, leaf morphology and chemistry) of western cottonwood and European aspen (*P. tremula*) can structure associated arthropod communities, producing high heritabilities for those communities ([Bangert et al., 2006](#); [Robinson et al., 2012](#); [DeWoody et al., 2013](#)). Also, in both *P. tremula* and *P. tremuloides*, specific genes have been linked to plant traits and associated insect species ([Bernhardsson et al., 2013](#); [Barker et al., 2019](#)). Yet, even when ITV is well characterized, residual variation can determine associations with herbivores and other species. In a common garden study of plant traits and insect communities of *Salix hookeriana*, all drawn from a small geographic area, [Barbour et al. \(2015\)](#) found high heritabilities for secondary chemistry and plant architecture. They also found that residual genotypic ITV played a major role in structuring associated insect communities, even after accounting for numerous chemical and physical traits.

In natural populations, the genotypic and ontogenetic sources of ITV in aspen will be accompanied by environmental variation. Environmental variation probably amplifies many of the trade-offs reported here, particularly when resources are limiting. While trees in our common garden were relatively widely spaced on nutrient-rich soils, growth–defence trade-offs in aspen become stronger when resources (light and nutrients) are limiting ([Osier and](#)

Lindroth, 2006) or trees are subject to competition with other species (Donaldson *et al.*, 2006a). Yet while we expect environmental variation to increase both expression and selection for ITV, it may also buffer ecological responses to ITV. In this experimental population, the strong patterns of individual trait ITV and the compound ontogenetic trajectories (Figs 6 and 7) contrast markedly with the relatively modest ontogenetic trends and genotypic trade-offs of herbivory and disease (Table 2; Supplementary data Fig. S8). The herbivory and disease trends reflect the interaction between these compound ontogenetic trajectories and environmental variation, both abiotic and biotic, most clearly including the growing resident population of insect herbivores (C. Morrow, C. Cole and R. Lindroth, unpubl. data).

A dominant message emerging from our work is that aspen possesses enormous levels of genotype-based ITV, even after accounting for the roles of ontogeny and trade-offs among growth, defence and reproduction (Table 2). The structure of this variation is distinctly different from that found in western *P. angustifolia*/*P. fremontii* populations, as it exists within a single species for multiple traits. This variation itself represents the fundamental pattern of the North American forests where aspen predominates – an architecture of community genetics representing high levels of variability enabling aspen to occupy diverse environments across a vast range. On this foundation arises further ITV from ontogeny and from environmental variability: climate extremes, fire and outbreaks of defoliators. Genotypic trade-offs such as those revealed here imply that selection for increased resistance to one factor comes with increased susceptibility to another. These patterns exemplify the ‘shifting balance’ model of Wright (1982), producing a landscape varied in space and time, hosting the diverse community of ecological associates both responding to and shaping aspen’s panoply of traits. Despite this wealth of phenotypic variation, the high heritabilities of traits closely associated with other species – notably defence traits – provide the foundation for connecting these traits to other organisms in the aspen community, as well as to the genes that shape them.

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Methods S1: methods for quantifying salicinoid PGs via HPLC-UV. Methods S2: modelling growth. Table S1: TOZ sex marker test for male, female and hermaphrodite trees. Table S2: flowering tree *t*-tests comparing size and age of male and female trees. Figure S1: violin plots of ontogenetic trajectories for individual phenolic glycosides. Figure S2: growth (basal area increment) vs. reproductive effort for different size trees. Figure S3: defence and leaf chemistry vs. leaf morphology. Figure S4: phenolic glycoside and condensed tannin concentrations vs. nitrogen, and CT concentrations vs. individual heterozygosity. Figure S5: flowering level vs. leaf morphology and individual heterozygosity. Figure S6: population flowering levels each year. Figure S7: reproductive level vs. size for female and male trees. Figure S8: herbivory and disease vs. size for different age trees. Figure S9: herbivory vs. condensed tannin and nitrogen concentrations. Figure S10: disease vs. phenolic glycoside and condensed tannin concentrations.

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