



Compromis écophysiologiques, stratégies d'utilisation des ressources et adaptation locale chez l'espèce modèle *Arabidopsis thaliana* (L.) Heynh

Kevin Sartori

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THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En écologie fonctionnelle

École doctorale GAIA (N°584)

Centre d'écologie fonctionnelle et évolutive

Compromis écophysiologiques, stratégies d'utilisation des ressources,
et adaptation locale chez l'espèce modèle *Arabidopsis thaliana* (L.) Heynh

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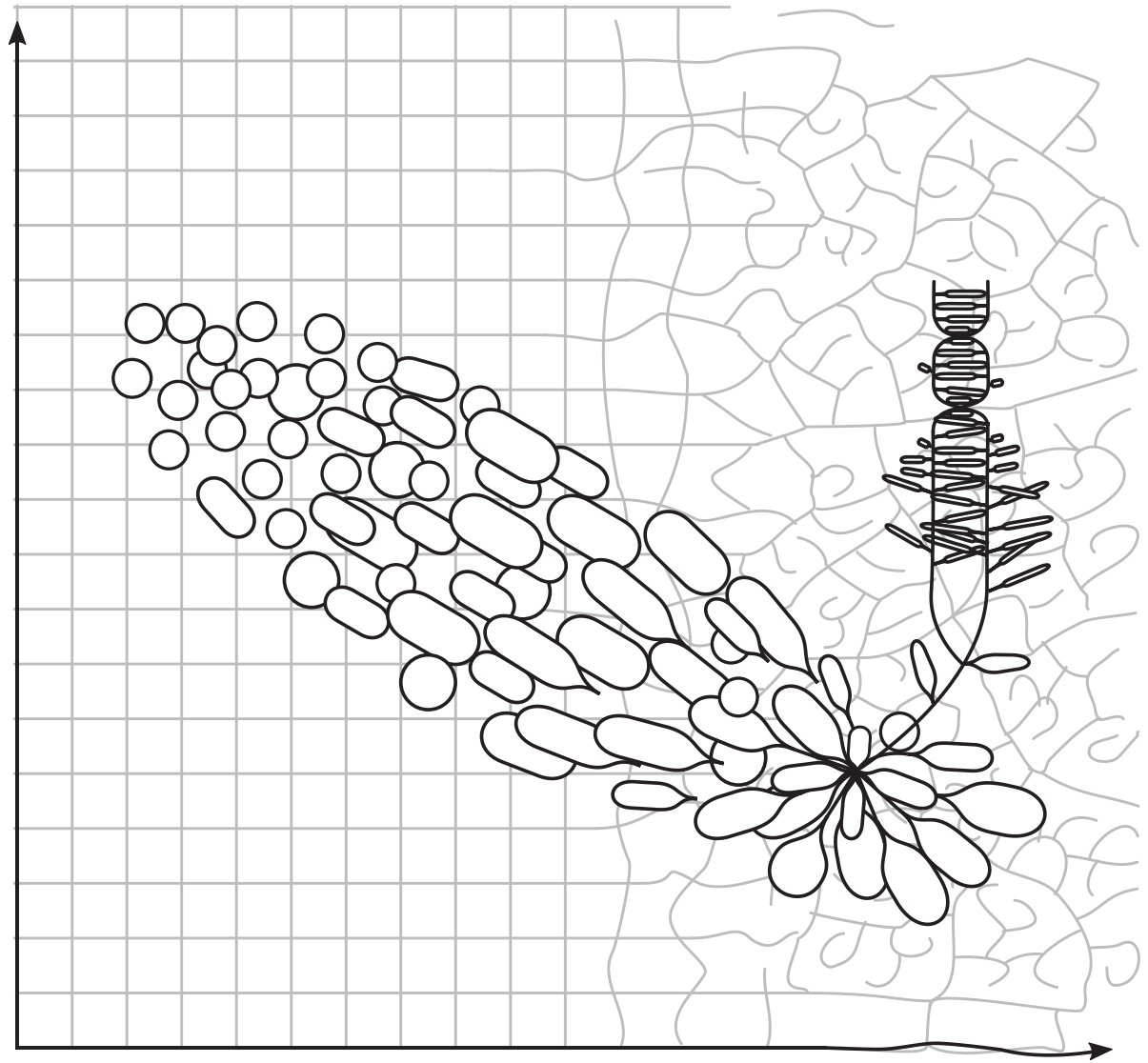
“I don’t believe in magic. I used to, but once I started taking introduction to life science,
I realized the logical explanation of practically every mystery in the world is even more
interesting than the supernatural one”

*Je ne crois pas en la magie. J’y croyais, mais une fois que j’ai commencé à prendre des cours
de science de la vie, j’ai réalisé que l’explication logique de presque tous les mystères du
monde est bien plus intéressante que son explication surnaturelle.*

-Wes Anderson, issu du livre fictif “the return of auntie Lorraine”, Moonrise kingdom, 2012.

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INTRODUCTION

Préambule historique

De l'histoire naturelle aux sciences de l'écologie

Comprendre les mécanismes de diversification du vivant est une entreprise relativement ancienne dans l'histoire des sciences. Les premiers philosophes et écologues, tels que Théophraste, ont commencé par classer les plantes en fonction de leur apparence générale, herbacée, buissonnante ou arborescente (Weiher *et al.*, 1999). Au cours des grandes explorations du 15^{ème} siècle, les naturalistes ont réalisé les premiers grands états des lieux documentés de la diversité des organismes vivants. C'est avec le travail considérable d'identification, de classification et de nomenclature de près de 6000 espèces végétales par Carl Von Linné (1707-1778) que le concept de biodiversité émerge au 18^{ème} siècle. La classification révèle une hiérarchie, prétendue d'origine divine, qui permet de classer les organismes des plus simples aux plus complexes. A la fin du 18^{ème} siècle, les Lumières développent une nouvelle démarche de recherche des connaissances libérée des contraintes théologiques. Dans ce cadre de pensée, Jean-Baptiste de Lamarck (1744-1829) postule que les organismes se transforment par complexification croissante en réponse à des variations de l'environnement. L'importance des facteurs environnementaux en interaction avec les organismes est affirmée par Ernst Haeckel (1834-1919), considéré comme le père de l'écologie. Enfin, Alfred Wallace (1832-1913), Charles Darwin (1809-1882) et Johann Gregor Mendel (1822-1884) théorisent l'évolution des organismes sous une forme proche de celle que nous connaissons aujourd'hui : les caractères héréditaires sont transmis entre générations via un support génétique altérable par la sélection naturelle.

Ce sont des grands naturalistes tels que Huxley, Wallace, Bates, Linné, Muller et Darwin qui ont initié une radiation des sciences de l'écologie (Peters, 1980; Travis, 2020). Cependant, la véritable distinction entre l'histoire naturelle (contemplation de la nature) et les sciences de l'écologie (compréhension des lois qui régissent la nature) n'est affirmée qu'un siècle plus tard. Ce qui rend un modèle intéressant pour les naturalistes est ce qui en fait un organisme rare. Les organismes et les caractères communs ont moins d'intérêt. La démarche naturaliste consiste à décrire les organismes, leur fonctionnement et leurs interactions avec l'environnement dans le détail. L'autoécologie constitue le cas extrême dans lequel une espèce est décrite pour l'ensemble de ses caractéristiques morpho-anatomiques distinguables (Fig. 1). Cette observation attentive de la nature est essentielle pour formuler des hypothèses en écologie (Travis, 2020). Cependant, les observations faites chez un organisme et dans un environnement

donné ont peu de chances de se répéter chez un organisme différent ou dans un environnement différent. A la fin du 20^{ème} siècle, une volonté croissante d'améliorer le pouvoir prédictif de l'écologie (Peters, 1980) conduit au développement de nouvelles approches de l'écologie.

L'originalité de l'écologie comparative

Deux approches ont été développées pour répondre aux besoins de prédiction. La première, l'approche hypothético-déductive, consiste à identifier le système (en général une espèce dans un environnement particulier) regroupant les caractéristiques permettant de tester une hypothèse formulée *a priori* (Travis, 2020). L'expérimentation et/ou la modélisation permettent de confronter les attentes théoriques. Si les données soutiennent la théorie, l'expérience constitue seulement une évidence de son exactitude et doit être testée sur de nouveaux systèmes pour attester de sa généralité. Si les résultats ne supportent pas l'hypothèse, cela n'invalide pas nécessairement la théorie. Il existe différentes issues, dont la reformulation du cadre expérimental, la reformulation de l'hypothèse, ou le changement de système (Peters, 1980). C'est l'approche généralement utilisée en sciences de l'écologie. La seconde, l'approche comparative, consiste à relever les similitudes de fonctionnement entre différents systèmes. Pour Peters (1980), comprendre la nature nécessite de la dépersonnaliser, de la quantifier et de la généraliser. Ce sont les phénomènes, les organismes et les caractères communs qui permettent de révéler les lois générales du vivant. La démarche de recherche associée consiste à étudier simultanément plusieurs espèces partageant un environnement à travers la mesure d'un ou quelques traits, afin de faire émerger des lois générales de fonctionnement (Keddy, 1992) (Fig. 1). Par exemple, en étudiant les dynamiques de populations insulaires, MacArthur & Wilson (1967) révèlent un compromis entre le taux de natalité et la probabilité de survie des individus d'une espèce. Les auteurs qualifient de stratégie *r* les espèces qui maximisent le nombre de leurs descendants au détriment de leur survie, tandis que les espèces dites *K* ont la stratégie inverse. De la même façon, en étudiant les communautés végétales du Royaume-Uni, Grime (1977) révèle l'existence de trois stratégies primaires chez les plantes. Elles sont définies par la propension des espèces à maximiser leur compétitivité, leur tolérance aux stress ou leur tolérance aux perturbations. Grime définit une liste de caractères morphologiques et phénologiques pour lesquels il existe des valeurs caractéristiques pour chacune de ces trois stratégies (Tableau 1). Chacune de ces stratégies est rencontrée dans des combinaisons particulières de niveau de perturbation et de disponibilité en ressources telles que présentées dans le Tableau 2.

Tableau 1. Les trois stratégies primaires des plantes vasculaires sont définies par des valeurs particulières de traits morphologiques et phénologiques.

Trait	Stratégie		
	Compétitrice (C)	Stress tolérante (S)	Rudérale (R)
Tiges	Hautes, ramifiées	Petites à hautes	Petites, peu ramifiées
Croissance	Rapide	Lente	Rapide
Forme de vie	Pérenne	Pérenne	Annuelle
Feuille	Robuste	Petite	Variable
Durée de vie des feuilles	Plutôt courte	Longue	Courte
Litière	Abondante et persistante	Rare plutôt persistante	Rare et non persistante
Floraison	Après des périodes de forte production de biomasse	Indifférent de la saison	Juste avant la période défavorable
Nombre de graines	Petit	Petit	Grand

Tableau 2. Les trois stratégies primaires des plantes vasculaires sont associées à des caractéristiques du milieu. Trois combinaisons de traits définissent trois stratégies écologiques : Compétitrice (C), Stress tolérante (S) et Rudérale (R). Deux axes environnementaux majeurs auxquels les plantes répondent, la disponibilité des ressources et l'intensité des perturbations, définissent les environnements viables dans lesquels évoluent les trois stratégies CSR.

Perturbations	Disponibilité des ressources	
	Faible	Forte
Faibles	Stratégies stress tolérantes (S)	Stratégies compétitrices (C)
Fortes	Pas de stratégie viable	Stratégies rudérales (R)

		Traits								
		1	2	3			<i>t</i>			
Espèces	1					comparative				
	2									
	3									
										
		autoécologie								
	<i>s</i>						écologie			

Figure 1 : L'écologie comparative et l'autoécologie diffèrent par la manière d'analyser un tableau traits-espèces. Adapté de Keddy 1992 Fig. 1.

L'écologie comparative comme cadre conceptuel

Objectifs de l'écologie comparative

Le cadre conceptuel de l'écologie comparative, dans lequel s'inscrit cette thèse, a été développé dans les années 1990 dans une série d'articles fédérateurs (Calow, 1987; Keddy, 1992, 1994; Westoby, 1998; Weiher *et al.*, 1999). La recherche en écologie souffre de trois principales difficultés : un nombre important d'espèces, un nombre encore plus important d'interactions envisageables (e.g. prédation, mutualisme, compétition) et une multitude d'habitats possibles (Keddy, 1994). Le triangle CSR est une première approche assez fructueuse pour résoudre ces problèmes, en classant les espèces selon des stratégies définies par leurs capacités compétitives et leur tolérance aux stress environnementaux et aux perturbations (Pierce *et al.*, 2013). Cependant, il est difficile à utiliser dans tous les contextes : il manque d'un protocole de mesures généralisables à toutes les espèces, la mesure de certains traits doit être faite en référence à l'environnement ou une autre espèce, et les définitions d'un stress et de compétition qu'il considère sont très critiquées (Westoby, 1998). Keddy (1994) propose de revenir à des descripteurs plus fondamentaux des plantes pour pouvoir apprécier les lois générales en écologie, en faisant référence à la classification des étoiles par Ejnar Hertzsprung et Henry Norris Russell. Le diagramme de Hertzsprung–Russell, ou diagramme H-R, est un graphique représentant la luminosité des étoiles visibles (magnitude absolue des étoiles) en fonction de leur température (Fig. 2). La plupart des étoiles observables se situent le long d'un seul axe, appelé séquence principale et allant des étoiles très lumineuses à couleur froide jusqu'aux étoiles les moins lumineuses à couleur chaude. Malgré la simplicité apparente de cette approche, elle a permis de prédire la mort des étoiles ou leur évolution en trou noir ou en naine blanche (Keddy, 1994). Cette analogie permet une certaine flexibilité dans ce qu'on pourrait appeler une loi en écologie puisqu'il existe des exceptions (ici les naines blanches et les géantes rouges) inhérentes à la complexité de la biologie. Une loi en écologie n'est donc pas universelle, elle doit pouvoir prédire le fonctionnement des organismes et des écosystèmes en général (Lawton, 1999).

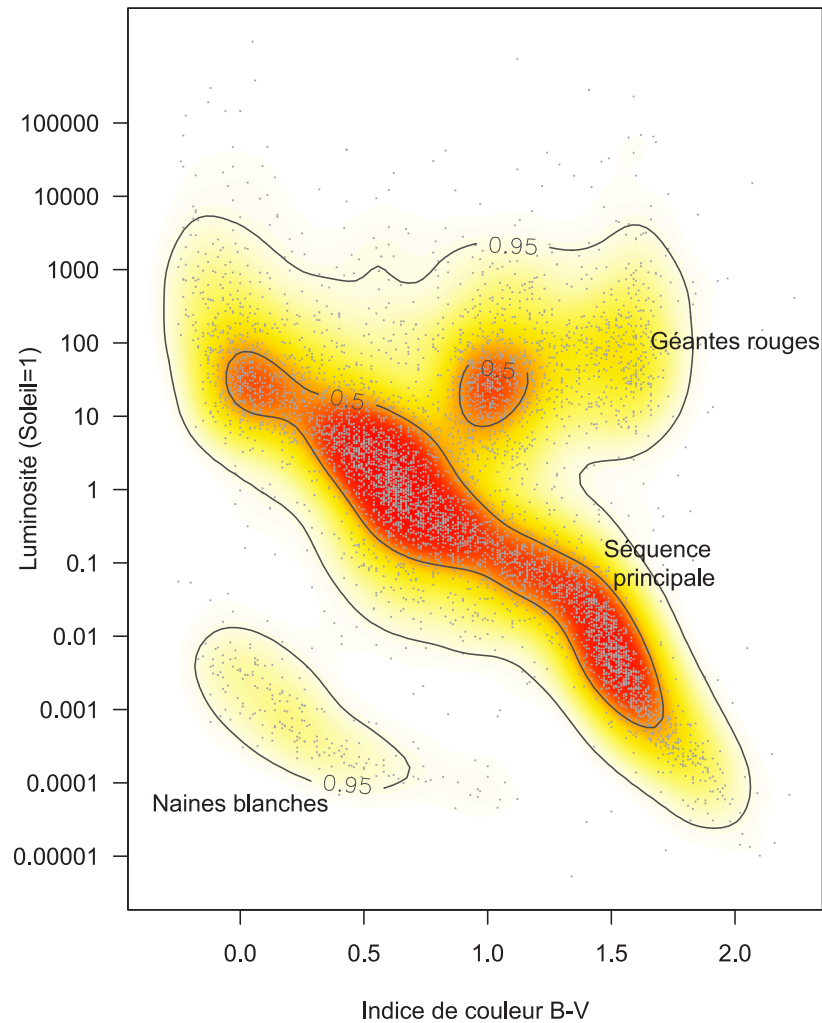


Figure 2 : Diagramme de Hertzsprung-Russell représentant la luminosité des étoiles en fonction de leur couleur. Chaque point gris représente une étoile visible depuis la terre. Le gradient de couleur de jaune à rouge représente un gradient de densité des points sur le graphique et permet de mettre en évidence les associations luminosité-couleur les plus fréquentes. La combinaison des ellipses annotées 0.5 (0.95) encerclent 50% (95%) des étoiles du jeu de données. Les données proviennent du département d'astronomie de l'université de Case Western Reserve, Yale, EU et sont disponibles via ce lien <https://rdrr.io/cran/GDAdata/man/HRstars.html>.

Outils de l'écologie comparative

Pour répondre aux besoins de simplification et de généralisation, l'écologie comparative combine deux outils : l'approche corrélative et le *screening* (Keddy, 1992). L'approche corrélative a pour objectif d'identifier les variables prédictives du fonctionnement des organismes et comprendre leurs réponses à l'environnement. L'approche corrélative a donc deux composantes, les relations trait-trait et les relations trait-environnement. En recherchant

les relations universelles entre des variables quantitatives dépendantes et indépendantes, l'exploration des relations trait-trait a pour ambition de prédire le comportement d'un système grâce à la mesure de quelques variables (Rigler, 1982). Il faut préciser qu'il s'agit d'aller au-delà de la simple prédiction d'une variable d'un système par une autre. En s'appuyant sur une théorie, l'écologie empirique doit pouvoir expliquer le fonctionnement du système. Ainsi, les enseignements acquis dans un écosystème donné doivent s'appliquer dans des contextes écologiques différents (Keddy, 1992; Westoby, 1998). Pour pouvoir formuler des lois indépendantes du contexte écologique, les théories et les modèles de prédiction doivent prendre en compte l'effet de l'environnement sur les traits eux-mêmes mais aussi sur les relations trait-trait. Les attributs sont les valeurs ou modalités prises par un trait (Lavorel *et al.*, 1997) et qui varient selon les individus et les espèces en fonction de l'environnement dans lequel ils sont mesurés. Il est attendu que certains attributs soient favorisés dans des environnements particuliers. Ainsi, un remplacement d'espèces le long de gradients environnementaux spatiaux et temporels en fonction de leurs attributs est attendu en écologie des communautés (Keddy, 1992b). Cela implique également que l'attribut puisse varier entre les individus d'une même espèce le long de ces mêmes gradients si le trait a une forte valeur adaptative. Ainsi, les informations relatives à l'environnement local dans lequel les traits ont été mesurés doivent être reportées avec les matrices espèces-trait dans le but d'interpréter la signification écologique et évolutive des traits. Le *screening* est la pierre angulaire de l'écologie comparative. Elle consiste à mesurer un caractère d'intérêt sur un très grand nombre d'espèces en même temps. L'objectif est d'être le plus exhaustif possible de la zone géographique étudiée. Les exemples de screening les plus célèbres rassemblent les attributs d'un à six traits de plusieurs milliers d'espèces distribuées tout autour du globe (Wright *et al.*, 2004, 2017; Díaz *et al.*, 2016). Plusieurs protocoles de mesures ont été publiés afin de standardiser les mesures effectuées en milieu naturel ou en milieu contrôlé (e.g. Cornelissen *et al.*, 2003; Pérez-Harguindeguy *et al.*, 2013). D'un côté, les campagnes de mesure de traits *in natura* rapportent les attributs réellement exprimés par les plantes, mais peuvent être biaisées par la plasticité éventuelle des traits en réponse à la variabilité de l'environnement. D'un autre côté, les expérimentations en milieu contrôlé permettent d'évaluer le potentiel intrinsèque des espèces étudiées en s'affranchissant des effets de l'environnement. Mais il est peu probable que des conditions expérimentales standardisées soient optimales pour le développement et le fonctionnement de toutes les espèces étudiées. D'une manière générale, les différences entre espèces sont supposées être suffisamment grandes pour que la variabilité intra-spécifique ou l'effet de l'environnement soient négligeables (Garnier *et al.*, 2001).

La notion de trait fonctionnel

Pour révéler des lois générales de fonctionnement, l'écologie comparative repose sur le choix du caractère, par opposition à l'autoécologie qui repose sur le choix du modèle (Fig. 1). Le choix des caractères est donc l'élément limitant de la méthode en écologie comparative, il doit être fait avec précaution. L'écologie comparative a défini une série de critères permettant d'orienter le choix des caractères, qui sont alors appelés *traits fonctionnels*. Pour être qualifié de trait fonctionnel, il y a deux aspects du caractère à clarifier : l'échelle d'organisation au niveau de laquelle la mesure est prise et la fonction que le trait occupe ou estime. Premièrement, un trait doit être mesurable à l'échelle de l'individu. C'est un caractère morphologique, physiologique ou phénologique mesurable de la cellule à l'organisme entier (Violle *et al.*, 2007). Le taux de croissance d'un individu est un trait fonctionnel tandis que le taux de croissance d'une population est un paramètre démographique. Le taux de fixation du carbone (ou taux d'assimilation photosynthétique) est un trait fonctionnel tandis que la productivité primaire nette est une propriété écosystémique. Deuxièmement, les premières définitions qualifient de fonctionnel, un trait qui estime une fonction de base des organismes (Calow, 1987). Une fonction de base désigne tout processus participant à l'économie des ressources et à l'homéostasie, le maintien du milieu intérieur de l'organisme. Il s'agit donc essentiellement des processus liés à l'acquisition des ressources et la résistance aux stress et aux perturbations biotiques et abiotiques (compétitivité, tolérance aux conditions environnementales extrêmes). La construction d'hypothèses robustes quant aux rôles d'un trait dans le fonctionnement et la performance de la plante est un aspect fondamental de la démarche. En effet, un caractère peut varier entre les espèces, être un bon descripteur des différences entre les espèces, mais avoir un rôle non démontré pour le fonctionnement. Un caractère peut également être intégré au fonctionnement via des contraintes physiologiques ou évolutives, sans y être directement impliqué. Ce problème a été souligné par Gould et Lewontin lors d'un article séminal commémorant les cent ans de la disparition de Darwin, en faisant référence au problème des écoinçons de la basilique Saint-Marc de Venise ('The spandrels of San Marco and the Panglossian paradigm', 1979). Certains caractères d'un organisme peuvent être une conséquence accidentelle du développement et du fonctionnement des organismes qui les portent. De la même façon, les écoinçons désignent un ouvrage de menuiserie ou de maçonnerie particulièrement esthétiques et souvent très décorés (Fig. 3). Ils donnent l'illusion d'avoir été délibérément construits alors qu'ils sont le résultat de contraintes architecturales et n'ont pas de fonction structurelle.

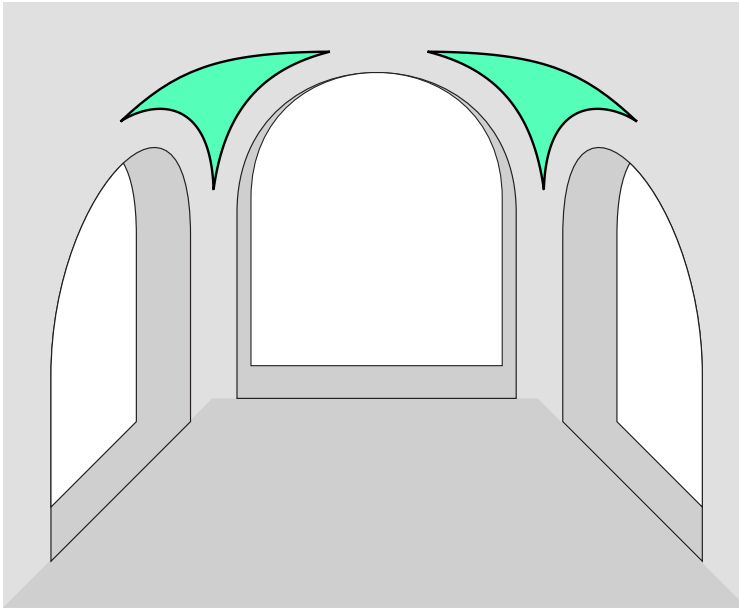


Figure 3. Les écoinçons sont le résultat de contraintes architecturales et n'ont pas de fonction structurelle. La construction d'arc est une solution architecturale permettant de réaliser des ouvertures dans des murs en transmettant les charges sur des appuis réduits. Un écoinçon (en bleu) désigne l'espace triangulaire compris entre deux arcs ou un arc et un encadrement.

Vers un diagramme H-R en écologie comparative

L'équivalent du diagramme H-R en écologie doit permettre d'organiser les espèces en catégories ou le long de syndromes en fonction des valeurs de leurs traits fonctionnels, pour former un schéma général des stratégies écologiques des plantes (Plant ecology strategy schemes, PESSs) (Westoby, 1998). Afin de fournir un langage commun pour comparer les espèces végétales et les types de végétation à travers le monde, un PESS doit avoir quatre propriétés. Premièrement, il doit révéler des différences de stratégies écologiques entre espèces. Deuxièmement, il doit être possible de placer n'importe quelle plante dans le schéma en mesurant uniquement des traits sur la plante elle-même. Troisièmement, il doit être construit grâce à des traits relativement simples à mesurer. L'objectif est de rendre la mesure systématique, même dans des études qui n'en ont pas une utilité directe, dans la perspective de méta-analyses. Enfin, les traits choisis doivent résumer toutes les principales dimensions de variabilité phénotypique des plantes.

Le schéma Leaf-Height-Seed

Le schéma LHS (Leaf-Height-Seed Scheme ; schéma Feuille-Taille-Graine) constitue un exercice théorique pour définir un PESS (Westoby, 1998) en s'appuyant sur les recherches en écologie comparative. Il est défini selon trois compromis, eux-mêmes définis par trois traits qui semblent résumer les principaux axes de fonctionnement des plantes : la surface spécifique foliaire d'une feuille (mature et développée en pleine lumière) (Specific Leaf Area, SLA), la hauteur de la canopée de la plante mature et la masse d'une graine. Le SLA est la surface déployée par une feuille par unité de masse sèche. Un fort SLA est associé à une maximisation de l'interception de la lumière et donc de la capacité photosynthétique. Un faible SLA est associé à une meilleure résistance des tissus foliaires aux dommages biotiques et abiotiques. La taille de la canopée à maturité renseigne sur les stratégies de succession. A partir d'un sol nu, qui se matérialise par exemple après la chute d'un grand arbre, les jeunes plantes se livrent à une compétition pour l'accès à la lumière. Le score compétitif des espèces est attribué en fonction de l'accumulation de biomasse. Une stratégie d'accumulation rapide est avantageuse en début de succession malgré une hauteur maximale faible. Une stratégie lente d'accumulation de biomasse est gagnante sur le long terme en permettant d'atteindre des hauteurs plus importantes. La taille des graines est inversement corrélée au nombre de graines que peut produire un individu, et donne une approximation de leurs chances de survie et d'établissement. Produire de petites graines en grand nombre donne une meilleure probabilité de survie quand les événements de mortalité sont aléatoires. Produire peu de grosses graines augmente la probabilité de survie individuelle des graines par une allocation accrue en ressources. Les grosses graines sont avantageuses quand les événements de mortalité dépendent des conditions, c'est-à-dire face à des stress comme la sécheresse, le manque de lumière voire la consommation des cotylédons. La pertinence et la simplicité de ce schéma ont pour objectif de fédérer suffisamment de chercheurs autour de la mesure des trois traits LHS et faciliter de futures méta-analyses (Westoby, 1998).

Le spectre général des formes et fonctions des plantes

Comme l'annonçait l'article fondateur des stratégies CSR, la démocratisation des ordinateurs a donné une nouvelle dimension à l'écologie comparative, grâce à une immense capacité de stockage et d'analyse (Grime, 1974). Les chercheurs ont formé des réseaux internationaux dont l'objectif était de regrouper les données expérimentales de milliers d'espèces. De nombreuses bases de données de traits de plantes ont été développées dans les

années 2000, au-delà des trois traits du LHS, avec une attention particulière portée sur certaines régions ou certains traits (Klotz *et al.*, 2002; Poschlod *et al.*, 2003; Wright *et al.*, 2004; Kleyer *et al.*, 2008; Green, 2009; Paula *et al.*, 2009; Baraloto *et al.*, 2010). La base de données TRY (Kattge *et al.*, 2011, 2020) regroupe des bases de données préexistantes et intègre un nombre croissant de jeux de données publiés avec pour objectif de collecter les mesures de traits de tous les organes des plantes en maximisant la couverture phylogénétique et géographique des espèces végétales. L'une de ses missions est de centraliser les données en écologie des plantes afin d'améliorer leur disponibilité et leur accessibilité (Kattge *et al.*, 2020). Une telle base de données a aussi permis de sonder les limites de l'espace phénotypique exploré par les plantes. Díaz *et al.* (2016) ont extrait les valeurs de six traits importants pour la croissance, la survie et la reproduction des plantes pour plusieurs milliers d'espèces. Il s'agit des trois traits du schéma LHS, le SLA, la hauteur et la masse d'une graine, ainsi que la densité spécifique des tiges, la surface d'une feuille et la concentration en azote d'une feuille. La densité spécifique des tiges reflète un compromis entre la vitesse de croissance et la résistance mécanique. La surface d'une feuille a une importance capitale pour la régulation de la température et l'équilibre osmotique des plantes. La concentration en azote des feuilles reflète en particulier la concentration en Rubisco, protéine essentielle de la chaîne photosynthétique. La concentration en azote des feuilles est donc soumise à un compromis, par son coût d'acquisition, et parce qu'elle augmente à la fois la capacité photosynthétique des feuilles et leur appétence pour les herbivores. L'analyse révèle que ces traits sont fortement corrélés à deux principales dimensions de variabilité phénotypique (Fig. 4). La première décrit un syndrome de taille allant d'espèces très grandes aux tiges denses, ayant des grandes feuilles et des grosses graines jusqu'aux petites espèces aux tiges peu denses, ayant des petites feuilles et des petites graines. La seconde décrit un syndrome foliaire allant de feuilles hautement concentrées en azote avec une grande capacité d'interception de la lumière, jusqu'à des feuilles moins concentrées en azote et plus robustes. Ces deux dimensions qui permettraient de résumer la diversité fonctionnelle des plantes définissent le spectre général des formes et fonctions des plantes (FFP). Cette analyse confirme l'importance des axes de taille et d'économie foliaire prédite par le schéma LHS (Westoby, 1998), à la différence que les axes de taille de la plante et de taille de la graine sont confondus. Récemment, Thomas *et al.* (2020) ont éprouvé la généralité du spectre FFP en effectuant une campagne de mesure de ses six traits dans des écosystèmes situés à l'extrême limite froide de la vie sur terre, les toundras. L'analyse démontre la robustesse du spectre FFP : les traits varient de façon très importante et respectent le patron de covariations attendu. Les auteurs suggèrent alors que ces syndromes pourraient constituer le schéma convoité par les fondateurs de

l'écologie comparative. Sans faire directement référence au diagramme H-R, les auteurs du spectre FFP terminent l'article par cette phrase : “The global spectrum of plant form and function is thus, in a sense, a galactic plane within which we can position any plant—from star anise to sunflower—based on its traits”.

