Convergent molecular evolution among ash species resistant to the emerald ash borer

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Recent studies show that molecular convergence plays an unexpectedly common role in the evolution of convergent phenotypes. We exploited this phenomenon to find candidate loci underlying resistance to the emerald ash borer (EAB, *Agrilus planipennis*), the United States’ most costly invasive forest insect to date, within the pan-genome of ash trees (the genus *Fraxinus*). We show that EAB-resistant taxa occur within three independent phylogenetic lineages. In genomes from these resistant lineages, we detect 53 genes with evidence of convergent amino acid evolution. Gene-tree reconstruction indicates that, for 48 of these candidates, the convergent amino acids are more likely to have arisen via independent evolution than by another process such as hybridization or incomplete lineage sorting. Seven of the candidate genes have putative roles connected to the phenylpropanoid biosynthesis pathway and 17 relate to herbivore recognition, defence signalling or programmed cell death. Evidence for loss-of-function mutations among these candidates is more frequent in susceptible species than in resistant ones. Our results on evolutionary relationships, variability in resistance, and candidate genes for defence response within the ash genus could inform breeding for EAB resistance, facilitating ecological restoration in areas invaded by this beetle.

Ash trees (*Fraxinus*) are key components of temperate forest ecosystems1,2, the health of which affects the provision of ecosystem services including climate change mitigation3. The continued survival of ash in North America and Europe is threatened by a highly destructive invasive insect4,5, the emerald ash borer (EAB, *Agrilus planipennis*). This wood-boring beetle has thus far proved to be highly destructive to the majority of *Fraxinus* species it has encountered outside of its native range in East Asia6,7. In North America, EAB has already killed hundreds of millions of ash trees and billions more are at risk8,9. In Europe, the beetle has established an invasive range in Moscow from which it is spreading5,7,10–12, and there is increasing concern about the threat posed to native species of ash13,14 or under drought conditions15.

EAB has killed hundreds of millions of ash trees and billions more are at risk. The United States Department of Agriculture, Forest Service, Northern Research Station, Delaware, OH, USA. 1United States Department of Agriculture, Agricultural Research Service, US National Arboretum, Washington, DC, USA. 6Present address: School of Life Sciences, The University of Warwick, Coventry, UK. ✉e-mail: l.kelly@kew.org; r.buggs@kew.org

Results

To better understand how resistance to EAB varies across the genus, and to examine evidence for convergent evolution of this trait, we assessed resistance to EAB for 26 *Fraxinus* taxa (Supplementary Table 1) representing four of the six taxonomic sections and 48% of species16. Tree resistance was scored according to the instar, health and weight of EAB larvae in the stems of inoculated trees 8 weeks after infestation16 (see Methods and Supplementary Table 1). In *F. baroniana*, *F. chinensis*, *F. floribunda*, *F. mandshurica*, *F. platypoda* and *Fraxinus* sp. D2006-0159, least-squares means (LSM) of the proportion of host-killed larvae (number of larvae killed by tree defence response divided by total larvae entering the tree) were >0.75 (Fig. 1a and Supplementary Table 2), and LSM estimate of the proportion of larvae successfully entering the tree and that reached the L4 instar was zero (Fig. 1b and Supplementary Table 2), indicating that these species are resistant to EAB. In contrast, all other taxa tested had a LSM proportion of larvae killed of 0.58 or less (Fig. 1a) and had LSM for L4 larvae proportion between 0 and 0.89 (Fig. 1b).

To infer a robust phylogenetic framework for *Fraxinus* within which to understand the evolution of EAB resistance and to allow analysis of evidence for molecular convergence, we sequenced and assembled the genomes of 28 individuals from 26 diploid taxa.
representing all six sections within the genus\textsuperscript{25}, including a common EAB-susceptible accession and a rare putatively EAB-resistant accession\textsuperscript{26} for \textit{F. pennsylvanica} (Supplementary Table 3). Estimated genome sizes (1C-values) of the individuals selected for sequencing range between \sim 700 and 1,100 Mb (Supplementary Table 4); for all individuals we generated \sim 35–85-fold of whole-genome shotgun coverage with Illumina sequencing platforms (see Methods and Supplementary Table 4). On assembly (Methods) these data generated 133,719–715,871 scaffolds for each individual, with the minimum contig length needed to cover 50\% of the assembly (N50) ranging from 1,987 to 50,545 base pairs (bp) (Supplementary Table 4); BUSCO analysis of the genome assemblies (Methods) found that 78.4–94.7\% of genes were present (either complete or fragmented; Supplementary Table 4). Therefore, despite some of the assemblies being highly fragmented, a sufficient proportion of the gene space had been assembled to facilitate testing for amino acid
convergence (see below). We annotated genes in these assemblies via a reference-based approach (Methods) using the published genome annotation of *F. excelsior*\(^2\). We clustered the protein sequences of these genes into putative orthologue groups (OGs; Methods), also including protein sequences from the *F. excelsior* reference genome and the published genome annotations of *Olea europaea*\(^3\), *Erythranthe guttata*\(^4\) and *Solanum lycopersicum*\(^5\). We found a total of 87,194 OGs, each containing sequences from between 2 and 32 taxa; 1,403 OGs included a sequence from all 32 taxa.

We generated multiple sequence alignments for the 1,403 OGs that included all taxa and inferred gene-trees for each (Methods). To generate a species-tree estimate for *Fraxinus*, we conducted Bayesian concordance analysis (Methods). This resulted in a tree based on 272 phylogenetically informative, low-copy genes (Fig. 2 and Supplementary Note 1). Within this tree, the EAB-resistant taxa identified from our bioassays occurred in three independent lineages: (1) *F. mandshurica* occurred within a clade corresponding to section *Fraxinus* that also included susceptible taxa; (2) *F. platypoda* was sister to a clade corresponding to section *Melioideae*, which includes most of the susceptible American species; and (3) *F. baroniana*, *F. floribunda* and *Fraxinus* sp. D2006-0159 clustered together, within a larger clade that included most species in section *Ornus*, including susceptible *F. ornus*. Thus, by combining phylogenetic data with the most highly evidenced phylogenetic hypothesis for *Fraxinus* to date, we show that EAB resistance has evolved convergently within the genus. A further resistant taxon identified from our bioassay, *F. chinensis*, was not included in the species-tree analysis because it is a polyploid\(^1\).

We searched for amino acid variants putatively convergent between the resistant lineages using an approach that identifies loci with a level of convergence in excess of that likely to be due to chance alone (grand-conv; Methods). We conducted three pairwise analyses of lineages, with each pair representing two of the three independent lineages of diploid EAB-resistant taxa identified from our egg bioassays and species-tree analysis: (1) *F. mandshurica* versus *F. platypoda*; (2) *F. mandshurica* versus *F. baroniana*, *F. floribunda* and *Fraxinus* sp. D2006-0159 clustered together, within a larger clade that included most species in section *Ornus*, including susceptible *F. ornus*. Thus, by combining pheno-

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Among our 53 candidate genes, seven have putative roles relating to the phenylpropanoid biosynthesis pathway (Supplementary Note 4). This pathway generates anti-feedant and cytotoxic compounds, as well as products involved in structural defence such as lignin\(^2\); it can contribute to indirect defence by producing volatiles that attract parasitoids or predators\(^3\). Loci OG15551, OG853 and OG16673 are of particular interest. Four convergent amino acids were identified in OG15551 (Fig. 3), a paralogue of CYP98A3 (Supplementary Fig. 2), which encodes a critical phenylpropanoid pathway enzyme\(^4\). Three of the four residues fall within CYP98A3 putative substrate-recognition sites, with two at positions predicted to contact the substrate\(^1\) including a leucine (sulfur-containing)/methionine (non-sulfur-containing) variant (Fig. 3), suggesting that these variants may affect the protein's function. OG853 is apparently orthologous to *RFR1* (also called *MED5a*; Supplementary Fig. 2), a known regulator of the phenylpropanoid pathway\(^5,6\) that seems to be involved in regulation of defence-response genes\(^6,7\). OG16673 is a probable glycoside hydrolase; putative *Arabidopsis thaliana* homologues of OG16673 belong to glycoside hydrolase family 1 and have beta-glucosidase activity, with functions such as chemical defence against herbivory, lignification and control of phytohormone levels\(^8\). A role for beta-glucosidases in defence against EAB in individual *Fraxinus* species has previously been suggested on the basis of chemical\(^9\) and transcriptomic\(^10\) data, and several metabolic studies have indicated that products of the phenylpropanoid pathway could be involved\(^11\).

We found 15 candidate genes (Supplementary Note 4) with possible roles in perception and signalling relevant to defence response against herbivorous insects\(^12\). OG4469 is a probable orthologue of *AG-LecRK*-1.6, a G-type lectin receptor kinase (LecRK) with ATP-binding activity (Supplementary Note 4.3). G-type LecRKs can act as pattern recognition receptors in the perception of feeding insects\(^12\); extracellular ATP is a damage-associated molecular pattern whose perception can trigger defence response-related genes\(^12,13\). OG38407 appears orthologous to *SNIPER4* (Supplementary Fig. 2), an F-box protein-encoding gene involved in regulating turnover of defence response-related proteins for optimal defence activation\(^11,14\); the convergent site is in a leucine-rich repeat region (Extended Data Fig. 1) involved in recognition of substrate proteins for ubiquitination\(^15,16\).

