

## FOCUSED REVIEW

# Using natural variation to achieve a whole-plant functional understanding of the responses mediated by jasmonate signaling

Rishav Ray , Dapeng Li, Rayko Halitschke  and Ian T. Baldwin\* 

Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, D-07745 Jena, Germany

Received 24 January 2019; revised 25 February 2019; accepted 27 February 2019; published online 29 March 2019.

\*For correspondence (e-mail [baldwin@ice.mpg.de](mailto:baldwin@ice.mpg.de)).

## SUMMARY

The dramatic advances in our understanding of the molecular biology and biochemistry of jasmonate (JA) signaling have been the subject of several excellent recent reviews that have highlighted the phytohormonal function of this signaling pathway. Here, we focus on the responses mediated by JA signaling which have consequences for a plant's Darwinian fitness, i.e. the organism-level function of JA signaling. The most diverse module in the signaling cascade, the JAZ proteins, and their interactions with other proteins and transcription factors, allow this canonical signaling cascade to mediate a bewildering array of traits in different tissues at different times; the functional coherence of these diverse responses are best appreciated in an organismal/ecological context. From published work, it appears that jasmonates can function as the 'Swiss Army knife' of plant signaling, mediating many different biotic and abiotic stress and developmental responses that allow plants to contextualize their responses to their frequently changing local environments and optimize their fitness. We propose that a deeper analysis of the natural variation in both within-plant and within-population JA signaling components is a profitable means of attaining a coherent whole-plant functional perspective of this signaling cascade, and provide examples of this approach from the *Nicotiana attenuata* system.

**Keywords:** Jasmonate signaling, natural variation, plant fitness, tissue specificity, MAGIC population, *Nicotiana attenuata*.

## INTRODUCTION

Plants, perhaps as a consequence of being sessile organisms, have evolved to be master chemists, synthesizing a plethora of natural products that allow them to solve the ecological challenges posed by their immobility. These natural products shape plant growth, development and Darwinian fitness, and are frequently produced in response to signals from the environment. Jasmonate (JA) signaling plays a central role in orchestrating the environmental responsiveness of a plant's repertoire of natural products, particularly with regard to the sectors that mediate herbivore and pathogen resistance (Wasternack and Hause, 2013; Howe *et al.*, 2018; Wasternack and Feussner, 2018).

Most of the work to understand the JA pathway has been carried out in *Arabidopsis*, primarily due to the advances that have been made in understanding the physiology of this model plant, advances enabled by a well-annotated and comparatively simple genome and impressive genomic

resources. Furthermore, the availability of mutants in *Arabidopsis*, tomato and rice has facilitated the detailed characterization of the individual enzymatic steps of JA biosynthesis (Li *et al.*, 2001; Browse, 2009; Dhakarey *et al.*, 2016). Through mutant screens, enzymes have been identified that provide the initial fatty acid substrates, catalyze the multiple biosynthetic steps in the octadecanoid pathway producing JA, conjugate JA to the active form, JA-Ile, or channel JA into degradation pathways that include methylation, glycosylation and hydroxylation or carboxylation reactions (Wasternack and Feussner, 2018). The availability of these biosynthesis and signaling mutants has fueled the discovery of an impressive catalog of traits which are regulated by JA signaling. However, the overall paucity of field studies with these JA signaling mutants has thwarted an organism-level functional understanding of these diverse traits, which is the objective of this review.

Amortizing natural variation in model species has proven to be one of the most powerful means of understanding the evolutionary significance of traits (Gasch *et al.*, 2016), and the considerable genetic resources available in *Arabidopsis* and other model crops, such as rice and tomato, have been leveraged to understand the responses mediated by JA signaling in genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping studies with JA-elicited plants, or more simply by comparing accessions with extreme phenotypes under the experimental conditions. In the next section, we review the traits that have been uncovered in these studies.

### NATURAL VARIATION IN JA-MEDIATED RESPONSES

Interactions between JA and other phytohormones are crucial factors which plants are likely to regulate to minimize growth and defense trade-offs. Proietti *et al.* (2018) performed GWAS on 349 natural accessions of *Arabidopsis* to dissect the crosstalk of salicylic acid (SA) and abscisic acid (ABA) with JA. The magnitude of change in JA-induced expression levels of the *PLANT DEFENSIN1.2* (*PDF1.2*) gene was calculated for each accessions 24 h after treating the leaves with methyl jasmonate (MeJA) alone or a combination of MeJA and SA or ABA, respectively, as a readout for GWAS and interpreted as evidence of phytohormonal 'crosstalk', without specifying the nature of the interaction. Through fine mapping and transfer DNA insertion mutant analysis, two genes, encoding a glyoxalase protein and a response regulator involved in cytokinin signaling, were found to be involved in the JA–SA interaction and also in resistance against *Botrytis cinerea*. Similarly, an uncharacterized cation efflux family protein was shown to affect the interaction of JA–ABA signaling by suppressing MeJA-induced expression of *PDF1.2* and *VSP2* and resistance against *Mamestra brassicae* (Proietti *et al.*, 2018). Although these studies highlight potential players in JA–SA and JA–ABA 'crosstalk', their functional roles in the interaction remain unclear as the phytohormone levels were not analyzed when the plants were challenged with a pathogen or herbivore. In another study, ethylene–JA interactions were found to affect the growth and elongation of rice mesocotyls and coleoptiles. Using publicly available resequencing data of 3000 rice accessions, the authors identified a gene, *gaoyao1* (*GY1*) which is homologous to two *Arabidopsis* lipases, *DONGLE* (*DGL*) and *defective in anther dehiscence1* (*DAD1*) (Xiong *et al.*, 2017). *DONGLE* is the first enzyme in JA biosynthesis, and an allelic difference in its homolog *GY1* suppresses JA biosynthesis in rice and promotes the elongation of mesocotyls and coleoptiles with increased ethylene production. A GWAS was performed on JA levels for 221 rice varieties and identified two pectin-modifying genes, *OsPME1* and *OsTSD2*, which are epigenetically regulated by a NAD(+)-dependent histone deacetylase gene, *OsSRT1*, indicating the interaction of methanol and JA

signaling in the regulation of senescence in rice (Fang *et al.*, 2016). These GWAS investigations identified previously uncharacterized genes and mechanisms that regulate JA signaling, presumably to maximize plant growth and fitness, with agronomic importance.

In another study, significantly elevated JA and sugar levels were found in eight *Arabidopsis alpina* accessions in response to cold stress, suggesting an interaction of JA and other phytohormones with sugar signaling in response to altitude and other environmental characteristics of the plant's native habitat in the French Alps (Wingler *et al.*, 2014). Furthermore, JA levels across accessions were negatively correlated with chlorophyll content, which the authors interpreted as suggesting a role for JA in acclimation and mediating plant responses to abiotic stresses associated with altitude.

Jasmonate signaling plays a pivotal role in orchestrating plant inducible defenses to herbivory. The ability to synchronize increases in resistance with herbivore attack has clear fitness benefits for plants in nature (Baldwin, 1998), yet we still have little understanding of the causes and fitness consequences of population-level genotypic variation in JA signaling. In a recent study of seven *Arabidopsis* accessions elicited by either insect attack or JA treatment, the overall inducible herbivore resistance was found to be highly variable across accessions and negatively correlated with constitutive resistance (Rasmann *et al.*, 2015). Such apparent trade-offs between constitutive and induced resistance suggest that the JA-mediated expression of resistance traits is costly or otherwise physiologically or evolutionarily constrained, generating patterns of heterogeneity within populations. Similar heterogeneity was reported in two maize accessions that differ in their resistance to herbivory – one of the accessions, Mp708, had higher constitutive transcript levels of JA biosynthesis and response genes, and performed better under herbivory compared with the susceptible genotype Tx601 (Shivaji *et al.*, 2010). Interestingly, another study investigating herbivory by spider mites on two extreme *Arabidopsis* accessions in a time series analysis found the overall initial transcriptional responses to herbivory to be similar in the two accessions (Zhurov *et al.*, 2014). The differentially expressed genes (DEGs) clustered together irrespective of genotype at the first time point (1 h after feeding) while later time points (3, 6, 12, 24 h) clustered by genotype. When enriched by their Gene Ontology (GO) terms, the early time point DEGs were involved in perception, signaling and transcriptional activation processes, whereas the DEGs in the later time points were involved in enzymatic activities for the production and alteration of defensive metabolites in response to herbivory and JA elicitation. The results were further validated using JA signaling mutants that performed significantly worse than wild-type plants when challenged with the herbivore. This study