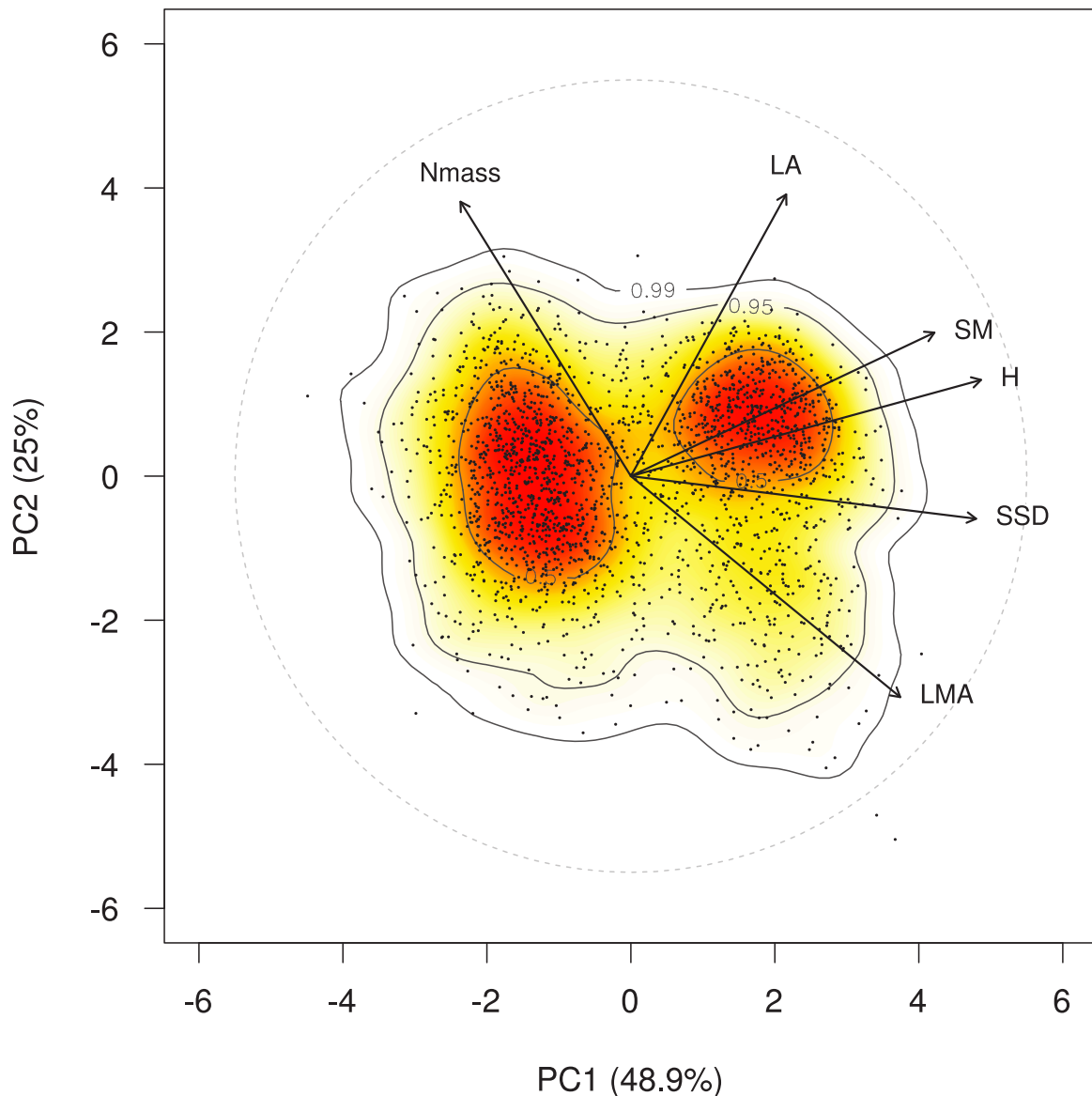


Figure 4 : Spectre général des formes et des fonctions des plantes. Chaque point noir représente une espèce de plante parmi les 2214 espèces ayant une mesure pour chacun des six traits suivants : la concentration en azote foliaire (nitrogen content per unit mass, Nmass), la surface d’une feuille (leaf area, LA), la masse d’une graine (seed mass, SM), la hauteur de la plante (height, H), la densité des tiges (specific stem density, SSD), et l’inverse de la surface spécifique foliaire (leaf mass per area, LMA). Les axes PC1 et PC2 représentent les deux principales composantes d’une analyse multivariée réalisée sur la matrice traits-espèces. La combinaison des ellipses annotées 0.5 (0.95, 0.99) encerclent 50% (95%, 99%) des espèces du jeu de données. Les données proviennent de la base de données TRY et la figure est téléchargeable via <https://shiny.cefe.cnrs.fr/PhenoSpace/>.

La place de l'organe foliaire en écologie comparative

Historiquement, l'écologie comparative a investi beaucoup d'effort dans l'étude de l'organe foliaire. La diversité des feuilles d'un point de vue de l'anatomie, de la physiologie mais aussi plus simplement de la morphologie, des formes et des couleurs est le résultat des nombreuses tentatives d'optimisation des nombreuses fonctions qu'elles remplissent (Garnier *et al.*, 2016). Le rôle primaire des feuilles est d'intercepter les photons en provenance du soleil pour transformer leur énergie en sucres via la photosynthèse. Cette énergie est d'abord transformée par la chaîne photosynthétique en molécules à haute énergie, l'adénosine triphosphate (ATP). L'assimilation du carbone, c'est-à-dire la transformation du CO₂ atmosphérique en sucres est effectuée par la Rubisco, protéine abondante et riche en azote, activée par l'ATP. Les feuilles sont également le lieu de l'évapotranspiration, qui constitue un mécanisme indispensable à l'extraction de l'eau et des nutriments essentiels du sol. L'évapotranspiration est également un processus important pour le refroidissement des feuilles en permanence exposées au soleil. Enfin cette situation à l'interface entre la plante et son environnement expose la feuille à des dommages mécaniques (ex. grêle, vent) et en fait une cible privilégiée des herbivores. Ces éléments font de la feuille un organe dont le fonctionnement est très contraint.

Le syndrome d'économie foliaire (WLES)

Dans ce contexte, il apparaît cohérent de trouver un compromis foliaire parmi les dimensions majeures du spectre général des formes et des fonctions des plantes. Ce compromis a initialement été détecté lors de la comparaison de nombreuses espèces vis-à-vis de la durée de vie et de la surface spécifique (SLA) de leurs feuilles (Poorter, 1994). Il s'agit d'un compromis entre la robustesse et la capacité d'interception de la lumière des feuilles. Ce compromis a initialement été interprété comme le résultat de deux stratégies économiques dans un contexte de ressources limitantes, théorisé par Bloom, Chapin et Mooney (1985). Ces stratégies visent à maximiser le retour sur investissement des ressources allouées à la fabrication des feuilles. Un SLA élevé permet un taux d'assimilation de carbone rapide et une compensation rapide de l'investissement initial pour la construction de la feuille. C'est une stratégie économique rentable à court terme qui n'implique pas une longue durée de vie. A l'opposé, un faible SLA est le résultat d'un investissement important dans la construction de la feuille, il est donc associé à des durées de vie plus longues, une meilleure résistance structurelle et parfois à la production de molécules de défense contre les herbivores (ex. tanins, phénols)

(Poorter, 1994). Une étude plus récente montre que ce compromis s'intègre à des stratégies plus générales du fonctionnement des feuilles. Wright et al. (2004) ont réuni, pour un peu plus de 2500 espèces, les valeurs de SLA, de durée de vie et de quatre autres traits clefs du fonctionnement foliaire, les concentrations en azote et phosphore, et les taux d'assimilation et de respiration. La concentration en azote foliaire (leaf nitrogen content, LNC) rend compte de l'investissement dans la machinerie photosynthétique. Le phosphore foliaire (leaf phosphorus content, LPC) est essentiellement retrouvé dans les acides nucléiques et les molécules énergétiques telle que l'ATP. Le taux maximal d'assimilation du carbone, standardisé par la masse de la feuille (A_{mass}) est le produit de la photosynthèse. La photo-respiration (R_{mass}) désigne la consommation de carbone nécessaire à la maintenance des feuilles et le transport des molécules néo-synthétisées. Les auteurs ont conduit une analyse multivariée, permettant de trouver les axes majeurs de variation phénotypique dans un espace multidimensionnel constitué des traits considérés. L'analyse révèle que la variabilité de ces propriétés physiologiques, structurelles et chimiques des feuilles peut être évaluée par un seul axe de variation résumant 74% de l'information. Indépendamment de la phylogénie, des formes de vie et de l'environnement, les feuilles sont contraintes le long d'un axe unique de variation : le syndrome d'économie foliaire (The Worldwide Leaf Economics Spectrum, WLES; Wright *et al.*, 2004). Dans une représentation en trois dimensions, quels que soient les traits considérés, l'ensemble des 2500 espèces étudiées n'explorent qu'une portion limitée de l'espace phénotypique possible (Fig. 5). Il ne s'agit pas d'un volume complexe ou d'un plan, mais d'un simple et unique axe de variation. Une extrémité de cet axe est occupée par des feuilles à grande capacité d'interception de la lumière (fort SLA), à taux élevé d'assimilation de carbone et concentration élevées en azote et en phosphore. A l'extrême opposé se trouvent des feuilles à masse sèche par unité de surface (faible SLA) élevée, à forte robustesse et longue durée de vie, et qui ont un coût de maintenance élevé (fort R_{mass}). Cet axe de variation est alors interprété comme un syndrome plus global de stratégies d'utilisation des ressources, allant de feuilles dites « acquises » jusqu'à des feuilles dites « conservatrices » des ressources.

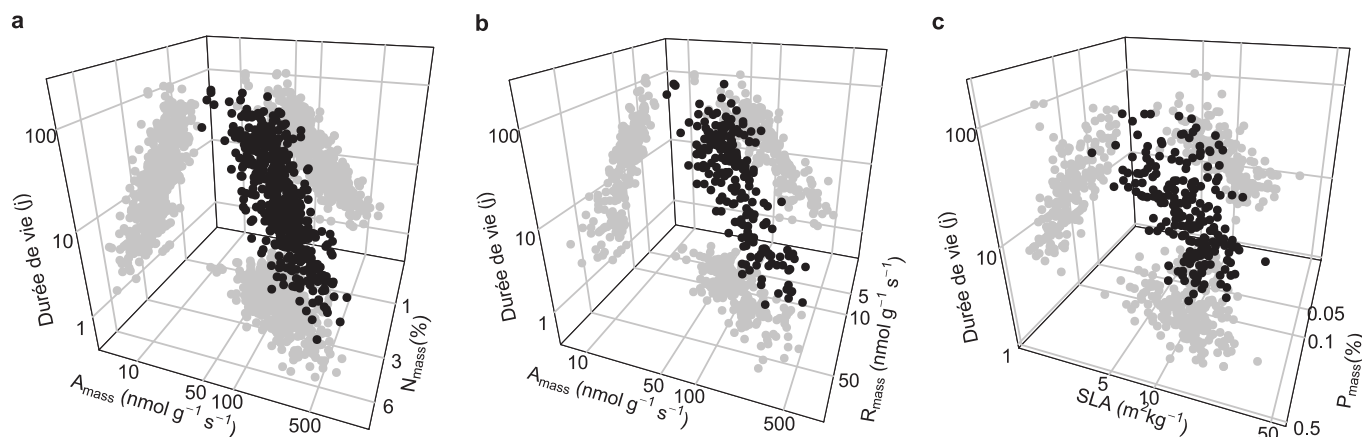


Figure 5 : Relations tridimensionnelles parmi les six traits du WLES. Chaque point noir représente une espèce. Les ombres (points gris) permettent de visualiser les relations bivariées et de se représenter le nuage de points dans l'espace. Le nombre de points dépend des données disponibles pour les combinaisons de variables considérées. Relation entre la durée de vie, le taux de photosynthèse par unité de masse (A_{mass}) et la concentration en azote (N_{mass}) ($N = 498$) (a), relation entre la durée de vie, A_{mass} et le taux de respiration par unité de masse (R_{mass}) ($N = 216$) (b), relation entre la durée de vie, la surface spécifique foliaire (SLA) et la concentration en Phosphore (P_{mass}) ($N=202$) (c). Reproduit à partir des données supplémentaires de l'article de Wright et al. (2004).

Origines éco-évolutives du syndrome d'économie foliaire

Le caractère universel de ces relations entre traits foliaires soulève une question qui reste encore irrésolue. Quelles sont les contraintes évolutives qui ont façonné l'espace phénotypique observé ? Cette question doit se décomposer en deux temps. Premièrement, quelles sont les forces de sélection qui délimitent l'espace phénotypique des possibles ? Deuxièmement, quelles sont les forces de sélection qui maintiennent la variation au sein de cet espace phénotypique ? Pour répondre à la première, il faut explorer les raisons pour lesquelles, non seulement des combinaisons de traits sont favorisées, mais aussi les raisons pour lesquelles des combinaisons de traits n'existent pas dans la nature. Une explication générale stipule que la sélection naturelle favorise les combinaisons de traits qui se trouvent le long du WLES et contre-sélectionne les combinaisons qui se trouvent en dehors (Donovan *et al.*, 2011). Par exemple, le bilan bénéfice/coût pour la plante de construire des feuilles à durée de vie courte et vitesse lente de photosynthèse est défavorable et la stratégie serait contre sélectionnée par manque de rentabilité. Cependant, cela n'explique pas pourquoi une vitesse élevée de photosynthèse ne puisse pas être associée à une durée de vie longue des feuilles (Blonder *et al.*, 2011). Dans ce cas, des contraintes de type anatomique ont été évoquées. Par exemple, l'épaisseur des parois cellulaires est positivement reliée à la robustesse des feuilles mais pourrait ralentir la circulation

du CO₂ au travers des tissus (Onoda *et al.*, 2017). Les nervures des feuilles, par leur rôle à la fois dans la structure des feuilles et le transport de l'eau et des nutriments, pourraient empêcher l'optimisation simultanée de la robustesse et de la photosynthèse (Blonder *et al.*, 2011). Cependant, les contraintes anatomiques n'empêchent pas la création de tous les phénotypes instables (Niklas, 1997). Par exemple, des feuilles à fort SLA pourraient hypothétiquement atteindre une longue durée de vie, mais les dommages mécaniques causés par le vent ou les herbivores agiraient comme agent de contre-sélection et éliminerait ces phénotypes des populations naturelles (Donovan *et al.*, 2011). Ainsi jusqu'à présent, les comparaisons entre espèces n'ont pas permis de démêler les rôles de la sélection et des contraintes dans l'évolution de l'espace phénotypique formé par le WLES. Enfin, la question du maintien de la variation au sein de l'espace phénotypique possible pourrait s'expliquer par la diversité des environnements terrestres. Par exemple on s'attend à ce que les environnements défavorables à la croissance, c'est-à-dire ayant des niveaux de ressources (eau, chaleur, nutriments) faibles, favorisent des stratégies de conservation des ressources (Borgy *et al.*, 2017). Cependant, les traits du WLES ne suffisent pas à évaluer spécifiquement la capacité des feuilles à conserver les ressources. Plus généralement, la valeur adaptative des traits du WLES et des traits associés le long de gradients de disponibilité de ressources n'a pas été clairement démontrée (Donovan *et al.*, 2011).

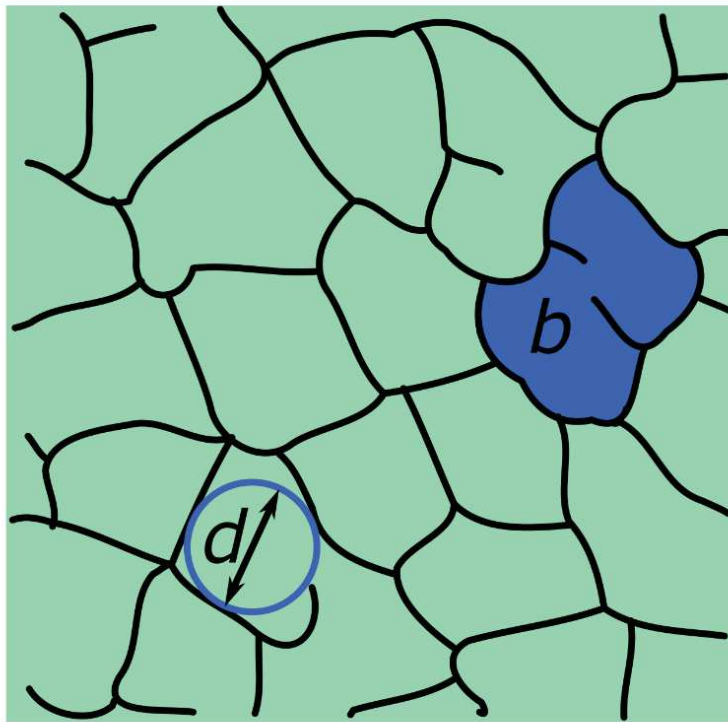
L'architecture des nervures, une contrainte à l'origine du WLES ?

Les nervures sont impliquées à la fois dans le transport de l'eau et des nutriments, processus essentiel à la réalisation de la photosynthèse, et dans le maintien et la résistance structurelle des feuilles, déterminant pour la durée de vie. A cet égard, l'architecture des nervures foliaires représente un marqueur possible des contraintes anatomiques à l'origine du WLES (Blonder *et al.*, 2011). L'architecture des nervures foliaires désigne la manière dont les segments de vaisseaux conducteurs sont agencés et connectés les uns par rapport aux autres pour former le système circulatoire des feuilles. La diversité des architectures rencontrées dans la nature est très grande (Niinemets *et al.*, 2007). La description de l'architecture des nervures a, dans un premier temps, été portée sur les premiers ordres de nervures, c'est-à-dire la ou les nervures principales qui émergent du pétiole des feuilles (ordre un), et les nervures qui leur sont directement connectées (ordre deux). Chez les Angiospermes, on distingue notamment les systèmes pennés (une nervure centrale et des nervures secondaires émergeant régulièrement le long de celle-ci), palmés (plusieurs nervures rayonnantes à partir de la base de la feuille) et

parallèles (des nervures issues du pétiole et se rejoignant à l'apex de la feuille). Les traits architecturaux sont utilisés depuis longtemps pour l'identification de taxons de plantes fossilisées (Trivett & Pigg, 1996). Cela a permis d'émettre des hypothèses quant au rôles de l'architecture des nervures dans la performance et l'adaptation des plantes à leur environnement biotique et abiotique (Carlquist, 1975; Niklas, 1997; Sperry, 2003). Des études de paléobotanique ont montré que les innovations architecturales des nervures des Angiospermes à partir du réseau ancestral ouvert et dichotomique est une étape décisive de leur succès sur les gymnospermes et les fougères au cours du Crétacée (Uhl & Mosbrugger, 1999; Roth-Nebelsick *et al.*, 2001; Sack & Frole, 2006; Brodribb *et al.*, 2007; Blonder *et al.*, 2011). Ces innovations impliquent essentiellement une augmentation de la densité du réseau de nervures (vein density, VD) (Roth-Nebelsick *et al.*, 2001; Brodribb & Feild, 2010; Blonder *et al.*, 2011), mesurée comme la longueur totale des segments de vaisseaux conducteurs divisée par la surface de la feuille (Fig. 6). C'est un trait intégratif de plusieurs fonctions intéressantes du réseau de nervures foliaire. Il reflète l'investissement dans la construction d'un réseau de tissus lignifiés dans les feuilles, et donc potentiellement dans leur résistance mécanique (Onoda *et al.*, 2011). Un réseau de nervures dense signifie également que tout point de la feuille se trouve à proximité immédiate d'un segment de vaisseau. Ceci se traduit par une réduction de la distance moyenne entre le lieu de la photosynthèse et le lieu de circulation des nutriments, supposée favoriser des taux élevés de photosynthèse (Brodribb *et al.*, 2007). Un réseau de nervures dense est aussi une solution pour multiplier les routes alternatives au transport de l'eau et des nutriments. Plusieurs événements peuvent être à l'origine de la rupture de la continuité hydraulique du réseau de nervures tels que des dommages causés par des herbivores, ou des phénomènes d'embolisme des vaisseaux pendant des périodes de froid ou de sécheresse (Brodribb *et al.*, 2016). Cependant, les explorations du rôle fonctionnel des nervures et de leurs implications dans la performance des plantes et leur valeur sélective sont rares dans la littérature (Niinemets *et al.*, 2007).

Blonder et collaborateurs (2011, 2013, 2015) ont construit un jeu d'équations capables de prédire les traits du WLES en fonction de trois descripteurs du réseau de nervure : la densité du réseau, la distance moyenne entre deux nervures et le nombre de boucles du réseau (Fig. 6). Ces traits renseignent sur les trois fonctions principales des nervures, telles que définies plus haut. Une forte densité du réseau de nervures (VD) implique un investissement coûteux en tissus lignifiés. Une courte distance inter-veineuse (d) optimise la vitesse des flux d'eau et de carbone. Les boucles (b) fournissent des routes alternatives en cas de dommage. La force de ce

modèle est de pouvoir expliquer l'ensemble des relations trait-trait du WLES, au-delà des mécanismes proposés par le passé qui ne pouvaient expliquer que certaines de ces relations (Brodribb *et al.*, 2007; Feng *et al.*, 2008; Niklas *et al.*, 2009; Brodribb & Feild, 2010). Le modèle a été confronté à des données réelles dans deux études expérimentales, chez *Arabidopsis thaliana* et chez *Populus tremuloides*, qui ont toutes les deux confirmé son pouvoir prédictif (Blonder *et al.*, 2013, 2015). Cependant, l'analyse de sensibilité des paramètres du modèle révèle que *b* n'a pas un fort pouvoir prédictif sur les traits du WLES (Blonder *et al.*, 2011). De plus, étant donnée la forte relation qu'il existe entre VD et *d* dans les systèmes naturels (Uhl & Mosbrugger, 1999), les relations entre les traits du WLES peuvent être prédites par un seul de ces deux traits. Ce résultat est en accord avec d'autres travaux montrant que le WLES pourrait avoir pour origine une contrainte structurelle imposée par un unique trait qui n'aurait pas été reporté dans l'article original du WLES. Shipley et collaborateurs (2006) stipulent que ce trait pourrait avoir un lien avec le volume relatif occupé par les parois cellulaires (rôle de soutien) par rapport au volume occupé par le cytoplasme des cellules (rôle métabolique). Les nervures étant composées à majorité de xylème, Blonder et collaborateurs suggèrent que le trait manquant serait la densité de nervures. Ces travaux en écologie comparative confirment l'importance de VD comme un trait central dans le fonctionnement de plantes comme l'ont suggéré les travaux en écologie et physiologie présentés plus haut. Cependant, l'implication des nervures dans le WLES a fait l'objet d'un vif débat dans la littérature (Blonder *et al.*, 2011, 2014; Sack *et al.*, 2013; Onoda *et al.*, 2017). De plus, rien n'indique que la diversité architecturale des nervures foliaires ne soit pas une conséquence secondaire du WLES, à la manière des écoinçons de Saint-Marc. En effet, malgré un fort potentiel adaptatif (Brodribb *et al.*, 2016), les démonstrations empiriques de l'importance de ces traits architecturaux dans la performance et la valeur sélective des plantes sont rares dans la littérature.



$$VD = \frac{\text{longueur du réseau}}{\text{SURFACE}}$$

Figure 6 : trois descripteurs du réseau de nervure : la densité du réseau (VD), la distance moyenne entre deux nervures (d) et le nombre de boucles (b) du réseau. La surface verte représente une portion de surface foliaire laissant apparaître le réseau de nervures (noir) par transparence. Adapté de Blonder et al. 2011 Fig. 2.

Le rôle du WLES dans la conservation des nutriments

Pour maximiser l'assimilation du carbone, la chaîne photosynthétique requiert de nombreuses protéines riches en azote (Chapin, 1980). Cependant, cet élément est limitant dans la plupart des écosystèmes et les formes les plus abondantes ne sont pas directement assimilables par les plantes (Berendse & Aerts, 1987; Aerts & Chapin, 1999). En conséquence, l'absorption de l'azote est un processus coûteux qui doit engendrer de fortes contraintes sur l'utilisation de l'azote dans les feuilles. Dans ce contexte il est attendu une sélection pour des traits réduisant les pertes et maximisant la conservation des nutriments plutôt que des traits améliorant l'acquisition de l'azote (Aerts & Chapin, 1999). La capacité des plantes à conserver les nutriments est évaluée classiquement à travers la durée de vie des feuilles et l'efficacité de résorption au moment de la sénescence des feuilles. Investir dans la construction de feuilles à durée de vie longue, c'est-à-dire protégées sur le long terme contre d'éventuels dommages biotiques ou abiotiques, permet de conserver longtemps les protéines riches en azote et maximiser leur utilisation pour la fixation du carbone (Chabot & Hicks, 1982). Mais dans certaines conditions environnementales particulières ou en fonction des stratégies déployées par la plante, il peut être plus avantageux de remobiliser ce qui a été investi dans une feuille pour le réinvestir dans de nouvelles feuilles, plutôt que de protéger cette feuille contre la sénescence (Havé *et al.*, 2017). La sénescence des feuilles est un processus dynamique complexe, qui va au-delà de la mort progressive des tissus (Chabot & Hicks, 1982). La résorption de l'azote définit le processus par lequel les feuilles sénescentes catabolisent les protéines, en particulier les protéines des chloroplastes (Havé *et al.*, 2017; Moison *et al.*, 2018), pour remettre l'azote en circulation et le réallouer aux tissus vivants en croissance (Killingbeck, 1986). Ce processus se traduit par un jaunissement progressif des feuilles (Fig. 7). L'efficacité de la résorption est calculée par la différence entre la quantité d'azote d'une feuille adulte et la quantité d'azote de cette feuille à la fin de la sénescence. Elle est rapportée à la quantité d'azote de la feuille adulte pour obtenir un pourcentage d'azote résorbé (Killingbeck, 1986). Des études comparatives interspécifiques montrent que l'efficacité de résorption peut varier de 50% à 90% (e.g. Aerts & Chapin, 1999; Drenovsky *et al.*, 2019). L'étude de la résorption chez des espèces modèles comme *Triticum* spp. et *Arabidopsis thaliana* montre que c'est un processus déterminant de la qualité des graines (Masclaux-Daubresse & Chardon, 2011; Vilnius *et al.*, 2014). Cependant, l'exploration des corrélations entre l'efficacité de résorption de l'azote et une mesure plus complète de la valeur sélective des plantes manque dans la littérature. Plus

généralement, le rôle de la résorption de l'azote foliaire dans le fonctionnement et la performance des plantes est encore mal connu.



Figure 7 : Photographie en vue plongeante d'un individu de l'espèce *Arabidopsis thaliana* cultivé en pot sous serre. Le jaunissement des feuilles lors de la senescence des tissus révèle le réseau de nervures par transparence. Crédit Photo : Kevin Sartori.

Une interprétation du WLES étendue aux stratégies d'acquisition et de conservation des ressources permet d'intégrer le processus de résorption au fonctionnement des plantes. Le faible coût de production des feuilles à fort SLA permet une production rapide de nouvelles feuilles. Associées avec une durée de vie courte, elle permet un renouvellement rapide des tissus qui pourrait se faire au détriment d'une résorption efficace. C'est une stratégie qui peut néanmoins permettre une certaine flexibilité dans des milieux où les ressources sont distribuées de manière hétérogène (Grime, 1994). D'autre part, les espèces à faible SLA ont une durée de vie des feuilles longue, permettant de séquestrer l'azote plus longtemps. Cependant, même si ces

mécanismes permettent de connecter les stratégies du WLES à la rétention des nutriments par la durée de vie des feuilles, rien n'indique qu'une grande efficacité de résorption soit également intégrée à une stratégie de conservation des ressources. L'efficacité de résorption pourrait agir comme une variable d'ajustement en fonction de la disponibilité en ressources du milieu. En effet, plusieurs études ont reporté une plasticité assez importante de ce trait dans des expérimentation à plusieurs niveaux de fertilisation (voir Drenovsky *et al.*, 2019 pour une méta-analyse). En conséquence, les rares études comparatives examinant le lien entre les traits du WLES et l'efficacité de la résorption ne rapportent pas de signal clair (Kazakou *et al.*, 2007; Freschet *et al.*, 2010).

La vitesse de résorption des nutriments est une dimension de la résorption qui a encore été très peu étudiée. Pourtant, des références historiques de l'étude de la sénescence la décrivent comme un processus dynamique complexe (Chabot & Hicks, 1982; Harper & Seltek, 1987). Des recherches plus récentes font état d'une importante variabilité de vitesse de jaunissement des feuilles entre géotypes de l'espèce modèle *Arabidopsis thaliana* (Diaz *et al.*, 2005, 2008) et de variation de la vitesse de concentration d'azote dans des feuilles de blé au moment du remplissage des graines (Vilmus *et al.*, 2014). Chez des espèces capables d'un renouvellement rapide de leurs feuilles, caractéristique des espèces à fort SLA (Grime, 1994), une vitesse de résorption rapide pourrait être avantageuse. A l'inverse, une stratégie de conservation des ressources, associée à des longues durées de vie et des renouvellements lents des feuilles, pourrait être associée à des vitesses de résorption plus lentes. En écologie comparative, l'approche classique pour caractériser la résorption de l'azote s'est concentrée sur des mesures instantanées des concentrations en azote, pendant le stade adulte de la feuille (LNC) et à la fin de la sénescence, permettant d'estimer l'efficacité de la résorption (Aerts & Chapin, 1999). Il apparaît nécessaire de développer des techniques de mesure de la vitesse de résorption des nutriments, afin de mieux comprendre le rôle de ce processus dans le fonctionnement des plantes, leur performance et leur adaptation le long de gradients environnementaux.