Several genes appear to relate to phytohormone biosynthesis and signalling, including those with putative functions in the biosynthesis of jasmonate (OG41448), brassinosteroid (OG43828), cytokinin (OG39275) and abscisic acid (OG47560), and Gene Ontology (GO) terms associated with hormone metabolism and biosynthesis are significantly enriched (*P* < 0.01) among our set of candidate genes (Supplementary Note 5 and Supplementary Table 7). Jasmonate signalling is the central regulatory pathway for defence response against insect herbivores\(^17,18\), whereas brassinosteroids and cytokinins can play important roles in insect resistance via modulation of the jasmonate pathway\(^19,20\). Abscisic acid is induced by herbivory and is a known modulator of resistance to insect herbivores\(^21,22\); OG11720 is putatively orthologous to *NRT1.5* (also known as *NPFP7.3*), a member of the NRT1/PT family\(^23,24\) involved in transport of multiple phytohormones (Supplementary Note 4.3); a transcript matching this
A gene family had decreased expression in response to both mechanical wounding and EAB feeding in *F. pennsylvanica*. Putative functions of further candidates relate to other signalling molecules involved in triggering of the defence response (Supplementary Note 4), including calcium (OG50989)\(^{33,48}\), nitric oxide (OG21033)\(^{49,50}\) and spermine (OG33348)\(^{51}\). Increased resistance to EAB can be artificially induced in *Fraxinus* species with otherwise high susceptibility\(^{52}\), leading to the suggestion that susceptible species may fail to recognize, or respond sufficiently quickly to, early signs of EAB attack\(^{41}\). Our identification of candidate genes putatively involved in perception and signalling underlines the possibility of differences between EAB-resistant and -susceptible *Fraxinus* species in both their ability to sense and react to attacking insects.

Hypersensitive response, involving programmed cell death (PCD), is associated with effector-triggered immunity in response to microbial pathogens\(^{53}\) but can also be induced by insect herbivory\(^{54}\) and oviposition\(^{55}\). OG16739 and OG37870 are candidates with putative roles related to hypersensitive response-like effects and PCD. OG16739 has homologues that control cell death in response to wounding, via the induction of ethylene and the expression of defence- and senescence-related genes\(^{56}\). OG37870 may be orthologous to genes that appear to play a role in controlling PCD of xylem elements\(^{57}\). Candidate loci whose putative functions lack an obvious link to plant defence response (Supplementary Note 4) could be involved in other phenotypic traits shared between EAB-resistant species or may play a role in defence response that is not yet understood.
We found that 19 of our 53 candidates match the same *A. thaliana* genes as transcripts that are differentially expressed in response to elm leaf beetle (either in response to simulated egg deposition, or larval feeding)58, including genes such as OG24969 whose putative *A. thaliana* homologues lack a clear defence-related function.

We analysed allelic variation at the 67 amino acid sites within the 53 candidate genes for all sequenced taxa assessed for resistance to EAB. Of the 67 sites, seven have the EAB resistance-associated state only in resistant taxa, and another is homozygous for the EAB resistance-associated state in only resistant taxa (Supplementary Table 8). Of the 53 candidate genes, four are homozygous only in resistant taxa for the EAB resistance-associated state at the candidate amino acid site(s) detected within them (OG853, OG21449, OG36502 and OG37560; Supplementary Table 8). If we omit the genomes of *F. nigra*, *F. excelsior* and the three *F. angustifolia* subspecies (section *Fraxinus*), for 24 of the 53 candidate genes we find the EAB resistance-associated states only in resistant taxa, for 48 genes they are found only in taxa with a LSM proportion of larvae killed of ≥0.25, and the remaining five genes are homozygous for the EAB resistance-associated states only in taxa with a LSM proportion of larvae killed of ≥0.25 (Supplementary Table 8).

Analysis of previously generated whole-genome sequence data for 37 *F. excelsior* individuals from different European provenances27 revealed that, for 50 of the 67 candidate amino acid sites (occurring in 41 of the 53 genes), the EAB resistance-associated state was present with evidence for polymorphism at seven of these sites
(Supplementary Table 9). None of the EAB resistance-associated amino acid changes were found in the putatively resistant *F. pennsylvania* genotype (Supplementary Table 8), suggesting that different genes, or different variants within these genes, are involved in the intraspecific variation in susceptibility of this species. Despite this, transcripts inferred to be from 11 of our candidate genes showed evidence for differential expression subsequent to EAB feeding in *F. pennsylvania* (Supplementary Note 6 and Supplementary Table 10), and two gene families that were highlighted as potentially important in response to tissue damage in *F. pennsylvania* are also represented among our candidates (see above).

**Discussion**

It has frequently been suggested that EAB has a co-evolutionary history with its native *Fraxinus* hosts within their shared geographic ranges in East Asia, during which defence mechanisms against EAB may have been selected for\(^4\). All six taxa identified as resistant to EAB on the basis of our egg bioassays are native to Asia\(^1\) (*Fraxinus* sp. D2006-0159 originates from material collected in northern China), including known natural EAB host species *F. chinensis* and *F. mandshurica*. In addition to *F. chinensis* and *F. mandshurica*, the native range of *F. platypoda* overlaps with that of EAB\(^4\),\(^2\),\(^9\). The current native ranges of *F. baroniana* and *F. floribunda*\(^10\) apparently do not overlap with the native range of EAB\(^4\),\(^5\), but we cannot discount the possibility that they did in the past and thus that these species also share a co-evolutionary history with EAB. Alternatively, it may be the case that the most recent common ancestor of *F. baroniana*, *F. chinensis* and *F. floribunda* was an EAB host species and that resistance has been retained in these extant descendants. Among the resistant Asian species are comparatively close relatives of all three major susceptible North American EAB hosts: *F. pennsylvania*, *F. americana* and *F. nigra*. It is known that the closely related *F. mandshurica* and *F. nigra* can produce hybrids\(^6\);\(^7\),\(^8\); the phylogenetic proximity of *F. platypoda* to *F. pennsylvania* and *F. americana* suggests that it may also be possible to increase resistance in these species via hybrid breeding.

By assessing resistance across the genus and testing for molecular convergence, we have provided evidence for candidate genes involved in EAB resistance in *Fraxinus*. Multiple loci, contributing to different defence responses, appear to underlie this trait. In less than 20 years since it was first detected in North America, EAB has caused devastating damage to native ash populations, to the point where *Fraxinus* risks being lost entirely as a functional component of forest ecosystems\(^9\). Our data may help to target future efforts to increase the resistance of North American and European ash species to EAB via breeding or gene editing, an intervention that could be required if these species are to persist in the face of the ongoing threat from this invasive beetle. Moreover, these results highlight the potential use of convergence analyses as an approach to identifying candidate genes for traits of interest in organisms where alternative strategies, such as genome-wide association studies or mapping of quantitative trait loci, may be less feasible.

**Methods**

**Data reporting.** For the EAB resistance assays, experiments were conducted using a randomized block design. No statistical methods were used to predetermine sample size. The investigators were not blinded to allocation during experiments and outcome assessment.

**Plant material.** All plant materials used in this study were sourced from living or seed collections in the United Kingdom or United States. Due to biosecurity measures, we were not able to move living materials between the two countries. In our initial selection of material we relied upon species identifications that had already been made in the arboretum or seed banks within which the materials were held. For each of the accessions included in this study, we PCR amplified and Sanger sequenced the nuclear ribosomal internal transcribed spacer (ITS) region, following standard methods; forward and reverse sequences were assembled into contigs using CLC Genomics Workbench v8.5.1 (QIAGEN). As far as possible, the identity of all materials was verified by E. Wallander using morphology and ITS sequences, according to her classification of the genus\(^1\). This led to some reclassification of samples of putative *F. mandshurica* and all resistant *F. mandshurica* genotype samples were originally sampled as *F. caroliniana*, and the accession that we originally sampled and genome sequenced as *F. bungeana* was determined to be *F. ornus* (Supplementary Table 3). However, subsequent phylogenetic analysis that included allele sequences for this latter individual (see Distinguishing between different underlying causes of convergent patterns) indicated that it is likely to be a hybrid between the *F. ornus* lineage and another lineage within section *Ornus*, and therefore we designate it as *Fraxinus* sp. 1973-6204. We also designated an accession that was originally sampled for genome sequencing as *F. chinensis* as *Fraxinus* sp. D2006-0159 due to uncertainty regarding species delimitation. Furthermore, for genotype vel-4, which was determined as *F. mandshurica*, we have maintained its original designation. A list of all materials used in the study is shown in Supplementary Table 3, along with initial identifications and subsequent identifications by E. Wallander, as well as details of voucher specimens.

**EAB resistance assays.** Twenty-six *Fraxinus* taxa (species, subspecies and one taxon of uncertain status) were collected for egg bioassay experiments (Supplementary Table 3). We aimed to test three clonal replicates (genotypes or cuttings) of at least two genotypes of each species. For some taxa, fewer than two genotypes were available in the United States, and occasionally genotypes did not propagate well by graft or cut so that seedlings from the same seedlot were used instead (details for each taxon are included in Supplementary Table 1). The majority of egg bioassays were conducted in 2015 and 2016 (Supplementary Table 1) with a small number of assays repeated in each week (susceptible *F. pennsylvania* genotype pe-37 and/or pe-39 and resistant *F. mandshurica* genotype ‘mancana’).