revealed that the different JA-elicited modules have different temporal dynamics in different accessions and suggested that the initial transcriptional reconfiguration in response to herbivory is broadly conserved across accessions. The evolution of this initial transcriptional reconfiguration was analyzed in a study of six *Nicotiana* species, in which plants were wounded and treated with oral secretion (OS) from specialist *Manduca sexta* or generalist *Spodoptera littoralis* larvae, respectively, and fatty acid–amino acid conjugates (FAC), a class of JA elicitors found in these larval OS (Halitschke *et al.*, 2001; Roda *et al.*, 2004). The study revealed different responses across species for the same elicitors, indicating their rapid evolution within the genus. The authors also reported a leucine-rich repeat receptor kinase, which functions independently of the JA signaling pathway but negatively regulates JA biosynthesis and the hydroxylation of JA-Ile. This modulation of JA signaling is likely to suppress defense elicitation effects, perhaps to regulate putative growth–defense trade-offs (Zhou *et al.*, 2016). It is tempting to speculate that similar mechanisms might be at play in the intra-species natural variation in JA signaling and hence would be testable with extant populations. In a meta-analysis, Bhosale *et al.* (2013) used 41 untreated leaves from three different *Arabidopsis* accessions originating from six different laboratories and used the residual expression, after removing the laboratory and accession effects, to uncover biologically relevant co-expression modules (Bhosale *et al.*, 2013). The residual variance accounted for an average of 52.5% for a single gene, which was substantially higher than the variance due to the accessions, lab and lab–accession interactions. When the gene modules were enriched for GO terms, ‘response to JA stimulus’ was one of the top-scoring GO modules in the network. As a proof of concept, the authors identified a previously uncharacterized component, ILL6 which acts as a negative regulator of JA accumulation and response, potentially as an amidohydrolase of JA-Ile. This result highlights the fact that variation in growing conditions alters regulatory mechanisms, and JA signaling is a top mediator of responses to subtle environmental changes. Whiteman *et al.* (2011) developed a plant–insect system with *Arabidopsis* and *Scaptomyza flava*, a drosophilid fly whose larvae feed on *Arabidopsis* in nature. Using different natural accessions, it was found that female flies caused significantly more feeding punctures and had higher oviposition rates on the Tsu-0 accession, which has been previously reported to be susceptible to attack from specialist herbivores (Pfalz *et al.*, 2007). Fly larvae also performed significantly better on the Tsu-0 accession than on the Col-0 accession. The leaf area mined by larvae on JA- and glucosinolate-deficient mutants was significantly greater than on Col-0 plants, indicating that the JA and glucosinolate defense pathways are important in

mediating quantitative resistance of the plant against *S. flava* herbivory (Whiteman *et al.*, 2011).

These studies highlight the importance of JA as a central mediator of the responses of plants to environmental stresses but fall short of understanding whether the elicited responses benefit plants by increasing their fitness and do not illuminate the reasons why so much natural variation exists in this signaling pathway. This limitation is partly due to the overall paucity of mechanistic understanding of the observed natural variation coupled with the fact that evaluating the fitness effects of JA-elicited responses without a deep understanding of the plant’s diverse natural histories is challenging.

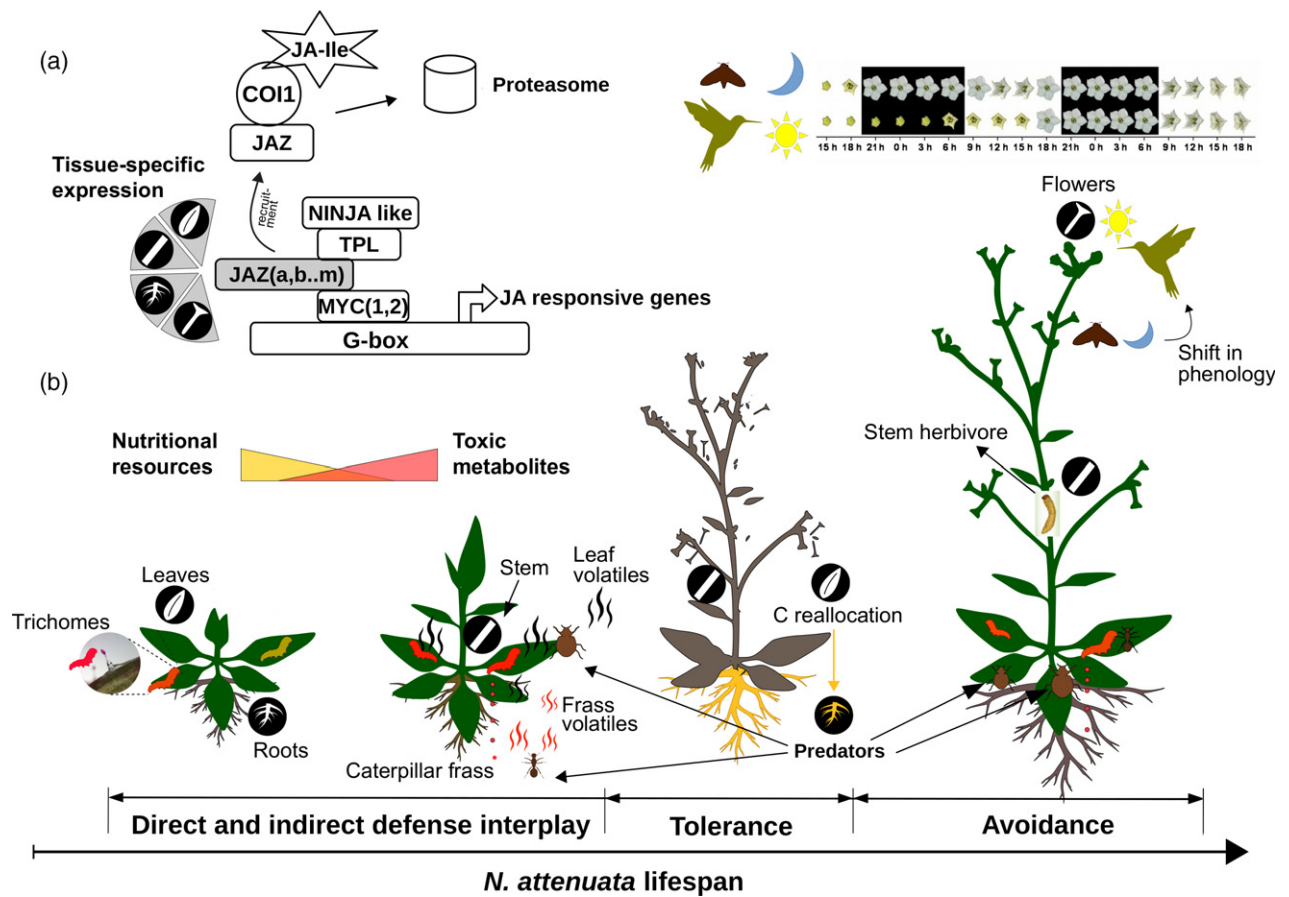
*Nicotiana attenuata*, an ecological model plant, is a diploid tobacco native to the Great Basin Desert of North America. It enjoys a rather unique position scientifically, because hundreds of transgenic lines have been studied in the field over the past two decades to understand the fitness consequences of precisely defined changes in gene expression for plants growing in their native habitats. Jasmonate signaling was found to be important for this plant’s performance in nature early in the research program (Baldwin *et al.*, 1994a, 1997; Baldwin, 1998). Several transgenic lines with impaired JA production through RNA interference (RNAi)-mediated silencing of JA biosynthetic genes (including *GLA1*, *LOX3*, *AOS*, *AOC*, *OPR3* and *JAR4/6*) or by creating ‘jasmonate sinks’ (by silencing *JME* and overexpressing *JMT*) have been released in the plant’s natural habitat to better understand the diverse functional consequences of JA signaling (see, for example, Kessler *et al.*, 2004; Stitz *et al.*, 2011; Kallenbach *et al.*, 2012; Machado *et al.*, 2016). Similarly, the function of signaling components up- and downstream of JA production have been characterized in field releases of plants silenced in the expression of *COI1* and *JAZ* genes, as well as in *WRKY* transcription factors and early signaling protein kinases (Skibbe *et al.*, 2008; Wu *et al.*, 2008; Kallenbach *et al.*, 2012; Oh *et al.*, 2012, 2013). This ‘sledgehammer’ reverse genetics approach has provided both mechanistic and functional understanding of within-plant JA signaling diversity in *N. attenuata* and has paved the way to map this diversity of responses at a population level to achieve a functionally coherent understanding of the pathway.

