

Plants and tortoises: mutations in the *Arabidopsis* jasmonate pathway increase feeding in a vertebrate herbivore

ALIA MAFLI,* JÉRÔME GOUDET* and EDWARD E. FARMER†‡

*Department of Ecology and Evolution, Biophore, University of Lausanne, 1015 Lausanne, Switzerland, †Department of Plant Molecular Biology, Biophore, University of Lausanne, 1015 Lausanne, Switzerland, ‡College of Science, King Saud University, P. O.Box 2455, Riyadh 11451, Saudi Arabia

Abstract

Photosynthetic tissues, the major food source of many invertebrates and vertebrates, are well defended. Many defence traits in leaves are controlled via the jasmonate signalling pathway in which jasmonate acts as a hormone by binding to a receptor to activate responses that lead to increased resistance to invertebrate folivores. We predicted that mutations in jasmonate synthesis might also increase the vulnerability of leaves to vertebrate folivores and tested this hypothesis using the Eastern Hermann's tortoise (*Eurotestudo boettgeri*) and an *Arabidopsis thaliana* (Brassicaceae) *allene oxide synthase* (*aos*) mutant unable to synthesize jasmonate. Tortoises preferred the *aos* mutant over the wild type (WT). Based on these results, we then investigated the effect of mutating jasmonate perception using a segregating population of the recessive *A. thaliana* jasmonate receptor mutant *coronatine insensitive1-1* (*coi1-1*). Genotyping of these plants after tortoise feeding revealed that the homozygous *coi1-1* receptor mutant was consumed more readily than the heterozygous mutant or the WT. Therefore, the plant's ability to synthesize or perceive jasmonate reduces feeding by a vertebrate herbivore. We also tested whether or not tortoise feeding behaviour was influenced by glucosinolates, the principal defence chemicals in *Arabidopsis* leaves with known roles in defence against many generalist insects. However, in contrast to what has been observed with such insects, leaves in which the levels of these compounds were reduced genetically were consumed at a similar rate to those of the WT.

Keywords: *Arabidopsis*, *Eurotestudo*, folivory, jasmonate, Jasmonic acid, *Testudo*

Received 1 November 2011; revision revised 3 February 2012; accepted 9 February 2012

Introduction

In terms of mass consumed, leaves are among the most important food sources for invertebrates and vertebrates alike (Walters 2011). However, many properties of these organs are known to influence herbivore feeding. For example, the feeding preferences of vertebrate leaf-eaters can relate to fibre content and overall leaf digestibility (Van Soest 1996), or to the leaf's contents in defence chemicals (Dearing *et al.* 2005; Rosenthal & Berenbaum 1991), and folivores constantly optimize

their leaf intake to get the best out of these seasonally variable food sources. In addition to seasonal changes in leaf quality, leaves are able to regulate their defence-related properties using hormone-based signal pathways that control gene expression. One such mechanism involves the jasmonate signal pathway, jasmonates being fatty acid-derived signals that control many defence responses in leaves (Browse 2009) including those of the genetic model *Arabidopsis thaliana* (Acosta & Farmer 2010). Extensive experimentation has shown that the jasmonate pathway greatly increases the resistance of leaves to certain invertebrates including many chewing insects (Howe & Jander 2008; Kessler & Baldwin 2002). However, despite intensive investigation

Correspondence: Edward E. Farmer, Fax: +41 21 692 4195; E-mail: edward.farmer@unil.ch

of the role of the jasmonate pathway in defence against invertebrates, equivalent efforts to understand the effects of the jasmonate pathway on folivory by vertebrates have not been reported. More generally, we have yet to take full advantage of plant genetic models to address questions such as how do defence signal transduction pathways affect feeding preferences in vertebrates.