Trees were treated as uniformly as possible before inoculation. Adult beetles were reared and used for egg production as previously described\(^1\). Inclusions were performed in a greenhouse to keep conditions uniform for the duration of the assay, and to minimize predation of the eggs. We followed the EAB egg bioassay methods developed by Koch et al.\(^2\), which had previously been used on genotypes of *F. pennsylvania* and *F. mandshurica*, with the changes noted below. The egg dose for each tree was determined according to the method of Duan et al.\(^2\);\(^5\), which takes into account the bark surface area. A target density of 400 eggs m\(^{-2}\) was used; this density is above that reported to allow host defences to kill larvae in green ash, but is within the range where competition and cannibalism are minimized\(^6\). Twelve individual eggs, on a small strip cut from the coffee filter paper on which they were laid, were taped to each tree. The spacing was varied between eggs to maintain a consistent target dose (for example, eggs placed 17.5 cm apart on a stem of diameter 1.0–1.1 cm, to placement 3 cm apart on a stem of diameter 2.5–2.6 cm). The portion of the tree where eggs were placed varied with species and subgenus, as it was expected from host trials that egg feeding would be expected to differ by host species and subgenus, with the presence of *E. quadrayangula* in *F. angustifolia* and *F. syriaca* and *E. candibida* in *F. ornus* (Supplementary Table 3). However, subsequent experiments have shown that egg assay results are not consistent on stems <1 cm in diameter. Due to size differences between some species, to achieve the target dose and avoid placing eggs where the stem diameter was <1 cm, occasionally <12 eggs were placed. A total of 2,199 egg bioassays (each egg represents one hatching event) were conducted on 63 different genotypes and a total of 206 ramets, cuttings or seedlings.

Occasional ramets, cuttings and/or seedlings were considered as assay failures if fewer than three larvae successfully entered the tree (that is, the effective egg dose was too low), or if there were other problems with the tree (too small diameter overall, cultivation issues and so on), and that replicate was excluded from analysis. The final number of ramets/cuttings/seedlings included for each taxon was as follows: *F. albicans* n=9, *F. americana* n=6, *F. angustifolia subsp. angustifolia* n=10, *F. angustifolia subsp. oxyarpa* n=6, *F. angustifolia subsp. syriaca* n=8, *F. apertusquimera* n=8, *F. baroniana* n=5, *F. bilmoreana* n=6, *F. chinensis* n=11, *F. cupidata* n=4, *F. excelsior* n=5, *F. floribunda* n=7, *F. lanigosa* n=3, *F. latifolia* n=3, *F. mandshurica* n=24, *F. nigra* n=4, *F. ornus* n=4, *F. paxiana* n=7, *F. pennsylvania* n=39, *F. platypoda* n=2, *F. profunda* n=12, *F. quadrayangula* n=6, *F. sieboldiana* n=5, *Fraxinus* sp. D2006-0159 n=2, *F. uldei* n=3, *F. velutina* n=6. Four weeks after egg attachment, each egg was inspected to determine whether it had successfully hatched and if there were signs of the larva entering the hole. Larval entry holes, when detected, were marked with a dot for future dissection. At 8 weeks, dissection of the entry site was performed and galleries made by larval feeding were carefully traced using a grafting knife to determine the outcome of each hatched egg. Health (dead or alive) and weight (in cases where larvae could be recovered intact) were recorded for each larva, and developmental instar was determined using measurements of head capsule length. Larvae that had been killed by host defence mechanisms were distinguished from those that had died from other causes, by examining the tissue immediately surrounding the larva for evidence of browning and/or callus formation (indicating a defence response), and by checking for the absence of evidence of any other
causes of death, including cannibalism, parasitism and fungal infection. The total number of eggs for which larvae successfully entered the tree and the outcome recorded was as follows: F. abies n=63, F. americana n=57, F. angustifolia subsp. angustifolia n=101, F. angustifolia subsp. oxycaerulea n=55, F. bimorpha n=83, F. chinensis n=43, F. cuspidata n=32, F. excelsior n=36, F. floridiana n=37, F. girondica n=24, F. latifolia n=31, F. mandshurica n=166, F. nigra n=30, F. orsaya n=27, F. paxiana n=59, F. pennsylvanica n=403, F. platypoda n=21, F. profunda n=110, F. quadrangulata n=50, F. sieboldiana n=26, Fraxinus sp. D2006-0159 n=25, F. shielii n=33, F. velutina n=42.

Preliminary flow cytometry data analysis indicated that the proportion of ‘tree-killed’ (that is, larval killed by tree defence response) and the proportion of live L4 larvae (number divided by the number of larvae that entered the tree) were the best variables to distinguish resistance versus susceptibility at the species level. We fitted a generalized linear mixed model to the proportion tree-killed and proportion L4 using the GLMMIX procedure in SAS v.9.4. The final model specification is: proportion as a binomial distribution with a logit link function, species as a fixed effect and block/replicate nested within sequential week (week within year) as a random effect (this allowed for comprehensive analysis over years and weeks). Due to problems with convergence, confidence intervals could not be accounted for dependence of eggs within tree and independence of trees in different blocks). Non-significant (P ≥ 0.05) predictors for propagule type and egg density were removed from the final model. Least-squares means of tree-killed or L4 proportion were calculated with confidence intervals on the data scale (proportion).

Genome size estimation by flow cytometry (FC). We used FC to estimate the genome size of individuals used for whole-genome sequencing (Supplementary Table 4). Fraxinus samples from UK collections were prepared and analysed as described in Pellier et al.8, with the exception that ‘general purpose isolation buffer’4, without the addition of 3% polyvinylpyrrolidone (PVVP 40) and LB1 buffer4 as used for Illumina samples. Oryza sativa (IR-36); DNA amounts in the unreplicated gametic nucleus (1C) = 0.50 pg; ref. 8) was used as an internal standard. For each individual analysed, two samples were prepared (from separate leaves or different parts of the same leaf) and two replicates of each sample run. Fraxinus samples from US collections were analysed using a Symex CyFlow System flow cytometer, as described in Whittemore and Xia70; ‘IR-36’ DNA amounts in the unreplicated gametic nucleus (1C) = 4.54 pg; ref. 70) and Glycine max (Williams 82); 1C = 1.13 pg; ref. 70) were used as internal standards. For each individual analysed, six samples were prepared (from separate leaves or different parts of the same leaf) and three samples run with each size standard.

DNA extraction. Total genomic DNA was extracted from fresh, frozen or silica-dried leaf or cambial material using either a cetyltrimethylammonium bromide extraction protocol, modified from Doyle and Doyle73, or a DNeasy Plant Mini or Maxi kit (QIAGEN).

Genome sequencing and assembly. For each of the 28 diploid individuals selected for whole-genome sequencing (Supplementary Table 3), sufficient Illumina sequence data were generated to provide a minimum of ~30-fold coverage of the IC genome size, based on the C-value estimates obtained for the same individuals (see Genome size estimation by flow cytometry (FC)), or those of closely related taxa. Libraries were prepared with insert size of 200 bp or 850 bp were prepared from total gDNA by the Genome Centre at Queen Mary University of London, and the Centre for Genomic Research at the University of Liverpool. Paired-end reads of 125, 150 or 151 nucleotides were generated using the Illumina NextSeq 500, HiSeq 2500 and HiSeq 4000 platforms (see Supplementary Table 4 for the exact combination of libraries, read lengths and sequencing platforms used for each individual). For selected taxa, chosen to represent different sections within the genus, we also generated data from long mate-pair (LMP) libraries (Supplementary Table 4). LMP libraries with an average insert size of 3 and 10 kb were prepared from total gDNA by the Centre for Genomic Research at the University of Liverpool, and sequenced on an Illumina HiSeq 2500 to generate reads of 125 nucleotides to a depth of approximately tenfold coverage of the IC genome size.

Initial assessment of sequence quality was performed for all read pairs from the short-insert libraries (300–850 bp inserts) using FastQC v0.11.3 or v0.11.5 (www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were clipped using the fastx_trimmer tool in the FASTX-Toolkit v.0.0.14 (http://hannonlab.cshl.edu/fastx-toolkit/index.html) to remove the first five to ten nucleotides of each read; for the NextSeq reads, the last five nucleotides were also clipped. Adapter trimming was performed using cutadapt v1.8.1 (ref. 7). With the ‘O’ parameter set to 5 and using option ‘b’, default settings were used for all other parameters. Quality trimming and length filtering were performed using Sickle v1.33 (ref. 7) with the ‘pe’ option and the following parameter settings: -i sample_name.fastq -o sample_name.trimmed.fastq for other parameters. This yielded quality-trimmed paired and singleton reads with a minimum length of 50 nucleotides; only intact read pairs were used for downstream analyses.

For the LMP libraries, duplicate reads were removed using NextClip v1.3.1 (https://github.com/richardmeggett/nextclip) with the –remove_duplicates parameter specified and default settings for all other parameters. Adapter trimming was performed using cutadapt v1.10 (ref. 7); junction adapters were removed from the start of reads by running option ‘g’, with the adapter sequence anchored to the beginning of reads. Default settings for the following settings for other parameters: -O 10 -m 25. Other adapter trimming was performed using option ‘a’, with further parameters set to the same values as specified above. Quality trimming was performed with PRINSEQ-lite v.0.20.4 (ref. 7), with the following parameter settings: --trim_qual_left 20 --trim_qual_right 20 --trim_qual_window 20 --trim_min_tail_left 101 --trim_min_tail_right 101 --trim_qual_left 1 --trim_qual_right 20 --out_format 3.