Dimension adaptative des traits fonctionnels

La description du WLES et des traits associés que nous venons de faire souffre d'une constante : les études en écologie comparative manquent de tests de la valeur adaptative des traits fonctionnels. La valeur adaptative du WLES a été suggérée très tôt (Reich *et al.*, 1997, 1999; Wright *et al.*, 2004; Westoby & Wright, 2006) et la littérature plus récente indique que la sélection naturelle est probablement le facteur le plus important à l'origine de l'évolution du WLES (Donovan *et al.*, 2011). Cependant, l'écologie comparative ne dispose pas d'outil pour tester correctement la valeur adaptative des traits. Pour y remédier, Violle et collaborateurs (2007) proposent de mesurer la force de la relation entre les traits et les trois composantes majeures de la valeur sélective : la survie, la fécondité et la croissance. La sélection naturelle tend à maximiser les valeurs de ces trois composantes (Calow, 1987). En pratique, ces composantes sont souvent inaccessibles par l'expérimentation du fait de la difficulté de mesure. Par exemple, évaluer la survie nécessiterait de suivre des individus parfois sur un temps très long, hors de contraintes temporelles raisonnables. Évaluer la fécondité nécessiterait de compter les descendants d'un individu et d'évaluer leur survie. En fait, plusieurs théories prédisent des compromis entre les composantes de la valeur sélective. Ces théories sont unifiées autour du syndrome du rythme de vie (Pace-of-life syndrome, Dammhahn *et al.*, 2018). Historiquement, les premières preuves de ce syndrome ont été constatées par MacArthur & Wilson (1967) au travers d'un compromis entre taux de natalité et probabilité de survie. Stearns (1983) explique que deux stratégies différentes d'allocations des ressources à la reproduction peuvent être avantageuses dans la nature. Une stratégie « rapide » consiste à atteindre la maturité sexuelle rapidement et avoir de nombreux descendants. Les individus sont alors de petite taille et peu résistants. Une stratégie « lente » permet de produire des individus plus grands en retardant l'allocation à la reproduction. Les individus sont moins nombreux mais leurs chances de survie individuelles plus importantes. De nombreuses études ont validé l'existence de ce syndrome chez les mammifères (Promislow & Harvey, 1990), les oiseaux (Sæther, 1988), les reptiles (Bauwens & Diaz-Uriarte, 1997), les poissons (Winemiller & Rose, 1992), les insectes (Johansson, 2000) et les plantes (Franco & Silvertown, 1996). Plus récemment, Ricklefs & Wikelski (2002) ont montré que de nombreuses adaptations physiologiques ont accompagné l'évolution de ces traits d'histoire de vie. Notamment, la variabilité des taux métaboliques serait à l'origine des différences d'âge de première reproduction, de fréquence de reproduction et de durée de vie des individus. En pratique, il est donc possible d'estimer les composantes de la valeur sélective par des traits plus accessibles tels que la vitesse de croissance et l'âge de

maturité sexuelle. Chez les plantes, Violle et collaborateurs (2007) proposent d'estimer les composantes de la valeur sélective (croissance, fécondité, survie) par la mesure de trois traits de performance (Fig. 8, flèches et boîtes noires). La biomasse végétative représente la capacité d'une plante à fixer le carbone. Le nombre de graines produites ou la biomasse d'une graine est une bonne approximation de la fécondité (nombre de descendants x probabilité de survie) chez les plantes (cf. Schéma LHS). Enfin la survie est estimée par une variable binaire de présence/absence de l'espèce dans le milieu considéré.

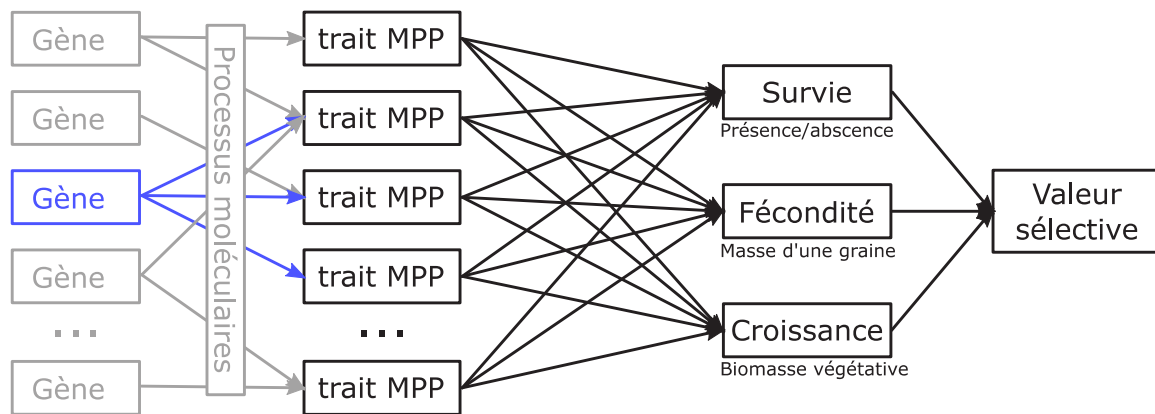


Figure 8. Déterminisme génétique et implication dans la valeur sélective des traits fonctionnels. Les traits Morpho-Physio-Phénologiques (trait MPP) peuvent être associés à l'une, les deux ou les trois composantes de la performance, elles-mêmes déterminant la valeur sélective de l'individu qui les porte. Les traits MPP peuvent être associés à l'action d'un ou plusieurs gènes (caractère polygénique) et un gène peut être associé à plusieurs traits MPP (gène pléiotrope). Les interactions entre gènes, entre traits et entre composantes de la valeur sélective ne sont pas représentées. Adapté de Violle *et al.* (2007), Fig. 3.

Une définition des traits fonctionnels qui fait référence à la valeur sélective pose le problème de l'unité de la sélection. La sélection a lieu au niveau de l'individu, au travers des performances individuelles (les composantes de la valeur sélective). Pourtant, ce sont les gènes qui perdurent entre les générations et sont considérés à ce titre comme l'unité de la sélection (Dawkins, 2007). L'effet direct de la sélection naturelle est le changement au cours du temps de fréquences alléliques d'une population (voir section Méthode). En d'autres mots, la valeur adaptative d'un trait s'observe dans le degré de différenciation des gènes qui le déterminent entre les populations d'une même espèce. Ce constat a une implication majeure pour l'écologie fonctionnelle : les traits fonctionnels ne sont que l'intermédiaire entre les gènes et la performance individuelle et la valeur sélective (Fig. 8, flèches et boîtes grises). Ainsi, pour

tester si la sélection a un effet sur le WLES et les traits fonctionnels en général, il faut quantifier l'héritabilité des traits ainsi que la force et le sens de la sélection (Donovan *et al.*, 2011). La force de la sélection peut être mesurée par la force de la relation trait-trait de performance, c'est-à-dire le rapport de la covariance et des variances des traits et traits de performance. L'héritabilité est la part de variance des traits expliquée par la variance génétique. La comparaison d'espèces au travers de la phylogénie des plantes ne permet pas d'établir des liens phénotypes-génotypes tant les structures génétiques sont variables entre espèces. En conséquence, l'approche traditionnelle de l'écologie comparative par des comparaisons interspécifiques ne permet pas d'évaluer l'héritabilité des traits mesurés. Les études intra-spécifiques des traits du WLES rapportent rarement cette mesure et, quand elles le font, se focalisent sur un trait en particulier sans considérer les syndromes globaux identifiés en écologie comparative (Donovan *et al.*, 2011). Dans une méta-analyse, Donovan et collaborateurs (2011) rapportent des valeurs significatives bien que modérément élevées d'héritabilité et de force de sélection pour les traits du WLES. Ces estimations sont probablement limitées par le manque de variation phénotypique dans ces études. En effet, dans un environnement donné, la sélection naturelle a pu éliminer les phénotypes peu performants ainsi réduire la variabilité des traits. Or, par construction l'héritabilité et la valeur sélective sont deux estimateurs très dépendants de la variabilité des traits. En complément, il serait intéressant d'utiliser les outils développés en génétique quantitative pour évaluer l'importance des contraintes génétiques dans ces compromis. En effet, des gènes pléiotropes (déterminant de l'expression de plusieurs caractères) peuvent être à l'origine de la corrélation entre deux traits phénotypiques (Fig. 7, flèches et boîte bleues). Il n'existe que peu d'études essayant de démêler l'effet des contraintes génétiques et des contraintes physiologiques dans les compromis identifiés en écologie comparative (Vasseur *et al.*, 2012). L'identification de gènes impliqués dans l'expression des traits du WLES et l'estimation de la valeur sélective et de l'héritabilité requiert de nouvelles explorations intra-spécifiques. Afin de comprendre le rôle des compromis physiologiques identifiés en écologie comparative pour l'adaptation locale des plantes, il apparaît nécessaire de changer d'échelle en appliquant la démarche de l'écologie comparative aux génotypes d'une espèce en particulier, distribuée le long de gradients environnementaux étendus. Plus généralement, les comparaisons interspécifiques ont permis d'identifier des patrons de fonctionnement valables à grande échelle au travers de la phylogénie et au cours de longues périodes géologiques, mais ne permettent pas de tester rigoureusement les mécanismes génétiques et physiologiques impliqués. Les manipulations expérimentales d'une espèce permettent de tester des hypothèses mécanistiques relatives aux contraintes physiologiques et

aux déterminants génétiques des patrons de covariation entre traits, mais souffrent souvent d'une impossibilité de généralisation. L'utilisation conjointe des comparaisons interspécifiques et de manipulations expérimentales à l'échelle d'une espèce est un moyen de générer puis d'évaluer des hypothèses causales en écologie évolutive (Weber & Agrawal, 2012). Ce schéma méthodologique cyclique permettant de générer et de tester de nouvelles hypothèses est résumé dans la figure 9.

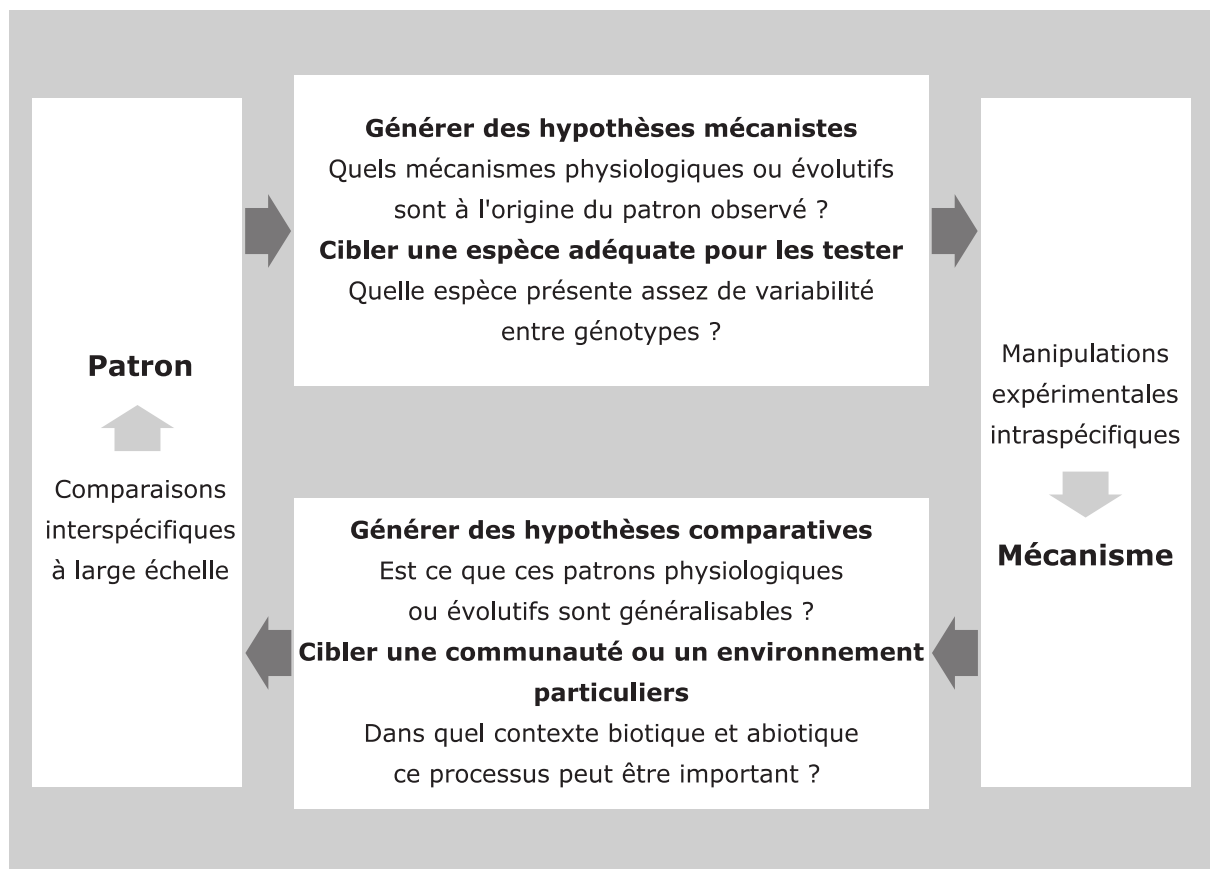


Figure 9. Schéma décrivant le processus itératif de création et de test d'hypothèses en écologie fonctionnelle. Adapté de (Weber & Agrawal, 2012).

Questions scientifiques

Le syndrome d'économie foliaire définit un axe de variation phénotypique préférentiel dont les mécanismes évolutifs et physiologiques sous-jacents et les implications pour le fonctionnement des plantes sont encore mal connus. En adaptant l'approche comparative à l'étude de nombreux génotypes d'une espèce modèle, cette thèse propose une exploration des causes et conséquences physiologiques du WLES et des déterminants environnementaux de sa variation en trois axes de recherche.

Axe 1. Exploration du rôle du WLES dans la performance des plantes

Questions : La contrainte du WLES s'applique-t-elle à la différenciation phénotypique des individus d'une même espèce ? Le WLES est-il intégré au fonctionnement de la plante entière, notamment à travers le syndrome du rythme de vie ? Existe-t-il des preuves génétiques du rôle adaptatif de ces contraintes phénotypiques ? Quels facteurs environnementaux sont à l'origine de la variabilité phénotypique observée entre individus d'une même espèce ?

Axe 2. Exploration de la valeur adaptative de l'architecture des nervures

Questions : l'architecture des nervures foliaires et la densité de nervure en particulier peuvent-elles expliquer l'adaptation d'une espèce à son environnement ? Existe-t-il des preuves génétiques du rôle adaptatif de ces traits des nervures ? Quels facteurs environnementaux sont à l'origine de la variabilité phénotypique observée entre individus d'une même espèce ?

Axe 3. Implication du WLES dans la résorption de l'azote.

Questions : Le WLES traduit-il un compromis général d'utilisation des ressources par les plantes ? Comment la capacité de résorption de l'azote est-elle intégrée au WLES ? Existe-t-il des preuves génétiques du rôle adaptatif de la résorption ? Quels facteurs environnementaux sont à l'origine de la variabilité phénotypique observée entre individus d'une même espèce ?

***Arabidopsis thaliana*, un modèle d'étude pour l'écologie comparative**

L'arabette des dames, *Arabidopsis thaliana* (L.) Heynh, est une espèce herbacée annuelle de la famille des Brassicaceae. Le stade végétatif ressemble à une rosette compacte de 5 à 10 cm de diamètre dont les feuilles sont initiées par un méristème central (Fig. 10). Le méristème a une croissance définie, ce qui signifie que la production de feuilles s'arrête par sa transformation en un méristème floral. Ce stade est facilement décelable car il s'accompagne d'une transformation du méristème en bourgeons floraux. L'inflorescence croît de dix à plusieurs dizaines de centimètres et peut ramifier à sa base ou le long de la tige. Les fleurs sont très réduites, conséquence d'un mode de reproduction essentiellement autofécondant. Les fruits sont des siliques longues d'un à deux centimètres et contiennent des graines d'un demi millimètre de diamètre. Le cycle de vie, de la germination à la fructification dure de 2 à 6 mois. Ces caractéristiques en ont fait un modèle de choix pour la biologie végétale : elle est à l'origine d'avancées considérables en biologie cellulaire, génétique et épigénétique et dans la compréhension des mécanismes moléculaires du développement, du métabolisme et de la physiologie (voir Krämer, 2015 pour une revue bibliographique). Son mode de reproduction autogame en fait un organisme essentiellement homozygote. Ceci suggère que les populations locales d'*A. thaliana* sont des clones génétiques, et que l'effet de la dépression de consanguinité a éliminé les gènes délétères. A ce titre, on considère qu'un génotype et le phénotype associé reflètent les adaptations locales de l'espèce, on parle alors d'écotype. Son aire de distribution native couvre la côte méditerranéenne et s'étend au Nord jusqu'au Nord de la Suède et à l'Est jusqu'en Asie. C'est une plante pionnière, qu'on retrouve essentiellement dans des sols perturbés et plutôt pauvres en nutriments. Il s'agit de la première plante à avoir été totalement séquencée en 2000 par le consortium *Arabidopsis Genome Initiative*. Depuis, plus de 1300 génotypes issus de populations couvrant son aire de distribution ont été séquencés (Alonso-Blanco *et al.*, 2016). Plusieurs banques de graines donnent accès à des stocks de graines qui sont régulièrement multipliées et les séquences génétiques associées aux écotypes sont libres d'accès (<https://www.arabidopsis.org/>). Les études de la structure génétique des populations d'*A. thaliana* montrent qu'elle corrèle avec les gradients climatiques couvrant la distribution de l'espèce, suggérant des phénomènes sous-jacents d'adaptation locale (Lasky *et al.*, 2012).

L'ensemble de ces éléments ont progressivement fait d'*Arabidopsis thaliana* un modèle intéressant en écologie et en évolution. Contrairement à la plupart de ses espèces cousines, *A. thaliana* a un potentiel d'acclimatation physiologique et d'adaptation à une large gamme d'environnements (Krämer, 2015) et les bases génétiques sous-jacentes à cette variabilité ont

commencé à être étudiées. Par exemple, le déterminisme génétique des traits d'histoire de vie ont été largement explorées, conduisant notamment à la découverte des gènes contrôlant les transition développementales majeures, telles que la floraison (Simpson, 2002). Des variations alléliques de ces gènes se sont révélées importantes pour l'adaptation des populations le long de gradients latitudinaux et altitudinaux (Mendez-Vigo *et al.*, 2011). Malgré un plan d'organisation simple, la grande variabilité phénotypique de l'espèce a été remarquée très tôt, lors de campagnes de récolte en milieu naturel (Somerville & Koornneef, 2002) (Fig. 11). L'étude de génotypes issus de croisements artificiels a révélé un fort potentiel de variabilité pour les traits du syndrome d'économie foliaire (Blonder *et al.*, 2015), la biomasse végétative et le métabolisme (Vasseur *et al.*, 2018). Des études ont montré des différences importantes pour des traits relatifs aux capacités hydrauliques (Stewart *et al.*, 2018). A cet égard, l'utilisation du modèle *A. thaliana* en écologie comparative est très récente, mais très prometteuse pour identifier les bases génétiques et la valeur adaptative des syndromes de traits identifiés par des comparaisons interspécifiques.

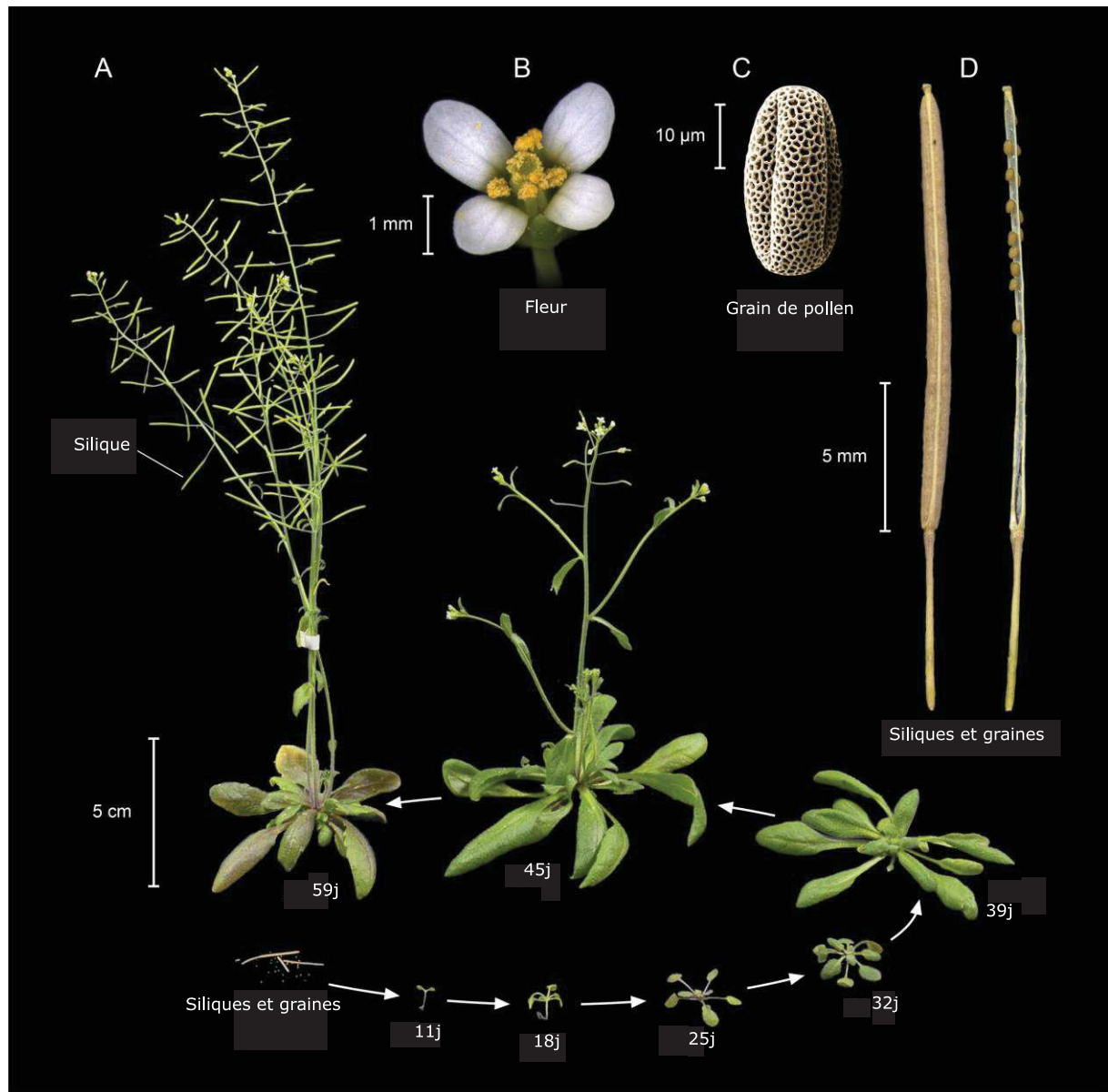


Figure 10 : Développement d'un individu d'*Arabidopsis thaliana*. Un individu du génotype Columbia-0 (Col-0) à différent stades de croissance (A), photographie d'une fleur (B) et d'un grain de pollen (C) par microscopie électronique à balayage et photographie du silique fermée (gauche) et ouverte (droite) (D). Adapté de Krämer (2015) Fig. 1.

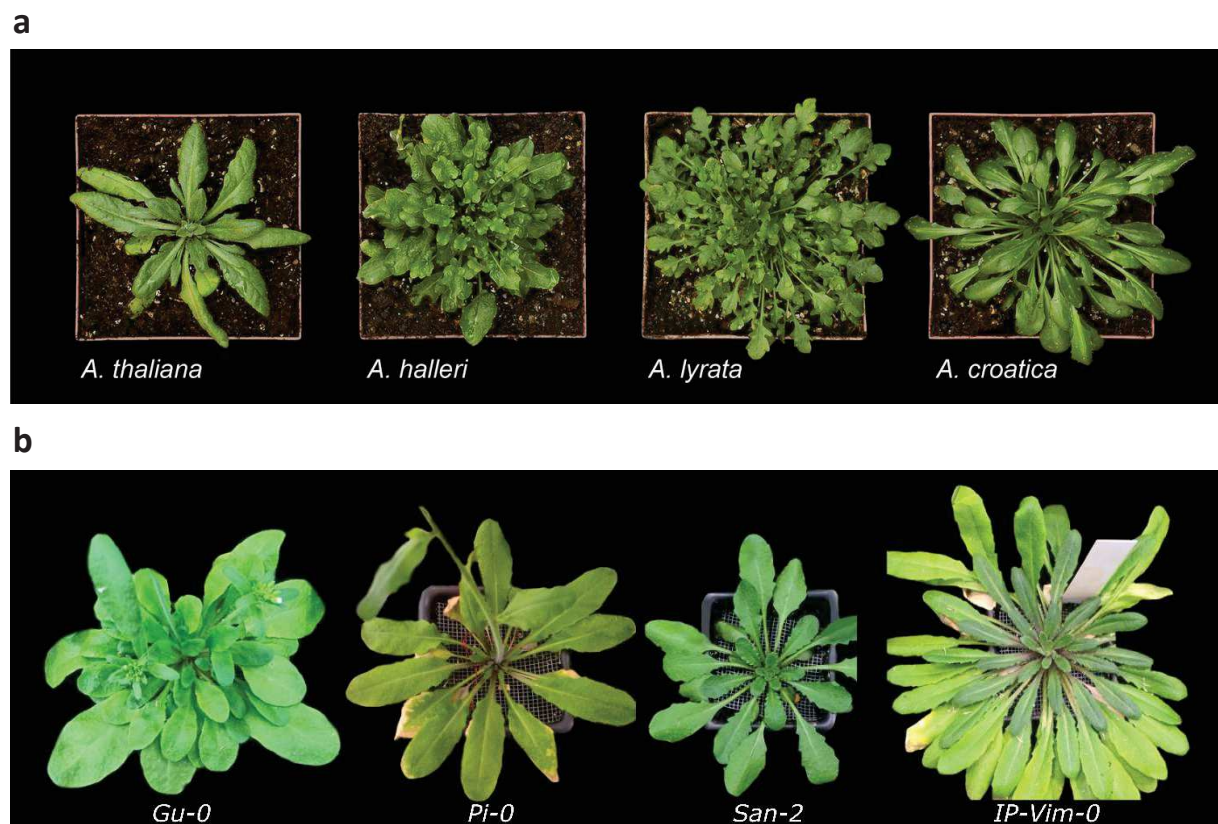


Figure 11 : Variabilité phénotypique de quatre génotypes de l'espèce *Arabidopsis thaliana* au regard de la variabilité phénotypique de quatre espèces du genre *Arabidopsis*. Photographies des espèces *A. thaliana* (génotype Col-0), *A. halleri*, *A. lyrata* et *A. croatica* (a). Adapté de Krämer (2015) Fig. 2. Photographies des génotypes *Gu-0*, *Pi-0*, *San-2* et *IP-Vim-0* (b). Crédit photo : Kevin Sartori.

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CHAPITRE I

Syndrome d'économie foliaire : variation intraspécifique et signification adaptative.

SCIENTIFIC REPORTS

OPEN

Leaf economics and slow-fast adaptation across the geographic range of *Arabidopsis thaliana*

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Life history strategies of most organisms are constrained by resource allocation patterns that follow a 'slow-fast continuum'. It opposes slow growing and long-lived organisms with late investment in reproduction to those that grow faster, have earlier and larger reproductive effort and a short longevity. In plants, the Leaf Economics Spectrum (LES) depicts a leaf-level trade-off between the rate of carbon assimilation and leaf lifespan, as stressed in functional ecology from interspecific comparative studies. However, it is still unclear how the LES is connected to the slow-fast syndrome. Interspecific comparisons also impede a deep exploration of the linkage between LES variation and adaptation to climate. Here, we measured growth, morpho-physiological and life-history traits, at both the leaf and whole-plant levels, in 378 natural accessions of *Arabidopsis thaliana*. We found that the LES is tightly linked to variation in whole-plant functioning, and aligns with the slow-fast continuum. A genetic analysis further suggested that phenotypic differentiation results from the selection of different slow-fast strategies in contrasted climates. Slow growing and long-lived plants were preferentially found in cold and arid habitats while fast growing and short-lived ones in more favorable habitats. Our findings shed light on the role of the slow-fast continuum for plant adaptation to climate. More broadly, they encourage future studies to bridge functional ecology, genetics and evolutionary biology to improve our understanding of plant adaptation to environmental changes.

Investigations of the genetic and phenotypic differentiation of plant lineages along environmental gradients is crucial for the understanding of plant evolutionary responses to current and future climate variations. As plants cannot simultaneously optimize competing eco-physiological functions, an important question is how plant adaptation occurs under the influence of major trade-offs between traits.

The slow-fast continuum is a pervasive trade-off between resource allocation to growth, reproduction and survival, spread across the tree of life¹. The slow end of this continuum is characterized by slow growing, long-lived species and low reproductive output, while species at the fast end reach reproductive maturity faster and produce more offsprings. In plants, the leaf economics spectrum (LES hereafter)^{2–4} is thought to reflect the physiological basis of the slow-fast continuum⁴. The LES arrays plant species along a continuum of leaf trait syndromes going from short-lived leaves with fast metabolism to the reverse syndrome³. Core LES traits include leaf dry mass per area (LMA), leaf lifespan (LLS) and net photosynthetic rate per mass unit (A_{mass})^{3,5–7}. LES traits are widely used in comparative ecology to infer whole-plant ecological strategies^{4,8–12}. However, the extent to which leaf-level resource economics reflects whole-plant physiology, performance, and ultimately fitness, is still under debate¹³. Many processes can lead to a mismatch between LES and whole-plant functioning¹⁴, including the impact of self-shading among leaves and resource allocation patterns, such as carbon investment in non-photosynthetic tissues^{15,16}. To gain insights into the robustness of the slow-fast continuum at different organizational levels, we need

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to examine how LES traits scale up to plant level resource-use strategies, life history and performance. However, it remains difficult to compare individual performance across species with different growth forms, phenology and dispersal strategies since cross-species comparisons impede a clear linkage between physiological and adaptive trade-offs^{17–19}.

The LES has been associated with differences in the ability of plants to adapt to more or less harsh environmental conditions^{4,12,20,21}: species displaying high photosynthetic, respiration and growth rates, are short-lived, with thin and nitrogen-rich leaves are preferentially found in nutrient-rich and/or growth-suitable climatic conditions. Those species are qualified as acquisitive species in contrast to conservative ones that exhibit the opposite set of traits. Despite these observations, functional ecology has no tools to test for adaptation, and empirical evidences of the adaptive value of being at one end or the other of the continuum in a given environment remain scarce (see ref.²² for a review). Furthermore, sampling procedure in field observation studies often impedes to disentangle the effects of plasticity vs. genetic differentiation on the emergence of the LES²³. Thus, comparative studies looking for plant adaptation are at best incomplete^{22–24}, and the role of selection in shaping the LES and driving adaptation to diverse environments is hardly understood. To fill this gap, intraspecific studies are encouraged since they can take benefit from tools developed in population ecology and genetics^{22,25,26}. The LES has started to be analyzed at the intraspecific level, with contrasting findings depending on the studied organism and type of study^{23,27–32}. LES relationships appeared consistent with cross-species ones when using species with broad environmental niche spectra^{31–33} and/or broad phenotypic variability²³, but inconsistent when using species with narrow phenotypic (and genetic) diversity³⁴. Genetic differentiation of LES strategies has been demonstrated among populations of *Helianthus anomalus* along a 400 km rainfall gradient³⁵. However, the question whether LES diversifies because of adaptation to climate among lineages spanning large geographic distribution remains open. Overall, we still miss a comprehensive understanding of within-species LES variation and the subsequent insights they can provide to well-described interspecific patterns from an evolutionary perspective.