Here, using both a jasmonate biosynthesis mutant as well as a population of plants that segregate for a single nonsense mutation in a jasmonate receptor gene, we have specifically targeted the processes of jasmonate synthesis and jasmonate receptor function in an investigation of whether a signal pathway that is known to regulate leaf defences against invertebrates can also affect the feeding of a vertebrate. Leaf-eating vertebrates are a diverse group, and they are represented among animals as different as primates, ungulates, rodents, marsupials etc. through to reptiles and other ectotherms. Reptiles including tortoises were a once far more species-rich, numerous and widespread group of folivores, but their populations have declined massively over the last few thousand years with tortoise species from Europe being no exception (Case *et al.* 1998). However, in some parts of the world tortoises still exert heavy pressure on plants, strongly modelling the flora of a few isolated islands (e.g. Grubb 1971). For the present study, we chose to use the Eastern Hermann's tortoise *Eurotestudo boettgeri* Bour. (synonyms: *Testudo boettgeri*, Bour; *Testudo hermanni boettgeri* Bour), a generalist herbivore that displays a high degree of folivory. It is not highly prone to stress when handled, can be bred readily in captivity and shows no obvious dietary sexual dimorphism. Moreover, this tortoise has a geographical range extending from Montenegro, Albania, East Serbia and Macedonia to Bulgaria, Romania and parts of western Turkey (Vetter 2006), overlapping with the distribution of *A. thaliana* (Koornneef *et al.* 2004). In captivity, *E. boettgeri* will eat the leaves of plants in the Brassicaceae family including radish (*Raphanus sativus*) and cabbage (*Brassica oleracea*; Vetter 2006). In this study, we found that *A. thaliana*, a plant from the same family, was consumed readily, although we are unaware of reports of the tortoise eating this plant in the wild.

As a first step, we investigated tortoise feeding on an *allene oxide synthase* (*aos*) mutant that lacks the ability to catalyse a vital step in jasmonate synthesis (the rearrangement of fatty acid 13-hydroperoxides into 12,13(S) allene oxides; Lee *et al.* 2008). Then, we asked whether a jasmonate receptor mutation in *Arabidopsis* affected tortoise feeding. For this, we employed the *A. thaliana* mutant *coronatine insensitive1-1* (*coi1-1*; Xie *et al.* 1998). A single mutation in the *COI1* gene inactivates the entire jasmonate perception complex, rendering plants fully insensitive to the jasmonates they produce them-

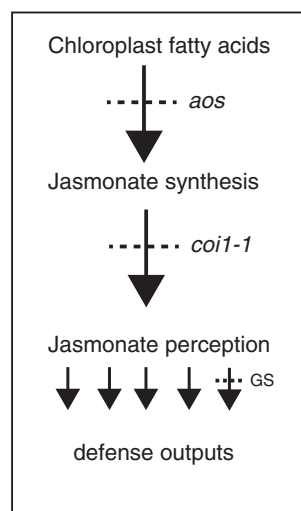


Fig. 1 Scheme showing the place of mutants in jasmonate synthesis and perception relative to downstream defence output in leaves. *aos*, *allene oxide synthase*; *coi1-1*, *coronatine insensitive1-1*. The jasmonate pathway controls the expression of multiple defence traits. One of these, leading to glucosinolate synthesis, is indicated with 'GS'.

selves as well as to any external source of jasmonate (Browse 2009; Acosta & Farmer 2010). The position of the jasmonate mutants used (*aos* and *coi1-1*) is shown in Fig. 1. The figure illustrates the fact that jasmonate synthesis and perception are upstream of diverse leaf defences only some of which have been characterized in *Arabidopsis*. Among these are nitrogen- and sulphur-containing chemicals known as glucosinolates that play important and varied roles in defence against diverse invertebrates (e.g. Müller *et al.* 2010). Two major families of these compounds are made in *Arabidopsis* leaves: those with aliphatic side chains and those with indole side chains (Wittstock & Burow 2010). Both groups of glucosinolates can act as feeding stimulants for insect herbivores that are specialists on crucifers. In contrast, the two families of glucosinolates found in *Arabidopsis* leaves have differential effects on generalist invertebrates. In some cases, both groups of compound reduce weight gain in generalist insects and in other cases only one of the two chemical groups does this (Müller *et al.* 2010). We used well-characterized mutants lacking the ability to produce one or both of these two subfamilies to further investigate the tortoises' feeding preferences.

Materials and methods

Plant genotypes and tortoises

The *Arabidopsis thaliana* (L.) Heynl. reference genotype was wild type (Col-0). We used the *allene oxide synthase*

(*aos*) mutant from Park *et al.* (2002). This original mutant is in a trichomeless (*glabrous1*) genetic background, and the plants we used had been backcrossed to our Col-0 reference line to eliminate this secondary mutation and to restore trichomes (Mène-Saffrané *et al.* 2009). The inability to synthesize jasmonate causes male infertility in *aos* although this can be restored by treating developing flowers with methyl jasmonate (Acosta & Farmer 2010; Park *et al.* 2002). In this way, *aos* was maintained as the homozygote. A feature of *aos* is that it grows faster than the wild type (WT) and it has been shown that, at 45 days, the *aos* mutant has an average five more leaves than the former (Yan *et al.* 2007). The planting of WT and *aos* was therefore staggered (*aos* were germinated 4 days after the WT) so that leaf number, size and overall appearance of the plants were similar. The *coi1-1* mutation (Xie *et al.* 1998) in the trichome-bearing Col-0 genetic background was used to eliminate the functional jasmonate receptor. In contrast to *aos*, homozygous *coi1-1/coi1-1* cannot be propagated from seed because its male sterility cannot be rescued with jasmonate treatments. We therefore used a population of the recessive *coi1-1* allele, which segregates in a simple Mendelian manner: WT (25%), heterozygotes (50%) and homozygotes (25%).