In this focused review, we discuss the within-plant diversity (tissue- and elicitation-specific changes with different dynamics) of JA signaling networks uncovered in *N. attenuata*, followed by within-population diversity (differences among individuals) and its ecological consequences. Finally, we propose how this multilevel variation can be utilized to attain a holistic functional perspective of JA signaling using sophisticated forward genetic tools that amortize the considerable natural variation in JA signaling that occurs in this species.

**FUNCTIONAL DIVERSITY OF JA SIGNALING IN *N. ATTENUATA***

The JA biosynthesis pathway has been intensively reviewed (Wasternack, 2015; Wasternack and Feussner, 2018), thus it is not the main scope of this paper. Briefly, in response to herbivore attack, which triggers a burst of accumulation of JA-Ile, JAZs are recruited by the F-box protein COI1 that binds JA-Ile and are subsequently ubiquitinated and degraded by the 26S proteasome. When this occurs, the inhibition of MYC transcription factors is released and transcription of early JA-responsive genes is activated. Tissue-specific transcription factors, the 13 JAZ proteins, and the variants of NINJA in the *N. attenuata* genome make up the within-plant diversity that enables JA responses to be rendered tissue-specific to tailor responses to different attackers (Figure 1a). Jasmonate signaling is strongly amplified when

herbivore-specific FAC elicitors are introduced into wounds (Halitschke *et al.*, 2001), which in turn activate mitogen-activated (SIPK and WIPK) and calcium-dependent (CDPK4/5) protein kinases and a lectin receptor kinase (LecRK1) which provide important context-dependent regulation of JA signaling and its outputs (Meldau *et al.*, 2009; Kallenbach *et al.*, 2010; Gilardoni *et al.*, 2011; Yang *et al.*, 2012). In this way, the JA signaling pathway can function with the versatility of a Swiss Army knife, providing context-dependent regulation for a host of different responses that function in direct and indirect defense, tolerance and avoidance responses (Figure 1b; Li *et al.*, 2016) that are hard to make sense of unless they are studied in a single species and in a plant's native environmental context. Broadly speaking, *N. attenuata* exhibits the following four classes of defense responses to herbivore attack, in which JA signaling plays a central role.



**Figure 1.** Schematic of the diversity of different defense responses mediated by jasmonate (JA) signaling in *Nicotiana attenuata*. (a) The canonical JA-Ile signaling cascade, as largely revealed by work in *Arabidopsis* and tomato, is fully operational in *N. attenuata* and includes the structural and functional diversity mediated by 13 JAZ proteins, but also a NINJA-like protein which provides floral-specific defense signaling (Li *et al.*, 2017) in *N. attenuata*. (b) Jasmonate signaling mediates a sophisticated six-layered suite of defense, tolerance and avoidance responses to herbivore attack in *N. attenuata* which are expressed at different times in the plant's life cycle and are described in the text. The up/down herbivory-regulated defensive and nutritional metabolites for the herbivore are shown as red and yellow bars indicating that as herbivore load increases the levels of defense-related metabolites generally increase while levels of nutritional metabolites for the herbivore decrease. However, when the attack comes from a specialized herbivore that can co-opt a plant defense mechanism for its own defense, as is the case with *Manduca sexta* attack and nicotine production, the production of defense metabolites can be strongly downregulated.

## DIRECT DEFENSES

Direct defense against herbivory involves a host of different metabolites such as nicotine, trypsin protease inhibitors, phenolamides and diterpene glycosides, that impair the growth and digestive capabilities of the herbivores and act as deterrents, thus reducing the probability of further damage. A majority of these defensive metabolites are regulated by JA signaling in some tissues (Halitschke and Baldwin, 2003; Heiling *et al.*, 2010; Kaur *et al.*, 2010; Kallenbach *et al.*, 2012; Machado *et al.*, 2016). Different variants of the canonical JA signaling cascade will activate different metabolites in a tissue-specific manner. For example, chlorogenic acid is specifically upregulated in the pith of stems when larvae of the pith-feeding weevil *Trichobaris mucorea* attack stems of *N. attenuata*, the regulation of which is locally mediated by JA (Lee *et al.*, 2017). Interestingly, this phenolic is neither regulated by JA nor elicited by herbivory in tissues other than the pith, such as leaves, where it functions as a 'sun screen' that accumulates in response to UVB exposure (Ballaré *et al.*, 1996; Dinh *et al.*, 2013). By comparing the field performance of the wild type, JA-signaling mutants and plants silenced in the expression of pathway-specific biosynthetic genes, the direct defensive function of particular sectors of JA-regulated secondary metabolism has been rigorously demonstrated.

## INDIRECT DEFENSES

Jasmonate signaling mediates different responses to herbivore attack in *N. attenuata* throughout the plant's life cycle. Glandular trichomes are one of the first physical barriers that the herbivore must overcome to feed on the leaves, and trichome development is regulated by JA signaling (Xu *et al.*, 2002; Paschold *et al.*, 2007; Yoshida *et al.*, 2009). The glandular trichomes of solanaceous taxa are also the sites of synthesis of many different secondary metabolites (Laue *et al.*, 2000; Kang *et al.*, 2010; Weinhold and Baldwin, 2011). One of the most abundant compound classes in *N. attenuata* glandular trichomes are the *O*-acyl sugars which are consumed by neonate caterpillars as their first meal after hatching. These ingested *O*-acyl sugars are rapidly saponified in the high-pH midguts of the larvae, releasing volatile short-chain fatty acids that impart a distinctive odor to larval bodies and frass. The fresh redolent caterpillar frass, as it falls to the ground, attracts the attention of ground-foraging predators, including ants (Weinhold and Baldwin, 2011) and possibly lizards (Stork *et al.*, 2011), and thereby functions as an indirect defense by tagging the larvae for predation. When herbivores attack leaves, plants employ other forms of indirect defenses by releasing herbivory-induced plant volatiles (HIPVs) which increase the foraging efficiency of higher trophic levels that prey upon herbivorous insects (Dicke

and Baldwin, 2010). In *N. attenuata*, these HIPV blends include terpenes, such as linalool and (E)- $\alpha$ -bergamotene (Halitschke *et al.*, 2000; Kessler and Baldwin, 2001), that are thought to function as long-distance cues and are released systemically from plants and require JA signaling and a pair of WRKY transcription factors (WRKY3/6) for their activation (Kessler *et al.*, 2004; Skibbe *et al.*, 2008). Interestingly, green leaf volatiles, that are released independently of JA signaling, are amplified when JAZh is silenced (Oh *et al.*, 2012), are under circadian control (Joo *et al.*, 2018) and are thought to function as short-distance cues for the predators as they are released more from attacked leaves than the entire plant (Kessler and Baldwin, 2001; Halitschke *et al.*, 2008; Allmann and Baldwin, 2010; Schuman *et al.*, 2012; Allmann *et al.*, 2013; Zhou *et al.*, 2017).