Within-species genetic and phenotypic diversity is driven by natural selection, genetic drift, mutation and migration. The measure of F_{ST} statistics among populations, i.e. a comparison of genetic or phenotypic differences among populations, is commonly used to distinguish neutral *versus* adaptive processes. At the genetic level, neutral loci are characterized by low F_{ST} values, i.e. low between-population differentiation due to the combined effect of migration, mutation and drift, while high F_{ST} values reflect genetic divergence as a consequence of directional selection in contrasted environments. From a phenotypic standpoint, adaptive divergence relies on the Q_{ST} statistics, which was developed, by analogy to F_{ST} , to evaluate the population structure for quantitative traits. Q_{ST} values above neutral F_{ST} are interpreted as a signature of diversifying selection on the underlying trait. For instance, Q_{ST} - F_{ST} comparisons have been successfully used in *Campanula rotundifolia*, *Arrhenatherum elatius*, *Quercus oleoides* and *Arabidopsis thaliana* to investigate the role of selection in the diversification of life-history traits, growth strategies and drought resistance among lineages at both local and global scales^{36–39}. This method is expected to be particularly powerful in model species where modern genomics have given access to high-throughput genotyping^{40,41}. By comparing genetic and phenotypic differentiation between populations or lineages, Q_{ST} - F_{ST} provides a powerful tool to infer adaptation in polygenic quantitative traits such as LES traits⁴¹.

The species *Arabidopsis thaliana* has been widely used in molecular biology, cell biology and quantitative genetics. Thanks to the efforts to characterize the genetic diversity in this species^{42–45}, it is also a model in population dynamics⁴⁶ and evolutionary ecology⁴⁷. For instance, the genetic determinism of *A. thaliana* life history has been extensively studied, notably with the discovery of genes that control major developmental transitions such as flowering time (see ref.⁴⁸ for a review). Allelic variation in these genes appears to be adaptive to climatic and altitudinal gradients⁴⁹. A recent study supports the hypothetical link between life history variation and the LES in *A. thaliana*, highlighted by strong genetic correlations between these traits²⁸. However, this analysis was performed on recombinant inbred lines used for genetic mapping. Made of artificial crosses, they preclude examining the relationships between LES and the natural environment. Interestingly, *A. thaliana* has recently gained a renewed interest in functional ecology and biogeography^{50–52}, notably due to the large panel of natural accessions that have been collected from contrasting climates, and genotyped at high density (e.g.^{42–45}). As genetic data in *A. thaliana* allow an unprecedented large-scale analysis of genetic variation among populations and lineages, this species is promising to investigate the extent of intraspecific diversity and its role for adaptation to contrasted climates.

In this study, we explored the evolutionary bases of intraspecific leaf and plant trait variation using a pan-European collection of 378 natural *A. thaliana* accessions from the RegMap panel⁴⁴. Specifically, we investigated whether plant adaptation to various climates is associated with genetic differentiation along the LES and the slow-fast continuum. To test this hypothesis, we first examined how the LES shapes phenotypic diversity across contrasted genotypes of *A. thaliana*, and tested whether LES traits scale up to plant level resource-use strategies and life history. Next, we took benefit from the large genomic information available in *A. thaliana* to evaluate to what extent phenotypic differences among lineages are attributable to adaptive processes such as adaptation to contrasted climates using Q_{ST} - F_{ST} comparisons.

Results

Geographic clustering of *A. thaliana* lineages. Following the Whittaker's biome classification method, two climatic variables, mean annual rainfall (MAR) and mean annual temperature (MAT), were used for study site classification into the major biome types. The range of biomes experienced by the sampled genotypes covers woodlands-shrublands and the less humid part of temperate forests, with a few extremes in boreal forests and deserts like environments (Fig. 1b). Using the 250 K SNPs data available from Horton *et al.*⁴⁴, we performed a genetic clustering of the genotype set, which revealed the presence of five genetic lineages. These lineages were moderately differentiated (mean F_{ST} = 0.11), geographically (Fig. 1a) as well as in the Whittaker's biome classification (Fig. 1b). The analysis revealed the existence of two genetic groups exclusively located in France in our sample (French 1 and French 2 hereafter) of 76 and 49 genotypes, respectively. Among the 20 genotypes of the

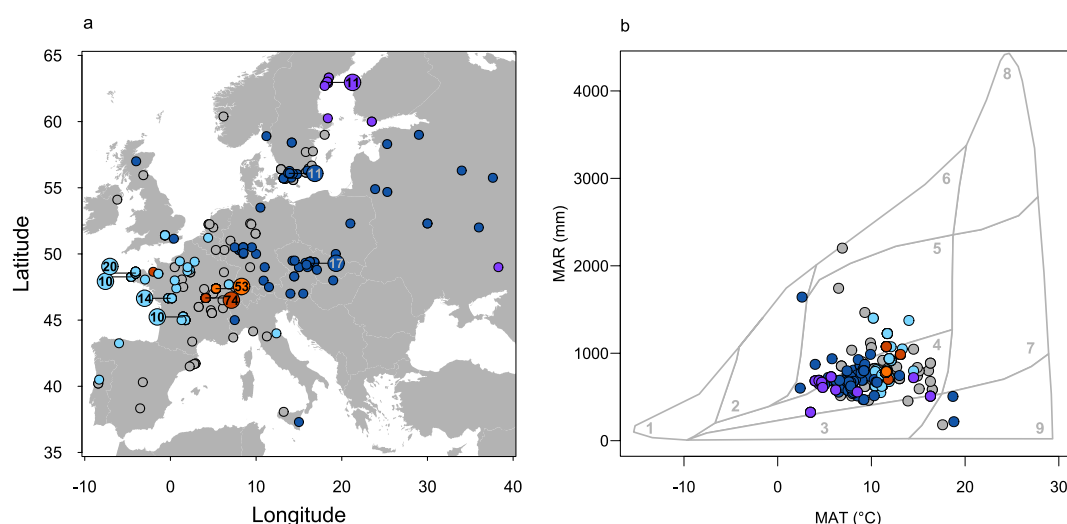


Figure 1. Location and climatic conditions of the genotype collecting sites. **(a)** Distribution of the 378 natural genotypes used in this study. The small points represent the collecting sites of genotypes and bigger points give the number of collecting sites overlapped at these positions. The colors represent the five genetic groups: Admixed (grey), French 1 (brown), French 2 (orange), Swedish (purple), Central Europe (dark blue), Western Europe (light blue). **(b)** Mean annual rainfall (MAR) and mean annual temperature (MAT) for the sites where genotypes were collected, in relation to major biome types of the world following Whittaker's classification. 1–9: Tundra, Boreal forest, Temperate Grassland Desert, Woodland Shrubland, Temperate Forest, Temperate Rain Forest, Tropical Forest Savana, Tropical Rain Forest, and Desert.

third group, seven were defined as North Swedish in the 1001 genomes dataset⁴⁵. Consistently, the 20 “Swedish” genotypes, although not all in Sweden (Fig. 1a), were mainly located in cold environments and woodland-shrubland in Whittaker's classification (Fig. 1b). We considered the 76 genotypes from group 4 as “Central European” (Fig. 1a), typically living at intermediate temperatures and rainfall (Fig. 1b). 83 genotypes composed the group 5, all located in Western Europe (Fig. 1a), in a range of relatively warm environments with intermediate rainfall.

Leaf economics of *A. thaliana*. Assimilation rate (A_{mass}) was the most variable trait among the leaf economics traits in our dataset (15-fold; from 40.9 to 608.9 $\mu\text{mol g}^{-1} \text{s}^{-1}$) while leaf mass per area (LMA) and leaf lifespan (LLS) varied 5 and 3.5 fold (from 18.7 to 101 g m^{-2} , and from 15 to 53.5 days), respectively. In regard to interspecific meta-analyses, variation in A_{mass} was wide (from 5 to 660 $\mu\text{mol g}^{-1} \text{s}^{-1}$) and was relatively weak for LMA (from 14 to 1,500 g m^{-2}) and for LLS (from 0.9 to 288 months³). Pairwise regression revealed strong correlations between traits, independently of the genetic structure of the sample ($r^2_{A_{\text{mass}}\text{-LLS}} = 0.32$, $r^2_{A_{\text{mass}}\text{-LMA}} = 0.73$, $r^2_{\text{LMA-LLS}} = 0.38$, all p -value < 0.01). The 3-dimension space shaped by traits covariations in *A. thaliana* was comparable to the interspecific spectrum previously observed³: genotypes are ranked from low A_{mass} and high LMA and LLS, toward high A_{mass} and low LMA and LLS (Fig. 2). A principal component analysis (PCA) showed that 78% of the covariation between these three traits was explained by a single Principal Component (PC1; Fig. S1a). Hereafter, we assigned a position along the LES for each genotype according to its score on PC1. A_{mass} was highly negatively correlated with PC1 ($r = -0.90$) while LMA and LLS were positively correlated with PC1 ($r = 0.91$ and 0.79 , respectively). Thus, high and low PC1 values are representative of genotypes located at the conservative and acquisitive side of the LES, respectively.

From the Leaf Economics Spectrum to the plant slow-fast continuum. Trait measurements at the plant level revealed that assimilation rate was again the most variable trait with a 68-fold variation (from 8.4 to 578.1 $\mu\text{mol g}^{-1} \text{s}^{-1}$), while plant mass per area and age of maturity (AM) both varied 5 fold (from 17.7 to 85.4 g m^{-2} and 22 to 111 d, respectively). Standardized major axis (SMA) regressions between traits measured at the leaf and plant levels were all significant. Leaf and whole plant-level LMA were highly correlated ($r = 0.89$; $P < 0.001$; Fig. S1e) and the slope was close to, but significantly different from 1 (95% Confidence Interval slope = [1.07, 1.18]), as well as for leaf-level and whole plant-level net assimilation rate ($r = 0.78$; $P < 0.001$; Fig. S1f, 95% CI slope = [0.78, 0.9]). Similarly, LLS and AM were significantly correlated with a slope below 1 (slope = 0.68 [0.55; 0.65], $r = 0.67$, $P < 0.001$; Fig. S1g). A single principal component explained 86% of the trait covariation at the plant level (Fig. S1b) and was highly correlated with PC1 at the leaf level ($r = 0.87$, $P < 0.001$; Fig. S1h). Furthermore, life history and performance at the plant level co-varied in parallel with this leaf level phenotypic variation. Relative growth rate (RGR) and age at maturity (AM) were negatively correlated (Fig. 3) in our dataset, consistently with the slow-fast continuum pattern: fast growing genotypes reproduced earlier than slow growing ones. As for the LES, we performed a PCA with slow-fast traits and we assigned a position along the continuum for each genotype according to its score on PC1. The positions of the genotypes along the leaf economics spectrum and the slow-fast continuum were tightly correlated (Fig. 4a) and the slope did not differ

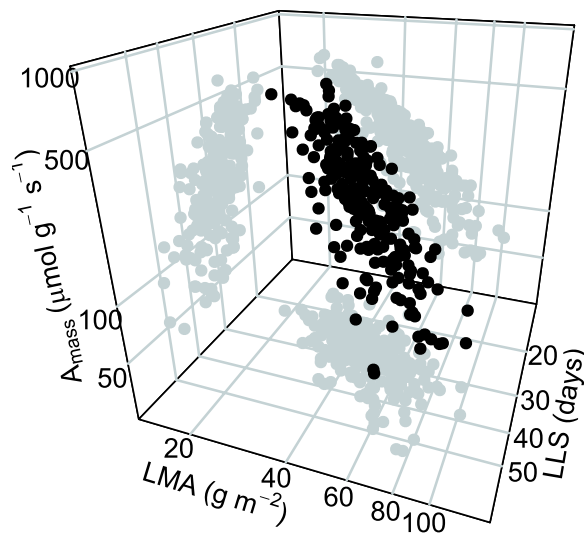


Figure 2. The leaf economics spectrum in *A. thaliana*. Three-way relationships among the main leaf economics traits: A_{mass} , mass based assimilation rate ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$); LMA, leaf mass per area (g m^{-2}); LLS, leaf lifespan (days). Each point represents a different genotype.

significantly when taking the kinship matrix of the genotypes as a covariate. In other words, slow growing and late reproducing genotypes have low leaf carbon assimilation rate and long leaf lifespan, whereas fast growing and early reproducing genotypes have high leaf assimilation rate and short leaf lifespan.

Adaptation cues of the slow-fast continuum. Consistently with the original description of the RegMap genotypes⁴⁴, the genetic differentiation among *A. thaliana* populations was substantial. Pairwise comparisons based on the whole genetic data indicated a strong genetic divergence between French 2 and the other groups ($F_{\text{ST}} > 0.19$). French 1 group was genetically closer to Western European group ($F_{\text{ST}} = 0.13$) than French 2 group ($F_{\text{ST}} = 0.20$). Interestingly, North Swedish lines showed strong phenotypic and genetic differentiation with other lineages (Fig. S4). We evaluated the significance of the differentiation of the slow-fast strategies performing $Q_{\text{ST}}-F_{\text{ST}}$ comparisons for both RGR and AM. Heritability was high for both traits ($h^2_{\text{AM}} > 0.95$, $h^2_{\text{RGR}} \sim 0.64$). P_{ST} is a good estimator of Q_{ST} when trait heritability (h^2) is high and the phenotypic between-populations component of variance genetically determined (c) is close to h^2 (see the Methods section). For the null hypothesis ($H_0: c = h^2 = 1$), P_{ST} values were high and significantly higher than the neutral F_{ST} for both traits ($P_{\text{ST AM}} \sim 0.35$, $P_{\text{ST RGR}} \sim 0.3$; see Table 1). However, further analyses revealed that the estimation of P_{ST} were highly sensitive to the ratio between the among-population (c) and overall heritability (h^2) of the trait. On the other hand, we showed that all LES traits, exhibited modest and non-significant P_{ST} values (Table 1) under the null assumption. Finally, we performed a P_{ST} analysis on PCA scores for the two set of traits. Despite a lower heritability ($h^2_{\text{SFC}} \sim 0.59$, $h^2_{\text{LES}} > 0.95$), the slow-fast continuum P_{ST} was high and significant compared to the leaf economics spectrum P_{ST} (Fig. 4b) ($P_{\text{ST SFC}} \sim 0.35$, critical $c/h^2 \sim 0.35$; $P_{\text{ST LES}} \sim 0.12$), although both were highly sensitive to c/h^2 . This suggests that slow-fast traits behaved like outlier variants that diverged among lineages due to the effect of diversifying selection. Our data do not support the same hypothesis for LES traits, despite a tight coordination with slow-fast traits.

Climatic drivers of *A. thaliana* phenotypes. We investigated whether annual rainfall and temperature, two key climatic variables in plant functional biogeography, explain the position of the genotypes along the slow-fast continuum and leaf economics spectrum axes. We extracted 19 climatic variables at the collecting sites of the genotypes on CHELSA website (www.chelsa-climate.org/) related to temperature and precipitation, their temporal variability and extremes. Consistently with the population structure analysis, only the slow-fast continuum axis was significantly correlated with MAR ($r = -0.18$, $P < 0.01$) and MAT ($r = -0.16$, $P < 0.01$) (Fig. 5). The correlation with the 17 other CHELSA variables revealed the same pattern: the slow-fast continuum axis and the related traits were more often significantly correlated with climatic variables (Table S1). Therefore, we investigated whether climatic variables at the collecting sites of the genotypes can predict the *A. thaliana* slow-fast strategies. Firstly, stepwise regressions revealed that position on the slow-fast continuum was best predicted by a subset of climatic variables, including the mean annual temperature and rainfall, and variables related to temperature variance and extremes throughout the year. We evaluated the accuracy of the model using a repeated cross-validation method, which revealed that phenotypes were more accurately predicted by the reduced model ($r^2 \sim 0.26$) than the formal model ($r^2 \sim 0.20$). Extrapolating prediction of phenotypes across Europe from climate variables, we showed that slow strategies, characterized by slow growth and late reproduction, were favored in North Europe and Central East of Spain and in the highest European reliefs (Fig. 6). In addition, fast strategies characterized by fast growth and early reproduction were found in Central Europe and near the coasts.

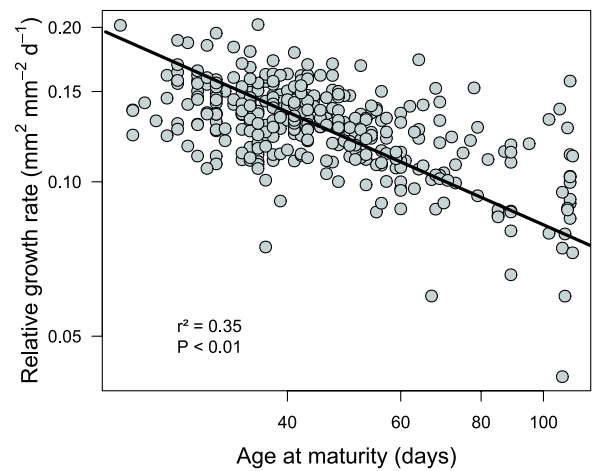


Figure 3. The slow-fast continuum in *A. thaliana*. Relationship between relative growth rate ($\text{mm}^2 \text{mm}^{-2} \text{d}^{-1}$) and age at maturity (days). Each point represents a different genotype. The solid line represents the output of a linear model, the slopes do not significantly differ while taking into account the genetic relatedness of genotypes or not.

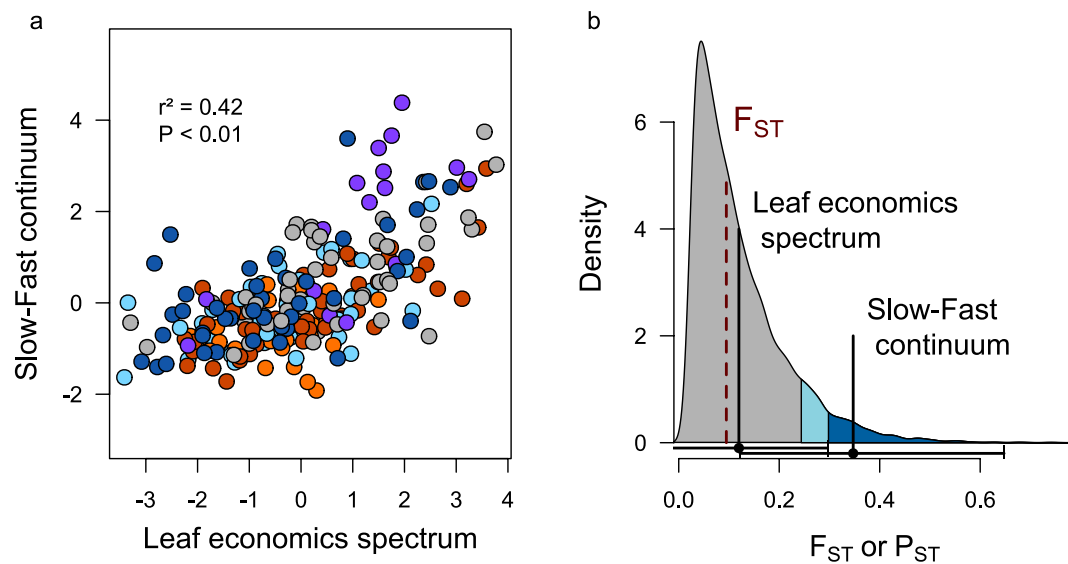


Figure 4. The Leaf Economics Spectrum and the slow-fast continuum covariate in *A. Thaliana* and are differentiated among populations. (a) Correlation between the position of each genotype along the leaf economics spectrum and the slow-fast continuum. Colors represent the five genetic groups (see Fig. 1). (b) Phenotypic differentiation (P_{ST} , black vertical lines) and their confidence intervals (horizontal brackets) relatively to the distribution of genetic differentiation (F_{ST}), its median (red dashed line), 90th quantile (light blue) and 95th quantile (dark blue).

	LES	SFC	A_{mass}	LLS	LMA	AM	RGR
h^2	0.99	0.59	0.70	0.66	0.88	0.99	0.64
P_{ST}	0.12	0.35	0.12	0.02	0.19	0.35	0.3
CI 95%	[−0.02;0.28]	[0.07;0.7]	[−0.01;0.29]	[−0.05;0.09]	[0.01;0.42]	[0.07;0.71]	[0.03;0.62]
Critical c/h^2	NA	1.25	NA	NA	NA	NA	1.49

Table 1. The phenotypic differentiation of *A. thaliana* regarding slow-fast and LES traits is genetically determined and structured among regional populations. Abbreviations: h^2 , narrow-sense heritability; P_{ST} , phenotypic differentiation statistic; CI, confidence interval; c, among-population heritability.

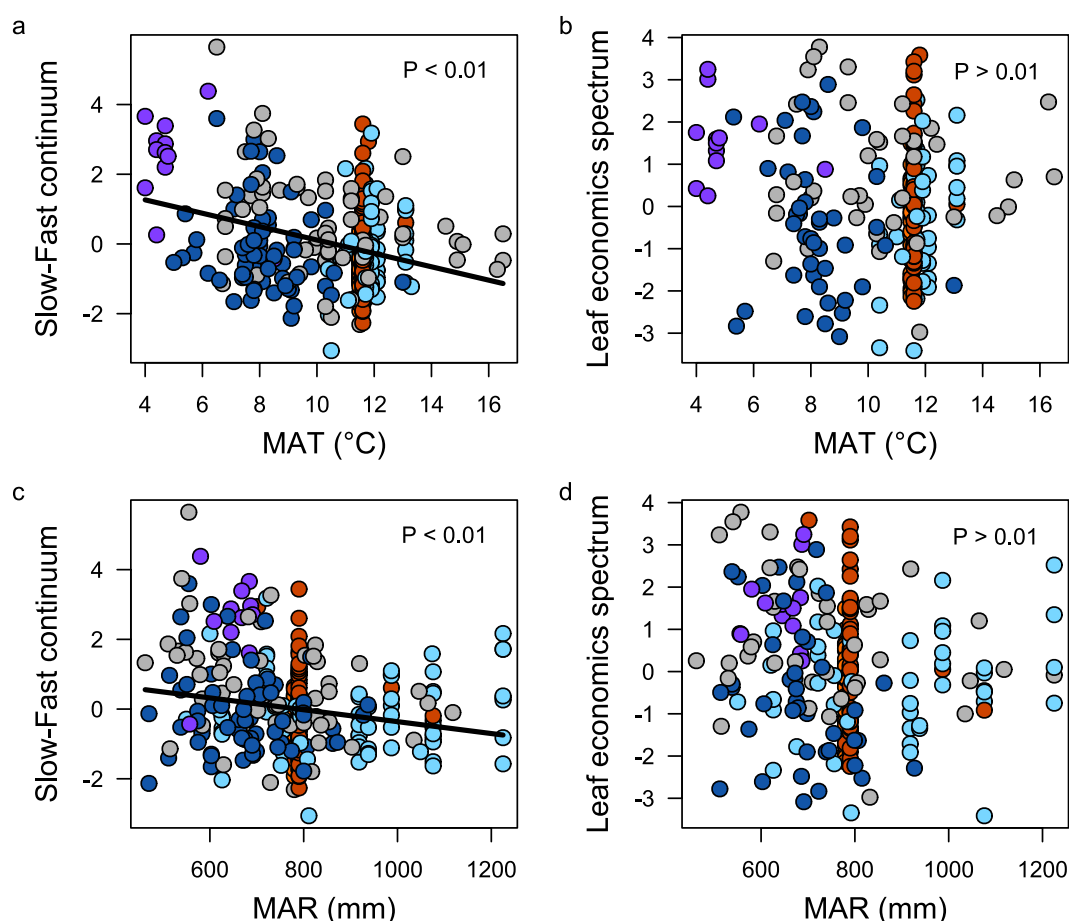


Figure 5. Slow-fast continuum, Leaf Economics Spectrum, and their relationships with climate. Position of genotypes along the slow-fast continuum as a function of (a) mean annual temperature (MAT) and (b) mean annual rainfall (MAR) and position of genotypes along the Leaf Economics Spectrum as a function of (c) MAT and (d) MAR. Linear regressions are represented by black lines when significant.

Discussion

The comparison of multiple species based on a few traits is the historical approach of functional ecology⁵³. While fruitful¹², such an approach impedes a deeper investigation of how evolutionary forces and trade-offs operate together to shape the observable phenotypic diversity^{24,26}. Notably, several trait-trait covariations have been discussed in functional ecology in the light of trade-off theories. One of the most prominent phenotypic pattern discussed in the last decades, the so-called Leaf Economics Spectrum (LES), is thought to reflect a trade-off between metabolic rate and lifespan at the leaf level^{3,5,54,55}. Plant species that exhibit long-lived leaves have been referred as resource conservative species. They optimize long-term carbon gain and extended nutrient residence time, as well as nutrient use efficiency⁵⁶. By contrast species with short-lived leaves sacrifice nutrient retention to maximize the rate of carbon fixation. The LES is expected to reflect an adaptive trade-off between fast and slow growth strategies across plant species⁴. Two assumptions underline this assertion: (i) the negative correlation between leaf photosynthetic rate and leaf lifespan is translated into a negative correlation between plant growth rate and the duration of the life cycle, (ii) particular combinations of slow-fast traits are selected in different environments. Both assumptions are difficult to test at the interspecific level. This has generated a living debate about the evolutionary causes of the LES^{22,28,57–60}. Taking benefit from a large collection of sequenced genotypes in a model species, our results show that LES traits are correlated with slow-fast strategies at the plant level, and that trait divergence among genetic lineages is non-neutral. This supports the idea that plant populations evolve different slow-fast strategies along with different LES traits in order to adapt to contrasting climates.

We showed that LES trait correlations in *A. thaliana* follow the interspecific pattern^{3,5}: individuals that invest a large amount of biomass per unit leaf area have a lower leaf assimilation rate and a longer leaf lifespan than plants that invest less biomass per unit leaf area. Moreover, the economics spectrum is still detected when scaling from leaf to whole-plant traits. This gives strong support to the idea that, at least in an herbaceous species such as *A. thaliana*, a trait value obtained on a single leaf using a standardized method, reflects the average phenotypic value expressed by all the leaves of an individual plant^{61,62}. Furthermore, our results showed that the ranking of genotypes was similar along the leaf economics spectrum and the slow-fast continuum, suggesting that carbon economy at the leaf level is connected to the slow-fast strategies at the plant level. Up to now, functional ecology has favored interspecific comparisons, focusing on species trait means⁶³, with the perspective of generalization

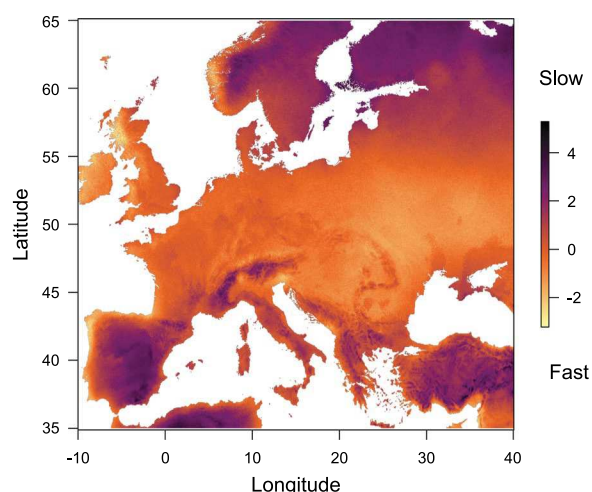


Figure 6. Slow strategy is favored in Mediterranean and Scandinavian regions while fast strategy is selected in the center of *A. thaliana* distribution. Map representing the prediction of slow-fast strategies based on climatic data: darker regions endure combination of climatic variables associated with slower strategies as opposed to faster strategies in lighter regions.

and prediction of the whole biota functioning⁶⁴. Conversely, model species and annual ones in particular, have often been considered as extreme and atypical strategies when examining the phenotypic space of the worldwide flora¹¹. Here we showed how the eco-physiological examination of these species enrich our interpretation of interspecific trait covariations. Our results thus encourage future studies analyzing intraspecific trait covariations using model species such as *A. thaliana* in plant functional ecology to further advance our understanding of their underlying origins and mechanisms. Nonetheless, it is also true that our findings can be specific to the relatively simple organization and functioning of an annual rosette species. In particular, the correlations between leaf-level and whole-plant traits are presumably strongly variable among species. This relation is notably expected to be weaker in woody species because of the varying proportion of non-photosynthesizing tissues⁶⁵. This result has a consequence for mass-based traits: when a given leaf trait increases by one unit, the same trait at the plant level increases by less than one unit. Our results illustrate this statement: leaf A_{mass} correlated with plant A_{mass} with a slope below of 1. Similarly, leaf lifespan varied less across genotypes than plant age at maturity. As a result, there is a room for a decoupling between leaf and plant life history, even though the ranking among genotypes is globally conserved at the two organizational scales. Further explorations of how much leaf-level trade-offs and plant functioning are coordinated in herbaceous and woody species are needed.

Despite the autogamous nature of *A. thaliana* which makes it peculiar in eco-evolutionary studies, its large geographic distribution allows to explore multiple trait-environment relationships at large scales. These relationships are still hardly known and quantified for most functional traits and in most biomes^{12,64} because of the myriad of species-specific responses that can blur the general interspecific trends. Again, our results illustrate how intraspecific comparisons can help elucidating the mechanisms underlying these relationships, notable putative adaptive ones. Using the Q_{ST} - F_{ST} comparisons, we brought evidence that *A. thaliana* regional populations are structured according to slow-fast traits, although better characterization of the genetic determinism of these phenotypes is needed. We reinforced this assertion identifying climatic factors related to this structure. The predicted distribution of slow-fast strategies across Europe revealed differential selection between roughly Norway, Sweden and Spain on one side, and central and Western Europe on the other. Selection for slow genotypes toward higher latitude in *A. thaliana*, specifically in North Swedish genotypes, is supported by previous findings on flowering time^{66,67}. More surprisingly however, our results suggest that similar trait combinations representative of slow strategies are selected in two contrasted climates: Spain and Scandinavia, which are at the opposite edges of the *A. thaliana* latitudinal range. This clustering of *A. thaliana* genotypes echoes a recent study showing fixation of drought-related alleles in both Scandinavian and Spanish *A. thaliana* populations⁶⁸. If we consider together the absence of significant effect of the kinship matrix on trait-trait relationships tested, the globally low average differentiation among genetic groups ($F_{\text{ST}} = 0.11$), and the phenotypic similarity observed at two distant locations, our results suggest that the genetic determinism of slow strategies as well as phenotypic differentiation could have occurred by convergence through adaptive processes. Thus, slow strategies could be selected in response to environmental stress in regions from nonetheless very different climates: low average temperature at Scandinavian sites and Mediterranean climate at Spanish sites. Interspecific studies at global scale revealed a negative relationship between conservative strategies and rainfall^{3,69}, possibly linked to a higher investment in cell wall complex macromolecules to face drought stress⁷⁰. Large-scale interspecific studies also reported a bias toward acquisitive strategies with increasing temperature in herbaceous species^{71,72}. Similarly, theoretical frameworks suggest that drought and cold favor slow growing individuals in environments limited by water or temperature⁷³. Together, this suggests a general selection pressure for slow strategies in stressful environments, as opposed to selection for fast strategies in non-stressing environments²³. A promising avenue for future studies is to perform reciprocal transplant experiments to test the effect of drought and high temperatures on *A. thaliana* genotypes distributed along the slow-fast continuum.