De novo genome assembly was performed for each individual using CLC Genomics Workbench v.8.5.1 (QIAGEN). All trimmed read pairs from the short-insert libraries were used for assembly under the following parameter settings: automatic optimization of word (k-mer) size; maximum size of bubble to try to resolve, 5,000; and minimum contig length, 200 bp. Assembled contigs were joined to form scaffolds using SSAPACE v.3.0 (ref. 7) with default parameters, incorporating data from mate-pair libraries with 3- and 10-kb insert sizes where available. Library insert lengths were specified with a broad error range (±40%). Gaps in the SSAPACE scaffolds were filled using GapCloser v1.12 (ref. 7) with default parameters. Average library insert lengths were specified using the estimates produced by SSAPACE during scaffolding. Scaffolding and gap filling were not performed for individuals lacking data from libraries with an insert size ≥800bp (only a single-insert size library was available for these taxa; Supplementary Table 4). We did not attempt extraction of sequences of organellar origin from the assemblies, or separate assembly of plastid and mitochondrial genomes.

Sequences within the assemblies corresponding to the Illumina PhiX control library were identified via BLAST. A PhiX bacteriophage reference sequence (GenBank accession no. CP0004084) was used as a query for BLASTN searches, implemented with the BLAST+ package v.2.5.0+ (ref. 7), against the genome assembly for each taxon. Each taxon-specific data set was compared to the PhiX reference sequence at this threshold were removed from the assemblies. We used the assemblathon_stats.pl script (https://github.com/ucdavis-bioinformatics/assemblathon-analysis/blob/master/assemblathon_stats.pl) with default settings to obtain standard genome assembly metrics, including N50, BUSCO v.2.0 (ref. 7) was used to assess the content of the genome assemblies. The ‘embryophyta_odb9’ lineage was used, and analyses run with the following parameter settings: --mode genome -c 8 -e 0.05 -s tomato.

Gene annotation and orthologue inference. To annotate genes in the newly assembled Fraxinus genomes, we used a similarity-based approach implemented in GeMoMa44 with genes predicted in the F. excelsior BATG05 assembly as a reference set. We used the ‘Full Annotation’ gff file for BATG05 (Fraxinus_excelsior_38873_TGAC_v2 longestIDTranscript.gff3; available from http://www.aschrogenome.org/transcriptomes), which contains the annotation for the single longest splice variant for each gene model. This annotation file also includes preliminary annotations for genes within the organellar sequences (gene models FRAEX88873_v2.0000370-FRAEX88873_v2.00041330) not reported in the publication of the reference genome44; none of the sets of putative orthologues used for the species-trees inference or molecular convergence analysis (see below) include these preliminary organellar gene models from the BATG05 reference assembly. The ‘embryophyta_odb9’ lineage was used, and analyses run with the following parameter settings: --min_ident 50 --min_idf 50 --s 250 --outfmt contigs.gff3. Other settings: --out ./blastdb --hash_index --dbtype nucl. tblastn was then run with the following parameter settings: -i sample_name.fasta -o sample_name.blastout --db ./blastdb --evalue 1e-5 -outfmt ‘6 std sallseqid score nident positive tident mismatch gapopen gapclose’ +

From the reference genome (gff and assembly files), we performed TBLASTN searches of individual downstream analyses. Because the presence of these redundant gene models does not, none of the sets of putative orthologues used for the species-trees inference or molecular convergence analysis (see below) include these preliminary organellar gene models from the BATG05 reference assembly. The ‘embryophyta_odb9’ lineage was used, and analyses run with the following parameter settings: --mode genome -c 8 -e 0.05 -s tomato.

To annotate the reference gene models and sequences in the newly assembled Fraxinus genomes, we performed TBLASTN searches of individual downstream analyses. Because the presence of these redundant gene models does not, none of the sets of putative orthologues used for the species-trees inference or molecular convergence analysis (see below) include these preliminary organellar gene models from the BATG05 reference assembly. The ‘embryophyta_odb9’ lineage was used, and analyses run with the following parameter settings: --mode genome -c 8 -e 0.05 -s tomato.

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We also repeated the species-tree inference using full-length sequences (that is, including exons, where present); alignment and gene-tree inference were carried out as described above, with the exception that the --seqType parameter in GUIDANCE2 was set to `nuc'. BUCKY analyses were performed as described above. The same PCT was obtained for all settings of α, with only minor differences in the mean sample-wide concordance factors. The PCT inferred from the full-length datasets was also identified from the CDS analyses; we based our final species-tree estimate on the output of the full-length analyses, due to the presence of a larger number of informative loci within these datasets.

One of the informative loci from the CDS analyses (that is, those with ≤2,000 distinct topologies within their gene-tree sample), and three of those from the full-length analyses, were subsequently found to be among our filtered set of 26 loci with evidence of molecular convergence between EAB-resistant taxa (see below). To test whether the signal from these loci had an undue influence on species-tree estimation, we excluded them and repeated the BUCKY analyses for the CDS and full-length datasets as described above, with the exception that only an α parameter setting of 1 was used. The PCTs obtained from these analyses were identical to those inferred when including all datasets, with minor (that is, 0.01) differences in CFs.

In addition to the analysis including all taxa, we also performed BUCKY analyses for 13 taxa selected for inclusion in the grand-conv analyses and for the subsets of 10–12 taxa for each of the three grand-conv pairwise comparisons (see Table 1). The same primary tree for each of the subsets was inferred by the full-length analyses. From these analyses, we excluded candidate loci with evidence of convergence between EAB-resistant taxa (see below). For the full set of 32 taxa, BUCKY was run separately with the MrBays tree samples for loci that included all 13 taxa and for additional loci that included taxa for each of the three smaller subsets for the grand-conv pairwise comparisons. BUCKY analyses were performed as described above, with the exception that no filtering of loci on the basis of number of distinct gene-tree topologies was performed, a value of between 2 and 35 was used for the -m parameter, and only a single α parameter setting, of 0.1, was used. Topologies for the PCTs for the set of 13 taxa and subsets of 10–12 taxa were congruent with each other and with the PCTs inferred from analyses including all taxa, for all nodes with a CF of ≥0.38.

Analysis of patterns of sequence variation consistent with molecular convergence. To test for signatures of putative molecular convergence in protein sequences, we used a set of diploid taxa representing the extremes of variation in susceptibility to EAB, as assessed by our egg bioassays. By limiting our analysis at this stage to this subset of taxa we could also maximize the number of genes analysed, because the increasing taxon sampling the number of OGs for which all taxa are represented decreases. This set comprised: five highly susceptible taxa (F. americana, F. latifolia, F. ornus, F. pennsylvanica (susceptible genotype) and F. velutina), five resistant taxa (F. baroniana, F. floribunda, F. mandshurica, F. platypoda and Fraxinus sp. D2006-0159) and three outgroups (O. europaea, E. guttata, E. densocarpa, E. lycopersicum).

Of the ten Fraxinus genome assemblies included in the convergence analysis, six were from genotypes that were also included in the EAB resistance assays outlined above (F. americana am-6, F. baroniana bar-2, F. floribunda flor-11s-2, F. pennsylvanica pe-48, F. platypoda sp-1 and Fraxinus sp. D2006-0159 F-unk-1). For the other four, we could not test the exact individual for which we sequenced the genome but, instead, relied on results of bioassays of other individuals in the same species. We used the following three pairwise comparisons to screen for loci showing amino acid convergence between resistant taxa:

1. F. mandshurica (section Fraxinus) versus F. platypoda (incertae sedis)
2. F. mandshurica (section Fraxinus) versus F. baroniana, F. floribunda and Fraxinus sp. D2006-0159 (section Ornus)
3. F. platypoda (incertae sedis) versus F. baroniana, F. floribunda and Fraxinus sp. D2006-0159 (section Ornus)

The more divergent homologous amino acid sequences are between species, the more likely it is that a convergent amino acid state will occur by chance. In order to account for this, we compared the posterior expected numbers of convergent versus divergent substitutions across all pairs of independent branches of the Fraxinus species-tree for the selected taxa using a beta-release of the software Grand-Convergence v0.8.0 (hereafter referred to as grand-conv; https://github.com/jenningslab/grandconv; based on PAAML v4.8.1 (ref. 5) and is a development of a method used by Castró et al.29 It has also been used recently, for example, to detect convergence among flowering plant lineages with crassulacean acid metabolism27.

For input into grand-conv, we used the same OGs that were the basis of the BUCKY analyses of the 13 taxa selected for the grand-conv analyses, and of analyses
of the subsets of taxa for the pairwise comparisons (see Species-tree inference). Therefore, as well as meeting the criterion of including all relevant taxa, the OGs analysed with grand-conv had also successfully passed the alignment, filtering and gene-tree inference filters. A set of input files were created in consistent order across all datasets (which is necessary for automating the generation of site-specific posterior probabilities for selected branch pairs with grand-conv), the phylip formatted files were sorted using the unix command ‘sort’ before input into grand-conv. Species-tree files for each of the pairwise comparisons were created from the Newick formatted PCTs for the relevant taxon sets generated with BUCKy, with the trees edited to root them on the hypothesized species.

For the grand-conv analysis, ‘gc-estimate’ was first run on the full set of input alignment files for each pairwise comparison, with the following parameter setting: --ngenome=0 --aamodeling=1 --free-bl=1, specifying the appropriate species-tree file for each of the pairwise comparisons. Next, ‘gc-discover’ was run to generate site-specific values for the posterior probability of divergence or convergence for the branch pairs of interest; the numbers for the branch pairs relating to the resistant taxa were established from an initial run of ‘gc-estimate’ and ‘gc-discover’ on a single input file, and then specified when running ‘gc-discover’ on all input files using the ‘--branch-pairs parameter. A custom Perl script was used to use the output files from the ‘gc-estimate’ tool to calculate the posterior probability of identity for loci with at least one amino acid site where the posterior probability of convergence was higher than divergence and passed a threshold of 0.9000. For this filtered set of datasets with a high posterior probability of convergence at least one site, we checked whether the ‘excess’ convergence, as measured from the residual values from the non-parametric errors-in-variables regression (Equation 1), was higher than for any other independent pair of branches within the species-tree (that is, the highest residual was found for the resistant branch pair). Only loci where the highest excess convergence was found in the resistant branch pair were retained for further analysis.