## TOLERANCE RESPONSES

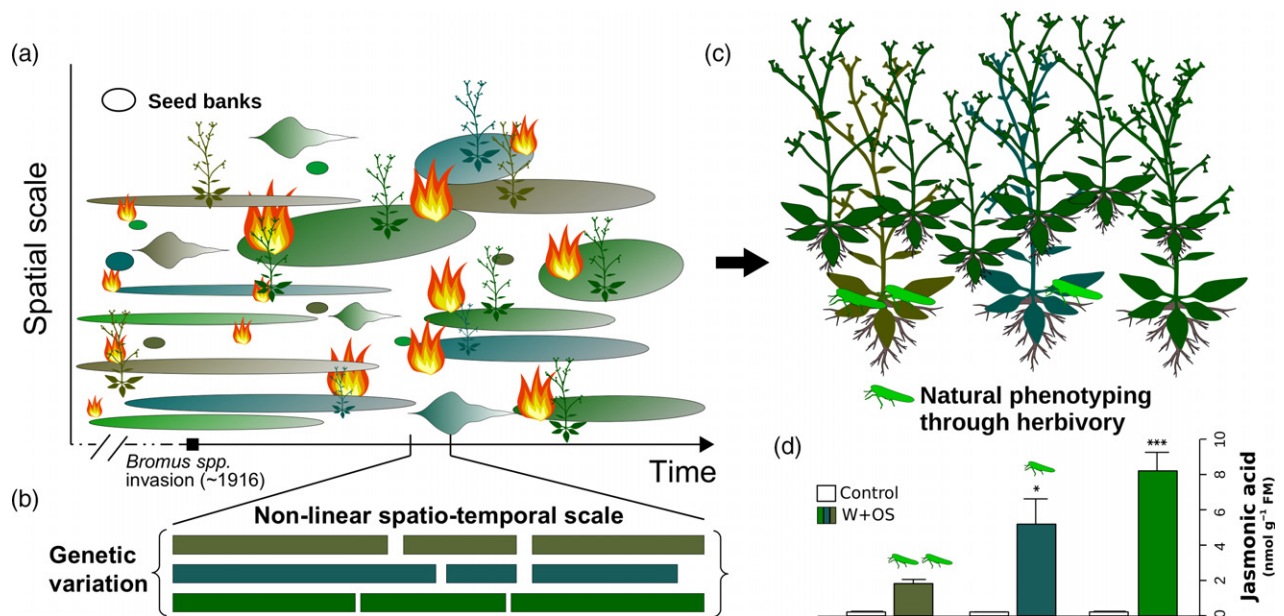
When consumed by highly adapted specialist herbivores that are able to sequester toxic compounds for their own defense, plants may rapidly downregulate the production of these toxins (e.g. nicotine; Winz and Baldwin, 2001) and also activate tolerance responses that translocate nutrient or carbon resources from attacked tissues (shoots) to spatially isolated belowground root sinks protected from leaf-feeding herbivores. These bunkered reserves can be rapidly remobilized to support regrowth and flowering after the herbivore pressure has subsided, and allow plants to tolerate attack from folivores and optimize fitness in the face of predictably varying herbivore loads. The mechanism of this transient reallocation of photoassimilates from shoots to roots is known to be independent of JA and requires the activity of the GAL83 subunit of a SNF1-related kinase (Schwachtje *et al.*, 2006). However, the remobilization of reserves from roots back to shoots to support regrowth requires JA signaling with a particular role for root-sequestered JA signaling, as shown by micrografting experiments with JA-deficient lines (Machado *et al.*, 2013). Regrowth after herbivore attack involves a complex suite of responses mediated by the intersection of JA signaling with many other phytohormone signaling systems, and will require more work to fully understand it (Machado *et al.*, 2013). While roots play a central role in tolerance responses to herbivore attack, they are also important players in the leaf defense responses discussed above. For instance, in *N. attenuata*, JA levels are highly increased in both shoots and roots in response to folivory, which consequently activates the synthesis of nicotine in the roots that is subsequently mobilized to the shoot for defense. Micrografting experiments with *N. attenuata* have revealed that intact JA signaling in both shoot and root compartments is required for expression of the complete nicotine defense response (Fragoso *et al.*, 2014).

## AVOIDANCE RESPONSES

Plants deficient in JA biosynthesis or perception are highly susceptible to attack from both invertebrate (Kessler *et al.*, 2004; Kallenbach *et al.*, 2012) (but not nematodes; Machado *et al.*, 2018) and vertebrate (Machado *et al.*, 2016) herbivores, as well as florivores (Li *et al.*, 2017). The floral tissues are a conduit of Darwinian fitness and hence are one of the most fitness-valuable tissues for plants in later developmental stages. Consequently, consistent with predictions of the optimal defense theory (McKey, 1974), flowers are highly provisioned with defenses. *Manduca sexta* moths commonly oviposit on leaves after pollinating and nectaring on flowers of *N. attenuata* (Kessler, 2012). The neonates hatching from these eggs grow into voracious leaf-eating caterpillars that can be devastating to the plant and against which most of the above-mentioned JA-regulated defenses are likely to have evolved. If these defenses are not effective and the larvae continue to consume leaf material, JA signaling activates a unique avoidance response, which entails interactions with components of the plant circadian clock, to switch the flowers' first opening time from night to day. This change in flower opening (and scenting) time allows the plant to switch

pollinators from moths to hummingbirds, thereby avoiding the collateral damage that results from attracting this moth to function as a pollinator (Kessler *et al.*, 2010). Silencing the expression of *ZEITLUPE* (*ZTL*), a clock component gene, alters flowering time and phenocopies the flower movement pattern for the first night when flowers are open, as is observed after herbivory (Yon *et al.*, 2016). A number of lines of evidence suggest that JA signaling is directly involved in altering the function of this component of the clock. Seven of the thirteen JAZ proteins (JAZa, JAZb, JAZd, JAZe, JAZj, JAZk and JAZl; Figure 2a) in *N. attenuata* are known to interact with ZTL in yeast two-hybrid assays (Li *et al.*, 2017). Furthermore, silencing ZTL expression causes a phase-shift in expression pattern of JA-responsive *MYC2a* transcripts in roots (Li *et al.*, 2018), which interestingly interact with JAZi that is only expressed in flowers and known to regulate floral defense (Li *et al.*, 2017). From these results, we infer that JA signaling transiently hijacks the clock to allow plants to switch pollinators, and thereby reduce future herbivore loads. This inference, however, will require additional research to place it on a stronger experimental footing.

Why plants continue to rely on the pollination services of the highly nicotine-tolerant moths and suffer the



**Figure 2.** Natural variation in jasmonate (JA) signaling in *Nicotiana attenuata* enables the genetic dissection of the regulation of the pathway.

(a), (b) The post-fire germination behavior of *N. attenuata* recruits plants from long-lived seedbanks separated in both time and space, resulting in natural populations that harbor a substantial amount of genetic variation in JA signaling. The circled areas indicate the spatial distribution and longevity of the seedbanks of *N. attenuata*. The size of the fire icons is proportional to the size of the fires, which in turn determines the amount of opened habitat and the opportunity to stimulate the germination of *N. attenuata* seeds from the seedbanks in the subsequent one to three growing seasons post-fire before the burned habitat is again recolonized by perennial plants. The seedbank colors reflect different genotypes and the color gradients reflect the density of viable seeds that wanes as the seedbank ages with time. The genetic variance that results from plant recruitment from multi-generational seedbanks is shown for a small subset of a population.

(c), (d) High-throughput phenotyping of natural variation in JA signaling using *Empoasca* spp. leaf hoppers which preferentially attack JA-deficient plants. The data are adapted from Kallenbach *et al.* (2012).

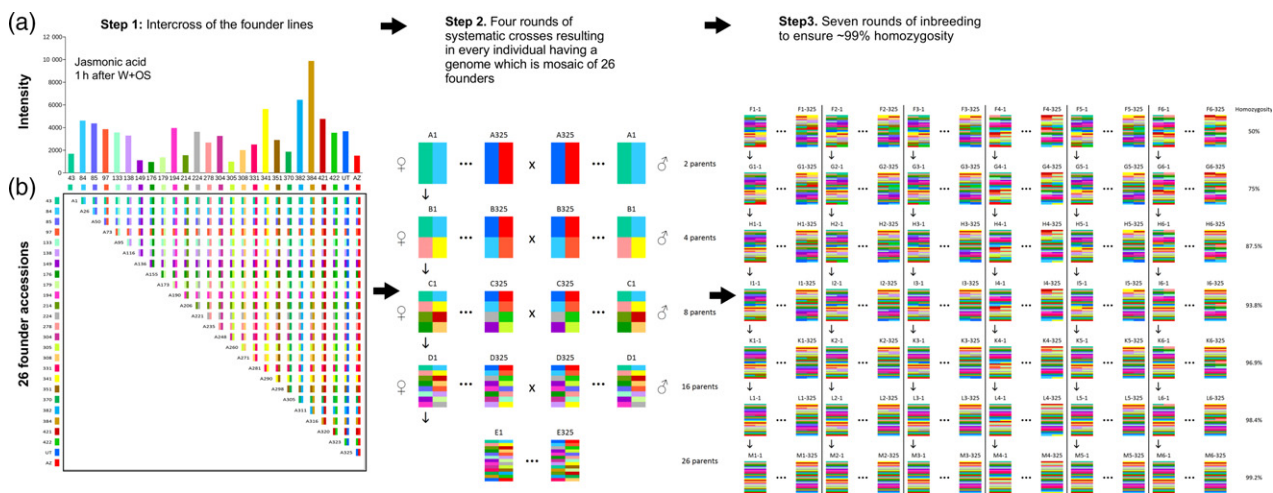
collateral damage that comes with their oviposition behavior remains an open question which deserves further experimental work. It might be that these moths, due to the greater distances they travel, provide plants with pollen loads that harbor a greater diversity of pollen genotypes than the locally foraging hummingbird pollinators. This diversity allows plants to select amongst potential mates in mixed pollen loads to increase the genetic diversity of their seedbanks (Bhattacharya and Baldwin, 2012). For a self-compatible species with a very long-lived seedbank, processes that increase the genetic diversity of offspring are likely to increase the chances of surviving the long dormancy periods between fires. At the time of *N. attenuata* flowering in Utah, hummingbirds visit nectar sources in the local areas of their nests, resulting in low rates of outcrossing. Interestingly, the plant then utilizes another JA-mediated defense, nicotine, in floral nectar to disrupt the trap-lining foraging behavior of hummingbirds to increase outcrossing rates with this pollinator (Kessler *et al.*, 2012).