Overall, our results suggest that slow-fast strategies are differentially selected in contrasted climates. Despite strong coordination of strategies from leaf level to plant level, slow-fast traits were significantly differentiated across populations and were associated with climatic conditions at the collecting sites, while significance was lacking for LES traits. By contrast, previous experiments under controlled conditions reported significant effects of temperature and soil water content on both whole plant and leaf traits in *A. thaliana*^{74,75}. Complex interactions between climatic variables could change their relationships with phenotype in natural environments. This echoes the long standing search for global pattern of covariation between climate and LES traits in functional ecology and biogeography^{3,71,76}. In addition to the expected anisometric relationship between plant and leaf traits (slope $\neq 1$), room is left for leaf level traits to desynchronize from individual strategy. This suggests a potential for leaf traits to adapt to microclimatic conditions, including those at the canopy level. More broadly, our results corroborate the weak predictive power of leaf-level traits only in functional ecology when searching for adaption to particular climatic variable combination^{12,13}. Indeed, even for an annual herb such as *A. thaliana*, whole-plant traits are more likely to capture adaptation to the environment compared to organ-level traits.

Using a model species, with large collections of well-characterized genetic material, appears particularly successful to go deeper into the evolutionary underpinning of major eco-physiological trade-offs, such as the LES and the slow-fast continuum. Combined with global climatic data, our findings notably revealed that adaptation to cold or dry habitats tends to favor slow strategies in *A. thaliana*. Next steps will be to merge approaches, and fully benefit from what a model species can provide both to genetics and ecophysiology. For instance, the climatic cues detected here despite the lack of climate data precision, is encouraging for the future of functional biogeography⁶⁴. There is also evidence that the connection between functional trait and environmental adaptation requires a better characterization of plant fitness through demographic measures²⁶. Comparative studies integrating demographic approach at population level are promising to understand how selection and macro-ecological gradients shape the evolutionary responses of plants to climate variation^{24,26}.

Materials and Methods

Plant material. We used a total of 378 natural genotypes of *A. thaliana* L. Heynh sampled from the worldwide lines of the RegMap population (<http://bergelson.uchicago.edu/wp-content/uploads/2015/04/Justins-360-lines.xls>), which were genotyped for 250 K bi-allelic SNPs⁴⁴. Seeds used in the present study were originally collected and multiplied by Fabrice Roux (UMR LIPM, Toulouse, France) and his collaborators.

Growth conditions. Phenotype characterization was performed under controlled conditions in the high-throughput PHENOPSIS phenotyping platform⁷⁷ to track daily growth. Seeds were kept in the dark at 4 °C for at least one week before sowing. Four to six seeds per genotype were sown at the soil surface in 225 ml pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost (Neuhaus N2). The soil surface was moistened with one-tenth strength Hoagland solution, and pots were kept in the dark during 48 h under controlled environmental conditions (20 °C, 70% air relative humidity). Then, pots were placed in the PHENOPSIS growth chamber at 20 °C, 12 h photoperiod, 70% relative humidity, 175 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. Pots were sprayed with deionized water three times per day until germination, and then soil water content was adjusted to 0.35 g H₂O g⁻¹ dry soil (−0.07 MPa soil water potential) to ensure optimal growth^{74,78}. After emergence of the fourth leaf, one individual plant was left in each pot.

Measurements of plant traits. In order to standardize measurements for all genotypes, all traits were quantified when flower buds were macroscopically visible (*i.e.* bolting stage), and leaf traits were measured on the last adult leaf, fully exposed to light.

Net photosynthetic rate, relative expansion rate, lifespan, vegetative dry weight, as well as leaf area were determined for the leaf and the plant canopy. Net photosynthetic rate was measured at leaf (leaf A, $\text{nmol CO}_2 \text{s}^{-1}$) and whole-plant levels (plant A, $\text{nmol CO}_2 \text{s}^{-1}$) under growing conditions using, respectively, the leaf cuvette provided with the infrared gas analyzer system (CIRAS 2, PP systems, USA), and a whole-plant chamber prototype designed for *A. thaliana* by M. Dauzat (INRA, Montpellier, France) and K. J. Parkinson (PP System, UK) (see²⁸). Leaf and whole-plant photosynthetic rates were both expressed on dry mass basis (leaf A_{mass} and plant A_{mass} , $\text{nmol CO}_2 \text{g}^{-1} \text{s}^{-1}$). Due to time constraints, we measured photosynthetic rates for 319 and 348 genotypes at the leaf and whole-plant levels (306 in common), respectively. We estimated the age of maturity by the number of days from germination to the appearance of the flower bud. Then, plants were harvested, and individual fresh weight was determined. The leaf used for photosynthetic measurements was identified and processed separately, and detached rosettes were kept in deionized water at 4 °C for 24 h, and water-saturated weight was determined. Individual leaves were then attached to a sheet of paper and scanned for subsequent determination of the leaf number and total leaf area using ImageJ⁷⁹. Dry weight of lamina and petioles were obtained after drying for 72 h at 65 °C. Rosette dry weight was expressed as the sum of lamina and petiole dry weights. Leaf mass per area was both calculated for the leaf used for photosynthetic measurements (LMA, g m^{-2}) and for the whole-rosette (plant LMA, g m^{-2}) as the ratio of lamina dry mass to lamina area. Relative growth rate (RGR, $\text{mm}^2 \text{mm}^{-2} \text{d}^{-1}$) and leaf lifespan (LLS, d) were estimated from automated daily pictures of the rosettes. More precisely, a sigmoid curve was fitted to rosette area as a function of time in order to extract growth parameters, where RGR was calculated as the slope at the inflection point^{80–82}. Using daily pictures, we tracked three consecutive leaves from birth (emergence) to death (full senescence). For each plant, leaf duration was calculated as the average number of days from leaf emergence to senescence.

F_{ST} and P_{ST} estimates. In order to perform population genetic analyses, genetic groups were identified by genetic clustering of 378 genotypes, using the 250 K SNPs data available from Horton *et al.*⁴⁴. Clustering was performed with ADMIXTURE⁸³ after linkage disequilibrium pruning ($r^2 < 0.1$ in a 50 kb window with a step size of

50 SNPs) with PLINK⁸⁴, resulting in 24,562 independent SNPs used for subsequent analyses. A cross-validation for different numbers of clusters ($k = 1$ to $k = 10$) showed that the set of studied genotypes can be separated into five groups representative of different genetic lineages (cross validation error = 0.89). Following the same approach as the 1001 genomes project⁴⁵, we assigned each genotype to a group if more than 50% of its genome derived from the corresponding cluster. The 74 genotypes not matching this criterion were labelled “Admixed” and were not used for the F_{ST} and P_{ST} calculation. The groups genetically defined were also geographically distinct as shown by early studies⁴⁴. We measured the genetic population structure using Weir and Cockerham F_{ST} statistic for all the 24,562 SNPs, as well as mean F_{ST} genome-wide. To determine the neutral F_{ST} value, we calculated the median of the significant F_{ST} values of the intergenic SNPs. More specifically, we filtered the SNPs dataset keeping the intergenic SNPs only, assuming that they are rather prone to endure neutral differentiation processes. We then calculated a F_{ST} value for each intergenic SNP with the attribution of population described above. To test for the significance of the F_{ST} values, we randomized 1000 times the population attribution to the genotypes in order to provide a null distribution of F_{ST} . F_{ST} values higher than the 95th quantile of their null distribution were stated as significant. The phenotypic population structure is ideally evaluated using Q_{ST} , an analogue of F_{ST} measure^{85,86}. We estimated Q_{ST} using a phenotype-based surrogate (P_{ST})⁸⁶ which depends on among-population and overall heritability of the trait (c and h^2 , respectively) computed as follow:

$$P_{ST} = \frac{c \sigma_B^2}{c \sigma_B^2 + h^2 \sigma_W^2}$$

where B and W are the between- and within-population part of variance, respectively. Note that within-population variance is not multiplied by 2 since *A. thaliana* is mainly autogamous and homozygote at all loci. Under the null assumption ($H_0: c = h^2 = 1$), P_{ST} and Q_{ST} are analogous. Sensitivity analysis consists in calculating the P_{ST} value and its 95% confidence interval for a gradient of hypothetical c and a given h^2 . P_{ST} is a robust estimator of Q_{ST} when the critical value of c/h^2 is low, i.e. the minimum value of c/h^2 for which the lower limit of P_{ST} 95% CI is higher than the neutral F_{ST} . Then, a value of Q_{ST} higher than neutral F_{ST} means that the phenotypic differentiation between populations is larger than expected by demographic events alone, in particular genetic drift, and is thus indicative of diversifying selection on traits^{41,87}. We used parametric bootstrap method to generate 95% CI around P_{ST} values with the package MCMCglmm in R (10,000 iterations).

Statistical analysis. Climate variables at the sampling sites of each genotype were extracted from the CHELSA database (<http://www.chelsa-climate.org/>), with a 2.5 arc-minutes resolution. The effect of climatic variables on traits was tested using linear model regressions. All analyses were performed in R 3.4.1 (R Core Team, 2017). Whittaker's biomes were plotted using the BIOMEplot function provided by G. Kunstler (<https://rdrr.io/github/kunstler/BIOMEplot/src/R/biomes-plot.R>). All leaf and plant traits, but RGR, were log₁₀ transformed when Gaussian distribution is required for statistical analyses. Principal component analysis (PCA) was performed using the package FactoMineR. The package nlme was used to perform linear models and phylogenetic generalized least squares regressions. We performed phylogenetic regressions including a relatedness matrix as covariance matrix, obtained after running the PLINK–make-rel command across the 250 K SNPs from the RegMap data. SMA regressions between leaf and plant traits were performed using the package SMATR⁸⁸, and phylogenetic SMA regressions using the Phyl.RMA function of the Phytools package. The phylogenetic tree required for SMA regression has been produced with Tassel using the RegMap SNPs data⁴⁴.

Data Availability

Data are deposited in the Dataverse repository: <https://doi.org/10.15454/B3W0OS>.

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Author Contributions

D.V., F.V. and C.V. designed the study, K.S., E.B., M.G., O.A.-G., A.C., L.G.D.J., D.M., E.H., M.D.R.G. and A.C. conducted the experiments. K.S. and F.V. performed statistical analyses. K.S. wrote the first draft of the manuscript, and K.S., F.V., C.V., E.B., M.G., N.R., O.A.-G., A.C., L.G.D.J., D.M., E.H., M.D.R.G., A.C., E.K. and D.V. contributed to revisions.

Additional Information

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Leaf economics and slow-fast adaptation across the geographic range of *Arabidopsis thaliana*

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Supplemental Information

Figure S1: Leaf economics spectrum is consistent from leaf to plant level as a result of strong trait covariation from leaf to plant level in *A. thaliana*. All traits were log10 transformed. Principal component analysis of LES traits (Amass, Assimilation rate; LLS, leaf lifespan; LMA, leaf mass per area): correlation circle of variable at the leaf (a) and plant levels (b), and individuals at the leaf (c) and plant (d) levels. Covariation between leaf and plant traits: Leaf mass per area (e), Assimilation rate (f), life history (g) and principal components (h). Dashed lines represent the identity relation and continuous lines represent standard major axis regression when significant.

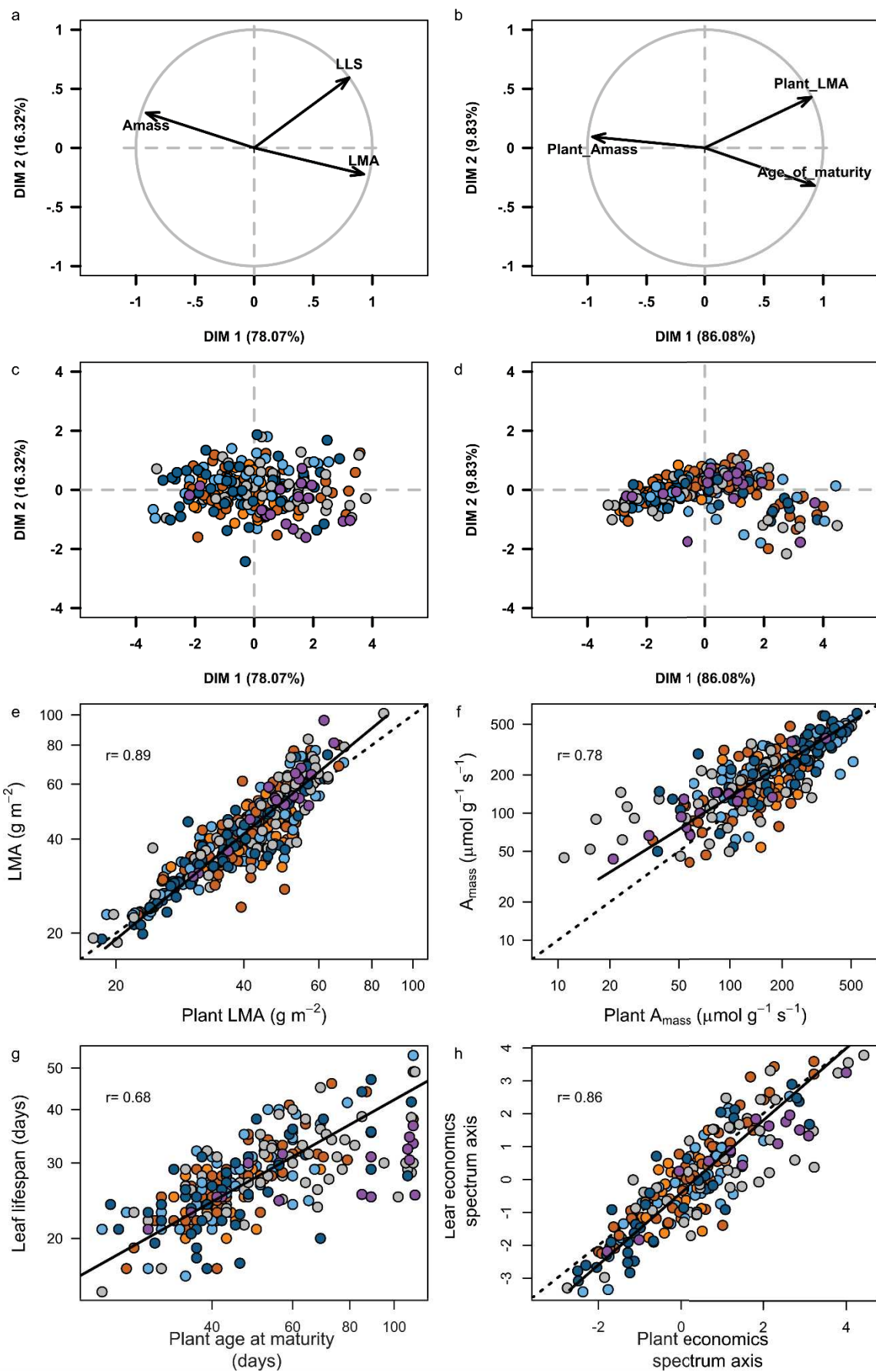


Figure S2: Influence of the c/h^2 ratio on the significance and robustness of the P_{ST} .

Phenotypic differentiation (P_{ST} , black vertical lines) relative to the distribution of genetic differentiation (F_{ST}), its median (red dashed line), 90th quantile (light blue) and 95th quantile (dark blue) (a). Sensitivity analysis: the red line indicates the F_{ST} median value, the black line indicates the P_{ST} values and the grey shape represents its 95% confidence interval as a function of the c/h^2 ratio. The phenotypic differentiation is significant and its estimator (P_{ST}) robust when P_{ST} confidence interval and F_{ST} overlap at low c/h^2 values. Relative growth rate ($\text{mm}^2 \text{mm}^{-2} \text{d}^{-1}$) (b), the age at maturity (days) (c), plant assimilation rate ($\text{nmol CO}_2 \text{g}^{-1} \text{s}^{-1}$) (d), plant leaf mass per area (g m^{-2}) (e) and position on the slow-fast continuum (f).

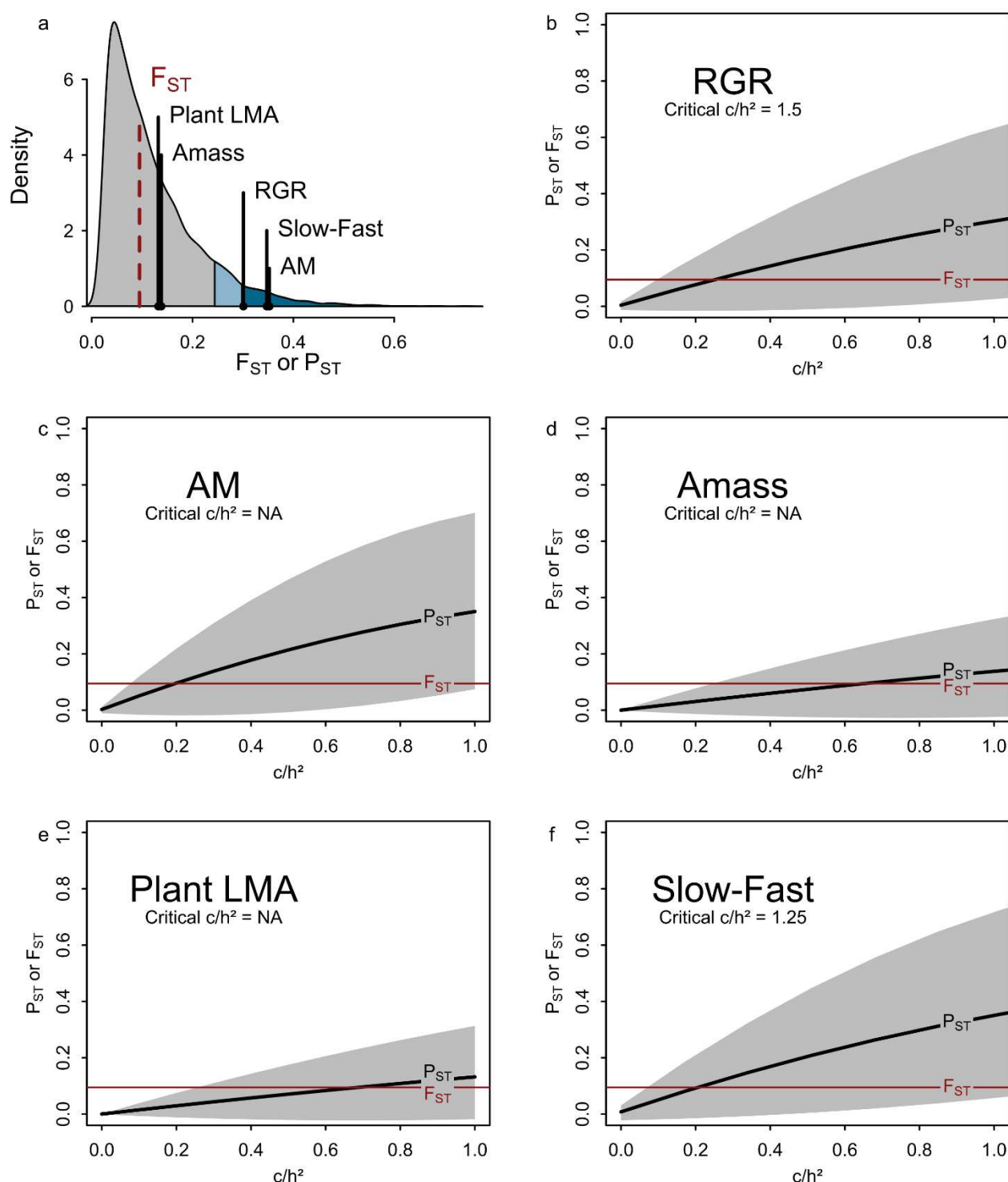


Figure S3: Influence of the c/h^2 ratio on the significance and robustness of the P_{ST} .

Phenotypic differentiation (P_{ST} , black vertical lines) relatively to the distribution of genetic differentiation (F_{ST}), its median (red dashed line), 90th quantile (light blue) and 95th quantile (dark blue) (a). Sensitivity analysis: the red line indicates the F_{ST} median value, the black line indicates the P_{ST} values and the grey shape represents its 95% confidence interval as a function of the c/h^2 ratio. The phenotypic differentiation is significant and its estimator (P_{ST}) robust when P_{ST} confidence interval and F_{ST} overlap at low c/h^2 values. Leaf lifespan (days) (b), leaf assimilation rate ($\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) (c), leaf mass per area (g m^{-2}) (d) and position on the leaf economics spectrum (e).

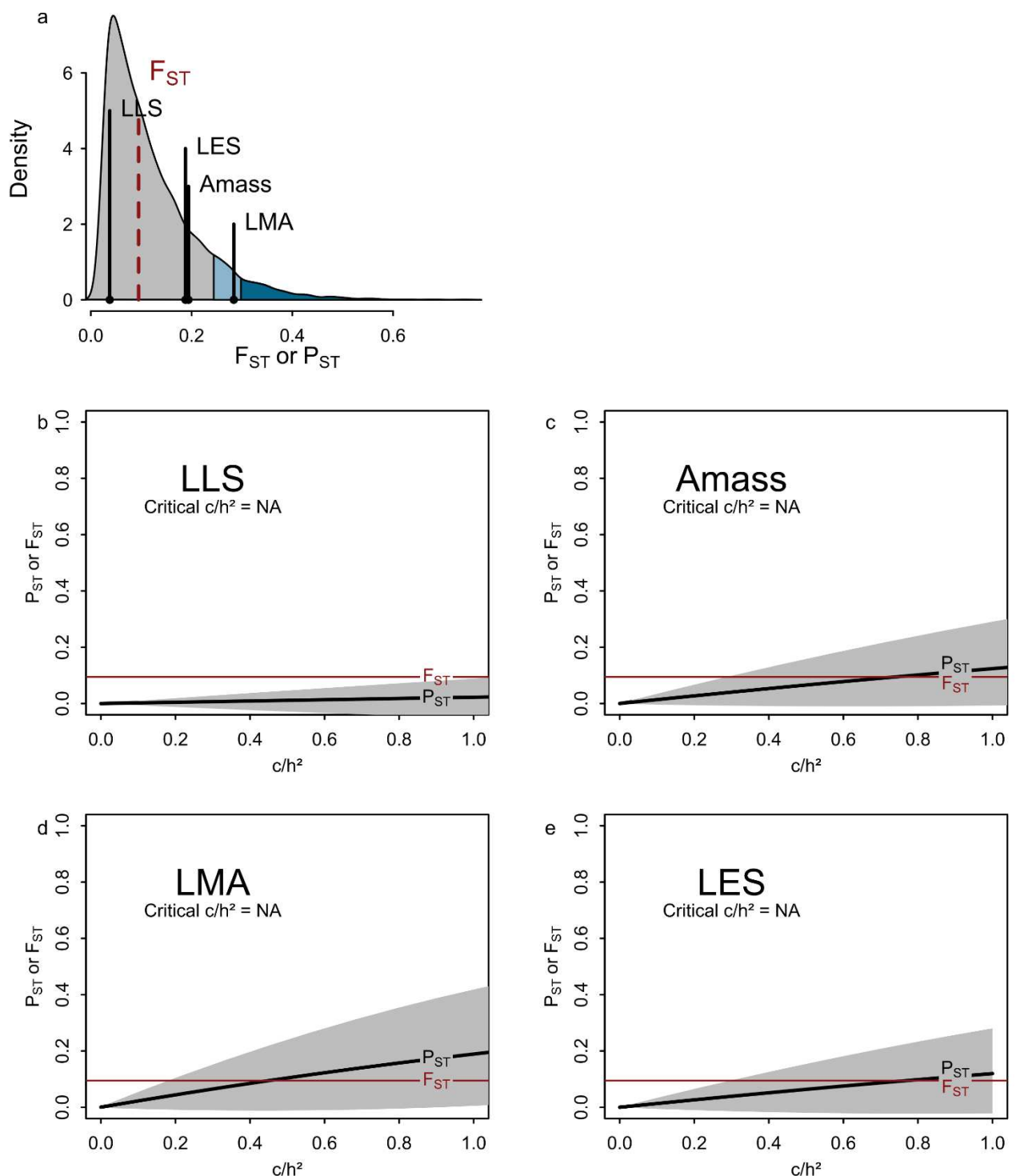
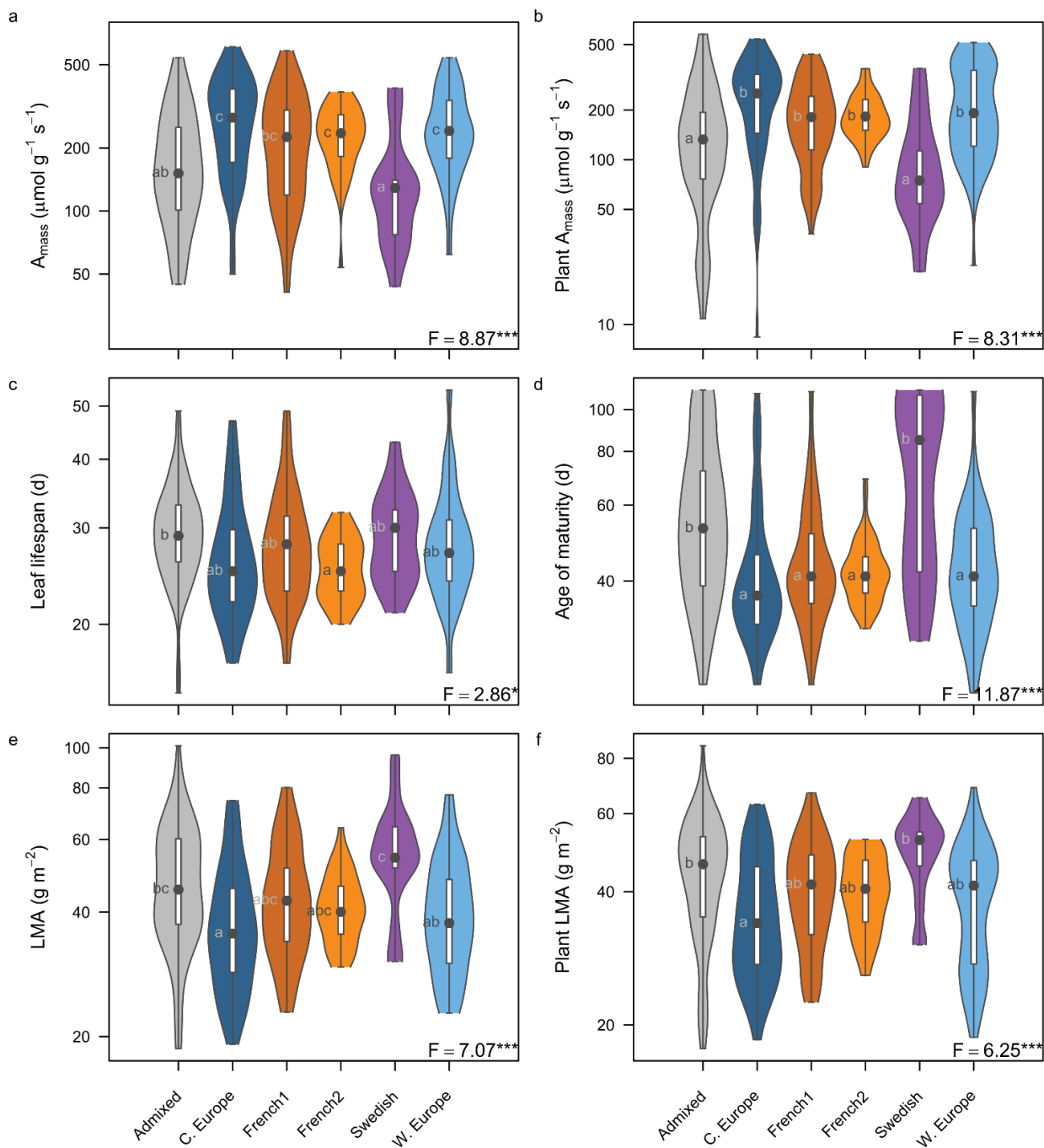
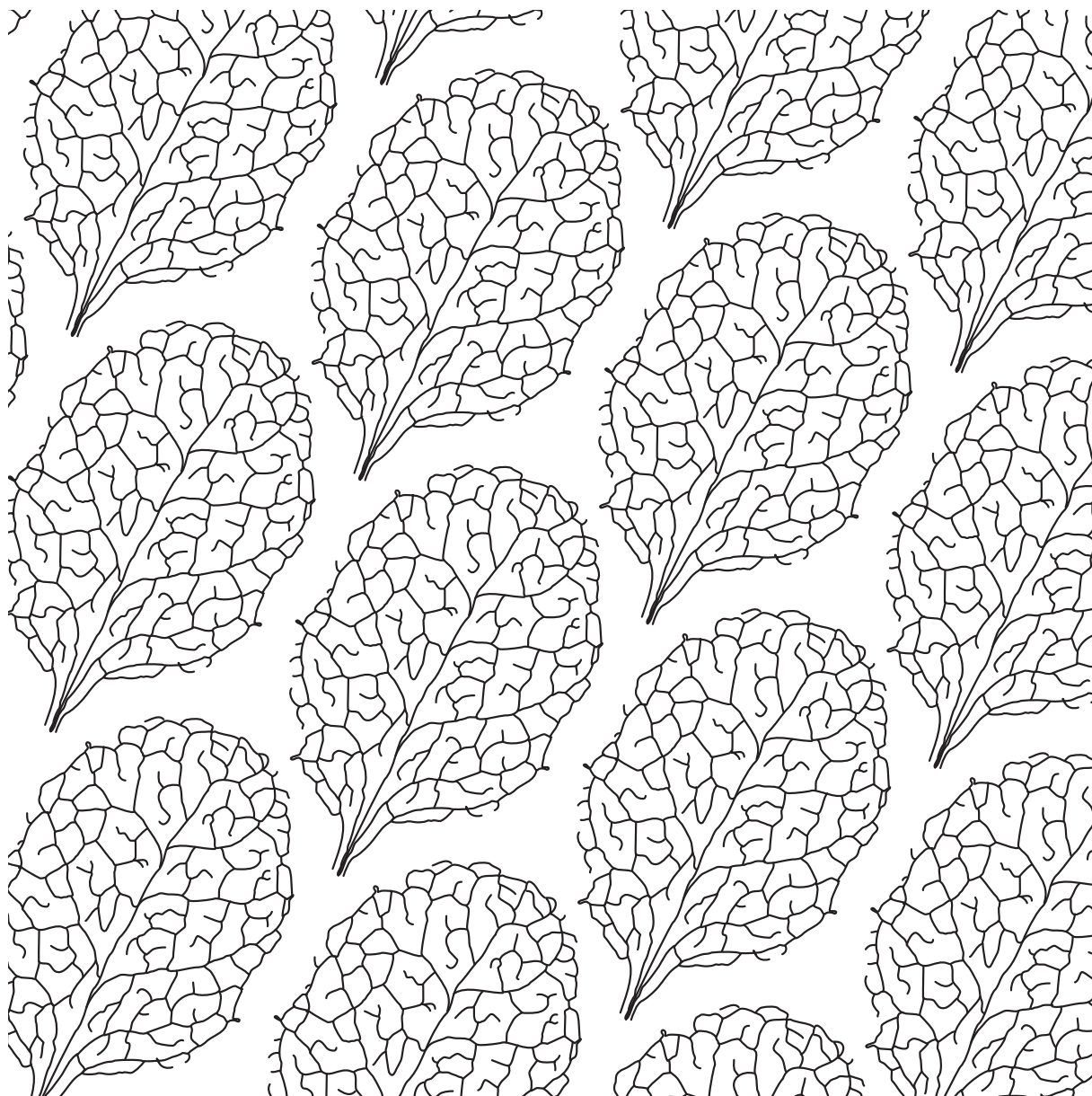


