this end, we are using a genetically tractable gnotobiotic animal model: *Drosophila melanogaster*, which is mono-associated with one of its natural dominant commensal, *Lactobacillus plantarum* (Fig. 1). We are developing multiscale functional approaches to identify the mechanisms that underlie their mutualistic relationship, which results in the promotion of host juvenile growth upon chronic undernutrition. Our approaches aim at identifying both the bacterial and host genetic networks required to sustain their mutualistic relationship.

**Disclosure of interest**  The author declares that he has no competing interest.

**Further reading**  M.E. Martino, P. Joncour, R. Leenay, H. Gervais, M. Shah, S. Hughes, B. Gillet, C. Beisel, F. Leulier, Bacterial Adaptation to the Host’s Diet Is a Key Evolutionary Force Shaping *Drosophila*-*Lactobacillus* Symbiosis, Cell Host Microbe 24. Published online 28 June 2018, 10.1016/j.chom.2018.06.001,


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17  What five insects told us about how a native plant copes with real-world problems  Ian Thomas Baldwin  Max Planck Institute for Chemical Ecology, Jena, Germany  E-mail address: baldwin@ice.mpg.de

Five native insects have taught us about traits that are essential for the survival of a native tobacco plant, *Nicotiana attenuata*, which lives in the Great Basin Desert of the USA. The five insects come from different feeding guilds, attack different tissues at different developmental stages and have revealed different traits that are essential for plant survival. For example, this plant recognizes attack from a specialized Lepidopteran herbivore (*Manduca sexta*) by the particular chemistry of the herbivore’s saliva, and uses this recognition to tailor a complex 6-layered suite of responses that involves a remodeled transcriptome, metabolome and proteome, as well as some of its life history traits. The plant traits revealed by the remaining four insects will be described below, and how these insects will be used as high-throughput (HTP) screeners of large plantations of transformed and recombinant inbred lines of this native plant, to reveal the function of genes required for survival in nature. Insects are some of the best plant biologists on our planet and it behooves plant biologists to rediscover the lost art of natural history discovery to take advantage of the unique skill-sets of insects.

**The plant and its insect herbivore community**  *Nicotiana attenuata* (coyote tobacco: Solanaceae) is a diploid tobacco native to the Great Basin Desert of North America. It enjoys a unique position scientifically, because hundreds of transgenic lines have been studied over the past two decades to understand the fitness consequences of precisely defined changes in gene expression for plants growing in their native habitats. For more than 30 years, the plant has been an ecological model system to study the complex plant-mediated interactions that occur in nature and to elucidate the genetic basis of traits important for the Darwinian fitness of plants. The coyote tobacco is diploid, largely selling plant as a model system is ground to its natural history and unusual germination behavior. Dormant seeds, survivors of decades or even centuries of microbial challenges in soil, germinate synchronously into the nitrogen-rich, competition-free environments that are commonly found after fires. Dense *N. attenuata* populations then develop, in which all plant taxa have not only conspecific competition, but also to attack from as many as 34 different herbivore and pathogen taxa that colonize these post-fire populations from adjacent unburned areas. These are the ecological conditions in which agriculture started, as slash-and-burn agriculture, and are the conditions that our crops face. However, most crop taxa have not originated from post-fire adapted native species and are unlikely to be pre-adapted to the conditions of the agricultural niche. By understanding the specific traits that this native tobacco plant has evolved to optimize its fitness in the post-fire environment in which it times its germination and growth from long-lived seedbanks, we hope to learn what will be required to make our crop plants more self-sufficient, less dependent on agricultural inputs.

*Nicotiana attenuata* hosts a diverse insect herbivore community that includes several specialists on the Solanaceae: *Corineura niscata* (Hemiptera), *Epitrix hirtipennis* and *E. subcinctria* (Coleoptera: Chrysomelidae), two sympatric sibling species of *Trichobasis*, *T. compacta*, and *T. mucorea* (Coleoptera: Curculionidae), *Tupiocoris notatus*, (Hemiptera: Miridae), *Manduca quinquemaculata*, and *M. sexta* (Lepidoptera: Sphingidae) as well as the generalist herbivores *Spodoptera spp.* (*Lepidoptera*: *Nocuidae*), *Trimerotropis sp.* (*Orthoptera*: *Acrididae*), and *Oecanthus spp.* (tree crickets (*Orthoptera*: * Gryllidae*) and the opportunistic generalist herbivores *Empoasca spp.* (Hemiptera: *Cicadellidae*) and *Heliothis spp.* (*Lepidoptera*: *Nocuidae*). *Geocoris pallens* is a common predator of herbivores on *N. attenuata*.