Refining the initial list of candidate loci identified with grand-conv. For the set of loci with evidence of convergence between at least one pair of resistant lineages from the grand-conv analyses, we applied additional tests to assess the robustness of the pattern of shared amino acid states. Specifically, we checked for the potential impact of alignment uncertainty on the orthology/paralogy confusion. For each candidate locus, we identified HOGs from the OMA analysis to which the sequences for the candidate locus belong. These HOGs include sequences for an expanded set of taxa (see Species-tree inference) and may represent a single gene for all taxa (that is, a set of orthologous sequences) or several closely related paralogues. Protein sequences for HOGs were aligned with GUIDANCE and gene-tree inference conducted with MrBayes, as described above for the OMA-putative orthologous groups, with the exception that the ‘--seqSubstitute parameter in GUIDANCE was set to ‘a’ and in MrBayes the preset parameter was set to ‘preset aamodelp = mixed. Any MrBayes analyses that had not converged after 5 million generations (ADSF ≥ 0.01) were run for an additional 5 million generations. The multiple sequence alignment and gene-tree estimates were then used to refine the initial list of candidate loci. Loci were dropped from the initial list of candidates if either of the following applied:

1. In the filtered MSA alignment generated by GUIDANCE, the site/sites where convergence was detected were not present, indicating that they were in a part of the protein sequences that cannot be aligned reliably.
2. In the consensus gene-tree estimated by MrBayes, there was evidence that the sequences within which convergence was initially detected (that is, those belonging to the ten Fraxinus species included in the grand-conv analysis) belong to different paralogues and that the pattern of convergence could be explained by sequences with the ‘convergent’ state belonging to one parologue and the ‘non-convergent’ state belonging to another parologue. We also included two loci belonging to large gene families (>10 copies) for which the MrBayes analysis failed to reach convergence within a reasonable time (≥10 million generations) and for which orthology/paralogy confounding could therefore not be excluded.

Additionally, for the set of loci remaining, we checked for errors in the estimation of gene models (including in the reference models from F. excelsior) that might impact the results of the grand-conv analyses. Specifically, we dropped from our list any loci where the amino acid sites with evidence of convergence were found to be outside of an exon, or outside of the gene itself, following manual correction of the gene model prediction.

Analysis of variants within candidate loci. To assess the possible impact of allelic variants (that is, those not represented in the genome assemblies) on patterns of amino acid variation associated with the level of EAB susceptibility in Fraxinus, we called variants (single-nucleotide polymorphisms and indels) and predicted their functional effects. For each sequenced Fraxinus individual, trimmed reads from the short-insert Illumina libraries were mapped to the de novo genome assembly for the same individual using Bowtie2 v.2.3.0 (ref. 20) with the ‘very-sensitive’ preset for 1.0-1.0× coverage at >200 libraries available for that individual. Read mappings were converted to BAM format and used to explore the sequence of the gene with the ‘H1’ and ‘H2’ variants. In cases where the selected phased block also spans unphased variants, both sequences output by bcftools will

For each candidate locus, we identified HOGs from the OMA analysis to which the potential impact of alignment uncertainty and orthology/paralogy confounding. Specifically, we dropped that might impact the results of the grand-conv analyses. Specifically, we dropped from our list any loci where the amino acid sites with evidence of convergence were found to be outside of an exon, or outside of the gene itself, following manual correction of the gene model prediction.
have the state found in the original genome assembly at these sites, as they will for any variants outside of the selected phased block. The revseq and descseq tools from EMBOSS v.6.6.0 (ref. 18) were used to reverse complement the sequences for any genes annotated on the minus strand and to rename the output sequences. Phased alleles were used for further phylogenetic analysis of candidate loci (see below); phasing results were also used to check loci with multiple potential loss-of-function mutations within a single individual, to establish whether the mutations are on the same or different alleles. We discounted any cases of potential loss-of-function mutations where multiple fragments occurred in close proximity on the same allele as a result of errors in the reference gene models, possibly arising from misassembly, which meant that the sites homologous to those with evidence of convergence between EAB-resistant taxa could not be identified (see Supplementary Table 5 for more details).

Distinguishing between different underlying causes of convergent patterns. To test whether evidence of convergence found by grand-conv might actually be due to taxa sharing the same amino acid variant as a result of introgression or ILS, we conducted phylogenetic analyses of coding DNA sequences for the candidate loci to test for shared gene-trees. We checked whether sequences with apparently convergent residues group together, even when nucleotides encoding those residues are removed. The CDSs for the refined set of candidate loci were aligned with MUSCLE via GUIDANCE2 and alignment files with unreliably aligned codons removed were used for downstream analyses, as described above (see Species-tree inference); none of datasets had sequences that were identified by GUIDANCE2 as being unreliably aligned. OG40061 failed to align due to the presence of an incomplete codon at the end of the reference gene model from F. excelsior; we trimmed the final 2 bp from the F. excelsior sequence and reran GUIDANCE2 using this modified file.

Phased allele sequences generated using the WhatHap results (see Analysis of variants within candidate loci) were added to the CDS alignments using MAFFT v.7.310 (ref. 189) with the options ‘--add’ and ‘-k’-lengthpath, in order to splice out any introns present in the phased sequences and maintain the original length of the CDS alignments. For any taxa for which phased sequences had been added, the original unphased sequence was removed from the alignment.

If intragenic recombination has taken place, gene-trees inferred from the CDS alignments may fail to group together the sequences with evidence of convergence even in cases where the convergent pattern is due to ILS or introgressive hybridization. This is because the phylogenetic signal from any non-recombining fragments of alleles derived from ILS or introgressive hybridization may not be sufficient to force the phylogenetic pattern that from fragments of alleles that have not been subject to these processes. To account for this possibility, we used hyphy v.2.3.1 4.20181030beta(MPI)190 to conduct recombination tests with GARD186 with the following parameter settings: 012345 ‘General Discrete’ 3. Where GARD found significant evidence for a recombination breakpoint (P < 0.05), we partitioned the alignment into non-recombining fragments for phylogenetic analysis. Alignment files were converted to nexus format and gene-trees estimated with MrBayes, as described above (see Species-tree inference). We checked the ASDSF and used Tracer v.1.6.0 (http://beast.bio.ed.ac.uk/Tracer) to inspect the ESS values for each parameter from the post-burn-in samples and to confirm that the burn-in setting (that is, discarding the first 10% of samples) was sufficient; in cases where runs had not converged on the same ESS value after 20 million generations, additional generations were run until ASDSF < 0.010 was reached. We examined the consensus trees generated by MrBayes to look for evidence that sequences sharing the amino acid states inferred as convergent by grand-conv cluster together in the gene-tree, in conflict with relationships inferred in the species-tree for Fraxinus. In cases where evidence of such clustering was found, the codon(s) corresponding to the amino acid site(s) at which evidence of convergence was detected were excluded and the MrBayes analysis repeated. In cases where sequences that have the ‘convergent’ amino acid group together in the gene-tree even after the codon(s) for the relevant site(s) have been excluded, we concluded that the evidence of convergence detected by grand-conv is more likely to be due to introgressive hybridization or ILS. To further test whether the protein topologies inferred from the refined set of candidate loci was tested. Fisher’s exact tests with the ‘weight’ and ‘elim’ algorithms, which take into account the GO graph topology110, were run using the topGO package111 (v2.32.0) in R v3.5.1 (ref. 112). We created a ‘genes-to-GOs’ file for the complete set of F. excelsior gene models included in the grand-conv analyses, we conducted GO enrichment tests. We used the ‘GO plot’ package113 to check for enrichment of terms within the biological process, molecular function and cellular component domains.

Further characterization of candidate loci. To identify the gene from A. thaliana that best matches each of the candidate loci in our refined set, we conducted a BLASTN search of the F. excelsior protein sequence belonging to the relevant OGs against the A. thaliana sequences in the nr/nt database in GenBank108 and selected the hit with the lowest e-value. In cases where the OG lacked a sequence from F. excelsior, we checked the protein sequence from F. mandshurica as the query for the BLASTN search instead.

We also checked for the presence of the F. excelsior sequences within the OrthoMCL clusters generated by Sollars et al.27 to determine whether they were associated with the same A. thaliana genes as identified by BLAST. We obtained evidence for the presence of the F. excelsior sequences in the function of the best-matching A. thaliana genes from The Arabidopsis Information Resource (https://www.arabidopsis.org) and the literature. The OrthoMCL analysis conducted by Sollars et al. also included a range of other plant species, including S. lycopersicum (tomato) and the tree species Populus trichocarpa (poplar). Because tomato is much more closely related to F. excelsior than is A. thaliana, and poplar is also a tree species, the function of the genes in other taxa may provide a better guide to the function of the F. excelsior genes. We therefore also checked the OrthoMCL clusters containing our candidate F. excelsior genes to identify putative orthologues, or close paralogues, from tomato and poplar. In cases where the OrthoMCL cluster included multiple tomato or poplar genes, we focused attention on the tomato sequence that also belonged to the OMA group as the putative orthologue in that species. For poplar, we looked for information on all sequences unless there were a large number in the cluster (more than four). We searched for literature on the function of the tomato and poplar genes, using the gene identifiers from the versions of the genome annotations used for the OrthoMCL analysis102, and also looked for information on PhytoMine, in Phytominer12 (ref. 2).