Jasmonate signaling clearly mediates a bewildering array of different responses in plants. When these responses are genetically dissected in a native plant which has not been subjected to rounds of artificial selection, and the genetically manipulated plants are released into the plant's native habitat, it is possible to obtain glimpses of a functionally coherent understanding of how these different responses work together to allow plants to contextualize their responses and maximize fitness. Clearly, JA signaling

regulates responses that can be understood as functioning as direct and indirect defenses against current and future attackers, enhancing a plant's tolerance of herbivore attack and avoiding future attack. However, these variants in JA signaling and response that have been generated by RNAi approaches do not necessarily represent natural variation in JA signaling, and hence the raw material from which natural selection could sculpt functional responses. In the next section, we summarize what we know about natural variation in JA signaling in *N. attenuata* and point to a way forward that amortizes this natural variation in a forward genetics approach to understand how evolution has shaped JA signaling networks to optimize plant defense in the face of varying environments.

**NATURAL VARIATION IN JA SIGNALING IN *N. ATTENUATA* POPULATIONS**

Natural accessions of *N. attenuata* collected from across the species' range in the Great Basin Desert and grown in a common glasshouse environment exhibit highly variable OS-elicited JA profiles and responses (Figures 2d and 3a) indicating a high within-population diversity in the species. Recent advances in untargeted mass spectrometry (MS)-based metabolomics have started to reveal the consequences of this within-population diversity in the layers of specialized metabolism differentially regulated by the JA signaling pathway among accessions (Li *et al.*, 2015). Advances in the speed and decreases in the costs of high-resolution MS instruments have made such analyses a



**Figure 3.** Creation of a Multiparent Advanced Generation Inter-Cross (MAGIC) recombinant inbred line (RIL) population for *Nicotiana attenuata* that captures the species' genetic variation in jasmonate (JA) signaling and other ecologically relevant traits. (a) Natural variation in herbivory-elicited JA signaling in the 26 founder accessions that were used for the construction of the MAGIC population; leaf JA levels were quantified 1 h after leaf puncture wounds were immediately treated with the oral secretions of *Manduca sexta* larvae (data adapted from Li *et al.*, 2015). (b)–(d) Construction of the MAGIC population. A complete unidirectional diallelic cross was performed with the 26 accessions, followed by four generations of systematic intercrossing to generate a 6 × 325 member RIL population that was inbred for six generations to create the final MAGIC RIL population which is about 99% homozygous and harbors different alleles from the 26 founders.

powerful high-throughput (HTP) means of phenotyping natural variation in JA signaling. In addition to this laboratory-based HTP phenotyping, we can utilize native herbivores as accurate JA phenotyping tools in field studies. *Empoasca* spp. leafhoppers, for example, can provide a HTP 'insect-guided' phenotyping tool to rapidly and efficiently screen native populations. The piercing-sucking *Empoasca* spp. leafhoppers apparently 'eavesdrop' on the JA-mediated signaling capacities of their host plants, preferentially selecting those hosts that are deficient in JA accumulation (Kessler *et al.*, 2004; Kallenbach *et al.*, 2012). Intriguingly, this natural phenotyping 'bloodhound' specifically targets JA signaling independently of the known downstream defense metabolites that are regulated by JA signaling, and is thereby a phenotyping tool for rapidly identifying JA-deficient accessions in natural populations [Figure 2c,d, adapted from Kallenbach *et al.* (2012)].

This observation raises the following question: why is there such extensive genetic diversity in natural *N. attenuata* populations? One contributing factor is that the plants largely occur for 2–3 years after fires and time their germination from long-lived seedbanks to initiate growth in the first growing season after a natural burn. Seeds can remain dormant in these seedbanks for hundreds of years and synchronize germination in response to smoke cues that are produced during fires (Baldwin and Morse, 1994; Baldwin *et al.*, 1994b; Preston and Baldwin, 1999). Since the invasion and spread of cheat grass (*Bromus* spp.) into the Great Basin Desert, the size of wildfires has grown substantially, as the dry grass readily spreads lightning-started fires amongst the widely distributed woody species that dominate this habitat. These larger fires are likely to have increased the genetic structure of post-fire populations as these large fires stimulate the germination of seeds from multiple seedbanks of different ages at the same time (Figure 2a). The genetic heterogeneity of these populations is probably maintained by the rapidly changing selection regimes that occur after fires. As one of the first plants to colonize post-fire habitats, *N. attenuata* enjoys low herbivore loads and open and nutrient-rich habitats. As these post-fire habitats are recolonized, herbivore loads and competition from other plant species for fire-released resources dramatically increase, and thereby change the selection for traits that are associated with JA signaling (Lynds and Baldwin, 1998; Baldwin, 2001). The adaptive phenotypic diversity that is associated with JA signaling, coupled with the ability to escape in time that the post-fire germination behavior facilitates, has probably allowed *N. attenuata* to evade selective sweeps that result from strong natural selection in small populations (Bahulikar *et al.*, 2004) and thereby maintain high genetic and metabolic heterogeneity in its natural populations (Figure 2b) (Wu *et al.*, 2008; Schuman *et al.*, 2009; Li *et al.*, 2015).

Population geneticists have long utilized the genetic variance derived from natural populations to query the genetic architecture underlying traits of interest. Recently, the allelic diversity in the genome of two natural accessions collected from Utah and Arizona (Glawe *et al.*, 2003; Wu *et al.*, 2008) has been utilized to create an advanced intercross recombinant inbred line (AI-RIL) population to identify genetic components of indirect defense (Zhou *et al.*, 2017) and arbuscular mycorrhizal interactions (Wang *et al.*, 2018). This AI-RIL approach has recently been extended by creating a 26-parent Multiparent Advanced Generation Inter-Cross (MAGIC) population which we describe in the next section.

### CAPTURING INTRA-SPECIES NATURAL VARIATION IN JA SIGNALING WITH A MAGIC POPULATION

Linkage mapping (or QTL mapping) and association mapping (or GWAS) are the two most commonly used statistical frameworks to query the genetic architecture underlying traits of interest. While GWAS can identify associative single nucleotide polymorphisms with 1-bp precision, the procedure has limitations that make it difficult to use with the highly structured natural populations of *N. attenuata*. One of the primary assumptions of GWAS is that there should be no structure in the population, that is, the population must interbreed freely. This is due to the fact that correlating patterns among loci and traits causing variation can create spurious associations between markers and traits where no actual causal relation exists. This problem has long been identified (Li, 1969; Lander and Schork, 1994) and statistical efforts help to address it (Devlin and Roeder, 1999; Pritchard *et al.*, 2000; Yu *et al.*, 2006). Given that *N. attenuata*'s post-fire germination behavior from long-lived seedbanks creates natural populations with substantial genetic structure (Figure 2b), thereby thwarting the use of GWAS, we started a 9-year effort to create a 26-parent MAGIC population that captures the natural variation of the species. MAGIC populations are one of the most powerful forward genetic tools but they also recapitulate aspects of natural *N. attenuata* populations and hence are ideal for planting into native habitats.

Approximately 400 natural accessions of *N. attenuata* have been collected over the past three decades of field work with this species. These accessions were grown in a glasshouse and screened for a panel of ecologically relevant traits for this species, which included JA signaling (Li *et al.*, 2015), *O*-acyl sugar content (Luu *et al.*, 2017) as well as volatile emissions. From this screening, 26 accessions that captured the majority of the phenotypic diversity of the approximately 400 accessions were selected as the founders of the MAGIC population (Figure 3a). Diallelic crossing was performed on the 26 founder lines (with each other) to obtain a set of intermediate crosses which had alleles from each of two parents (Figure 3b). Systematic



intercrossing of the intermediate lines was performed for four generations, resulting in a population of 325 lines,  $(26 \times 25)/2$ , each harboring genetic contributions from all the 26 founder lines in their genome. Two of six replicate populations were selected and inbred for six subsequent generations to ensure approximately 99% homozygosity across all loci. While phenotyping this large MAGIC population for JA levels can be onerous, the job can be readily 'outsourced' to herbivores such as *Empoasca* leafhoppers, which can identify the JA-deficient lines in a HTP manner under field conditions as described earlier.