To reduce the levels of glucosinolates, we used plants with lower-than-WT levels of aliphatic (*myb28/myb29K*; Sønderby *et al.* 2007) or indole glucosinolates (*cyp79B2 cyp79B3*; Zhao *et al.* 2002) and the quadruple mutant (*myb28/29K cyp79B2cyp79B3*) strongly depleted in both classes of chemical (Sun *et al.* 2009). All plants were soil-grown under short-day conditions (10 h light, 100 $\mu\text{E}/\text{m}^2/\text{s}$). All experiments used rooted 35- to 42-day old plants and, at this age, some leaves in the rosette exceeded the pot diameter (7 cm). Glucosinolate mutants have visible phenotypes and were, as far as possible, size-matched with the WT. The enclosure boxes used for experiments were filled to a depth of 10 cm with soil into which the pots were sunk so that rosettes were level with the soil surface. Tortoises (*Eurotestudo boettgeri* Bour.) weighing between 75 and 177 g were maintained outside, allowed to forage on a grass lawn and fed a supplement of *Taraxacum officinale* leaves. During maintenance, the tortoises did not have access to Brassicaceae plants.

Experiments

Experiments with *aos* plants lacking the ability to make jasmonates were conducted in boxes of the following internal dimensions: 30 (height) \times 56.5 \times 76.5 cm containing three plants of each of the two genotypes (WT and *aos*) spaced 13 cm apart and displayed at random in a different pattern in each replicate. Each replicate

used an individual tortoise (89–177 g; four females, three males) and, in total, eight replications were performed using seven individual tortoises (i.e. one tortoise was used twice) for data shown in Fig. 2A. Therefore, 24 plants of each genotype were tested in total. Each of the experiments with *coi1-1* and with glucosinolate mutants were conducted with 20 plants that were displayed at 20 cm intervals to single tortoises in a box of internal dimensions 30 (height) \times 100 \times 110 cm. For jasmonate receptor mutant experiments, the 20 plants consisted of a population segregating for *COI1/COI1*, *coi1-1/COI1* and *coi1-1/coi1-1* mutant plants. This experiment was repeated three times, each with a different tortoise (83–125 g; two females, one male), that is, a total of 60 plants were analysed for data in Fig. 2B. As the different genotypes in this *coi1-1* experiment were visually indistinguishable, plants were genotyped with

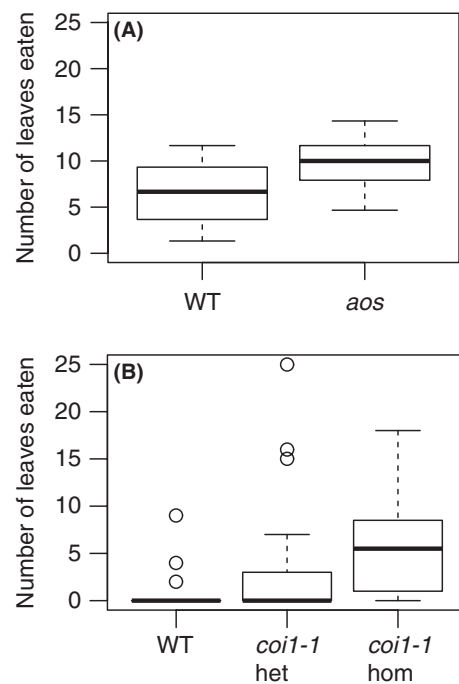


Fig. 2 Mutants in jasmonate synthesis and jasmonate receptor function are eaten more readily than the WT by tortoises. (A) Boxplot showing that *Eurotestudo boettgeri* shows a preference for the leaves of the jasmonate synthesis mutant *aos* over those of the WT. The ordinate gives the mean number of leaves per plant that had >50% of the lamina eaten. Data are from 8 replicated trials each with three plants of each genotype. Tortoises showed a significant preference for *aos* ($P < 0.05$). (B) Tortoises showed a preference for jasmonate perception mutants in a plant population segregating for the *coi1-1* allele. Boxplot showing the number of severely damaged leaves (>50% of the lamina eaten) per plant for the following genotypes. WT, wild type; het, heterozygote; hom, homozygous *coi1-1* mutant. Feeding on the WT and homozygous *coi1-1* differed significantly, while feeding on the heterozygote showed no significant difference with the WT and only a marginal difference with the homozygote.