To further clarify the orthology/paralogy relationships between our candidate genes and genes from other species, we conducted phylogenetic analyses of the relevant OrthoMCL clusters from Sollars et al.27 for selected loci. Protein sequences belonging to each OrthoMCL cluster were aligned and gene-trees inferred using GUIDANCE2 and MrBayes, respectively, as described above for OGs and HGT analyses (see whether sequences within the same OrthoMCL cluster were included in the analysis) and OG46977 were excluded from this analysis due to errors in the reference gene model. We aligned the CDSs of all sequences shared by the OrthoMCL clusters generated by Sollars et al.27 and removed two incomplete sequences (Mign00792.1.p and GSVIVT01025800001, which were missing >25% of characters in the alignment) and two divergent sequences from A. thaliana (AT1G74540 and AT1G74550), which are known to derive from a Brassicaceae-specific retroposition event and subsequent specific tandem duplication114; the alignment and phylogenetic analysis was then repeated for the reduced dataset.

For OG15551, we generated a sequence logo for regions of the protein containing sites at which evidence of convergence was detected. We obtained putatively homologous sequences by downloading the fasta file for the OMA group (OMA Browser fingerprint YGPIYSF108) containing the A. thaliana CYCP643 gene (AT2G40890); the sequences were filtered to include only those from angiosperms, with a maximum of one sequence per genus retained (29 genera in total). To this dataset, we added the OG15551 protein sequences for F. mandshurica and F. pennsylvanica pe-48 and manually aligned the regions containing the relevant sites (positions 208–218 and 474–482 in the F. excelsior PREAX38873_v2_00261700 reference protein). We used WebLogo v.3.7.3 (ref. 115) without compositional adjustment to generate logos for each of these regions.

GO term enrichment analysis. To test for the possibility of over-representation of particular functional categories among the candidate loci in our refined set, we considered genes that were present in the OMA groups as input for the signal peptide analyses; we concluded that a signal peptide was present only if it was predicted by both methods. The NetPhos 3.1 (http://www.cbs.dtu.dk/services/NetPhos/) was used as input for the signal peptide analyses; we concluded that a signal peptide was present only if it was predicted by both methods. The NetPhos 3.1 Server (http://www.cbs.dtu.dk/services/NetPhos/) was used with default settings.

**Protein modelling.** The servers SignalP 5.0 (ref. 116) and Phobius117 (http://phobius.sbc.su.se/index.html) were used to detect the presence of signal peptides; for SignalP the organism group was set to ‘Eukarya’ and for Phobius the ‘normal prediction’ method was used. All Fraxinus sequences belonging to the OMA groups were used as input for the signal peptide analyses; we concluded that a signal peptide was present only if it was predicted by both methods. The NetPhos 3.1 Server (http://www.cbs.dtu.dk/services/NetPhos/) was used with default settings.
to identify candidate phosphorylation sites for loci where the amino acid variant observed at a site with evidence of convergence included a serine, threonine or tyrosine. The same protein sequences for resistant and susceptible taxa as used for protein modelling (see below) were input to an initial run of NetPhos where evidence for phosphorylation site presence/absence was detected with this initial sequence pair (that is, present in the sequence with the convergent state and absent from that with the non-convergent state, or vice versa), we reran NetPhos 3.1 on all Fraxinus sequences from the relevant OMA groups to test whether this difference was consistently associated with the convergent/non-convergent state. We counted as potential phosphorylation sites only those for which the NetPhos score for phosphorylation potential was 20.900 for all sequences with the putative site. RaptorX-Binding (http://raptorx.uchicago.edu/BindingSite/) was used to generate predicted protein models for each of the candidate genes in our refined set, as well as to outline possible binding sites and candidate ligands. Protein sequences for gene models from the F. excelsior reference genome were used for initial protein model and binding site prediction, except in cases where F. excelsior was not present in the OMA group or where comparison with the other ingroup and outgroup taxa indicated that the F. excelsior gene model may be incorrect/incomplete; for these loci, the F. mandshurica sequences were used instead. After the alignment, the genome assembly for this taxon is among the highest quality available. For those loci for which a binding site could be successfully predicted (that is, with at least one potential binding site with a pocket multiplicity value of ≥2.40), additional models were generated for representative resistant (F. mandshurica or F. platypoda) and susceptible (F. ornus or susceptible F. pennsylvanica) taxa using Swiss-model (https://swissmodel.expasy.org) and Phyre2 (ref. 10) intensive mode), with the exact taxon selection depending on which grand-conv pairwise comparison the locus was detected in (see Analysis of patterns of sequence variation consistent with molecular convergence) and which taxa had complete gene models. Where errors were detected in the predicted protein sequences for resistant or susceptible taxa (that is, due to errors in the predicted gene model that were detected through comparison with sequence from other species, including those from outgroups), these were corrected before modelling (for example, by trimming extra sequence resulting from incorrect prediction of the start codon). Models predicted by the three independent methods (RaptorX-Binding, Swiss-modeller and Phyre2) were compared by aligning them using PyMOL v2.0 with the align function to check for congruence; only those loci whose models displayed congruence and where the convergent site was located within/close to the putative active site were taken forward for predictive ligand docking analysis (using the Phyre2 and RaptorX-Binding models for the docking itself). In addition, any loci with congruent models where the site with evidence of convergence is also a putative phosphorylation site presence/absence variant, or that are within a putative functional domain, were analysed further. Ligand candidates were selected based on relevant literature and/or the RaptorX-Binding output, with SDF files for each of the molecules being obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov). SDF files were converted to three-dimensional programme database files using Online SMILES Translator and Structure File Generator (https://cactus.nci.nih.gov/), so that these could be used with AutoDock. Docking analysis was carried out using AutoDock Vina v1.1.2 (ref. 14) with the GUG PyRx v0.8 (ref. 15). Following docking, ligand binding site coordinates were exported as SDF files from PyRx and loaded into PyMOL with the corresponding protein model file for the resistant and susceptible taxa. Binding sites were then annotated and the residues at which evidence for convergence had been detected with grand-conv were labelled.

Evidence for differential expression of candidate loci in F. pennsylvanica. We used published transcriptome assembly and expression data from F. pennsylvanica14 to look for evidence of differential expression of our candidate loci in response to EAB larval feeding. This dataset comprised six genotypes of F. pennsylvanica, four putatively resistant to EAB and two susceptible to EAB. To identify the orthologues of our genes in the protein sequences of this independently assembled transcriptome, we searched the OMA clustering analysis (see Gene annotation and orthologue inference) with the addition of these data, available as ‘Fraxinus_ pennsylvanica_120313_peptides’ at the Harwood Genomics Project website (https://harwoodgenomics.org). OMA was run as described above, with SpeciesTree parameter set to ‘estimate’, because we intended to use only the results for the OGs and not the HOGs from this analysis, we did not repeat the clustering with a modified species-tree as was done for our main OMA analysis. Having identified the probable orthologous loci from the F. pennsylvanica transcriptome, we used the results of the differential expression analysis11 to check whether our candidate loci had significantly (P < 0.05) increased or decreased expression post-EAB feeding in this dataset.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Underlying data for Fig. 1 are available in Supplementary Tables 1 and 2. All trimmed read data and genome assemblies have been deposited in the European Nucleotide Archive under accession no. PRJEB20151. The genome assemblies are also available to download at: http://www.ashtagome.org.

Code availability
The custom scripts used is this study have been deposited in GitHub: https://github.com/kelly3/ebm-ms-scripts.