Once the variation is quantified, it can be mapped to the identified JA networks mentioned above, much like mapping genomic sequences onto a reference genome, allowing for the discovery of network variants that are of ecological significance. Although there is compelling evidence that the core JA signaling module has remained highly conserved throughout the evolution of vascular plants (Howe *et al.*, 2018; Monte *et al.*, 2018), the elicitation of the core JA module by environmental signals and the resulting physiological responses are highly variable (Li *et al.*, 2016), which in turn might help explain the heterogeneity maintained at the population level. Whether natural variation in the up- and downstream components of the core JA signaling pathway can explain the highly variable distribution of specialized metabolites in *N. attenuata* populations remains an open question. The natural variation in JA-mediated metabolites quantified within *N. attenuata* populations often exceeds the between-population diversity (Li *et al.*, 2015), a pattern consistent with the polymorphic genetic structure of the species (Bahulikar *et al.*, 2004). This coupled variation pattern in the natural populations suggests that the phenotypic variation results from genetic variation in JA accumulation and perception. These polymorphic genetic loci could include regulatory genes that shape downstream responses to JA, potentially including small and long non-coding RNAs. The genetic variation might also be involved in the JA activation/deactivation pathways through mechanisms such as hydroxylation, carboxylation, glycosylation or methylation, which in turn could be subjected to layers of regulation, fine tuning the amplitude, duration and timing of JA-mediated responses. This fine-tuned JA signaling readout, which includes both spatial and temporal variations, mediates various physiological trade-offs and interactions with other signaling pathways.

Given the quantitative variation in JA-induced metabolic profiles and defense phenotypes, it is likely that a transcriptomic analysis of these natural accessions would provide excellent opportunities for identifying previously uncharacterized genes associated with various ecological traits regulated by JA signaling and extending our understanding of JA signaling networks. Moreover, with current

advances in unbiased metabolomic techniques the previously inaccessible layers of JA-regulated defense metabolites that are central to plant fitness are now readily accessible and quantifiable. These processes can be conceptualized as adding extra dimensions to existing biological networks at the organism level, dimensions that can be extended to the level of populations. In addition to JA-related traits, the MAGIC RIL population presents an excellent tool with which to uncover the genetic underpinnings of other important ecological traits, including, for example, the recruitment of the plant's root microbiome, mate selection, etc.

We propose that such an unbiased forward genetic approach will pave the way towards a coherent understanding of the molecular mechanisms responsible for plant fitness in natural environments. The approach does not obviate the need for reverse genetic tools, as it is essential to dissect imputed genetic loci to manipulate these molecular mechanisms and evaluate their fitness consequences, both under field and more controlled laboratory conditions. The objective would be an organism-level understanding of the function of this 'Swiss Army knife' of phytohormones.

#### Summary Box

- Within-plant variation in JA signaling is mediated by a diverse array of proteins such as JAR/JIH which activate/deactivate the bioactive form of JA (JA-Ile), JAZ, NINJA-like proteins, transcription factors, etc. that can mediate various growth and defense phenotypes in a tissue-specific manner when challenged with various abiotic and biotic stress factors.
- Within-population variation in JA occurs when plants face heterogeneous environments and evolve the ability to contextualize their response to these varying factors in order to maximize their fitness; we know very little about the mechanisms responsible for this type of variation.
- The population-level variation can be leveraged to genetically map these variable traits, using increasingly complex AI-RIL and MAGIC mapping populations that harness the power of forward genetics for a species.
- Identifying the genetic components harboring the variable traits in JA signaling that can be dissected from genetically linked QTLs and genomic islands extracted from QTL mapping and GWAS studies will greatly advance our understanding of the evolutionary processes that maintain the natural variation in this important signaling pathway.

### Open Questions

- What are the genetic mechanisms responsible for natural variation in JA signaling, particularly in the control of JA-Ile levels?
- How does natural variation in JA signaling at the molecular level influence phenotypic variation and Darwinian fitness at the organismal level?
- Despite the clear advantages of strong JA signaling, which selective forces maintain variation in JA signaling in natural populations of *N. attenuata*?
- If fitness costs of JA-mediated responses are at play in maintaining the natural variation, can these costly responses be uncoupled from the advantageous defenses mediated by JA signaling?
- To what extent do environmentally mediated epigenetic factors affect the genetic configuration and evolution of JA signaling networks?

### ACKNOWLEDGEMENTS

The authors thank the Max Planck Society, and the Deutsche Forschungsgemeinschaft Grant, 'Chemical Mediators in Complex Biosystems – ChemBioSys' (SFB 1127) for funding.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### AUTHOR CONTRIBUTIONS

All authors contributed to the writing and revising of the paper.

### REFERENCES

- Allmann, S. and Baldwin, I.T. (2010) Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. *Science*, **329**, 1075–1078.
- Allmann, S., Späthe, A., Bisch-Knaden, S., Kallenbach, M., Reinecke, A., Sachse, S., Baldwin, I.T. and Hansson, B.S. (2013) Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. *eLife*, **2**, e00421.
- Bahulikar, R.A., Stanculescu, D., Preston, C.A. and Baldwin, I.T. (2004) ISSR and AFLP analysis of the temporal and spatial population structure of the post-fire annual, *Nicotiana attenuata*, in SW Utah. *BMC Ecol.* **4**, 12.
- Baldwin, I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc. Natl Acad. Sci. USA*, **95**, 8113–8118.
- Baldwin, I.T. (2001) An ecologically motivated analysis of plant-herbivore interactions in native tobacco. *Plant Physiol.* **127**, 1449–1458.
- Baldwin, I.T. and Morse, L. (1994) Up in smoke: II. Germination of *Nicotiana attenuata* in response to smoke-derived cues and nutrients in burned and unburned soils. *J. Chem. Ecol.* **20**, 2373–2391.
- Baldwin, I.T., Schmelz, E.A. and Ohnmeiss, T.E. (1994a) Wound-induced changes in root and shoot jasmonic acid pools correlate with induced nicotine synthesis in *Nicotiana sylvestris* Spegazzini and Comes. *J. Chem. Ecol.* **20**, 2139–2157.
- Baldwin, I.T., Staszak-Kozinski, L. and Davidson, R. (1994b) Up in smoke: I. Smoke-derived germination cues for postfire annual, *Nicotiana attenuata* Torr. Ex. Watson. *J. Chem. Ecol.* **20**, 2345–2371.