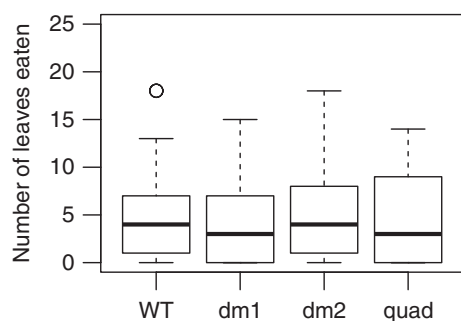


Fig. 3 Tortoises do not show a strong preference for plants lacking glucosinolates. The boxplot shows the number of leaves (per plant) on which >50% of the lamina was eaten. WT, wild type; dm1, plants lacking aliphatic glucosinolates; dm2, plants lacking indole glucosinolates; quad, quadruple mutant lacking both classes of compound.

a cleaved amplified polymorphic sequence (CAPS) procedure for the *coi1-1* allele described in Xie *et al.* (1998). The primers used in this test amplify DNA that, when cut with the *Xcm1* restriction enzyme, yields a unique 1.5 kb DNA fragment from *coi1-1* and not from the WT. This fragment, as well as smaller fragments from the WT *COI1* allele, can be visualized after electrophoresis using ethidium bromide staining. In this way, all genotypes (WT, heterozygote and homozygote) can be distinguished.

For testing glucosinolate mutants, five plants of each genotype (WT, *myb28/29K*, *cyp79B2cyp79B3* and *myb28/29K cyp79B2cyp79B3* i.e. 20 plants in total) and placed at random were offered to individual tortoises. The experiment was repeated five times, each time with a different tortoise (75–153 g, one female, four males) so that data in Fig. 3 represent 100 plants. All experiments were terminated when approximately 20% of plant material had been consumed. WT, the *aos* mutant, (*COI1/COI1*), *coi1-1/COI1* and *coi1-1/coi1-1* had similar growth forms, but the leaves of all glucosinolate mutants were found to overlap more than those of the WT, *aos* and *coi1-1*. That is, when viewed from above, the laminas of the expanded leaves in the glucosinolate mutants slightly occluded one another. This became more pronounced with leaf age and therefore precluded using image analysis to assess leaf damage. Therefore, for all experiments, we chose to score individual leaves that had lost >50% of lamina surface with respect to undamaged reference plants. Leaves that were broken off but not consumed were not counted.

Statistical analyses

Statistical analyses were conducted using the R program (R Development Core Team, 2011). Data from the first experiment comparing WT and *aos* were analysed using

a paired *t*-test, the response variable being the log of the number of leaves eaten more than 50% and tortoises representing the pairing unit. For the second and third experiments, the effect that the *COI* genotype (second experiment) or the three glucosinolate mutants (third experiment) had on the log of the number of leaves eaten more than 50% (*Y*) was analysed using a two-way crossed ANOVA, with tortoise (*T*) as a random effect and genotypes (*g*) as a fixed effect using the model ($Y_{ijk} = \mu + g_i + T_j + (gT_{ij}) + e_{ijk}$). The mean square of the genotype effect was compared with the mean square of the interaction between tortoise and genotype while the mean square of the random effect (tortoise) was tested against the error mean square using the *lm* function of R. As we found significant genotype effects, we used a Tukey honestly significant difference test (Yandell 1997) to delineate how the genotypes affected the feeding behaviour.

Results

Under the plant growth conditions we employed (10-h day length), the plants used produced only vegetative tissue. All experiments were conducted during daylight hours in a greenhouse. When offered these plants, the tortoises fed only on rosette leaves and were not observed to excavate and consume root material. During the experiments, the tortoises typically alternated between short periods of feeding punctuated by longer periods of stasis. Most feeding was on fully expanded leaves at the periphery of the rosettes with the tortoises often consuming one or a few of these larger leaves before moving to other plants. The duration of the experiments was variable depending on tortoise activity and ranged from 30 min to 8 h 30 with a mean of approximately 4 h. In the first series of experiments we compared feeding on WT and on *aos*, a mutant that cannot synthesize jasmonates. A one-sided paired *t*-test of the null hypothesis that tortoises eat WT and *aos* leaves equally was rejected with the tortoises showing a preference for *aos* leaves over the WT ($T = 2.1098$, $df = 6$, P -value = 0.04; Fig. 2A).