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References
1. Pautasso, M., Ass, G., Queloz, V. & Holderegger, R. European ash (Fraxinus excelsior) dieback – a conservation biology challenge. Biol. Conserv. 158, 37–49 (2013).
2. MacFarlane, D. W. & Meyer, S. P. Characteristics and distribution of potential tree host trees for emerald ash borer. For. Ecol. Manage. 213, 15–24 (2005).
3. Boyd, I. L., Freer-Smith, P. H., Gilligan, C. A. & Godfray, H. C. J. The consequence of tree pests and diseases for ecosystem services. Science 342, 1235773 (2013).
4. Herms, D. A. & McCulloch, D. G. Emerald ash borer invasion of North America: history, biology, ecology, impacts, and management. Annu. Rev. Entomol. 59, 13–30 (2014).
5. Orlova-Bienkowskaja, M. J. Ashes in Europe are in danger: the invasive range of Agrilus planipennis in European Russia is expanding. Biol. Invasions 16, 1345–1349 (2014).
6. McCulloch, D. G. Challenges, tactics and integrated management of emerald ash borer in North America. Forestry 93, 197–211 (2019).
7. Drogafulenco, A. N., Orlova-Bienkowskaja, M. J. & Bienkowski, A. O. Record of the emerald ash borer (Agrilus planipennis) in Ukraine is confirmed. Insects 10, 338 (2019).
8. Semizer-Cuming, D., Krutovsky, K. V., Baranchikov, Y. N., Kjær, E. D. & Williams, C. G. Saving the world’s ash forests calls for international cooperation now. Nat. Ecol. Evol. 3, 141–144 (2019).
9. Evans, H. F., Williams, D., Hoch, G., Loomans, A. & Marzano, M. Developing a European toolbox to manage potential invasion by emerald ash borer (Agrilus planipennis) and bronze birch borer (Agrilus anxius), important pests of ash and birch. Forestry 93, 187–196 (2020).
10. Baranchikov, Y., Mozolevskaya, E., Yurchenko, G. & Kenis, M. Occurrence of the emerald ash borer, Agrilus planipennis in Russia and its potential impact on European forestry. Bull. OEPP 38, 233–238 (2008).
11. Zhao, T. et al. Induced outbreaks of indigenous insect species by exotic tree species. Acta Entomol. Sin. 50, 826–833 (2007).
12. Liu, H. et al. Exploratory survey for the emerald ash borer, Agrilus planipennis (Coleoptera: Buprestidae), and its natural enemies in China. Great Lakes Entomol. 36, 191–204 (2003).
13. Wei, X. et al. Emerald ash borer, Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), in China: a review and distribution survey. Acta Entomol. Sin. 47, 679–685 (2004).
14. Orlova-Bienkowskaja, M. J. & Volkovitsh, M. G. Are native ranges of the emerald ash borer, Agrilus planipennis and its putative ancestor, Agrilus anxius, native to North America? Evolutionary, ecological and phylogeographic implications. Invasions 10, 1275–1286 (2018).
15. Showalter, D. N., Villari, C., Herms, D. A. & Bonello, P. Drought stress increased survival and development of emerald ash borer larvae on coevolved Manchurian ash and implicates phloem-based traits in resistance. Agric. For. Ecol. 200, 170–179 (2018).
16. Whitehill, J. G. A. et al. Interspecific proteomic comparisons reveal ash phloem genes potentially involved in constitutive resistance to the emerald ash borer. PLoS ONE 6, e24863 (2011).
17. Whitehill, J. G. A. et al. Interspecific comparison of constitutive ash phloem phenolic chemistry reveals compounds unique to Manchurian ash, a species resistant to emerald ash borer. J. Chem. Ecol. 38, 499–511 (2012).
18. Lane, T. et al. The green ash transcriptome and identification of genes responding to abiotic and biotic stresses. BMC Genomics 17, 702 (2016).
19. Sackton, T. B. et al. Convergent regulatory evolution and loss of flight in paleognathous birds. Science 364, 74–78 (2019).
20. Arnold, B. J. et al. Borrowed alleles and convergence in serpentine adaptations. Proc. Natl Acad. Sci. USA 113, 8320–8325 (2016).
21. Hu, Y. et al. Comparative genomics reveals convergent evolution between the bamboo-eating giant and red pandas. Proc. Natl Acad. Sci. USA 114, 1081–1086 (2017).
22. Yang, X. et al. The Kalanchoe genome provides insights into convergent evolution and building blocks of crassulacean acid metabolism. Nat. Commun. 8, 1899 (2017).
23. Hill, J. et al. Recurrent convergent evolution at amino acid residue 261 in fish rhodopsin. Proc. Natl Acad. Sci. USA 114, 18473–18478 (2017).
24. Zhen, Y., Aardema, M. L., Medina, E. M., Schumer, M. & Andolfatto, P. Parallel molecular evolution in an herbivore community. Science 337, 1634–1637 (2012).
Hilker, M. & Fatouros, N. E. Resisting the onset of herbivore attack: A recent paper discusses the role of early signalling events in plant–insect interactions. *Nature* 541, 212–216 (2017).

Cruz, F. et al. Genome sequence of the olive tree, *Olea europaea*. *Gigascience* 5, 29 (2016).

Hellesten, U. et al. Fine-scale variation in meiotic recombination in *Mimulus* inferred from population shotgun sequencing. *Proc. Natl. Acad. Sci. USA* 110, 19478–19482 (2013).

Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* 485, 635–641 (2012).

Wright, J. W. Nitrogen content in *Acer* and *Mimulus*. *Arb. Arh.* 8, 33–34 (1957).

Bernards, M. A. & Bästrup-Spohr, L. in *Tsuga canadensiscomplexity to breed disease-resistant crops*. *Nat. Rev. Genet.* 110, 19478–19482 (2013).

Wallander, E. Systematics and floral evolution in *Belg.* 10, 176–184 (2012).

Koch, J. L., Carey, D. W., Mason, M. E., Poland, T. M. & Knight, K. S. Intraspecific variation in *Tsuga canadensis* and *Olea europaea*. *Environ. Entomol.* 43, 251–262 (2014).

Doyle, J. L. Strategies for selecting and breeding EAB-resistant ash. In *22nd US Department of Agriculture Interagency Research Symposium on Invasive Species* (eds McManus, K. A. & Gottschalk, K. W.) 33–35 (US Department of Agriculture, Forest Service, Northern Research Station, 2011).

Duan, J. J., Larson, K., Watt, T., Gould, J. & Leitlo, J. P. Effects of host plant and larval density on intraspecific competition in larvae of the emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (Fraxinus spp.). *Environ. Entomol.* 42, 1193–1200 (2013).

Cappaert, D., McCullough, D. G., Poland, T. M. & Siegert, N. W. Emerald ash borer in North America: a research and regulatory challenge. *Am. Entomol.* 51, 152–165 (2005).

Chamorro, M. L., Volkov, M. G., Poland, T. M., Haack, R. A. & Lingafelter, S. W. Preimaginal stages of the emerald ash borer, *Agrilus planipennis Fairmaire* (Coleoptera: Buprestidae): an invasive pest on ash trees (*Fraxinus*). *PLOS ONE* 7, e33185 (2012).

Pellicer, J., Kelly, L. J., Leitch, I. J., Zomlefer, W. B. & Fay, M. F. A universe of dwarfs and giants: genome size and chromosome evolution in the monoeccious Melanithraceae. *New Phytol.* 201, 1484–1497 (2014).

Loureiro, I., Rodriguez, E., Dolezel, J. & Santos, C. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Ann. Bot.* 100, 875–888 (2007).

Dolezel, J., Binarová, P. & Lucretti, S. Analysis of nuclear DNA content in plant cells by flow cytometry. *Biol. Plant.* 31, 113–120 (1989).

Bennett Michael, D. & Smith, J. B. Nuclear DNA amounts in angiosperms. *Plant J.* 110, 58–59 (2016).

Bennet, D. et al. ‘Northern Treasure’ and ‘Northern Gem’ hybrid ash. *Environ. Entomol.* 39, 273–279 (2010).

Roettner, M., Henkel, C. V., Jansen, H. J., Butler, D. & Pirovano, W. Scaffolding pre-assembled contigs using SPACe. *Bioinformatics* 27, 578–579 (2011).

Luo, R. et al. SOPA/Spiewok: an empirically improved memory-efficient short-read de novo assembler. *Gigascience* 1, 18 (2012).

Camacho, C. et al. BLAST+: architecture and applications. * BMC Bioinform.* 10, 421 (2009).

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V. & Zdobnov, E. J. *Novel gene prediction* with single-copy orthologs. *BMC Bioinform.* 12, 421 (2011).

Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for genomic analysis. *Bioinformatics* 26, 870–872 (2010).

Kemp, J. G. & Waterhouse, R. M. *Cutadapt* removes adapter sequences from high-throughput sequencing reads. *EMBNet J.* 17, 10–12 (2011).

Joshi, N. A. & Fass, J. N. Sickle: a sliding-window, adaptive, quality-based trimming tool for fastq files; https://github.com/najoshi/sickle

Schneider, R. & Edwards, R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27, 863–864 (2011).

Roettner, M., Henkel, C. V., Jansen, H. J., Butler, D. & Pirovano, W. Scaffolding pre-assembled contigs using SPACe. *Bioinformatics* 27, 578–579 (2011).

Luo, R. et al. SOPA/Spiewok: an empirically improved memory-efficient short-read de novo assembler. *Gigascience* 1, 18 (2012).

Camacho, C. et al. BLAST+: architecture and applications. *BMC Bioinform.* 10, 421 (2009).

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V. & Zdobnov, E. J. *Novel gene prediction* with single-copy orthologs. *BMC Bioinform.* 12, 421 (2011).

Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for genomic analysis. *Bioinformatics* 26, 870–872 (2010).

Kemp, J. G. & Waterhouse, R. M. *Cutadapt* removes adapter sequences from high-throughput sequencing reads. *EMBNet J.* 17, 10–12 (2011).

Joshi, N. A. & Fass, J. N. Sickle: a sliding-window, adaptive, quality-based trimming tool for fastq files; https://github.com/najoshi/sickle

Schneider, R. & Edwards, R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27, 863–864 (2011).

Roettner, M., Henkel, C. V., Jansen, H. J., Butler, D. & Pirovano, W. Scaffolding pre-assembled contigs using SPACe. *Bioinformatics* 27, 578–579 (2011).

Luo, R. et al. SOPA/Spiewok: an empirically improved memory-efficient short-read de novo assembler. *Gigascience* 1, 18 (2012).

Camacho, C. et al. BLAST+: architecture and applications. *BMC Bioinform.* 10, 421 (2009).
88. Hinsinger, D. D. et al. The phylogeny and biogeographic history of ashes (Fraxinus, Oleaceae) highlight the roles of migration and vicariance in the diversification of temperate trees. *PLoS ONE* **8**, e80431 (2013).

89. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797 (2004).

90. Sela, I., Ashkenazy, H., Katoh, K. & Pupko, T. GUIDANCE2: accurate detection of unreliable alignment regions accounting for the uncertainty of multiple parameters. *Nucleic Acids Res.* **43**, W7–W14 (2015).

91. Rice, P., Longden, I. & Bleasby, A. EMBOSS: the European Molecular Biology Open Software Suite. *Trends Genet.* **16**, 276–277 (2000).