- Baldwin, I.T., Zhang, Z.-P., Diab, N., Ohnmeiss, T.E., McCloud, E.S., Lynds, G.Y. and Schmelz, E.A. (1997) Quantification, correlations and manipulations of wound-induced changes in jasmonic acid and nicotine in *Nicotiana sylvestris*. *Planta*, **201**, 397–404.
- Ballaré, C.L., Scopel, A.L., Stapleton, A.E. and Yanovsky, M.J. (1996) Solar ultraviolet-B radiation affects seedling emergence, DNA integrity, plant morphology, growth rate, and attractiveness to herbivore insects in *Datura ferox*. *Plant Physiol.* **112**, 161–170.
- Bhattacharya, S. and Baldwin, I.T. (2012) The post-pollination ethylene burst and the continuation of floral advertisement are harbingers of non-random mate selection in *Nicotiana attenuata*. *Plant J.* **71**, 587–601.
- Bhosale, R., Jewell, J.B., Hollunder, J., Koo, A.J., Vuylsteke, M., Michoel, T., Hilson, P., Goossens, A., Howe, G.A. and Maere, S. (2013) Predicting gene function from uncontrolled variation among individual wild-type *Arabidopsis* plants. *Plant Cell*, **25**, 2865–2877, tpc-113.
- Browse, J. (2009) The power of mutants for investigating jasmonate biosynthesis and signaling. *Phytochemistry*, **70**, 1539–1546.
- Devlin, B. and Roeder, K. (1999) Genomic control for association studies. *Biometrics*, **55**, 997–1004.
- Dhakarey, R., Kodackattumanni Peethambaran, P. and Riemann, M. (2016) Functional analysis of jasmonates in rice through mutant approaches. *Plants*, **5**, 15.
- Dicke, M. and Baldwin, I.T. (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the “cry for help”. *Trends Plant Sci.* **15**, 167–175.
- Dinh, S.T., Galis, I. and Baldwin, I.T. (2013) UVB radiation and 17-hydroxygeranylinalool diterpene glycosides provide durable resistance against mirid (*Tupiocoris notatus*) attack in field-grown *Nicotiana attenuata* plants. *Plant, Cell Environ.* **36**, 590–606.
- Fang, C., Zhang, H., Wan, J. et al. (2016) Control of leaf senescence by an MeOH-jasmonates cascade that is epigenetically regulated by *OsSRT1* in rice. *Mol. Plant*, **9**, 1366–1378.
- Fragoso, V., Rothe, E., Baldwin, I.T. and Kim, S.-G. (2014) Root jasmonic acid synthesis and perception regulate folivore-induced shoot metabolites and increase *Nicotiana attenuata* resistance. *New Phytol.* **202**, 1335–1345.
- Gasch, A.P., Payseur, B.A. and Pool, J.E. (2016) The power of natural variation for model organism biology. *Trends Genet.* **32**, 147–154.
- Gilardoni, P.A., Hettenhausen, C., Baldwin, I.T. and Bonaventure, G. (2011) *Nicotiana attenuata* LECTIN RECEPTOR KINASE1 suppresses the insect-mediated inhibition of induced defense responses during *Manduca sexta* herbivory. *Plant Cell*, **23**, 3512–3532.
- Glawe, G.A., Zavala, J.A., Kessler, A., Van Dam, N.M. and Baldwin, I.T. (2003) Ecological costs and benefits correlated with trypsin protease inhibitor production in *Nicotiana attenuata*. *Ecology*, **84**, 79–90.
- Halitschke, R. and Baldwin, I.T. (2003) Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. *Plant J.* **36**, 794–807.
- Halitschke, R., Kessler, A., Kahl, J., Lorenz, A. and Baldwin, I.T. (2000) Ecophysiological comparison of direct and indirect defenses in *Nicotiana attenuata*. *Oecologia*, **124**, 408–417.
- Halitschke, R., Schittko, U., Pohnert, G., Boland, W. and Baldwin, I.T. (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. *Plant Physiol.* **125**, 711–717.
- Halitschke, R., Stenberg, J.A., Kessler, D., Kessler, A. and Baldwin, I.T. (2008) Shared signals—“alarm calls” from plants increase apparency to herbivores and their enemies in nature. *Ecol. Lett.* **11**, 24–34.
- Heiling, S., Schuman, M.C., Schoettner, M., Mukerjee, P., Berger, B., Schneider, B., Jassbi, A.R. and Baldwin, I.T. (2010) Jasmonate and ppHsystemin regulate key malonylation steps in the biosynthesis of 17-hydroxygeranylinalool diterpene glycosides, an abundant and effective direct defense against herbivores in *Nicotiana attenuata*. *Plant Cell*, **22**, 273–292.
- Howe, G.A., Major, I.T. and Koo, A.J. (2018) Modularity in jasmonate signaling for multistress resilience. *Annu. Rev. Plant Biol.* **69**, 387–415.
- Joo, Y., Schuman, M.C., Goldberg, J.K., Kim, S.-G., Yon, F., Brütting, C. and Baldwin, I.T. (2018) Herbivore-induced volatile blends with both “fast”

- and "slow" components provide robust indirect defence in nature. *Funct. Ecol.* **32**, 136–149.
- Kallenbach, M., Alagna, F., Baldwin, I.T. and Bonaventure, G. (2010) *Nicotiana attenuata* SIPK, WIPK, NPR1, and fatty acid-amino acid conjugates participate in the induction of jasmonic acid biosynthesis by affecting early enzymatic steps in the pathway. *Plant Physiol.* **152**, 96–106.
- Kallenbach, M., Bonaventure, G., Gilardoni, P.A., Wissgott, A. and Baldwin, I.T. (2012) *Empoasca* leafhoppers attack wild tobacco plants in a jasmonate-dependent manner and identify jasmonate mutants in natural populations. *Proc. Natl Acad. Sci. USA*, **109**, E1548–E1557.
- Kang, J.-H., Liu, G., Shi, F., Jones, A.D., Beaudry, R.M. and Howe, G.A. (2010) The tomato *odorless-2* mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. *Plant Physiol.* **154**, 262–272.
- Kaur, H., Heinzl, N., Schöttner, M., Baldwin, I.T. and Galis, I. (2010) R2R3-NaMYB8 regulates the accumulation of phenylpropanoid-polyamine conjugates, which are essential for local and systemic defense against insect herbivores in *Nicotiana attenuata*. *Plant Physiol.* **152**, 1731–1747.
- Kessler, D. (2012) Context dependency of nectar reward-guided oviposition. *Entomol. Exp. Appl.* **144**, 112–122.
- Kessler, A. and Baldwin, I.T. (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, **291**, 2141–2144.
- Kessler, A., Halitschke, R. and Baldwin, I.T. (2004) Silencing the jasmonate cascade: induced plant defenses and insect populations. *Science*, **305**, 665–668.
- Kessler, D., Diezel, C. and Baldwin, I.T. (2010) Changing pollinators as a means of escaping herbivores. *Curr. Biol.* **20**, 237–242.
- Kessler, D., Bhattacharya, S., Diezel, C., Rothe, E., Gase, K., Schöttner, M. and Baldwin, I.T. (2012) Unpredictability of nectar nicotine promotes outcrossing by hummingbirds in *Nicotiana attenuata*. *Plant J.* **71**, 529–538.
- Lander, E. and Schork, N. (1994) Genetic dissection of complex traits. *Science*, **265**, 2037–2048.
- Laue, G., Preston, C.A. and Baldwin, I.T. (2000) Fast track to the trichome: induction of *N*-acetyl nornicotines precedes nicotine induction in *Nicotiana repanda*. *Planta*, **210**, 510–514.
- Lee, G., Joo, Y., Kim, S.-G. and Baldwin, I.T. (2017) What happens in the pith stays in the pith: tissue-localized defense responses facilitate chemical niche differentiation between two spatially separated herbivores. *Plant J.* **92**, 414–425.
- Li, C. (1969) Population subdivision with respect to multiple alleles. *Ann. Hum. Genet.* **33**, 23–29.
- Li, L., Li, C. and Howe, G.A. (2001) Genetic analysis of wound signaling in tomato. Evidence for a dual role of jasmonic acid in defense and female fertility. *Plant Physiol.* **127**, 1414–1417.
- Li, D., Baldwin, I.T. and Gaquerel, E. (2015) Navigating natural variation in herbivory-induced secondary metabolism in coyote tobacco populations using MS/MS structural analysis. *Proc. Natl Acad. Sci. USA*, **112**, E4147–E4155.
- Li, D., Baldwin, I. and Gaquerel, E. (2016) Beyond the canon: within-plant and population-level heterogeneity in jasmonate signaling engaged by plant-insect interactions. *Plants*, **5**, 14.
- Li, R., Wang, M., Wang, Y., Schuman, M.C., Weinhold, A., Schäfer, M., Jiménez-Alemán, G.H., Barthel, A. and Baldwin, I.T. (2017) Flower-specific jasmonate signaling regulates constitutive floral defenses in wild tobacco. *Proc. Natl Acad. Sci. USA*, **114**, E7205–E7214.
- Li, R., Llorca, L.C., Schuman, M.C., Wang, Y., Wang, L., Joo, Y., Wang, M., Vassão, D.G. and Baldwin, I.T. (2018) ZEITLUPE in the roots of wild tobacco regulates jasmonate-mediated nicotine biosynthesis and resistance to a generalist herbivore. *Plant Physiol.* **177**, 833–846.
- Luu, V.T., Weinhold, A., Ullah, C., Dressel, S., Schoettner, M., Gase, K., Gaquerel, E., Xu, S. and Baldwin, I.T. (2017) *O*-acetyl sugars protect a wild tobacco from both native fungal pathogens and a specialist herbivore. *Plant Physiol.* **174**, 370–386.
- Lynds, G.Y. and Baldwin, I.T. (1998) Fire, nitrogen, and defensive plasticity in *Nicotiana attenuata*. *Oecologia*, **115**, 531–540.
- Machado, R.A., Ferrieri, A.P., Robert, C.A., Glauser, G., Kallenbach, M., Baldwin, I.T. and Erb, M. (2013) Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. *New Phytol.* **200**, 1234–1246.
- Machado, R.A., McClure, M., Herve, M.R., Baldwin, I.T. and Erb, M. (2016) Benefits of jasmonate-dependent defenses against vertebrate herbivores in nature. *eLife*, **5**, e13720.
- Machado, R.A., Arce, C.C., McClure, M.A., Baldwin, I.T. and Erb, M. (2018) Aboveground herbivory induced jasmonates disproportionately reduce plant reproductive potential by facilitating root nematode infestation. *Plant, Cell Environ.* **41**, 797–808.
- McKey, D. (1974) Adaptive patterns in alkaloid physiology. *Am. Nat.* **108**, 305–320.
- Meldau, S., Wu, J. and Baldwin, I.T. (2009) Silencing two herbivory-activated MAP kinases, SIPK and WIPK, does not increase *Nicotiana attenuata*'s susceptibility to herbivores in the glasshouse and in nature. *New Phytol.* **181**, 161–173.
- Monte, I., Ishida, S., Zamarreño, A.M. et al. (2018) Ligand-receptor co-evolution shaped the jasmonate pathway in land plants. *Nat. Chem. Biol.* **14**, 480.
- Oh, Y., Baldwin, I.T. and Galis, I. (2012) NaJAZh regulates a subset of defense responses against herbivores and spontaneous leaf necrosis in *Nicotiana attenuata* plants. *Plant Physiol.* **159**, 769–788.
- Oh, Y., Baldwin, I.T. and Galis, I. (2013) A jasmonate ZIM-domain protein NaJAZd regulates floral jasmonic acid levels and counteracts flower abscission in *Nicotiana attenuata* plants. *PLoS One*, **8**, e57868.
- Paschold, A., Halitschke, R. and Baldwin, I.T. (2007) Co(i)-ordinating defenses: NaCOI1 mediates herbivore-induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. *Plant J.* **51**, 79–91.
- Pfalz, M., Vogel, H., Mitchell-Olds, T. and Kroymann, J. (2007) Mapping of QTL for resistance against the crucifer specialist herbivore *Pieris brassicae* in a new *Arabidopsis* inbred line population, Da (1)-12xEi-2. *PLoS One*, **2**, e578.
- Preston, C.A. and Baldwin, I.T. (1999) Positive and negative signals regulate germination in the post-fire annual, *Nicotiana attenuata*. *Ecology*, **80**, 481–494.
- Pritchard, J.K., Stephens, M. and Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Proietti, S., Caarls, L., Coolen, S., Van Pelt, J.A., Van Wees, S.C. and Pieterse, C.M. (2018) Genome-wide association study reveals novel players in defense hormone crosstalk in *Arabidopsis*. *Plant, Cell Environ.* **41**, 2342–2356.
- Rasmann, S., Chassin, E., Bilat, J., Glauser, G. and Reymond, P. (2015) Trade-off between constitutive and inducible resistance against herbivores is only partially explained by gene expression and glucosinolate production. *J. Exp. Bot.* **66**, 2527–2534.
- Roda, A., Halitschke, R., Steppuhn, A. and Baldwin, I.T. (2004) Individual variability in herbivore-specific elicitors from the plant's perspective. *Mol. Ecol.* **13**, 2421–2433.
- Schuman, M.C., Heinzl, N., Gaquerel, E., Svatos, A. and Baldwin, I.T. (2009) Polymorphism in jasmonate signaling partially accounts for the variety of volatiles produced by *Nicotiana attenuata* plants in a native population. *New Phytol.* **183**, 1134–1148.
- Schuman, M.C., Barthel, K. and Baldwin, I.T. (2012) Herbivory-induced volatiles function as defenses increasing fitness of the native plant *Nicotiana attenuata* in nature. *eLife*, **1**, e00007.
- Schwachtje, J., Minchin, P.E., Jahnke, S., van Dongen, J.T., Schittko, U. and Baldwin, I.T. (2006) SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. *Proc. Natl Acad. Sci. USA*, **103**, 12935–12940.
- Shivaji, R., Camas, A., Ankala, A., Engelberth, J., Tumlinson, J.H., Williams, W.P., Wilkinson, J.R. and Luthe, D.S. (2010) Plants on constant alert: elevated levels of jasmonic acid and jasmonate-induced transcripts in caterpillar-resistant maize. *J. Chem. Ecol.* **36**, 179–191.
- Skibbe, M., Ou, N., Galis, I. and Baldwin, I.T. (2008) Induced plant defenses in the natural environment: *Nicotiana attenuata* WRKY3 and WRKY6 coordinate responses to herbivory. *Plant Cell*, **20**, 1984–2000.
- Stitz, M., Baldwin, I.T. and Gaquerel, E. (2011) Diverting the flux of the JA pathway in *Nicotiana attenuata* compromises the plant's defense metabolism and fitness in nature and glasshouse. *PLoS One*, **6**, e25925.
- Stork, W.F., Weinhold, A. and Baldwin, I.T. (2011) Trichomes as dangerous lollipops: do lizards also use caterpillar body and frass odor to optimize their foraging? *Plant Signal. Behav.* **6**, 1893–1896.
- Wang, M., Schäfer, M., Li, D. et al. (2018) Blumenlons as shoot markers of root symbiosis with arbuscular mycorrhizal fungi. *eLife*, **7**, e37093.
- Wasternack, C. (2015) How jasmonates earned their laurels: past and present. *J. Plant Growth Regul.* **34**, 761–794.
- Wasternack, C. and Feussner, I. (2018) The oxylipin pathways: biochemistry and function. *Annu. Rev. Plant Biol.* **69**, 363–386.