After these two-genotype (WT and *aos*) experiments, we switched to more complex experimental designs using either three or four genotypes simultaneously. First, to assess the effects of a mutation that affects jasmonate perception we used the mutant *coi1-1* (Xie *et al.* 1998). Tortoises were allowed to feed on a segregating population of WT (*COI1/COI1*), *coi1-1/COI1* and *coi1-1/coi1-1* plants, then the plants were recovered and genotyped to reveal their identity. Cleaved amplified polymorphic sequence genotyping for the *coi1-1* mutation revealed that the populations of plant used contained the following numbers of genotypes: WT, 15;

heterozygotes, 29; homozygous *coi1-1/coi1-1*, 16, closely fitting the Mendelian segregation known for this allele (Xie *et al.* 1998). The *coi1-1* experiments (Fig. 2B) indicated a clear preference for *coi1/coi1* over the WT and also showed that *E. boettgeri* consumes tissue of the homozygous jasmonate perception mutant more readily than plants containing a single WT allele (heterozygotes). An ANOVA on the log transformed number of leaves eaten showed no block or block \times genotype effects (where a block represents one experiment with one individual tortoise) but did show a strong genotype effect $F_{2,51} = 5.49$, $P = 0.007$. A *post hoc* Tukey honestly significant difference test showed that the homozygote mutant and the WT differed significantly ($d = 1.12$, P adjusted = 0.0047), while heterozygous mutants and the WT did not differ ($d = 0.46$, $P = 0.28$). A marginal difference could be seen between the homozygous and heterozygous mutant ($d = 0.66$, $P = 0.07$).

Finally, a similar experimental set-up examined feeding on the WT and on three glucosinolate mutants that reduce the foliar levels of indolic glucosinolates, aliphatic glucosinolates or both. In these experiments, we did not detect any difference on feeding on the four genotypes (Fig. 3, ANOVA $F_{3,96} = 0.402$, $P = 0.7519$) and no block or block \times genotype effects were observed.

Discussion

Through the use of genetics and the selection of candidate genes, it is possible to manipulate the signal pathways that control multiple defensive traits in plants or, instead, to alter specific defence traits, for example, the levels of defence chemicals (Rasmann & Agrawal 2009; Anderson & Mitchell-Olds 2011). Here, using a reptile/plant interaction, we have taken both approaches to study a signal pathway of known ecological importance (Kessler & Baldwin 2002; Rasmann & Agrawal 2009). Both herbivorous lizards and tortoises have been observed to be selective feeders and diet optimization has been studied, for example, in herbivorous whiptail lizards (Dearing & Schall 1992). Wild tortoises, where studied, have also been found to be selective leaf feeders. This was the case for giant tortoises (*Geochelone gigantea*, now *Aldabrachelys gigantea*) on Aldabra. In that case, the tortoises ignored certain plants completely, occasionally fed on others, but exerted considerable feeding pressure on some plant species (Grubb 1971). Characteristic of this later category was strong and repetitive injury to *Commiphora marchandii*, which led to browsing lines on these plants.

In the present study, we used the Eastern Hermann's tortoise (*Eurotestudo boettgeri* Bour.), a relatively small animal that lends itself well to work with plants like *Arabidopsis* for which useful and well-characterized

jasmonate mutants exist. The first set of feeding experiments revealed that the tortoises preferred *aos* over the WT, therefore jasmonate synthesis reduces leaf predation by these reptiles. The *aos* mutation cuts off jasmonate synthesis at its root, eliminating the production of allene oxides that give rise to all jasmonates including jasmonic acid and its multiple precursors (Acosta & Farmer 2010). However, in *Arabidopsis*, some cyclopentenone jasmonate precursors are incorporated into secondary metabolites called arabinosides (Böttcher & Weiler 2007). Recently, these compounds were found to weakly reduce feeding of the generalist lepidopteran *Spodoptera littoralis* (Glauser *et al.* 2009). This left open the question of whether or not the presence of jasmonates and/or arabinosides reduced the palatability of leaves, or whether jasmonate functioned as a signal to reduce tortoise feeding.