Table S1: Correlation coefficient between plant traits and climatic variables at the collecting sites. All traits but RGR were log10 transformed. Significance code: ***, p<0.001, **, p<0.01, *, p<0.05, ns; non-significant. Abbreviations: RGR; Relative Growth Rate, AM; Age at maturity, Amass; mass based assimilation rate, LMA; Leaf Mass per Area, LLS; leaf lifespan, SFC; slow-fast continuum, LES; leaf economics spectrum, BIO1; Annual Mean Temperature, BIO2; Mean Diurnal Range, BIO3; Isothermality, BIO4; Temperature Seasonality, BIO5; Max Temperature of Warmest Month, BIO6; Min Temperature of Coldest Month, BIO7; Temperature Annual Range, BIO8; Mean Temperature of Wettest Quarter, BIO9; Mean Temperature of Driest Quarter, BIO10; Mean Temperature of Warmest Quarter, BIO11; Mean Temperature of Coldest Quarter, BIO12; Annual Precipitation, BIO13; Precipitation of Wettest Month, BIO14; Precipitation of Driest Month, BIO15; Precipitation Seasonality, BIO16; Precipitation of Wettest Quarter, BIO17; Precipitation of Driest Quarter, BIO18; Precipitation of Warmest Quarter; BIO19; Precipitation of Coldest Quarter

	RGR	AM	Amass	LMA	LLS	SFC	LES
BIO1	0.34 ***	-0.22 ***	ns	-0.13 *	ns	-0.18 **	-0.13 *
BIO2	0.12 *	-0.17 **	ns	ns	ns	-0.16 **	ns
BIO3	0.23 ***	-0.29 ***	ns	-0.14 *	ns	-0.31 ***	-0.2 **
BIO4	-0.16 **	0.12 *	-0.15 *	0.14 **	ns	0.15 *	0.14 *
BIO5	0.2 ***	-0.14 *	ns	ns	ns	ns	ns
BIO6	0.28 ***	-0.17 **	ns	-0.14 *	ns	-0.16 **	ns
BIO7	ns	ns	-0.13 *	ns	ns	ns	ns
BIO8	ns	ns	ns	ns	ns	ns	ns
BIO9	ns	ns	ns	ns	ns	ns	ns
BIO10	0.25 ***	-0.13 *	ns	ns	ns	ns	ns
BIO11	0.3 ***	-0.2 ***	ns	-0.14 *	ns	-0.18 **	-0.14 *
BIO12	0.15 **	-0.16 **	ns	-0.12 *	-0.16 **	-0.16 **	-0.14 *
BIO13	ns	-0.14 **	0.12 *	-0.15 **	-0.13 *	-0.14 *	-0.14 *
BIO14	0.16 **	-0.16 **	ns	ns	ns	-0.15 *	ns
BIO15	-0.11 *	ns	ns	ns	ns	ns	ns
BIO16	ns	-0.15 **	0.13 *	-0.16 **	-0.14 *	-0.15 *	-0.15 *
BIO17	0.16 **	-0.16 **	ns	ns	ns	-0.15 *	ns
BIO18	ns	-0.11 *	ns	ns	-0.21 ***	ns	ns
BIO19	0.19 ***	-0.15 **	ns	-0.11 *	ns	-0.14 *	ns

90 **Figure S4: Mean trait comparison between genetic groups.** Leaf (a) and plant (b)
 91 assimilation rate per unit mass (A_{mass}), leaf (c) and plant (d) life history trait, leaf mass per
 92 area (LMA) (e), plant level LMA (f). F statistics and letters are given from Tukey tests, all
 93 traits were log-transformed.





CHAPITRE II

Exploration de la valeur adaptative de l'architecture des nervures.

A within-species analysis reveals the adaptive value of vein density along climatic gradients

En préparation.

Summary

Interspecific variability in vein density (VD) has been attributed to the diversity of the strategies of plant adaptation to their environment. However, the lack of within-species variability studies impedes a thorough evaluation of the role of VD in plant adaptation. This study brings new evidences of the role of vein architecture in plant adaptation to climate. We explored the morphological, genetic and environmental determinants of natural variation of leaf vein density among 169 *Arabidopsis thaliana* genotypes grown under controlled experimental conditions. Contrary to the global interspecific pattern, vein density was mainly explained by leaf size variation and increased with decreasing temperature. Nonetheless, we detected a genetic basis for VD variation with genes previously associated with VD variation as well as new genes not previously identified in VD studies. We further detected selection cues on the genome that collocated with the loci associated with VD variation. The model species *Arabidopsis thaliana* proves to be an attractive model to test hypotheses regarding the implication of functional traits for the plant adaptation to climate, merging approaches such as functional ecology, evolutionary biology and omics.

Keywords

Genetic differentiation, intraspecific variability, local adaptation, leaf vein density, leaf area

Introduction

Functional ecology holds promise for revealing the phenotypic determinants of the adaptation of organisms to their local environment thanks to the comparison of plant functional traits across species or genotypes on a physiological basis (Calow, 1987; Keddy, 1992; Violle *et al.*, 2007; Garnier *et al.*, 2016). In that respect, plant ecology has gained tremendous progress in our understanding of local adaptation through structure-function analyses at the leaf level (e.g., REF Kikuzawa, wright et al. 2004, un papier de Reich). Notably, leaf vein density (VD) has a strong physiological basis, and has been advanced as a key trait to explain both leaf-level variation in metabolism (Brodribb *et al.*, 2007; Brodribb & Feild, 2010) and plant-level adaptation to climate (Blonder *et al.*, 2018). This echoes macroevolution studies that have long emphasized the complexification of leaf vein architecture as a major morphological innovation in the success of Angiosperms over Gymnosperm and ferns (Roth-Nebelsick *et al.*, 2001; Boyce C. Kevin *et al.*, 2009; de Boer *et al.*, 2012; Simonin & Roddy, 2018). In addition, leaf vein traits have been proposed as a palaeoclimate and palaeoenvironmental proxy (Uhl & Mosbrugger, 1999; Blonder *et al.*, 2014). However, most of these studies are based on the environment-leaf venation-fitness linkage that is in fact still poorly known.

Establishing trait-environment relationships (TERs) is a foundation stone of functional ecology and functional biogeography (Violle *et al.*, 2014; Garnier *et al.*, 2016), but many of them have been hardly examined in plants (Shipley *et al.*, 2016). This is especially true for VD-climate relationships even if theoretical expectations have been provided. Physiological models hypothesized that VD is positively linked to growing season temperature, based on an assumed coupling between leaf transpiration rate and the maximum potential water demand of the environment (potential evapotranspiration) (Blonder & Enquist, 2014). Higher VD strategies should also be advantageous in arid environments given the putative role of leaf venation in withstanding hydraulic continuity failure (i.e. embolism) resulting from soil water depletion (Brodribb *et al.*, 2016). Some studies indeed highlighted a positive relationship between VD and temperature or aridity (Zhu *et al.*, 2012)(Blonder *et al.*, 2018)(Schneider *et al.*, 2017). However, many others also showed a lack of climate signal on VD variation (e.g., Jordan *et al.*, 2013). Overall, VD-climate relationships appeared to be taxon-specific, which can blur cross-species explorations. The comparison of intraspecific and interspecific TERs has gained momentum in functional ecology (Siefert *et al.*, 2015), and has allowed to reveal physiological mechanisms at play. More generally, cross-species TERs are sensitive to many sampling bias

(Borgy *et al.*, 2017), and phylogeny-controlled TERs can only partly capture their underlying mechanisms (Violle *et al.*, 2014).

TERs are classically built trait-by-trait, i.e. by overlooking trait covariation. Such a single-trait approach can lead to spurious physiological response curves if one or several traits covary with the trait and the environmental factor under scrutiny (Wüest *et al.*, 2018). This could be particularly true for VD that is computed as the total path length of vein conduit divided by leaf area. By construction, it is a leaf area-based trait, which makes it a scale-dependent parameter (Price *et al.*, 2014a). If VD is under the control of leaf area, TERs can be quite complex because the latter is already known to display a strong environmental signal at both local and global scales (Moles *et al.*, 2014; Wright *et al.*, 2017). In this context, the scaling relationship for vascular architecture with leaf size is pivotal to examine (Sack *et al.*, 2012). Sack *et al.* (2012) advocated that the relationship between VD and leaf area depends on the developmental stage of the leaves, and by consequence, on the different orders of veins that are used to compute VD. For full mature leaves, the authors did not expect any relationship between the density of minor veins as well of total VD and leaf area. Global cross-species analyses indeed revealed a lack of relationship between total VD and leaf area (Price *et al.*, 2012; Sack *et al.*, 2012). On the opposite, a negative relationship was found in several taxa and in more local studies (Roth-Nebelsick *et al.*, 2001). The divergence between global interspecific patterns and local and/or intraspecific patterns is common in functional ecology (Price *et al.*, 2014b; Messier *et al.*, 2017; Anderegg *et al.*, 2018; Osnas *et al.*, 2018). It can translate differential actions of evolutionary, ecophysiological and biophysical constraints at different scales and biological organizational levels.

Within-species studies are required to go deeper into the characterization of the constraints at the origin of trait-trait relationships. In particular, allometric scaling relationships are expected to hold within species if they result from fundamental biophysical (Witting, 1998; Shoval *et al.*, 2012) and/or evolutionary (Donovan *et al.*, 2011) constraints. More broadly, it is the time for functional ecology to meet molecular ecology in order to reveal the adaptive meaning of plant functional traits. In particular, model species for which genetic information is available are the best candidates to examine whether VD is linked to local adaptation. The co-localization (or lack of) of genes involved in both VD and leaf area could further be very promising to identify pleiotropic effects and explain the interdependency of both traits. Among model species, *Arabidopsis thaliana* has been used for decades in molecular biology due to its

short life cycle and small genome (Krämer, 2015). The natural distribution of the species covers large climatic gradients that led to a strong genetic structure of the populations, suggesting underlying local adaptation (Lasky *et al.*, 2012). Recently, *A. thaliana* has been used in functional ecology to quantify the heritability of functional traits and plant ecological strategies (Vasseur *et al.*, 2018b; Kazakou *et al.*, 2019) as well as to identify the mechanisms at the origin of functional tradeoffs and allometric scaling relationships (Vasseur *et al.*, 2012, 2018a; Blonder *et al.*, 2015; Sartori *et al.*, 2019). Surprisingly, the analysis of the natural variability of VD in *A. thaliana* remains scarce (Rishmawi *et al.*, 2017), as well as the exploration of VD-environment linkage and the underlying local adaptation (Stewart *et al.*, 2015, 2016). The combined use of the unique genetic data available for the species and the newly developed fast and efficient genome analysis methods (Zhou & Stephens, 2012; Luu *et al.*, 2017) allows an unprecedented exploration of the VD genetic determinism and adaptive value across the distribution range of *A. thaliana*. Specifically, following a recent study that highlights drought resistance-associated alleles at both Northern and Southern extremes of *A. thaliana* distribution (Exposito-Alonso *et al.*, 2018), we expect genes conferring high vein density to be under selection at these margins, too.

Material and Methods

Plant material and growth conditions

We selected a set of 169 *A. thaliana* genotypes covering the natural distribution of the species (Fig. 1) and loaded the genetic sequences from the 1001 genome website (1001genomes.org). Seeds were sown in moist organic compost and stratified in a cold chamber at 4 °C for four days. Four seedlings per genotype were then transferred in individual pots filled up with organic compost (Neuhaus N2). Pots were randomly distributed on four tables, i.e. blocks, with one replicate per genotype per table. Tables were placed in a greenhouse with temperature maintained at 18°C during the day and 16°C during the night and with a supplemental lighting to maintain a constant 12.5 h day length. Plants were watered twice a week. Tables were rotated daily to reduce block effects within the greenhouse. The experiment lasted 140 days, from sowing (1st of December 2015) to the last harvest (19th of April 2016).

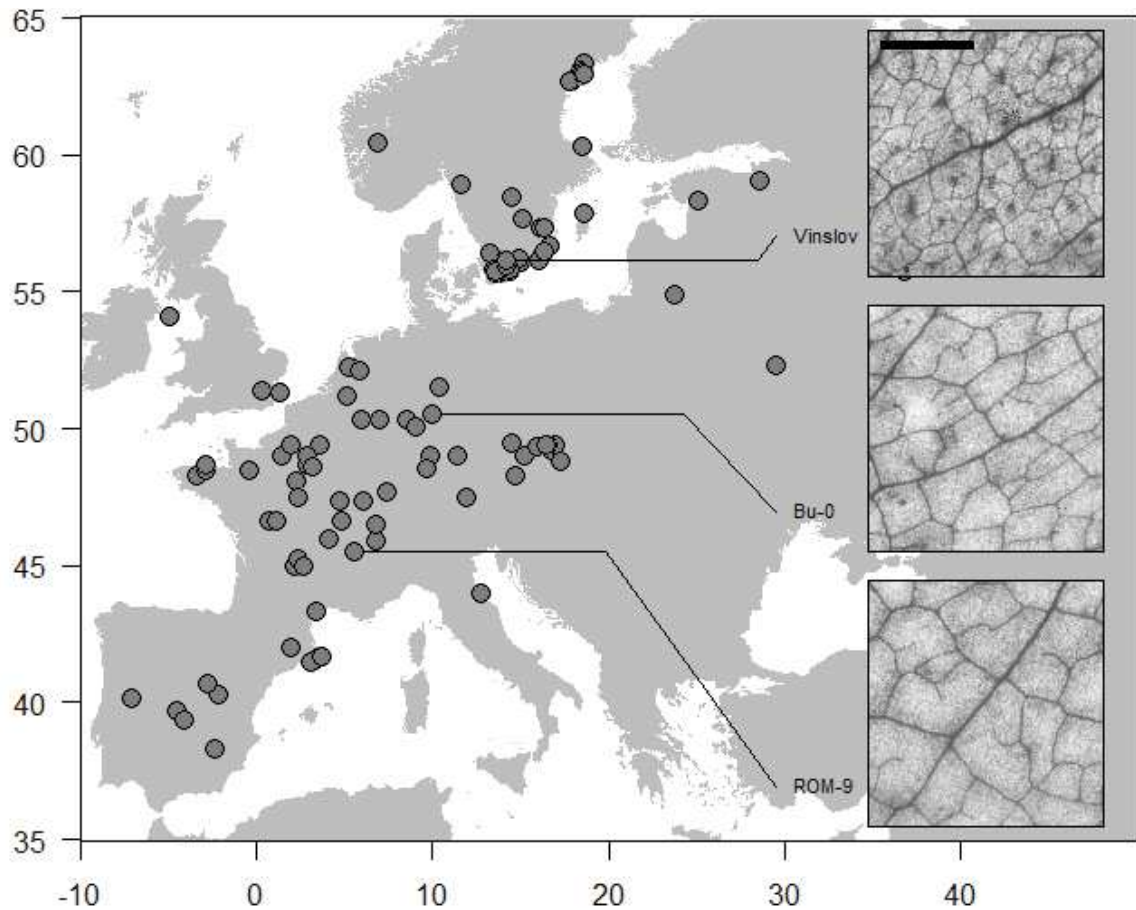


Figure 1: Geographic distribution of the European genotypes grown in this study and examples of contrasted leaf vein densities measured ($VD_{Vinslov} \sim 7 \text{ mm mm}^{-2}$, $VD_{Bu-0} \sim 5 \text{ mm mm}^{-2}$, $VD_{ROM-9} \sim 4 \text{ mm mm}^{-2}$). Scale bar on leaf vein network image is 2mm long.

Vein density measurement

We harvested the last developed leaf which was fully expanded and fully exposed to light, at the bolting stage of each individual plant. Doing so, we aimed to measure traits expressed by adult leaves and avoid bias potentially caused by ontogenic trait variations (Vasseur *et al.*, 2018b). Leaf tissues were fixed by placing the leaves in individual micro-tubes filled with Formalin–Acid–Alcohol for at least two days. Leaves were then cleared by a solution of 95% ethanol and 5% glacial acetic acid for 24 h. To ensure a high contrast between the vein network and other leaf tissues, the solution was supplemented with 0.001% of safranin powder. Then, leaves were dipped one hour in pure glycerol before mounting between two glass blades. We took pictures of the samples using a backlight device and a digital camera (Nikon D300s) equipped with a macro lens, at a 100 pixel per millimeter resolution. Leaf veins were manually

traced using the Gimp software (The GIMP Development Team, 2019) and the vein networks were analyzed using MATLAB (Thompson & Shure, 1995) with the code provided in Blonder *et al.* (2018). Vein density was computed as the ratio of total vein length to leaf area. Leaf area were measured using the *ImageJ* software (Schneider *et al.*, 2012). We compared the VD range in our dataset to the range reported in a previous *A. thaliana* study and global interspecific study using published datasets (Sack *et al.*, 2012; Rishmawi *et al.*, 2017).

Statistical analyses

Statistical analyses were performed with the R software (R Core Team, 2019, version 3.6.1). We calculated the genotype means of both VD and LA by estimating the marginal means of the variables from linear mixed models. The linear mixed models included the genotype as a random effect and the experimental block as a fixed effect. The linear mixed models were performed with the *lme* function from the *nlme* package (Pinheiro *et al.*, 2020). The marginal means were computed with the *emmeans* function from the *emmeans* package (Searle *et al.*, 1980). We evaluated the part of VD variation explained by LA and by the genotypes using a partial regression model, performed using the *varpart* function from the *vegan* package. We explored the relationships between leaf traits and mean annual temperature and precipitation using spearman correlation test. When the correlation was significant but the distribution of the trait were asymmetric, we fitted 5th and 95th quantile linear regressions. The mean annual temperature and mean annual precipitation of genotypes' collecting sites were extracted from the CHELSA database (chelsa-climate.org). The 5th and 95th quantile regressions and their slope comparison were performed using the *quantreg* package.

Genotype-phenotype associations

Taking advantage from the genetic data available for *A. thaliana*, we estimated the narrow sense heritability (h^2) of the traits, i.e. the phenotypic variance due to the additive effect of the alleles. We used the Bayesian sparse linear mixed model using a Markov chain Monte Carlo method performed by the GEMMA software (Zhou *et al.*, 2013). The model takes the genetic structure into account by computing the relatedness matrix of the genotypes. The model compute the overall additive effect of all the single nucleotide polymorphisms (SNPs) on the phenotype. To look for specific association between particular SNPs and the phenotype, and detect candidate genes, we performed genome wide association studies (GWAs) for both VD and LA. We used a linear mixed model performed with GEMMA (Zhou & Stephens, 2012) that

controls for the genetic structure of the dataset by using the genetic relatedness matrix. In this case, we calculated the relatedness matrix using the *-gk* GEMMA function. We ensured that the tests were not liberal looking at the histograms of significance values (François *et al.*, 2016) and calculated q-values (corrected p-values for the false discovery rates) using the Bioconductor's q value R package when required (Storey, 2002). Using a Bonferroni significance threshold, we spotted sequences significantly associated with the phenotype (quantitative trait loci, QTL). To account for the effect of linkage disequilibrium, we extended the targeted sequences by 10 Kb upstream and downstream significant sequences (Kim *et al.*, 2007). Using the Arabidopsis Information Resource database (www.arabidopsis.org), we extracted the list and functions of genes carried by these QTL. The final filter consisted in considering the relevance of the gene functions for the studied phenotype.

Selection cues

We scanned the genome for selection cues using the *PCAdapt* R package (Luu *et al.*, 2017). The algorithm assesses the importance of each SNP on the genetic structure of the dataset. It postulates that SNPs under selection contribute more to the population structure than expected by neutral processes, such as genetic drift. The method uses a multivariate analysis that is well suited to study the large regional continuous populations of *A. thaliana* (Horton *et al.*, 2012). It identifies the principal components (PC) of genetic variation, i.e. the genetic structure, and measures the contribution of each SNPs on their construction. The contributions of the SNPs are processed as p-values and filtered for false discovery rates. Using this method, we conducted an analysis with a genotype set independent from the GWAS' genotype sets that are limited by the available phenotypic data. We started with the full set of 1135 genotype sequences available on the 1001 genomes project website (1001genomes.org) containing 12,883,854 variant loci. The distribution of *P*-values computed by *PCAdapt* from this dataset did not show the expected uniform distribution due to correlations between genotypes and between SNPs. We reduced the dataset until the uniform distribution assumption was correct (François *et al.*, 2016). We pruned the data by keeping only the Single Nucleotide Polymorphisms, and loci and genotypes having less than 10% of missing data, which resulted in a 1,032 genotypes by 6,385,774 SNPs dataset. We then filtered the genotypes by the genetic distance using a 0.075 correlation coefficient limit. Final data contained 222 genotypes and 6,385,774 SNPs. As for GWAs, the p-values produced by *PCAdapt* were controlled for false discovery rates using the Bioconductor's q value R package and the subsequent q-values were used to scan for adaptive peaks using a Bonferroni threshold.

Results

Vein density variability among genotypes

The mean ($\pm$ sd) leaf VD was 5.27 ± 0.85 mm mm⁻² and varied by 2.5-fold among the genotype set, from 3.4 to 8.3 mm mm⁻² (Fig. 1, Fig. 2a). It is higher in mean but similar in variance than previously shown in *A. thaliana* (between 1.54 and 3.46 mm mm⁻², Rishmawi *et al.*, 2017). Our range of VD is located at the low part of the VD variability recorded in large interspecific comparisons (0.5 to 25 mm mm⁻², Sack *et al.*, 2012). The mean ($\pm$ sd) of LA was $423.41 (\pm 123.31)$ mm² on average and varied from 44.3 to 715.3 mm². LA was significantly negatively correlated with VD ($R^2 = -0.77$, $P < 0.01$; Fig. 2b). The variance partitioning showed that 41% of VD variation was explained by LA versus 17% by the genotype, and 20% was explained jointly by VD and LA.

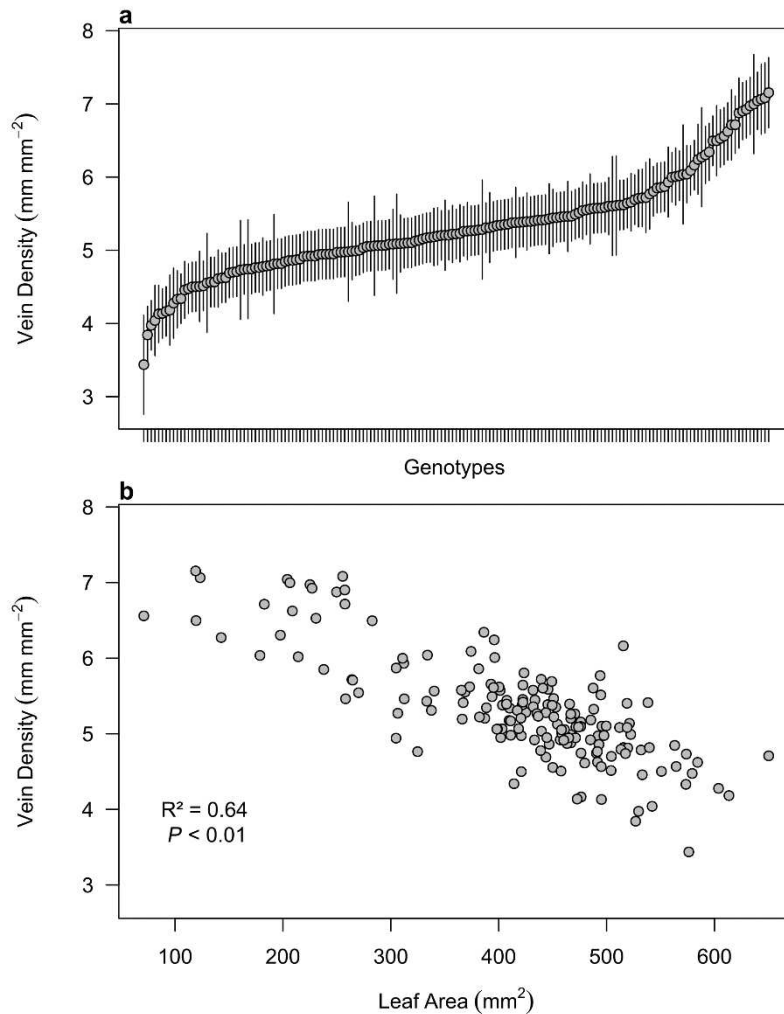


Figure 2: Leaf area variation explained a large part of the vein density variation among *Arabidopsis thaliana* genotypes. Variation of vein density across genotypes (a) and relationship between vein density and leaf area (a). Each dot represent one genotype ($n = 169$) and straight bars represent standard errors.

Traits-climate relationships

VD decreased with increasing mean annual temperature (MAT) at genotype's collecting site ($r = -0.24$, $P < 0.01$, Fig. 3a). LA increased on average with increasing MAT ($r = 0.22$, $P < 0.01$, Fig. 3a). However, LA distribution was asymmetric and the quantile regression revealed a triangular relationship between LA and MAT (Fig. 3c). The 5th and the 95th quantile regression slopes were significantly different ($P < 0.01$). Large to small leaves were encountered at low temperatures while rather large leaves at higher temperatures (Fig. 3c). VD and LA varied independently from the mean annual precipitations (MAP) at the genotype's collecting site ($P > 0.05$, Fig. 3b,d).

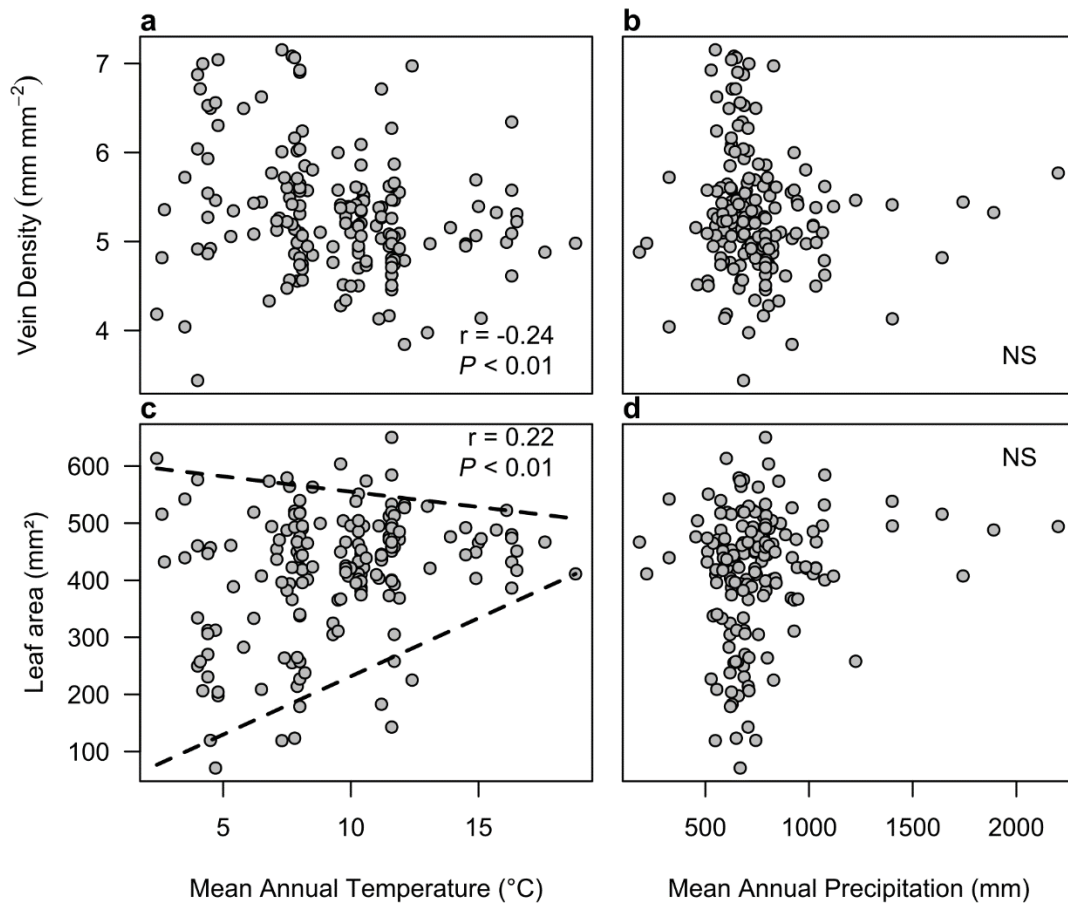


Figure 3: Traits-climate relationships show influence of temperature on vein density and leaf area. Relationships between vein density and mean annual temperature (a) and mean annual precipitation (b), and relationships between leaf area and mean annual temperature (c) and mean annual precipitation (d). Each dot represent one genotype ($n=169$), dashed lines represent 5th and 95th quantile regression fits.

Genetic determinism of VD

The cumulative allelic additive effect explained 26% of the natural variation of VD in our dataset. The genome wide association study revealed that four QTLs were significantly associated with VD variation. The significant QTLs are illustrated by the four association peaks on the Manhattan plot (Fig. 4a,b): three peaks were located on the first chromosome and the last peak was located on the fourth chromosome. The first QTL covers a region gathering three genes involved in cell wall modification; XTH8, PMEPCRA and PME19. In the second QTL, three genes attracted our attention: AT1G12440, CDI3 and DDF1. AT1G12440 is a gene from the zinc finger family reported to interact with the vascular-specific adaptor proteins VIT and VIK that influence leaf venation patterning (Ceserani *et al.*, 2009). CDI3, is involved in water homeostasis, regulation of stomatal closure and opening, response to abscisic acid, response to carbon dioxide and response to humidity (Saito & Uozumi, 2019). DDF1, is a transcription factor that causes dwarfism and delays flowering when overexpressed (Kang *et al.*, 2011). Overexpression of this gene is triggered in response to freezing, heat, salt stress and water deprivation. The third QTL covers a region including LSM1A, a gene involved in cold acclimation and response to water deprivation (Perea-Resa *et al.*, 2016), and ARF11, a gene involved in the auxin-activated signaling pathway. Finally, the fourth QTL covers a region including two genes involved in response to stress: REIL1, involved in acclimation to cold and ABC1K1 involved in the response to water deprivation, and a gene involved in cell wall deposition (FLA5), reported to contribute to the biomechanical resistance of vascular tissues (MacMillan *et al.*, 2010).