Five of these insects (four herbivores and one predator) have proven to be very useful in eliciting and responding to plant traits, characteristics that make them very useful as High-Through-Put (HTP) screeners and sentinel insects for large forward-genetic Recombinant Inbred Line (RIL) populations that will be described after these five insects are introduced. The herbivores: *Manduca, Empoasca, Tupiocorus, and Trichobasis spp.*

*Manduca* spp. The tobacco and tomato hornworms (*M. sexta* and *M. quinquemaculata*) have evolved an unprecedented capacity to detoxify the signature defense metabolite of the genus, nicotine. *M. sexta* larvae excrete the vast majority of the nicotine that they ingest with their food, but also co-opt a small fraction of the ingested nicotine for their own defense [1]. *M. sexta* larvae are found on *N. attenuata* plants because the adult moths oviposit while nectaring on the flowers. Hence, this insect plays dual roles in the biology of *N. attenuata* plants, as a devastating herbivore that elicits a complex suite of defensive responses on the larval stages and as an important pollinator as an adult, vectoring gametes among the spatially separated plant populations that occur after fires, likely adding important genetic diversity to the seeds that must survive the decades of dormancy in the seed banks. Attack from these nicotine-adapted larvae is significantly recognized by the plant, when fatty acid amino acid conjugates (FACs) in the larval oral secretions are introduced into wounds when the larvae feed. This FAC-mediated recognition by the plant results in a six-layered suite of defense, avoidance, and tolerance responses (summarized in an *iBiology* talk available at https://www.ibiology.org/plant-biology/studying-plants-ecological-interactions-genomics-era-story-nicotiana-attenuata/#part-2), which engages all aspects of the plant’s physiology. As such, *Manduca* larvae are ideal elicitors of ecologically important plant phenotypes and these phenotypes can be elicited by introducing larval oral secretions into plant wounds that can be readily accomplished in a HTP manner for the large numbers of plants in a MAGIC population.