To resolve this question, we next tested whether the plant's ability to perceive jasmonate affected tortoise feeding. When given the choice between three genotypes (*COI1/COI1*, *COI1/coi1-1* and *coi1-1/coi1-1*) the tortoises fed more rapidly on the latter. As *coi1-1* does not lack the ability to produce jasmonic acid (Glauser *et al.* 2009) and must therefore be able to make its precursors, we conclude that it is impairment in jasmonate signal transduction that causes the tortoises to prefer the jasmonate mutants over the WT. The COI-1 protein is part of the jasmonate receptor, so the results show that the presence of the functional jasmonate receptor reduces the damage rate to plants. For the first time to our knowledge, these results show that jasmonate synthesis and perception slow feeding in a vertebrate folivore. Hormonal signal pathways in plants can control whole suites of defence genes affecting numerous different facets of the defence response against herbivores. This is the case for the jasmonate pathway that is known to control the basal- and damage-inducible expression of hundreds of genes in *Arabidopsis* leaves (e.g. Reymond *et al.* 2004; Yan *et al.* 2007), so it is probable that a variety of leaf properties are modified in both jasmonate synthesis and jasmonate perception mutants.

What makes the leaves of *aos* or *coi1-1* more palatable than those of the WT? Two possibilities exist. Either the initial palatability of the undamaged mutant leaves differed from that of the WT, or during the experiments, inducible defences were strongly activated in the WT and not, or less so, in the mutants. Distinguishing between these two scenarios is, however, difficult, and this is a central challenge encountered in investigations of signal transduction pathways that control both basal and inducible defences. The leaf is a constantly changing substrate in all experiments with pathogens and herbivores, and as soon as the first damage is inflicted,

inducible defences can be activated. This issue has not been resolved in the present study (although it could be addressed in the future with inducible downregulation of defence signalling). However, we noted that, in some experiments, the tortoises quickly (i.e. in <30 min) showed a preference for the jasmonate mutants. We therefore think it likely that the initial palatability of leaves was a major factor in feeding choice in these experiments. This still leaves open the question of what biochemical or structural differences maintained or induced in the WT through activity of the jasmonate pathway could affect tortoise feeding.

One obvious candidate is defence chemicals that, in general, are known to reduce either feeding or can affect leaf digestibility (Dearing *et al.* 2005). In his classic monograph entitled 'Plants and snails', Ernst Stahl suggested that glucosinolates ('scharfen Stoff'), the defence chemicals most characteristic of the Brassicaceae, were deterrents to 'higher animals' (Stahl 1888). Today we know that these compounds play roles in defence against a variety of invertebrates (e.g. Kliebenstein *et al.* 2005; Müller *et al.* 2010). Furthermore, the levels of these chemicals (especially indole glucosinolates) in leaves are controlled in part by jasmonate signalling (Mikkelsen *et al.* 2003) and basal levels can increase modestly over several days during invertebrate herbivory (Agrawal & Kurashige 2003). However, such increases in glucosinolate levels in plants upon herbivory do not appear to be universal and were not observed when a vertebrate (the groundhog *Marmota monax*) fed on the leaves of a wild mustard (*Brassica kaber*) (Cipollini & Sipe 2001). In the present experiments, we used well-characterized *Arabidopsis* mutants in which the levels of glucosinolates are known to be reduced drastically (Sønderby *et al.* 2007; Sun *et al.* 2009; Zhao *et al.* 2002). Nevertheless, these plants were consumed as fast as was the WT. The failure to observe strong effects of eliminating glucosinolate pools means that other properties of *Arabidopsis* leaves must be altered in the *aos* and *coi1-1* mutants. Perhaps, the effects of jasmonate perception relate to physical properties of the leaves, and/or to other factors such as available protein content.

The experiments we describe have investigated whether a signal pathway that is known to provide resistance to a number of invertebrates can also impact vertebrate feeding and this turned out to be the case. Are fundamental differences to be expected in the pressures that invertebrates and vertebrates exert on basal and inducible defences controlled by the jasmonate pathway? It has been argued that inducible leaf defences will be of benefit to plants (and are therefore likely to evolve) if damage to the plant is sustained (Karban *et al.* 1999). This is the case, for example, with

caterpillars that spend their entire larval lives on single plants, constantly inflicting damage to them. While many vertebrate browsers are unlikely to do this there are examples of strongly maintained pressures exerted by generalist vertebrates on plants, the tortoises on Aldabra being just one example. Therefore, in some cases, vertebrates may have acted to select for inducible plant defences. In any case, further experiments on the effects of jasmonate signalling on vertebrate herbivores are now justified.