Testing the specificity of the QTL associated with VD

The cumulative allelic additive effect explained 59% of the natural variation of LA in our dataset. The genome wide association study revealed that two QTLs were significantly associated with LA variation. As illustrated by the Manhattan plot (Fig. 4c,d), the QTLs highlighted in this analysis corresponded to the first and third QTL associated with VD variation. Thus, the effects of the second and fourth QTLs were specific to VD variation.

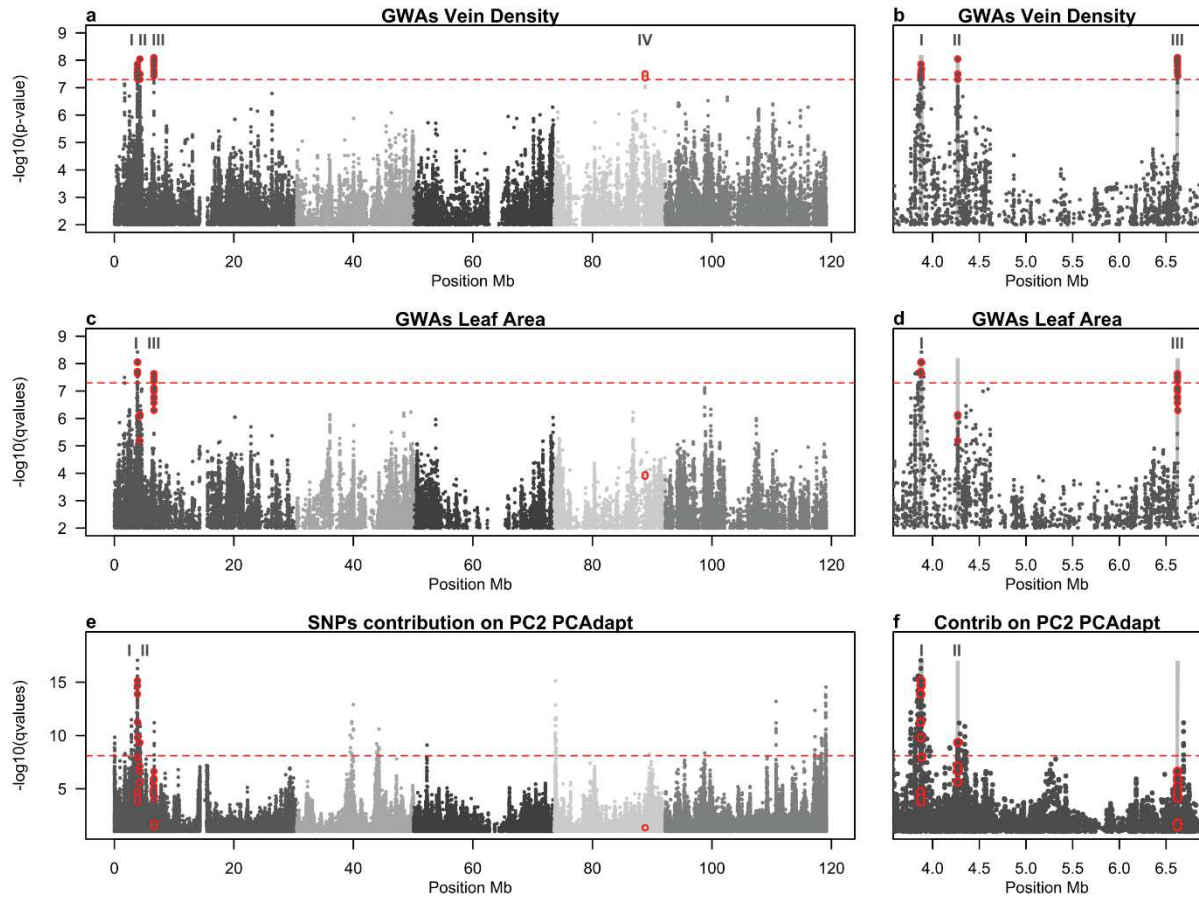


Figure 4: Manhattan plots highlighting the QTL significantly associated with vein density, leaf area, and the North-South differentiation of *A. thaliana* populations. Genome wide association study (GWAs) of vein density (VD) (a), and a zoom on the first chromosome (b). GWAs of leaf area (c), and a zoom on the first chromosome (d). Contribution of each SNPs on the construction of the second axis of *A. thaliana* genetic differentiation (e), and a zoom on the first chromosome (f). Each grey dot represent a single nucleotide polymorphism (SNP), dots surrounded by red are the significant SNPs of the vein density genome wide association study. The five shades of grey materialize the five *A. thaliana* chromosomes. The red dashed lines represent the Bonferroni significance threshold.

Adaptation cues of VD variation

The analysis of the genetic structure of the 1001 genomes accession set showed that three principal components (PCs) structured the dataset. Given the high number of PCs (6,385,774), the proportion of variance explained by each principal component was small, around 0.6% for the three firsts. Yet, the three first PCs captured 47% of the variance explained by the ten first components (Fig. S1). The PC 1, 2 and 3 were representative of the population differentiation along an East-West axis, a South-North axis going from central Europe to Scandinavia and a North-South axis going from central Europe to Spain, respectively (Fig. S2). Among the SNPs that contributed more than expected to the construction of the three PCs, a

few of them were located on the QTL identified by the GWAs performed on VD. More precisely, the first and second QTL associated with VD were strong contributors to the population differentiation along the South-North axis going from central Europe to Scandinavia (Fig S3).

Discussion

Global patterns of vein architecture reported a fifty-fold magnitude variation of vein density across species and sites. In addition, vein density was independent from leaf size despite its scale-dependent nature, suggesting that natural selection operates independently on leaf area and vein density (Sack *et al.*, 2012). By contrast, our results indicated a 2.5-fold variation in vein density and a strong negative relationship between vein density and leaf area in *A. thaliana*. This inconsistency with the global pattern might be explained by the natural history of the species. Despite its large phenotypic variability and its wide distribution, *A. thaliana* is mainly a ruderal-stress tolerant species (May *et al.*, 2017; Vasseur *et al.*, 2018b) that explores a few diverse environmental contexts. In addition, the rosette body plan reduces the constraints on the structural role of the vein architecture, compare to erected plant form exposed to wind. Thus, the evolutionary constraints acting on vein density and leaf area might not be conflicting, if not parallel. Beyond that, this result suggests that the leaf vein density is not under stabilizing selection and might be correlated with environmental drivers. Moreover, a significant part of the vein density variation was explained by the genotype suggesting underlying genetic structure that might arise from adaptation to climate.

We initially expected higher vein densities to occur in both the Mediterranean and the Scandinavian extremes of the *A. thaliana* distribution, where drought is more likely to occur due to low amounts of precipitation and low temperature, respectively (Exposito-Alonso *et al.*, 2018). However, our data did not support any relationship between vein density and precipitation, or any indices of aridity (data not shown), and Mediterranean genotypes did not exhibit high leaf vein densities. By contrast, the main climatic driver of vein density across *A. thaliana* populations was temperature: vein density increased linearly with decreasing mean annual temperature. Interestingly, the response of leaf area to temperature corresponded to the pattern observed in large-scale interspecific studies (Wright *et al.*, 2017): large sized leaves were favored at high temperature while small to large leaves occurred at low temperature. This result indicates that high vein densities are selected under low temperatures in both large and small leaves, despite the overall coordination between leaf area and vein density. The water

viscosity increases at low temperatures, which further slowdown the flux of water from soil to leaves and through the leaves (Richardson, 2000). In this context, three mechanisms may favor high vein densities under cold climates. Firstly, high vein density reduces the distance between veins and transpiration sites, which might compensate for such reduction of hydraulic conductance. Secondly, high vein density makes leaf water potential less negative, reducing the tensions in the vascular network (Roth-Nebelsick *et al.*, 2001). These tensions are susceptible to cause embolism, i.e. generate air bubbles obstructing vessels and tracheids (Tyree *et al.*, 1994). Finally, a denser leaf vein network guaranties the hydraulic continuity in case of embolism events by providing alternative routes (Brodribb *et al.*, 2016). This might be of particular evolutionary advantage for the Scandinavian *A. thaliana* populations that grow during the cold season, in some cases under snow covers (Lewandowska-Sabat *et al.*, 2017).

The cumulative allelic additive effect on vein density was weak in our dataset, around 26%, while it explained 59% of leaf area variation. Nonetheless, the genome wide association study uncovered four loci significantly associated with vein density variation, including the two associated with leaf area variation. This result presupposes a strong effect of a few genes on the genetic vein density variation. By contrast, leaf area is determined by numerous small effects that did not reached the detecting threshold of the method. Not surprisingly, leaf area is the result of many developmental processes including cell growth and cell division, which implies complex gene expression patterns (Tsiantis & Hay, 2003). Two loci associated with vein density variation were also among the few loci excessively related to the differentiation of *A. thaliana* populations toward the North of its distribution. The first one refers to the gene cluster of cell wall modifiers, common to both vein density and leaf area genome wide association studies. This result gives strong support to the crucial role of cell wall in shaping the major physiological constraints in leaves and the plant adaptation to their environment (Onoda *et al.*, 2017). The second one was specific to vein density; it refers to a factor influencing leaf patterning identified in previous molecular study (Ceserani *et al.*, 2009). This is a rare convergence of findings between a pure genomics study comparing mutants to a reference genotype and a large-scale study screening the natural variability of a trait.

Among the loci identified in our dataset, the first one gathered three genes involved in cell wall modification. Their proximity suggests the existence of a cluster of genes performing a similar function and tightly linked along the genome. Previous work has shown how variation in cell wall thickness alters the development and final features of vein architecture (Bourquin,

2002). The modification of cell wall properties could influence the mechanical structure of the leaf through reinforcement of vascular tissues. This property has been described by (MacMillan *et al.*, 2010) for FLA5, a gene identified here in the fourth QTL. Our results show how cell wall properties and vein architecture can be genetically and developmentally linked, bringing evidences of the role of cell wall on leaf hydraulic and mechanic properties through vein network alteration. The second QTL contained an unlabeled gene coding for a zinc finger protein (At1g12440). The role of this protein family is to make tandem contact with other molecules, promoting or repressing their activity. Ceserani *et al.* (2009) reported a strong interaction signal between the protein encoded by this gene and the VH1/BRL2 receptor-like kinase. Mutations of VH1/BRL2 cause vein density reduction and vein gapping. While Ceserani *et al.* (2009) did not retain At1g12440 as a major promotor of VH1/BRL2 in Col-0 mutants; our results suggest reconsidering its importance on the natural variation of vein density. Interestingly, a set of genes located in the second, third and fourth loci were associated with plant resistance to drought. For instance, the CDI3 gene, involved in guard cells homeostasis, regulates the stomatal closure and opening. Stomata and veins share a common evolutionary history: modification of stomata properties in leaves and thus the evaporative demand implies a coordinated modification of the leaf water supply and conductivity, achieved by vein density adjustments (Schneider *et al.*, 2017). The role of vein density variation in drought adaptation might explain this coordination. The genome wide association studies highlighted both genes directly influencing venation patterns and co-varying genes involved in drought adaptation.

The species *Arabidopsis thaliana* appears to be an attractive model to merge disciplines such as population genetics, molecular biology and functional ecology through innovative approaches. The large distribution of the species covering contrasted environments coupled to an extensive genetic characterization of the populations allow an unprecedented exploration of how functional constraints guide the genetic differentiation of plant species (Krämer, 2015; Baron *et al.*, 2015; Vasseur *et al.*, 2018a; Sartori *et al.*, 2019). Next steps would be to extend the exploration of the natural variation and functional roles of vein architecture to other metrics. Notably, the differentiation of vein orders might be of differential importance depending on leaf size (Sack *et al.*, 2012). In addition, the functional role of free ending veinlets remains to be elucidated. Nonetheless, interpretation of our results should be made considering the peculiarities of the species. As a ruderal species, *Arabidopsis thaliana* is characterized by a small size, simple organization, fast growing and short life cycle. We still need intraspecific explorations of the importance of vein density for tree adaptation.

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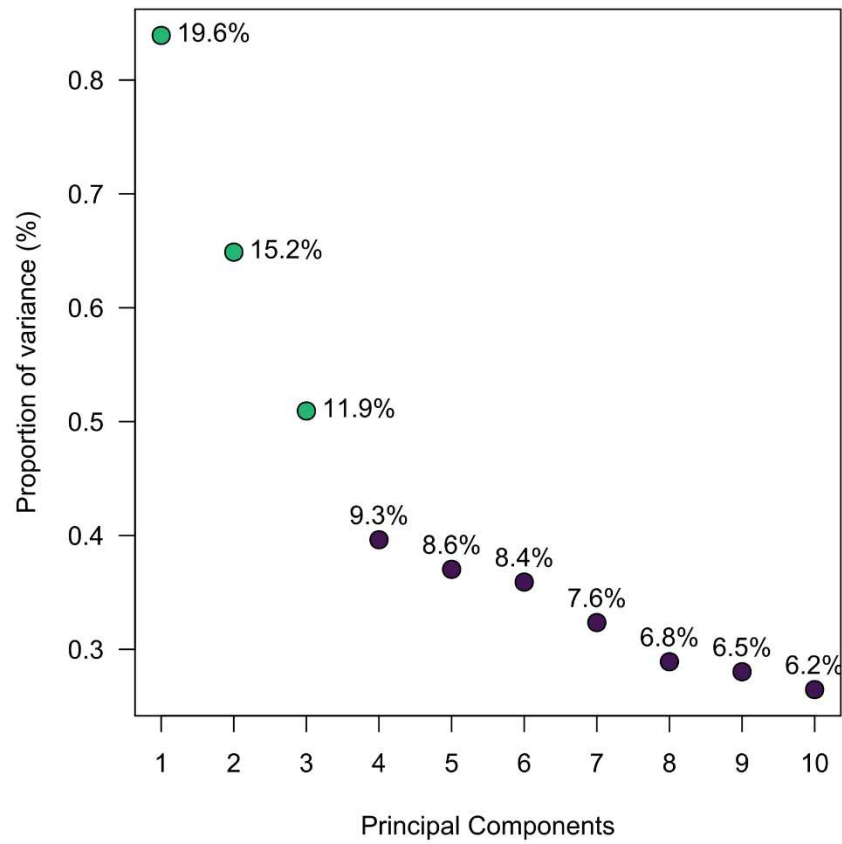
Supplemental Information

Figure S1: Proportion of genetic variance explained by the ten first principal components (PCs). The broken stick method (blue vs purple) indicates that the three first PCs are valuable to explain the *A. thaliana* genetic structure. Percentages on the plot indicate the proportion of variance explained by each PC relatively to the proportion of variance explained by the ten first PCs.

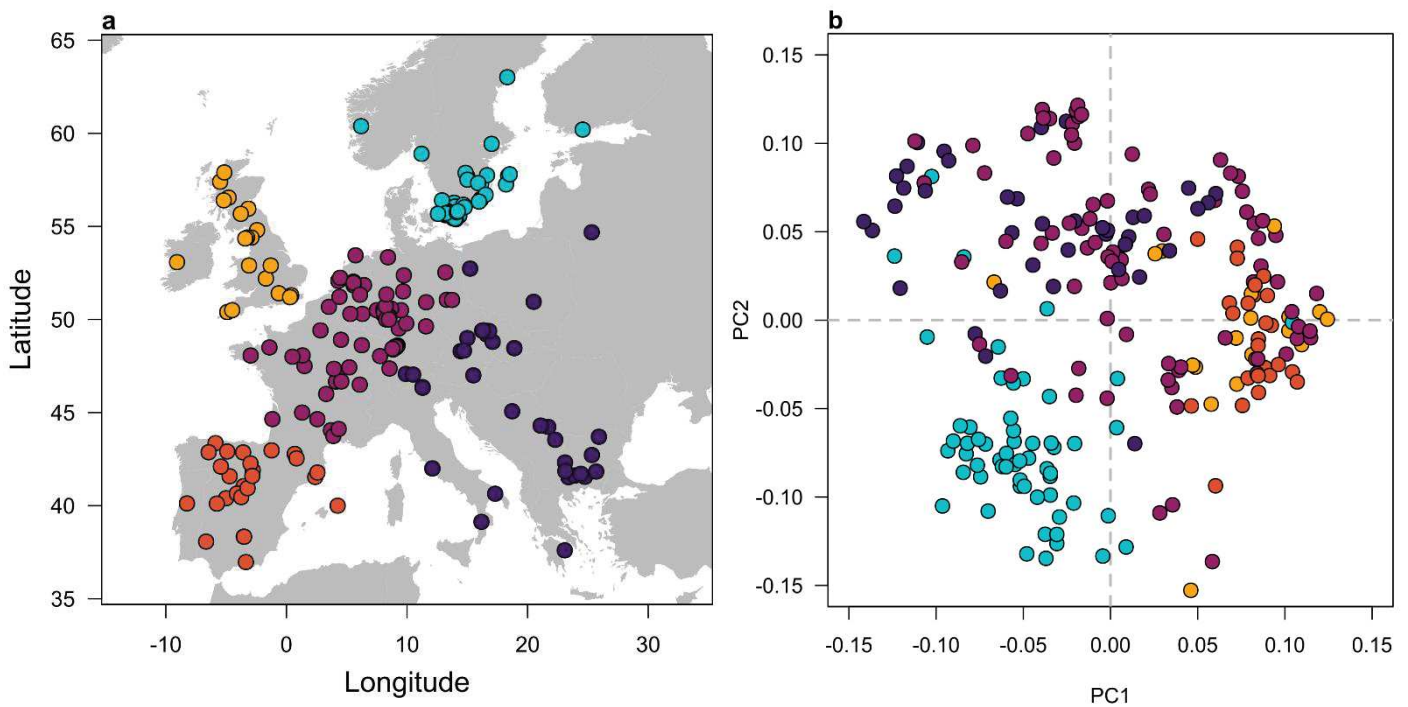


Figure S2: The genetic differentiation echoes the geographic distribution of *Arabidopsis thaliana* genotypes. Position of the genotypes on the map of Europe (a). The colors define geographic groups attributed using the 1001 GENOMES genotype information and allow visualizing the genotype origin on the multivariate analysis (b): Iberian Peninsula; orange, Western Europe; purple, central Europe; dark blue, Ireland and United Kingdom; yellow and Scandinavia; light blue. Position of 222 *A. thaliana* genotypes representatives of the genetic diversity of the species on a plane constituted by the two principal components (PC) of genetic variability (b).

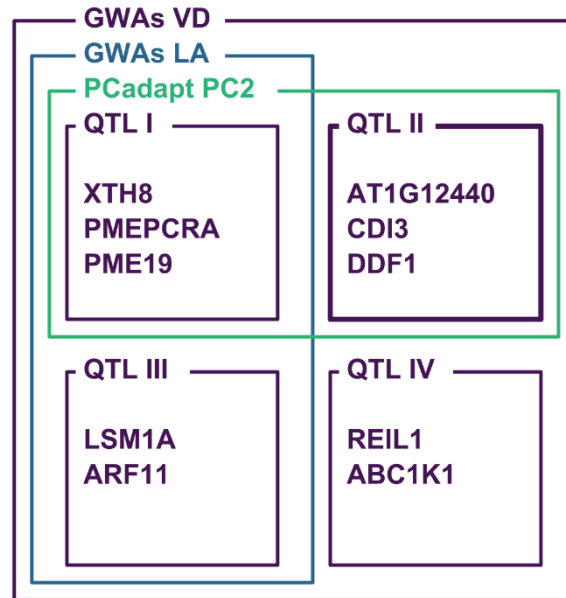
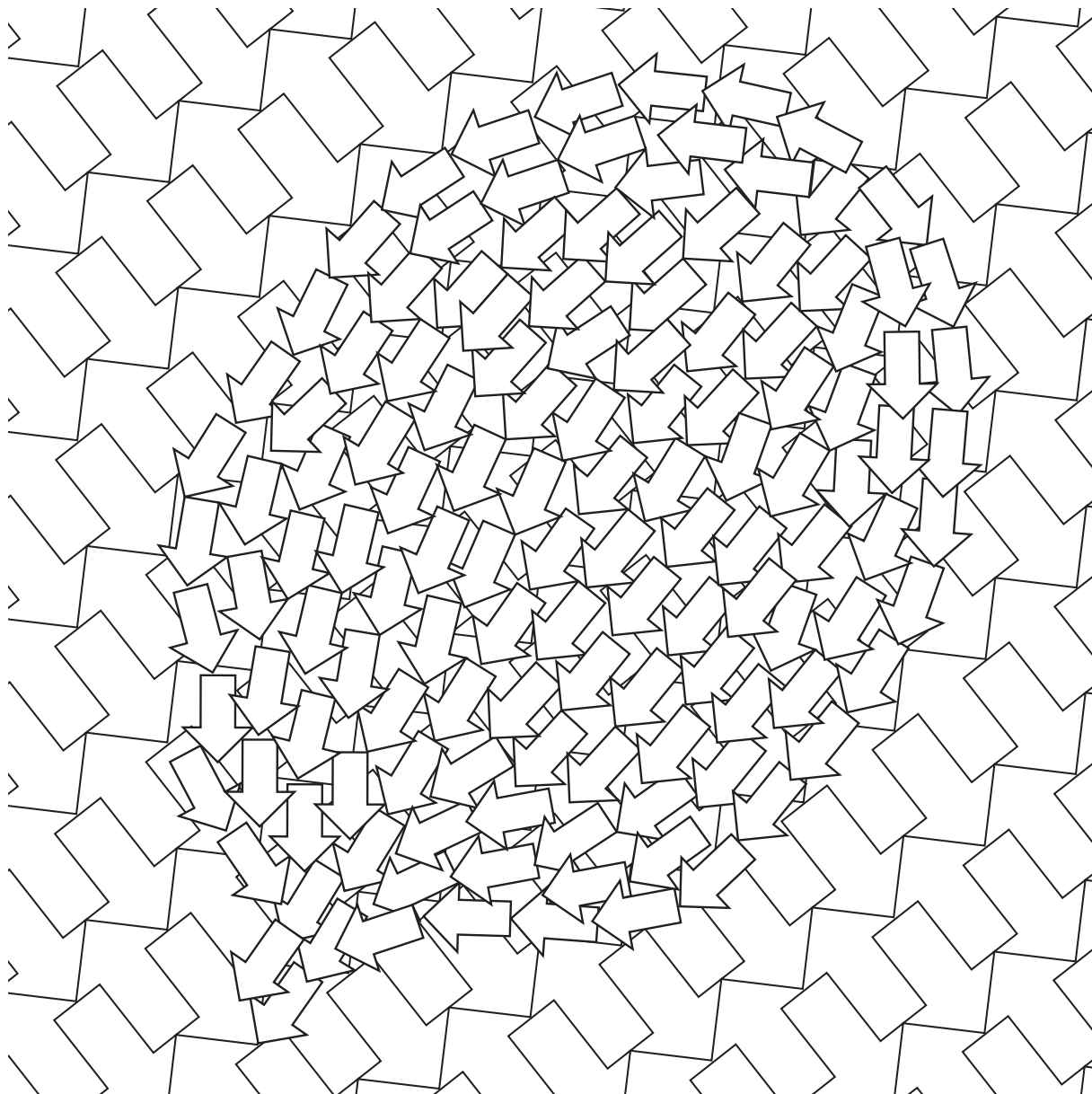


Figure S3: Summary of the results from the genome wide association studies and the scan for selection. QTLs associated with vein density variation and the genes they code for (purple), including the QTLs associated with leaf area variation (blue) and QTLs under selection (green). QTL II was specific to vein density variation and adaptive along the South-North axis going from central Europe to Scandinavia (PCadapt principal component 2).



CHAPITRE III

Implication du Syndrome d'économie foliaire dans la résorption de l'azote.

Leaf nitrogen resorption dynamics within the slow-fast continuum in an annual species

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Soumis à *The New Phytologist*

Summary

Few studies have tested the links between leaf nitrogen resorption and whole-plant resource use strategies and performance. Indeed, tests of such potential linkages are hampered by the classical evaluation of plant nitrogen resorption capacity based on ‘snapshots’ of leaf nitrogen concentration from adult and senescent leaves (the nitrogen resorption efficiency, RE_N).

We greatly increased the resolution for measuring nitrogen resorption by tracking time courses of leaf nitrogen concentration in 121 natural *Arabidopsis thaliana* genotypes native to a wide range of climates across Europe, grown in a greenhouse. In addition to the classical measurement of resorption efficiency measurement, we computed the absolute nitrogen resorption rate (RR_N), i.e., the amount of nitrogen remobilized by a leaf per unit time.

Across genotypes, high rates and efficiency of nitrogen resorption were associated with low leaf photosynthetic capacity ($r = -0.77$, and $r = -0.28$, $P < 0.01$) and long plant lifespan ($r = 0.75$, and $r = 0.23$, $P < 0.01$). The RR_N showed significant heritability, genetic associations, selection, and was negatively correlated across genotypes with the mean annual temperature of the native population. By contrast, RE_N showed low heritability, no evidence of genetic association, and no relationship with climatic variables.

Our results suggest a much stronger adaptive role for leaf nitrogen resorption than previously uncovered.

Introduction

Leaf nitrogen resorption plays a major role in the plant nutrient budget, remobilizing and relocating nitrogen from senescing organs to surviving tissues (Killingbeck, 1986). This process increases nitrogen mean residence time, enabling plants to mitigate the nitrogen limitation that exists in most natural ecosystems (Berendse & Aerts, 1987; Aerts & Chapin, 1999). Despite the tremendous importance of nitrogen resorption for plant physiology and ecology, its contribution to species local adaptation remains unresolved. Nitrogen resorption is indeed a complex, dynamic, and multifaceted molecular choreography barely captured by ‘snapshots’ of leaf trait measurements (Harper & Sellek, 1987; Reich *et al.*, 1991) as has been the state of the art for comparative ecology (Aerts & Chapin, 1999). In this study, we greatly improved the resolution of nitrogen resorption measurements by recording daily changes in nitrogen concentration and measuring rates of resorption. In addition, we explored the intrinsic and extrinsic drivers of nitrogen resorption within a model species, to improve our understanding of the adaptive nature of this pivotal process.

The search for global patterns in nutrient use strategies in functional ecology has generally focused on variations in leaf lifespan and nutrient concentrations during the “green phase” of the leaf. Leaf lifespan and leaf nutrient concentrations, taken into account alongside specific leaf area (SLA), assimilation, and respiration rates, define a pervasive leaf-level trade-off, the “leaf economics spectrum” (LES) (Wright *et al.*, 2004). The LES describes inter- or intraspecific leaf trait covariations as a slow-fast continuum. On one extreme, species express leaf traits related to rapid metabolism (high photosynthetic rate) over a short lifespan and low investment in structural tissues, and on the other extreme, species express leaf traits related to long-lived leaves with slow metabolism. This leaf-level trade-off may indeed reflect a whole-plant trade-off (Reich, 2014; Salguero-Gómez *et al.*, 2016). Thus, species characterized by “slow” LES traits would grow slowly, reach maturity later, live longer, and more effectively conserve resources over their lifespan (conservative strategy). By contrast, species with “fast” LES traits should grow faster, reach maturity earlier, have shorter lifespans, and more effectively acquire resources (acquisitive strategy). Empirical evidence of the connection between leaf-level and plant-level resource use strategies has been supported by recent intraspecific studies (Sartori *et al.*, 2019). Given that nitrogen mean residence time is a determinant of operational nitrogen concentration (Aerts & Chapin, 1999) one might thus expect that plant resource-use strategies, and thus LES traits would be associated with nitrogen

resorption rate (Kazakou *et al.*, 2007; Freschet *et al.*, 2010). However, the few studies examining the coupling between nitrogen resorption and LES across species did not show consistent patterns (Kazakou *et al.*, 2007; Freschet *et al.*, 2010; Yuan & Chen, 2010; Campanella & Bertiller, 2011; Achat *et al.*, 2018). Higher resolution intraspecific comparisons are needed to control for variation across other traits, and species-specific biochemical and biophysical determinants of nitrogen resorption that may blur the LES traits – resorption efficiency relationship. Moreover, comparing ecotypes within a species can be crucial to understand the role of nitrogen resorption in plant performance and local adaptation given its influence on plant fitness.

Traditional comparative approaches for measuring nitrogen resorption have focused on the proportion of nitrogen resorbed between the adult stage and the end of the leaf life cycle (namely resorption efficiency, RE_N) and the leaf nitrogen concentration at the end of the leaf life cycle (namely nitrogen resorption proficiency) (Aerts & Chapin, 1999). However, there are many ways to obtain comparable nitrogen resorption efficiencies. The dynamics of nitrogen resorption can be informative of the overall metabolic machinery of the leaf and provide a more comprehensive understanding of plants' nutrient-use strategies (Fig. 1). Non-destructive measurements are now possible using near infrared spectroscopy (NIRS) that enables the estimation of material properties from light absorbance (Ecartot *et al.*, 2013) and can help monitor the temporal variation of leaf nitrogen concentration (Vilmus *et al.*, 2014). We define the absolute nitrogen resorption rate (RR_N) as the amount of nitrogen remobilized by a leaf per unit time. We expect slow-growing plants to exhibit low RR_N over a long leaf lifespan, whereas fast-growing plants should display high RR_N over a short period of time. This is consistent with the expected higher resorption efficiency of slow-growing plants: low rates of resorption over a long period would enable a higher proportion of nitrogen remobilization (Fig. 1).

According to ecology and functional biogeography studies, species or genotypes adapt to harsh environmental conditions with conservative trait values (e.g. Borgy *et al.*, 2017). Thus, we expect higher rates and lower efficiency of resorption for genotypes native to environments that are more favorable. Empirical tests of this assumption remain scarce and limited to local and site-specific studies. Yuan & Chen (2009) performed a meta-analysis, gathering resorption efficiencies across species distributed worldwide. The authors reported a significant decrease in nitrogen resorption efficiency with increasing latitude, temperature, and precipitation. The

comparison of populations of a single species distributed along environmental gradients is now essential to test whether natural selection underlines these patterns.