*Empoasca* spp. These piercing–sucking leafhoppers “eavesdrop” on the jasmonate (JA)-mediated signaling capacities of their host plants, preferentially selecting those hosts that are deficient in jasmonate accumulations [2]. Given that JA signaling mediates the vast majority of plant defenses, it is particularly intriguing that this natural phenotyping “bloodhound” specif-
ically targets JA signaling, apparently independently of the downstream defense metabolites that are regulated by JA signal-
ring. Hence this insect can be used as a phenotyping tool to rapidly
identify JA-deficient accessions in natural populations and
the large number of plants in MAGIC populations. All that
is required is an accurate quantification of Em poasca feeding
damage after an experimental release of these herbivores into
a population. T upio ricor is n otatus. These free-living mirid herbivores manip-
ulate the plant’s cytokinin signaling, and in the process have
lifted a page from the playbook of endophytic insects. Endo-
phytic insects, because they are embedded within plant tissues,
cannot easily move to other tissues, but instead have evolved
the ability to modify their hosts’ physiology to create metabolic
“sinks” in the very tissues they infest. They do this in part
by manipulating cytokinin signaling, which can transform a
“source”, which is a plant organ that normally produces
sugars—like a mature leaf—into a “sink”, where sugars are stored
or consumed, as in fruits and young leaves. These mirids pro-
duce their own cytokinins, transferring these phytohormones
to plants during feeding to increase food quality and minimize
the activation of senescence processes that are part of a plant’s
generalized defense response [3]. In the field, T. notatus prefer-
entially attacks leaves with transgenically increased CK levels,
and plants with transgenically abrogated CK-perception are
less tolerant of T. notatus feeding damage. Hence, by monitoring
mirid feeding damage on the many plants of MAGIC popula-
tions, we would be able to rapidly identify RILs altered in
cytokinin production, detoxification and perception. Again, all
that is required is an accurate quantification of T upio ricor is
feeding damage after an experimental release of these herbivores
into a population. T richo baris m ucorea. The adult females of this weevil species
oviposit their eggs into basal parts of elongating stems, early
in the growing seasons as plants begin their transitions from
vegetative to reproductive growth. The hatched neonate weev-
ils burrow into stems to feed on the pith of elongating stems,
all without damaging the plant’s vasculature. These weevils
manage to complete their life cycles with few negative conse-
cuences for their hostplant’s fitness, and adults emerge after
overwintering in completely senescent stems, to mate and
oviposit on a next generation of plants. Larvae move up and
down the stem, elicting the production of phenolic defenses in
the pith, eating fresh pith as the stems grow, and consuming
their own frass in the process [4–6]. While unstudied, the lar-
vae’s coprophagic behavior is likely important for the larva’s
ability to complete all four instars and pupate into an adult
while only feeding on the limited amount of pith available
in plant stems. Recent unpublished data suggests that the larvae
use a bacterial microbiome to combat fungal infections that
are readily acquired while feeding in stems, in a manner reminis-
cent of that of its host plant’s use of bacterial microbiomes to
combat fungal phytopathogens [as described in [7] and in Lee
et al., unpublished]. The larvae’s bacterial microbiome appears
to be transferred to the pith via the egg, and eggs are readily
sterilized by a superficial bleach treatment. Hence, by compar-
ing the fate of experimental ovipositions of both sterile and
nonsterile eggs into the stems of the plants of a MAGIC pop-
ulation, one could potentially identify genetic loci involved in
the recruitment of entomopathogenic fungi as plant defenses,
and as well as loci that facilitate the coprophagic behaviors of
the larvae, which in turn likely allow plants to tolerate Trichobaris
infestations without significant fitness consequences. The predators: Geocorus spp. G. pallens and G. punctipes can be
found on N. attenuata or neighboring plant species through-
out N. attenuata’s habitat in the Great Basin Desert. The adults and
larvae are acutely responsive to the volatiles emitted from N. attenuata after herbivory, learning to associate the
release of particular mono- and sesquiterpenes and green leaf
volatiles with the presence of feeding herbivores (their prey),
and use this information to optimize their hunting behavior
and thereby mediating an important indirect defense for the
plant [8,9]. A simple, HTP egg predation bioassay has been
developed that provides quantitative information about the
feeding behavior of these otherwise difficult-to-observe “bi-
ed” hemipteran predators in the field, and this assay was
recently used to successfully input the genomic location for a
linalool-synthase from a RIL population [Li et al., in review]. We plan to use this egg-predation assay to assess the feeding
behavior of native Geocorus predators with MAGIC populations planted
into natural habitats to identify genes that regulate this impor-
tant means of indirect defense for plants. In summary, these four herbivorous and one carnivorous insect species respond to and elicit remarkably complex traits in
plants, traits that would be onerous, if not impossible, to iden-
ify in a HTP manner. Forward-genetic tools that capture the
natural genetic variation of a species are one of the most suc-
cessful means of identifying genetic controls over phenotypes
that are useful for phenotype manipulations. This is because
natural selection selects for traits that increase an organisms’
Darwinian fitness, and the genes responsible for natural varia-
tion have already been tested under real-world conditions. By
providing HTP means of screening, the large number of plants
required by these forward-genetics tools, sentinel insects can
provide plant biologists with a means of understanding the
microcosms behind the regulation of complex traits responsible
for the ecological sophistication of plants. The forward-genetics tool: The magic population
Linkage or QTL mapping, and association mapping or GWAS
are the two most commonly used forward-genetics statistical
frameworks to query the genetic architecture of underlying traits. While GWAS can identify associative SNPs with one
base pair precision, the procedure has limitations that make it
difficult to use with the highly structured natural popu-
lations 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. A decade ago,
a nine-year effort was initiated to create a 26-parent Multi-
parent Advanced Generation Inter Cross (MAGIC) population
that captures the natural variation of the N. attenuata species.
More than 230 accessions from seed collections representa-
ing 60 sites across the species’ range were phenotyped in
a glasshouse common-garden experiment, using comprehen-
sive metabolomics, growth and morphology screens to identify
26 parental accessions that captured the species ecological
phenotypic diversity. These diverse founder plants were used to
create the MAGIC population, which involved all possible

![Image](331x175 to 547x361)

Fig. 1 Five entomological screeners of a native plant. Nico-
tiana attenuata plants growing in a native population in the
Great Basin Desert (bottom photo: G. Baldwin) are regu-
larly attacked by Em poasca spp., Tupiocor is n otatus, M anduca
spp., and Tricho baris spp. insect herbivores (middle photos: A.
Kessler), which in turn are attacked by Geocorus spp. (top photo:
A. Kessler) hemipteran predators.)
unidirectional binary matings, four generations of intercrossing and six generations of inbreeding of 1950 RILs to ensure ~99% homozygosity across all loci.

Pending successful funding, we plan to plant this MAGIC population into two field sites in the Great Basin Desert, and recruit the services of the above-mentioned five entomological screeners (Fig. 1) to identify genetic loci that regulate the complicated ecological traits that native plants use to optimize their fitness in natural environments.

Disclosure of interest The author declares that he has no competing interest.