- Wasternack, C. and Hause, B.** (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *annals of botany*. *Ann. Bot.* **111**, 1021–1058.
- Weinhold, A. and Baldwin, I.T.** (2011) Trichome-derived *O*-acyl sugars are a first meal for caterpillars that tags them for predation. *Proc. Natl Acad. Sci. USA*, **108**, 7855–7859.
- Whiteman, N.K., Groen, S.C., Chevasco, D., Bear, A., Beckwith, N., Gregory, T.R., Denoux, C., Mammarella, N., Ausubel, F.M. and Pierce, N.E.** (2011) Mining the plant–herbivore interface with a leafmining *Drosophila* of *Arabidopsis*. *Mol. Ecol.* **20**, 995–1014.
- Wingler, A., Juvany, M., Cuthbert, C. and Munné-Bosch, S.** (2014) Adaptation to altitude affects the senescence response to chilling in the perennial plant *Arabidopsis alpina*. *J. Exp. Bot.* **66**, 355–367.
- Winz, R.A. and Baldwin, I.T.** (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. IV. Insect-induced ethylene reduces jasmonate-induced nicotine accumulation by regulating putrescine *N*-methyltransferase transcripts. *Plant Physiol.* **125**, 2189–2202.
- Wu, J., Hettenhausen, C., Schuman, M.C. and Baldwin, I.T.** (2008) A comparison of two *Nicotiana attenuata* accessions reveals large differences in signaling induced by oral secretions of the specialist herbivore *Manduca sexta*. *Plant Physiol.* **146**, 927–939.
- Xiong, Q., Ma, B., Lu, X. et al.** (2017) Ethylene-inhibited jasmonic acid biosynthesis promotes mesocotyl/coleoptile elongation of etiolated rice seedlings. *Plant Cell*, **29**, 1053–1072.
- Xu, L., Liu, F., Lechner, E., Genschik, P., Crosby, W.L., Ma, H., Peng, W., Huang, D. and Xie, D.** (2002) The SCF<sup>COI1</sup> ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis*. *Plant Cell*, **14**, 1919–1935.
- Yang, D.-H., Hettenhausen, C., Baldwin, I.T. and Wu, J.** (2012) Silencing *Nicotiana attenuata* calcium-dependent protein kinases, *CDPK4* and *CDPK5*, strongly up-regulates wound- and herbivory-induced jasmonic acid accumulations. *Plant Physiol.* **159**, 1591–1607.
- Yon, F., Joo, Y., Cortés Llorca, L., Rothe, E., Baldwin, I.T. and Kim, S.-G.** (2016) Silencing *Nicotiana attenuata* *LHY* and *ZTL* alters circadian rhythms in flowers. *New Phytol.* **209**, 1058–1066.
- Yoshida, Y., Sano, R., Wada, T., Takabayashi, J. and Okada, K.** (2009) Jasmonic acid control of *GLABRA3* links inducible defense and trichome patterning in *Arabidopsis*. *Development*, **136**, 1039–1048.
- Yu, J., Pressoir, G., Briggs, W.H. et al.** (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet.* **38**, 203.
- Zhou, W., Brockmüller, T., Ling, Z., Omdahl, A., Baldwin, I.T. and Xu, S.** (2016) Evolution of herbivore-induced early defense signaling was shaped by genome-wide duplications in *Nicotiana*. *eLife*, **5**, e19531.
- Zhou, W., Kügler, A., McGale, E. et al.** (2017) Tissue-specific emission of (*E*)- $\alpha$ -bergamotene helps resolve the dilemma when pollinators are also herbivores. *Curr. Biol.* **27**, 1336–1341.
- Zhurov, V., Navarro, M., Bruinsma, K.A. et al.** (2014) Reciprocal responses in the interaction between *Arabidopsis* and the cell-content-feeding chelicerate herbivore spider mite. *Plant Physiol.* **164**, 384–399.