92. Ronquist, F. et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012).

93. Ané, C., Larget, B., Baum, D. A., Smith, D. S. & Rokas, A. Bayesian estimation of concordance among gene trees. *Mol. Biol. Evol.* **24**, 412–426 (2007).

94. Larget, B. R., Kotha, S. K., Dewey, C. N. & Ané, C. BUCKy: gene tree/species tree reconciliation with Bayesian concordance analysis. *Bioinformatics* **26**, 2910–2911 (2010).

95. Castoe, T. A. et al. Evidence for an ancient adaptive episode of convergent molecular evolution. *Proc. Natl Acad. Sci. USA* **106**, 8986–8991 (2009).

96. Yang, Z. PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* **24**, 1586–1591 (2007).

97. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**, 357–359 (2012).

98. Li, H. et al. The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).

99. McKenna, A. et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303 (2010).

100. Cingolani, P. et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly* **6**, 80–92 (2012).

101. Martin, M. et al. WhatSap: fast and accurate read-based phasing. Preprint at bioRxiv https://doi.org/10.1101/085050 (2016).

102. Milne, I. et al. Using Tablet for visual exploration of second-generation sequencing data. *Brief. Bioinform.* **14**, 193–202 (2013).

103. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).

104. Pond, S. L. K., Frost, S. D. W. & Muse, S. V. HyPhy: hypothesis testing using phylogenies. *Bioinformatics* **21**, 676–679 (2005).

105. Kosakovsky Pond, S. L., Posada, D., Gravenor, M. B., Woelk, C. H. & Frost, S. D. W. Automated phylogenetic detection of recombination using a genetic algorithm. *Mol. Biol. Evol.* **23**, 1891–1901 (2006).

106. Benson, D. A. et al. GenBank. *Nucleic Acids Res.* **41**, D36–D42 (2013).

107. Liu, Z. et al. Evolutionary interplay between sister cytochrome P450 genes shapes plasticity in plant metabolism. *Nat. Commun.* **7**, 13026 (2016).

108. Allenhoff, A. M. et al. The OMA orthology database in 2018: retrieving evolutionary relationships among all domains of life through richer web and programmatic interfaces. *Nucleic Acids Res.* **46**, D477–D485 (2018).

109. Crooks, G. E., Hon, G., Chandonia, J.-M. & Brenner, S. E. WebLogo: a sequence logo generator. *Genome Res.* **14**, 1188–1190 (2004).

110. Alexa, A., Rahnenführer, J. & Lengauer, T. Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. *Bioinformatics* **22**, 1600–1607 (2006).

111. Alexa, A. & Rahnenführer, J. topGO: enrichment analysis for gene ontology R Package v2.32.0 (2016).

112. R Core Team et al. R: a language and environment for statistical computing (R Foundation for Statistical Computing, 2013).

113. Petersen, T. N., Brunak, S., von Heijne, G. & Nielsen, H. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat. Methods* **8**, 785–786 (2011).

114. Käll, L., Krogh, A. & Sonnhammer, E. L. J. Advantages of combined transmembrane topology and signal peptide prediction—the Phobius web server. *Nucleic Acids Res.* **35**, W429–W432 (2007).

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Author contributions

R.J.A.B conceived and oversaw the project. I.J.K and R.J.A.B wrote the manuscript, with input from J.L.K., W.J.P and S.J.R. L.J.K. conducted gene annotation, orthologue orthology analysis and high throughput. J.L.K. and R.J.A.B. wrote the manuscript, R.J.A.B. and L.J.K. acknowledge additional support from the Erica Waltraud Albrect Endowment Fund. W.J.P was funded by the Walsh Scholarship Programme of the Department of Agriculture, Food and the Marine, Ireland. E.D.C was supported by the Marie Skłodowska-Curie Individual Fellowship ‘FraxiFam’ (grant agreement no. 660003).

Competing interests

The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Predicted protein structures for selected candidate loci.  

**a**, Predicted protein structure for OG36502, modelled using the protein sequence for *Fraxinus platypoda*. The serine/asparagine variant at the site where convergence was detected is highlighted; the serine is a putative phosphorylation site.  

**b**, Predicted protein structure for OG40061, modelled using the protein sequence for *F. mandshurica*. The asparagine/serine variant at the site where convergence was detected is highlighted; the serine is a putative phosphorylation site. The putative substrate, NADP, is shown docked within the predicted active site.  

**c**, Predicted protein structure for OG38407, modelled using the protein sequence for *F. mandshurica*. The aspartic acid/asparagine variant at the site where convergence was detected is highlighted; the site falls within a leucine rich repeat region (LRR; shaded blue) which is predicted to span from position 111-237 within the protein sequence (detected using the GenomeNet MOTIF tool [www.genome.jp/tools/motif/], searching against the NCBI-CDD and Pfam databases with default parameters; the LRR region was identified as positions 111-237 with an e-value of 1e-05).  

**d**, Predicted protein structure for OG21033, modelled using the protein sequence for *F. platypoda*. The lysine/glutamine at the site where convergence was detected is highlighted. The putative substrate, \( \beta-D\text{-Glc}\text{-p(1 \rightarrow 3)}\beta-D\text{-GlcA}\text{-p(1 \rightarrow 4)}\beta-D\text{-Glc} \), is shown docked within the predicted active site.
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Underlying data for Figure 1 are available in Supplementary Tables 1 and 2. All trimmed read data and genome assemblies have been deposited in the European Nucleotide Archive under accession number PRJEB20151 [https://www.ebi.ac.uk/ena/browser/view/PRJEB20151]. The genome assemblies are also available to download at: http://www.ashgenome.org.

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Sample size
No statistical methods were used to predetermine sample size. The number of samples used for whole genome sequencing was determined by the availability of suitable material; all recognised diploid species of Fraxinus for which DNA suitable for whole genome sequencing could be obtained were included in the study. The number of Fraxinus samples used for the emerald ash borer resistance assays was determined by the availability of material for which suitably sized individual trees (stem diameter of at least 1cm) could be cultivated; all Fraxinus genotypes for which material potentially suitable to support the normal growth and development of emerald ash borer larvae was available were included in the study. Adequacy of sample size was inferred based upon successful previous bioassays with a more limited number of species using the same protocols.

Data exclusions
Illumina sequence data were excluded from further analysis if they did not meet the predefined quality thresholds stated in the Methods. Data from individual ramets, cuttings and/or seedlings included in the emerald ash borer resistance assay were excluded from analysis if the effective egg dose was too low (<3 larvae successfully entered the tree), or if there were other problems with the growth of the tree (e.g. cultivation issues). Individual EAB eggs were excluded if the did not hatch and the neonate did not enter the tree.

Replication
No replication of whole genome sequencing was performed. For the emerald ash borer resistance assay, we aimed to test three clonal replicates (grafts or cuttings) of at least two genotypes of each of the 26 Fraxinus taxa included in the experiments. Three replicate grafted ramets (or occasionally three seedlings as noted in Supplementary Tables 1) were assayed for each genotype. Each experiment contained three replicates (blocks) and one ramet was included in each block.

Randomization
For the emerald ash borer resistance assays, experiments were conducted using a randomised block design; groups of approximately 20 Fraxinus genotypes were included in each set of bioassays with one grafted ramet, cutting or seedling in each block and all ramets, cuttings and seedlings within the block randomised to location/order of assay. Eggs batches were applied to blocks arbitrarily and recorded, and post-setup quality control (hatch test) showed no egg batch quality differences.

Blinding
Blinding was not used during data acquisition or analysis. Blinding was not relevant to the majority of analyses conducted, because data from all samples were treated equally, according to predefined thresholds, irrespective of their source. Blinding was not possible during the convergence analyses because it required samples with the phenotypic trait of interest (i.e. resistance to emerald ash borer) to be defined during analysis. In the emerald ash borer resistance assays, Tree ID was not blinded as species differences are readily apparent and all assay workers were familiar with the different species. Replicates of each genotype were dissected by different assay workers to reduce bias.

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### Methods

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| ChIP-seq               |
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| MRI-based neuroimaging |

### Animals and other organisms

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| Laboratory animals |
|---------------------|
| Eggs and larvae (up to the L4 instar) of the beetle species Agrilus planipennis were used in experiments. Agrilus planipennis eggs, which had been produced in a breeding facility at the USDA Northern Research Station and provided by Therese Poland, were allowed to hatch and develop within different species of Fraxinus; the sex of the resulting larvae was not determined. |

| Wild animals |
|-------------|
| The study did not involve wild animals. |

| Field-collected samples |
|-------------------------|
| The study did not involve samples collected from the field. |

| Ethics oversight |
|------------------|
| Ethical guidance on the study protocol was provided by Ohio State University, which does not require approval for protocols involving the use of insects. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### Flow Cytometry

**Plots**

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
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  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

| Sample preparation |
|--------------------|
| Fresh leaf material was chopped with a new razor blade in an isolation buffer and then passed through a nylon filter before staining with propidium iodide. |

| Instrument |
|------------|
| CyFlow Space flow cytometer (Sysmex) |

| Software |
|----------|
| FloMax v.2.4 |

| Cell population abundance |
|---------------------------|
| Flow cytometry was solely used for the purposes of estimating approximate 2C genome size, to allow for generation of appropriate amounts of genome sequence data; the abundance of different cell populations and purity of the samples was not determined. |

| Gating strategy |
|-----------------|
| Flow cytometry was solely used for the purposes of estimating approximate 2C genome size, to allow for generation of appropriate amounts of genome sequence data, and a specific gating strategy was not defined. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.