In contrast to the results with jasmonate mutants, our results with glucosinolates were negative and these chemicals did not appear to affect tortoise feeding on *Arabidopsis*. However, the glucosinolate content of leaves can strongly affect the feeding of generalist invertebrate herbivores (e.g. Mikkelsen *et al.* 2003). Might there be differences in the way plant defence chemicals affect the feeding of invertebrates and vertebrates? Numerous plant chemicals can affect both groups of organisms, for example, respiratory poisons such as rotenone, or cyanide derived from cyanogenic glucosides. One would expect that such chemicals would be avoided by generalists from both groups. Nevertheless, some differences between the attractiveness of toxin-containing plant material to invertebrates and vertebrates might occur. For example, some tortoise species are known to feed on toxin-rich plant species that are avoided by ungulates (Lagarde *et al.* 2003). Indeed, Hermann's tortoise displays this behaviour and may deliberately ingest plant chemicals to reduce parasite loads (Meek 2010). It is conceivable that such behaviour may have impacted our experiments with glucosinolate mutants.

In conclusion, the results show that the jasmonate pathway can affect the palatability of leaves to a vertebrate, the tortoise *E. boettgeri*. This means that plant genes with roles in controlling both jasmonate synthesis and perception may have important roles in defence against vertebrate. More experiments with a wider variety of animals are needed to test the generality of our observations. The findings open the way to using plant populations segregating for genetically defined mutants in signal transduction pathways in experiments designed to probe the characteristics of plant organs that affect vertebrate herbivore feeding preferences.

Acknowledgements

Alexander Roulin (University of Lausanne) is thanked for help with the obtention of authorization 2184.1 from the *Service de la consommation et des affaires vétérinaires du Canton de Vaud*. We thank Jean-Marc Ducotterd (Centre de Protection et Récupération de Tortues, Chavornay, Switzerland) for supplying tortoises, Aurore Chételat (University of Lausanne) for genotyping, Blaise Tissot and Nathalie Bataillard (University of Lausanne)

for technical help and Sergio Rasmann for many critical comments. John Celenza (Boston University), P. Reymond, F. Schweitzer (University of Lausanne) and J. Turner (University of East Anglia) kindly provided mutant seeds. Supported by Swiss National Science Foundation Grant 3100A0-122441 to EEF.