References

Further reading
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18
Évolution et spécificité des interactions insectes hôtes–insectes parasitoïdes
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Charles Darwin parlait ainsi de certains insectes hyménéop- tères parasitoïdes: « Je ne peux pas me persuader qu’un Dieu bénévole et omnipotent aurait créé volontairement les Ich- neumonidae avec l’intention exprès qu’ils se nourrissent du corps vivant des chenilles. » Mais qu’est-ce qu’un parasitoïde? Il s’agit d’un organismue au mode de vie à moitié parasite – il se développe au contact et aux dépens de son hôte – et à moitié prédateur, car son développement conduit généralement à la mort de l’hôte, dont il consomme les tissus. Les parasitoïdes sont pour la majorité d’étre insectes. On les rencontre dans six ordres d’insectes différents, mais plus particulièrement dans l’ordre des Hyménoptères, au sein duquel 80 % des espèces seraient des parasitoïdes. Ils parasitent des hôtes arthropodes et sont donc des régula- teurs naturels de leurs populations ainsi que des auxiliaires importants en lutte biologique. Ils on en effet l’avantage d’être plus spécifiques que les prédateurs. Les parasitoïdes peuvent se développer à la surface de l’hôte (« ectoparasitoïdes ») ou à l’intérieur du corps de leur hôte (« endoparasitoïdes »). Ils peuvent être solitaires (un seul parasitoïde adulte émergera de l’œuf du prédateur) ou grégaires (plusieurs parasitoïdes se développeront dans un même hôte).

Ce résumé présente trois aspects des recherches de l’équipe sur les interactions immunitaires entre des guêpes endopara- sitoïdes et leurs hôtes drosophiles. La réponse immunitaire au parasitisme est décrite dans un premier temps pour apporter le contexte nécessaire.

Face à un corps étranger de grande taille comme un œuf d’endoparasitoïde, la plupart des insectes mettent en place une réponse d’encapsulation. Elle met en jeu des cellules circu- lantes ou hémocytes, parmi lesquelles les plasmatocytes qui formeront une première couche de cellules autour de l’œuf du parasitoïde. Sur cette couche cellulaire viendront s’agréger les lamellocytes qui formeront les couches cellulaires suivantes. Les lamellocytes sont typiques du sous-groupe melanogaster de drosophiles et ils sont produits essentiellement en réponse au parasitisme. L’encapsulation implique aussi la mélanisa- tion de la capsule qui fait intervenir des enzymes appelées phénol oxydases (PO; proenzymes PPO). PPO3 est produite par les lamellocytes, PPO1 et PPO2 étant stockées sous forme de cristaux dans les cellules à cristaux qui font partie des hémoco- ytes, PPO1 et PPO2 sont activées par une cascade de protéines à séries régulées par des serpines. Les radicaux cytosotiques produits par la cascade PO (conduisant à la mélanisation) sont supposés tuer l’œuf ou la larve du parasitoïde. Les par- asitoïdes du genre Leptopilina, qui se développent dans des drosophiles, peuvent cibler ces différents composants immu- nitaris. Ils injectent par exemple des molécules (Rho GAPs) capables d’inhiber les GTFases Rac1 et Rac2 de D. melanogaster, qui sont nécessaires à la réaction d’encapsulation, ou des serpines qui inhibent l’activation des phénol oxydases. Dans la larve de drosophile, stade parasité par les Leptopilina, les hémocytes sont présents dans deux compartiments: (i) des ilots sessiles sous-cuticulaires au niveau de chaque segment larvaire, dans lesquels on trouve des plasmatocytes et des cel- lules à cristaux–les lamellocytes peuvent se transdifférencier à partir des plasmatocytes sessiles mis en circulation après parasitisme; (ii) la glande lymphatique, composée d’une série de lobes postérieurs contenant des prohémocytes et de deux lobes antérieurs contenant des hémocytes dans la zone médullaire et des hémocytes différenciés dans la zone corti- cale. La différenciation est contrôlée par des signaux produits par un groupe de cellules formant le PSC (Posterior Signaling Center).

Notre équipe s’intéresse aux bases génétiques de la résistance de D. melanogaster à un type de souche du parasitoïde Lep- topilina bouardi. Nous avons pu identifier un candidat potentiel dans le gène majeur de résistance caractérisé (un gène à deux allèles, l’allèle résistant étant dominant sur l’allèle sensible), edl/mae, qui code pour une protéine contenant un domaine SAM d’interaction protéine-protéine. La protéine Edl/mae est un interactant et un régulateur de Pointed P2 et Aop/Van, deux protéines jouant un rôle dans le développement et la différenciation/prolifération cellulaire. L’analyse du phéno- type immunitaire entre souche résistante et sensible met en