References

- Acosta I, Farmer EE (2010) Jasmonates. *The Arabidopsis Book*. 8:e0129 (DOI: 10.1199/tab.0129).
- Agrawal AA, Kurashige NS (2003) A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. *Journal of Chemical Ecology*, **29**, 1403–1415.
- Anderson JT, Mitchell-Olds T (2011) Ecological genetics and genomics of plant defenses: evidence and approaches. *Functional Ecology*, **25**, 312–324.
- Böttcher C, Weiler EW (2007) *cyclo*-Oxylipin-galactolipids in plants: occurrence and dynamics. *Planta*, **226**, 629–637.
- Browse J (2009) Jasmonate passes muster: a receptor and targets for the defense hormone. *Annual Review of Plant Biology*, **60**, 183–205.
- Case TJ, Bolger DT, Richman AD (1998) Reptilian extinctions over the last ten thousand years. In: *Conservation Biology for the Coming Decade* (eds Fiedler PL, Kareiva PM), pp. 157–186. Chapman & Hall, New York, USA.
- Cipollini DF, Sipe ML (2001) Jasmonic acid treatment and mammalian herbivory differentially affect chemical defenses and growth of wild mustard (*Brassica kaber*). *Chemoecology*, **11**, 137–143.
- Dearing MD, Schall JJ (1992) Testing models of optimal diet assembly by the generalist herbivorous lizard *Cnemidophorus murinus*. *Ecology*, **73**, 845–858.
- Dearing MD, Foley MJ, McLean S (2005) The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. *Annual Review of Evolution and Systematics*, **36**, 169–189.
- Glauser G, Dubugnon L, Mousavi SA, Rudaz S, Wolfender JL, Farmer EE (2009) Velocity estimates for signal propagation leading to systemic jasmonic acid accumulation in wounded *Arabidopsis*. *Journal of Biological Chemistry*, **284**, 34506–34513.
- Grubb P (1971) The growth, ecology and population structure of giant tortoises on Aldabra. *Philosophical Transactions of the Royal Society of London Series B*, **260**, 327–372.
- Howe GA, Jander G (2008) Plant immunity to insect herbivores. *Annual Review of Plant Biology*, **59**, 41–66.
- Karban R, Agrawal AA, Thaler JS, Adler LS (1999) Induced plant responses and information content about risk of herbivory. *TREE*, **14**, 443–447.
- Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: The Emerging Molecular Analysis. *Annual Review of Plant Biology*, **53**, 299–328.
- Kliebenstein D, Kroymann J, Mitchell-Olds T (2005) The glucosinolate-myrosinase system in ecological and evolutionary context. *Current Opinion in Plant Biology*, **8**, 264–271.
- Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annual Review of Plant Biology*, **55**, 141–172.
- Lagarde F, Bonnet X, Corbin J, Henen B, Mardonov B, Naulleau G (2003) Foraging behaviour and diet of an extothermic herbivore: *Testudo horsfieldi*. *Ecography*, **26**, 236–242.
- Lee D-S, Nioche P, Hamberg M, Raman CS (2008) Structural insights into the evolutionary paths of oxylipin biosynthesis enzymes. *Nature*, **455**, 363–368.
- Meek R (2010) Nutritional selection in Hermann's tortoise, *Testudo hermanni*, in Montenegro and Croatia. *Testudo*, **7**, 88–95.
- Mène-Saffrané L, Dubugnon L, Chételat A, Stolz S, Gouhier-Darimont C, Farmer EE (2009) Nonenzymatic oxidation of trienoic fatty acids contributes to reactive oxygen species management in *Arabidopsis*. *Journal of Biological Chemistry*, **284**, 1702–1708.
- Mikkelsen MD, Petersen BL, Glawischnig E, Jensen AB, Andreasson E, Halkier BA (2003) Modulation of CYP79 genes and glucosinolate profiles in *Arabidopsis* by defense signaling pathways. *Plant Physiology*, **131**, 298–308.
- Müller R, de Vos M, Sun JY *et al.* (2010) Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. *Journal of Chemical Ecology*, **36**, 905–913.
- Park JH, Halitschke R, Kim HB, Baldwin IT, Feldmann KA, Feyereisen R (2002) A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. *Plant Journal*, **31**, 1–12.
- R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from: <http://www.R-project.org/>.
- Rasmann S, Agrawal AA (2009) Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits. *Current Opinion in Plant Biology*, **12**, 473–478.
- Reymond P, Bodenhausen N, Van Poecke RMP, Krishnamurthy V, Dicke M, Farmer EE (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell*, **16**, 3132–3147.
- Rosenthal GA, Berenbaum MR, eds. (1991) *Herbivores: Their Interactions with Secondary Plant Metabolites. Vol. I. The Chemical Participants*. Academic Press, San Diego, California.
- Sønderby IE, Hansen BG, Bjarnholt N, Ticconi C, Halkier BA, Kliebenstein DJ (2007) A systems biology approach identifies a R2R3 MYB gene subfamily with distinct and overlapping functions in regulation of aliphatic glucosinolates. *PLoS ONE*, **2**, e1322.
- Stahl E (1888) *Pflanzen und Schnecken: Biologische Studie über die Schutzmittel der Pflanzen gegen Schneckenfrass*. Gustav Fischer, Jena.
- Sun JY, Sønderby IE, Halkier BA, Jander G, de Vos M (2009) Non-volatile intact indole glucosinolates are host recognition cues for ovipositing *Plutella xylostella*. *Journal of Chemical Ecology*, **35**, 1427–1436.
- Van Soest PJ (1996) Allometry and ecology of feeding behavior and digestive capacity in herbivores. *Zoo Biology*, **15**, 455–479.
- Vetter H (2006) *Tortue d'Hermann, Tortue de Boettger et Tortue d'Herzegovine, Testudo boettgeri, hercegovinensis et hermanni*, 2nd edn. Editions Chimaria, Frankfurt.

- Walters DR (2011) *Plant Defense: Warding off Attack by Pathogens, Herbivores and Parasitic Plants*, 1st edn. Wiley-Blackwell, UK.
- Wittstock U, Burow M (2010) *Glucosinolate Breakdown in Arabidopsis: Mechanism, Regulation and Biological Significance. The Arabidopsis Book*. 8:e0134 (DOI:10.1199/tab.0134).
- Xie D-X, Feys BF, James S, Nieto-Rostro M, Turner JG (1998) *COI1* : an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science*, **280**, 1091–1094.
- Yan Y, Stolz S, Chetelat A *et al.* (2007) A downstream mediator in the growth repression limb of the jasmonate pathway. *Plant Cell*, **19**, 2470–2483.
- Yandell BS (1997) *Practical Data Analysis for Designed Experiments*. Chapman & Hall, New York, USA.
- Zhao Y, Hull AK, Gupta NR *et al.* (2002) Trp-dependent auxin biosynthesis in *Arabidopsis*: involvement of cytochrome

P450s CYP79B2 and CYP79B3. *Genes & Development*, **16**, 3100–3112.

A.M. is a teaching student with a degree in biology and a commitment to tortoise conservation. J.G. is a statistical geneticist working on population structures and selection. E.E.F., a plant molecular biologist, works on leaf defence mechanisms.

Data accessibility

Tortoise feeding results available from Dryad, doi: 10.5061/dryad.jf2vg06r.