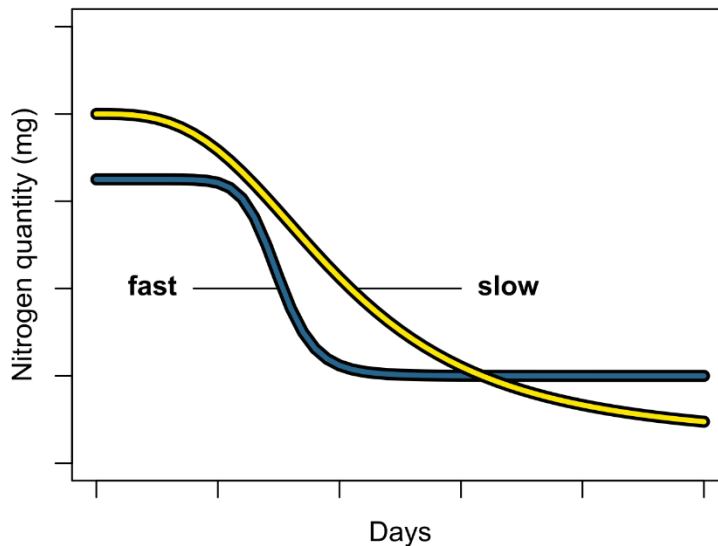


Figure 1: Expected leaf nitrogen dynamics for slow-growing (yellow) versus fast-growing (blue) plants.

Arabidopsis thaliana is a model species for not only genetic studies, but increasingly for ecological studies given its broad climatic range, and large variation in life-history traits (e.g. Mitchell-Olds, 2001; Brachi *et al.*, 2012) and functional traits, notably those related to the LES (Vasseur *et al.*, 2018b; Sartori *et al.*, 2019). *A. thaliana* is an herbaceous annual species with a large distribution spanning from the Mediterranean coast to Northern Sweden and across Asia. This distribution covers considerable variation of climatic conditions that are correlated with the genetic structure of *A. thaliana*'s populations, suggesting underlying local adaptation (Lasky *et al.*, 2012). The genetic determinism of the life cycle length, most often measured as the germination-flowering time interval, has been particularly well explored given its key role in *A. thaliana* adaptation to contrasting environments (e.g., Mendez-Vigo *et al.*, 2011; Lovell *et al.*, 2013; Schmalenbach *et al.*, 2014). Further, recent studies uncovered common genetic bases for flowering time and LES traits (Vasseur *et al.*, 2012), and evidenced that the differentiation of these traits is mediated by abiotic stresses (Vasseur *et al.*, 2018a). Substantial variability in resorption efficiency has been detected among a few genotypes (Masclaux-Daubresse & Chardon, 2011), reflecting variation in the genetic and metabolic pathways

involved in proteolysis and transport of N-bearing molecules (Havé *et al.*, 2017). However, the broader range of natural variability of nitrogen resorption requires assessment for a large panel of genotypes from contrasting environments, including elucidation of its genetic determinants and their importance for local adaptation.

We quantified the phenotypic and environmental variation of nitrogen resorption, and performed a genetic association study using common garden-grown ecotypes representing 121 *A. thaliana* populations sampled across the bulk of their native climate range. We hypothesized (i) strong variability in nitrogen resorption across ecotypes spanning this native climatic range, (ii) that rate and efficiency of leaf nitrogen resorption would be associated with the conservation-acquisition trade-off, (iii) that the natural variation of resorption traits has strong genetic bases that have been selected in contrasting environments. The latter implies that resorption traits display high heritability and significant associations with genetic variation.

Materials and methods

Plant material and growth conditions

We selected 121 natural genotypes from the 1001 GENOMES PROJECT list (Alonso-Blanco *et al.*, 2016), maximizing the geographic distribution and variance of the life cycle duration of the *A. thaliana* populations selected (Vasseur *et al.*, 2018a; Sartori *et al.*, 2019) (Supporting Information Table S1). We compiled 19 bioclimatic variable values to characterize the climate from the collection site of each genotype using the CHELSA database (<http://chelsa-climate.org/>). Eight times 121 pots (5×5×12 cm) were filled with a 1:1 mixture of sand and soil collected from the experimental field of the Centre d'Écologie Fonctionnelle et Évolutive (CEFE, Montpellier, France). This soil has a relatively low total nitrogen concentration ($1.38 \pm 0.11 \text{ mg g}^{-1}$) (Kazakou *et al.*, 2007). A layer of 2-3 mm of organic compost (Neuhaus N2) was added to the soil surface in each pot to improve germination and seedling survival. Three to five seeds were sown on December 8th and 9th of 2016 on the soil surface of each pot. Pots were placed in the dark at 8°C for one month to ensure seed vernalization and then placed in a greenhouse at 18/15°C day/night with a 12.5 h photoperiod. Eight individual plants per genotype were randomly placed, spaced apart in a checkerboard to avoid self-shading, on forty-four trays split across four tables. Tables were rotated daily to reduce block effects within the greenhouse. Measurements started at bolting stage, i.e. apparition of the flowering bud, for each individual plant. At this moment, we marked the largest leaf fully exposed to light of each

individual plant. Aboveground parts of four replicates per genotype were harvested at bolting and kept in deionized water at 4°C for one night before conducting destructive measurements (see below). The four remaining replicates were used to perform non-destructive measurements on the marked leaf until its complete senescence (see below). We used the flowering time (FT), i.e. the number of days from germination to opening of the first flower as a proxy of the individual life cycle length.

Destructive measurements

The marked leaf of each plant individual was harvested at bolting, after plant rehydration, and placed in a test tube with deionized water for rehydration 24 h at 4°C. Marked leaves were then scanned to determine the leaf area (LA, mm²). Marked leaves were oven-dried at 70°C for 72 h to determine the leaf dry mass (DM, mg). Specific leaf area (SLA, m² kg⁻¹) was calculated as the ratio between leaf area and leaf dry mass. A subset of 126 randomly selected dry leaves were ground individually to assess leaf nitrogen concentration (LNC, %) at bolting using the Pregl-Dumas method performed with a CHN Elemental Analyzer (Flash EA1112 Series, Thermo Finnigan, Milan, Italy).

Non-destructive measurements

Leaf area of the marked leaf of each tracked individual was determined two to three times a week from pictures taken with a smartphone (Microsoft Lumia 540®) equipped with an 8 Mpx camera and white plastic cube sticks on the flashlight as a light diffuser. Using an aluminum board, we made a small lightbox attached to the smartphone, designed to squeeze the leaves between a white support and a transparent piece of plastic (Fig. S1). Images were calibrated and leaf area was determined using ImageJ (Schneider *et al.*, 2012). Time-course measurements of the marked leaf optical properties were performed using a Near Infra-Red Spectrometer (LabSpec 4, ASD Inc.). Light absorbance of leaf tissues was recorded for the spectral region 350-2500 nm near the tip and at one edge of the leaves avoiding the midrib. Data represent leaf light absorbance for 2150 successive wavelengths, averaged for tip and edge (Fig S2). Marked leaves were harvested at complete senescence and all of them were ground individually (459 leaves in total) to assess leaf nitrogen concentration (LNC, %) as described above.

Leaf nitrogen dynamics

The procedure for N time course estimation is presented in Fig. S3. The first step was predicting SLA and LNC from spectral records. Wavelengths from the spectral records are considered as independent variables for the following analysis, despite the strong association between two successive wavelengths. We checked the presence of outlier spectra by computing a Mahalanobis distance on the coordinates of a principal component analysis (Whitfield *et al.*, 1987) and removed any spectrum exceeding the 95th quantile of the distances (11,250 remaining spectra). Samples collected at bolting and at complete senescence of the marked leaves were used as reference values to calibrate the models that predict leaf trait values at intermediate ages (LNC, 585 spectra; SLA, 882). We performed locally weighted partial least square regressions (Zavala-Ortiz *et al.*, 2020) using the measured SLA or LNC value as the predicted variable and the wavelength absorbance as the predictive variable. To select the best model parameters and spectra pre-treatments, we used a cross-validation method, which consisted in training the model on 70% of the reference values, then predicting the values of the remaining 30%. Best model pretreatments for both SLA and LNC were a Savitzky-Golay smoothing (Savitzky & Golay, 1964) with a 42 wavelengths window, a second order polynomial and first order derivative. Accuracy of the models was high for both SLA and LNC: the correlation coefficients of the predicted vs. measured values regression were higher than 0.95 (Fig. S4).

Data exploration suggested that temporal trajectories of SLA and LNC with leaf age followed a decreasing sigmoidal trend and leaf area followed a bell-shaped curve (Fig. 2). We therefore fitted time-course data using three different functions: gaussian, decreasing logistic and polynomial. Time-course trait variation was modeled using the function that minimized the error, estimated with the least square method. If none of the three functions fitted the data with a correlation coefficient higher than 0.75, the individual was removed from the analysis. Time-course of leaf dry mass (DM) and leaf nitrogen quantity (qN) were calculated as follows:

$$\text{Eq.1} \quad \text{DM} = \text{Area} / \text{SLA};$$

$$\text{Eq.2} \quad \text{qN} = \text{LNC} \times \text{DM}.$$

We searched for outliers using a shape-clustering algorithm. Virtually all marked leaves showed decreasing sigmoidal trends of qN decrease that differed in amplitude and rate (N = 407, Fig. S5b). We observed a few leaves with sudden increases in qN at end of the leaf lifespan, a behavior that could arise from accumulating error through the estimation procedure or from

drastic changes in leaf properties; those individuals were removed from the klmShape clustering analysis (N=34, Fig. S5a). For each marked leaf we measured the maximum nitrogen resorption rate (RR_N , mg d^{-1}) as the maximum absolute value of the local slopes of qN dynamics obtained from the first derivative value at each time point. Second, we extracted the day and value of leaf maximum nitrogen quantity (dN_{max} , d; qN_{max} , mg) to estimate the nitrogen resorption efficiency (RE_N , %), i.e. the proportion of total nitrogen resorbed. Since a single leaf was tracked through time, there is no need for area and mass standardization (Heerwaarden *et al.*, 2003), and RE_N can be directly calculated as:

$$\text{Eq.3} \quad RE_N = (qN_{\text{max}} - qN_{\text{min}}) / qN_{\text{max}}.$$

Overall, the method we used to measure qN variation and related traits controls for dilution effects that may arise from leaf thickening, or from leaf mass and area increasing due to spectral pretreatments and precise daily mass and area tracking, respectively. Thus, any change in leaf N status reflects leaf N in and out fluxes.

Genome-wide association study

We performed a genome wide association study (GWAS) using the GEMMA software (Zhou & Stephens, 2012). The method uses the single nucleotide polymorphisms (SNPs) as predictors of the traits in a linear mixed model. A relatedness matrix was added to the model in order to account for population structure. From the genetic data available on the 1001 genomes project website (1001genomes.org), we excluded all variants with more than two possible alleles and all variants with more than 10% of missing values. Bonferroni significance threshold of $\alpha = 0.05$ was used to identify SNPs associated with the traits.

Genome scan for selection

We scanned for SNPs under selection using an F_{ST} -like method (Luu *et al.*, 2017). This method postulates that SNPs under selection contribute more to population structure than expected by neutral processes, such as genetic drift. The method uses a principal component analysis (PCA) to detect the population structure based on the genetic information. The scores of the variables (the SNPs here) reflect their contribution to the construction of the principal components of the PCA and are processed as p-values. Outliers in the contribution distribution are considered highly differentiated between populations and, by extension, under selection. This method had the advantage of considering the genotypes as part of large regional and continuous populations, well suited for *A. thaliana* genotypes (Horton *et al.*, 2012). We started

with the full set of 1,135 genotype sequences available on the 1001 genome project website (1001genomes.org) containing 12,883,854 variant loci. We pruned the data by keeping only the SNPs, loci and genotypes having less than 10% of missing data, which resulted in a 1,032 genotypes by 6,385,774 SNPs dataset. We then filtered genotypes by genetic distance using a 0.075 correlation coefficient limit. Final data contained 222 genotypes and 6,385,774 SNPs. We spotted outlier SNPs that reached the Bonferroni significance threshold of $\alpha = 0.05$. The SNPs that were significant in both the GWAS and the selection scan delineated relevant regions of the genome for further analyses. To account for the effect of linkage disequilibrium, we extended the detected windows by 10 Kb upstream and downstream significant SNPs (Kim *et al.*, 2007). Using The Arabidopsis Information Resource database (www.arabidopsis.org), we extracted the list and functions of genes carried by those sequences.

Statistical analysis

All statistical analyses but GWAS were performed using the R software (R Core Team, 2019, version 3.6.1).

The chemometric analyses were performed using the *rnirs* package (<https://github.com/mlesnoff/rnirs>). A clustering of N dynamics was performed using the *kmlShape* package (Genolini *et al.*, 2016).

We estimated trait broad-sense heritability (H^2) as the part of variance explained by the genotype by performing linear mixed models using the package *nlme* (Pinheiro *et al.*, 2020). We calculated trait genotype means by estimating the marginal means of the variables from linear mixed models including the genotype identity as a random effect and the experimental trails as fixed effects. The models were performed with the *lme* function from the *nlme* package, and the marginal means were computed with the *emmeans* function from the *emmeans* package (Searle *et al.*, 1980).

Mean comparisons were performed using a Student's *t*-test test with the *t.test* function. The scaling of RR_N to qN was tested using a standardized major axis (SMA) regression using the *smatr* package (Warton *et al.*, 2006). Given the importance of the life cycle length in driving adaptive strategies in *A. thaliana*, we analyzed the RR_N -SLA and RE_N -SLA relationships by controlling for the flowering time using partial correlations performed using the *ppcor* R package (Kim, 2015).

Genome scans for selection were performed using the *PCAdapt* package (Luu *et al.*, 2017) and q-values were calculated using the *q-value* package (Storey *et al.*, 2004).

Results

Leaf trait dynamics

On average ($\pm$ se), the marked leaves reached their maximum area 16.7 ± 9.4 days after bolting (Fig. 2). This indicates that measurements started before leaves were fully expanded. Leaf area decreased until complete senescence, following a bell-shaped curve. Specific leaf area (SLA) and leaf nitrogen concentration (LNC) reached their maximum values significantly earlier than leaf area (both $P < 0.01$) and then decreased following a sigmoidal curve. The N peak occurred on average 10.3 ± 6.7 days after bolting. The LA peak occurred significantly later than the N peak ($P < 0.01$) and on average 16.7 ± 9.4 days after the onset of measurements. The maximum qN decrease (RR_N measurement day) occurred significantly later than the LA peak ($P < 0.01$) and on average 21.9 ± 9.7 days after bolting. Finally, the peak of dry mass occurred significantly later than maximum leaf area ($P < 0.01$) and on average 23.4 ± 9.8 days after bolting.

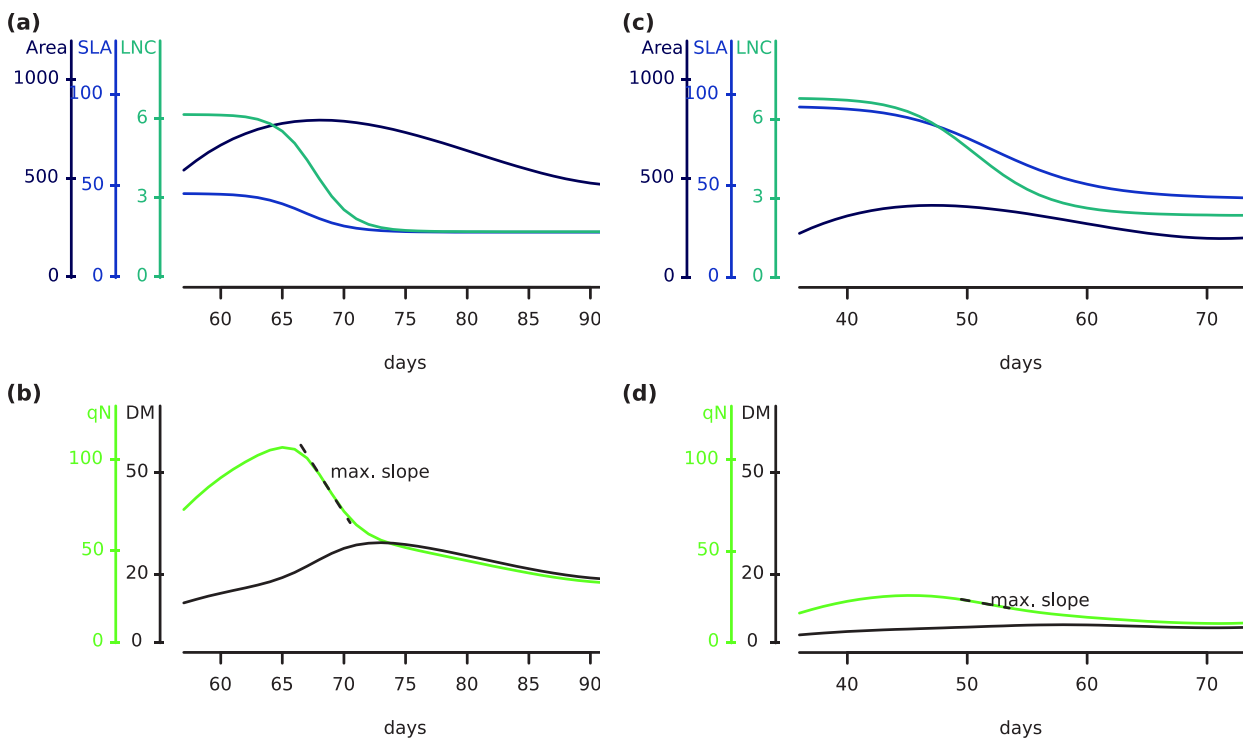


Figure 2: Observed temporal variability of leaf properties of a slow-growing plant (a, b) versus a fast-growing plant (c, d). The Y-axes represent the following traits: Area, leaf area (mm²); SLA, specific leaf area (m² kg⁻¹); LNC, leaf nitrogen concentration (%); qN, nitrogen quantity (mg); DM, leaf dry mass (mg). The X-axes represent the days after germination along which the onset of trait recording vary, depending on plant slow-fast strategies.

Trait variability and heritability

N resorption efficiency (mean $RE_N = 64.9 \pm 14.5 \%$) and resorption rate (mean $RR_N = 4.1 \pm 3.5 \text{ mg d}^{-1}$) displayed significant variation among natural genotypes and had a broad-sense heritability (H^2) of 0.20 and 0.76, respectively. Traits related to the structure and composition of the leaves were highly heritable: DM ($12.4 \pm 6.2 \text{ mg}$; $H^2 = 0.64$), LA ($496 \pm 169 \text{ mm}^2$; $H^2 = 0.61$), SLA ($55.7 \pm 13.6 \text{ mm}^2 \text{ mg}^{-1}$; $H^2 = 0.67$), maximum nitrogen quantity ($48.4 \pm 20.9 \text{ mg}$, $H^2 = 0.72$). Conversely, LNC heritability was surprisingly low ($5.4 \pm 0.7\%$, $H^2 \sim 0.11$). Flowering time (FT) was the most heritable trait ($52.6 \pm 13.8 \text{ d}$, $H^2 \sim 0.89$).

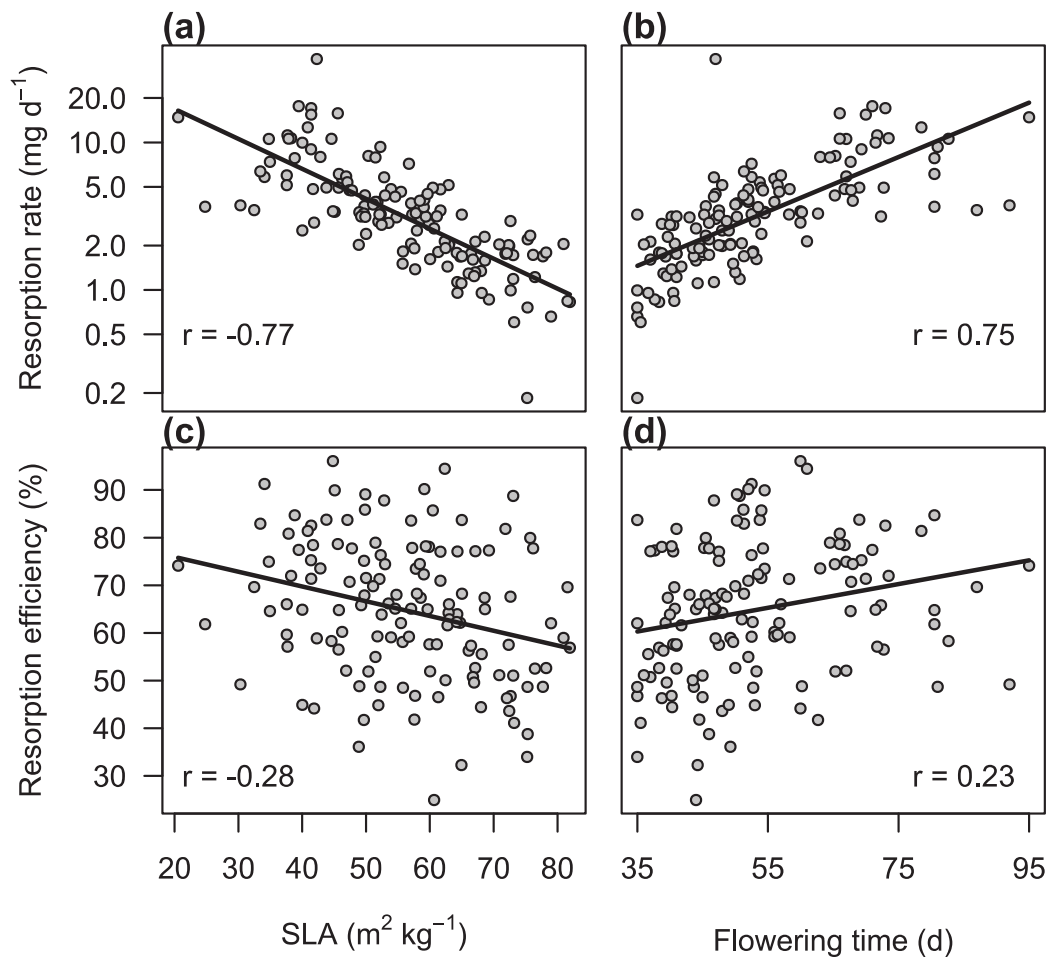


Figure 3: Relationships between nitrogen resorption, specific leaf area, and flowering time across natural genotypes of *A. thaliana*. Each dot represents the average trait value of a natural genotype (grey circles; $n = 121$). Lines represent significant linear relationships at $P < 0.05$. Pearson's correlation coefficients are reported for each relationship. Resorption rate is represented on a $\log_{10}$ -scale.

Relationships between nitrogen resorption and other plant traits

Across ecotypes, RR_N was negatively associated with SLA ($r = -0.77$, $P < 0.01$) and positively associated with FT ($r = 0.75$, $P < 0.01$) (Fig. 3a, b). The negative relationship of RR_N with SLA was independent of FT (partial correlation $r = -0.31$, $P < 0.001$). Further, RE_N was negatively associated with SLA ($r = -0.28$, $P < 0.01$) and positively associated with FT ($r = 0.23$, $P < 0.01$) (Fig. 3c,d), and the relationship of RE_N and SLA was also independent of FT (partial correlation $r = -0.16$, $P = 0.05$).

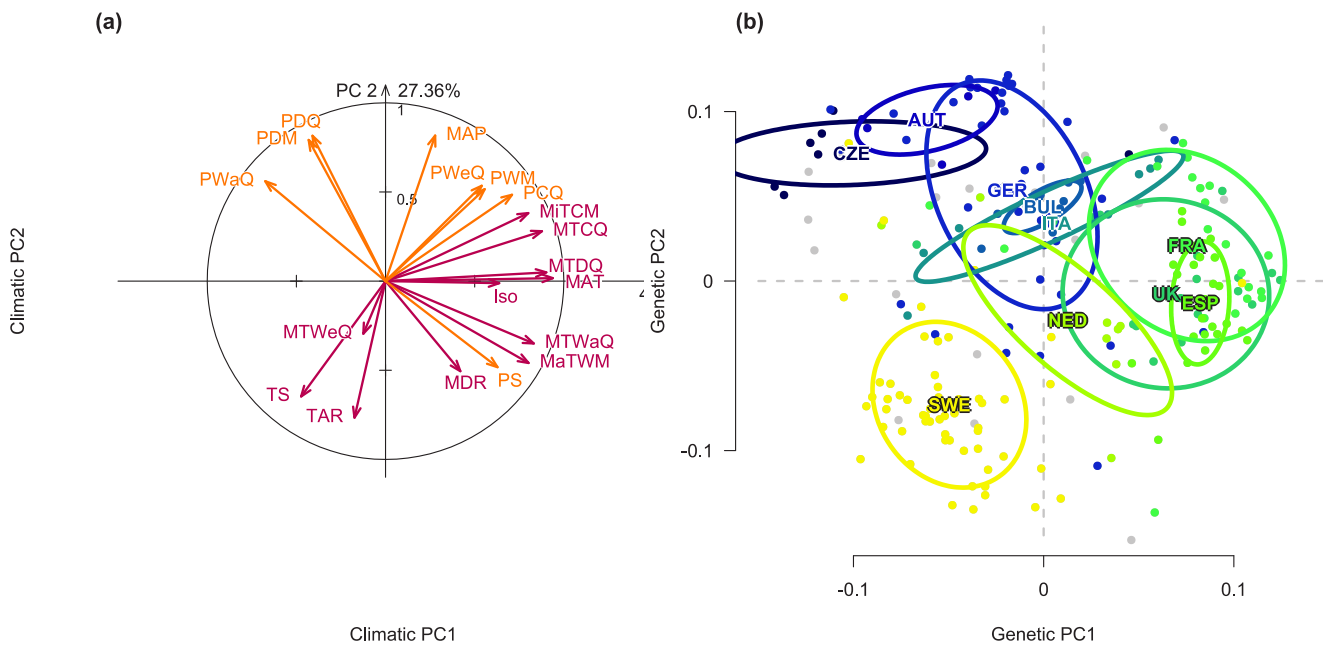


Figure 4: Climatic and genetic differentiation of *Arabidopsis thaliana* populations. (a) Correlation circle of a principal component analysis performed on a matrix composed of the 19 climatic variables obtained from the CHELSA database and the genotype collecting sites ($n = 140$). Climatic variables related to temperature are represented in red and those related to precipitation are represented in orange. (b) Score plot of a principal component analysis performed on the genetic data obtained from the 1001 genomes project database and the genotypes ($n = 222$). Each dot represents a genotype, and ellipses represent bivariate normal density contour of the best represented countries. Abbreviations: MAP; Mean Annual Temperature, MDR; Mean Diurnal Range, Iso; Isothermality, TS; Temperature Seasonality, MaTWM; Max Temperature of Warmest Month, MiTCM; Min Temperature of Coldest Month, TAR; Temperature Annual Range, MTWeQ; Mean Temperature of Wettest Quarter, MTDQ; Mean Temperature of Driest Quarter, MTWaQ; Mean Temperature of Warmest Quarter, MTCQ; Mean Temperature of Coldest Quarter, MAP; Mean Annual Precipitation, PWM; Precipitation of Wettest Month, PDM; Precipitation of Driest Month, PS; Precipitation Seasonality, PWeQ; Precipitation of Wettest Quarter, PDQ; Precipitation of Driest Quarter, PWaQ; Precipitation of Warmest Quarter, PCQ; Precipitation of Coldest Quarter; CZE, Czech Republic; AUT, Austria; GER, Germany; BUL, Bulgaria; ITA, Italy; UK, United Kingdom; FRA, France; ESP, Spain; NED, Netherlands; SWE, Sweden.

Axes of climatic and genetic population differentiation

The two first principal components of the climatic PCA accounted for 68% of the variation of the 19 CHELSA climatic variables (Fig. 4a). The first axis was mainly driven by the mean annual temperature, the mean temperature of the driest and the warmest quarter, and overall increased with increasing temperature. The second axis was mainly driven by the mean annual precipitation and the precipitation of the driest month and driest quarter, and it increased overall with increasing precipitation. The genetic PCA (PCAdapt) revealed that *A. thaliana* genotypes were differentiated along two major axes of variation following their geographical location (Fig. 4b). The first axis (Genetic PC1) described the Eastern Europe-Western Europe differentiation, and the second axis (Genetic PC2) described the Southern Europe-Northern Europe differentiation of the regional populations.

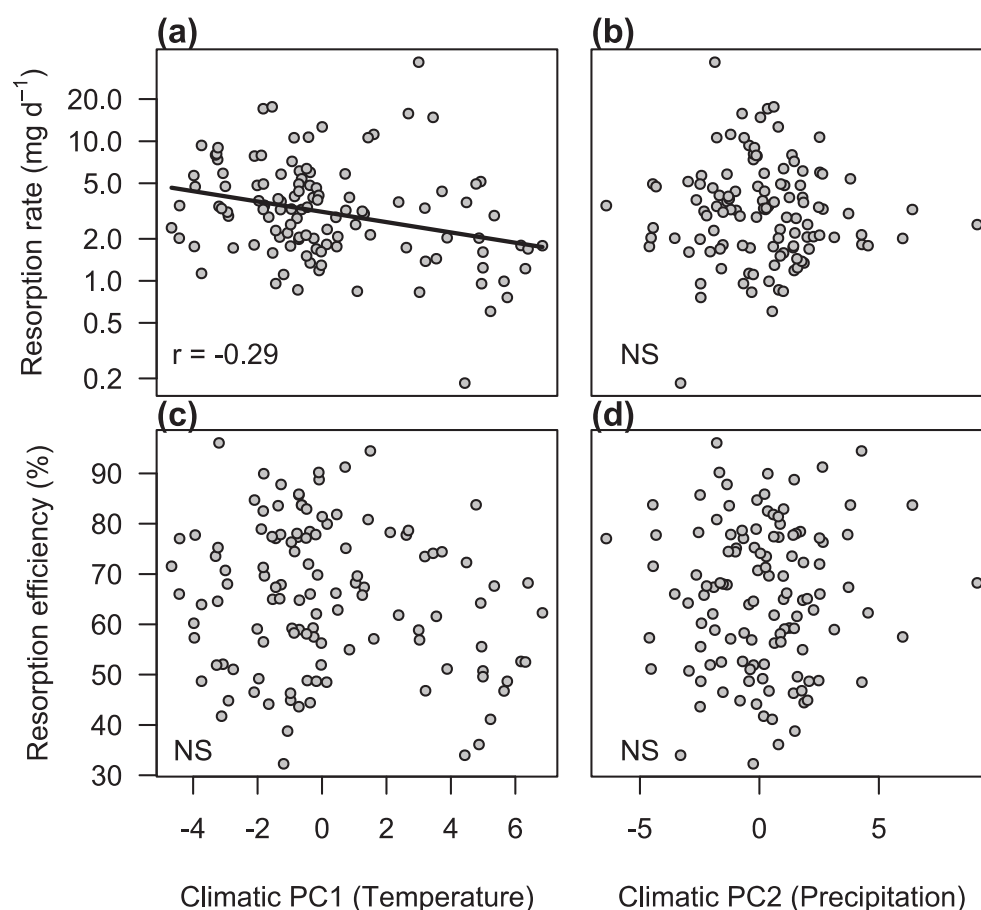


Figure 5: Relationships between the first principal components of climate variation of the collecting sites and resorption. Each dot represents the average value of a natural genotype ($n = 121$) of (a, b) resorption rate ($\log_{10}$ -scale) and (c, d) resorption efficiency as a function of climatic PCs scores from a principal component analysis performed on 19 climatic variables of the genotypes collecting sites (Fig. 4a). The values of PC1 and PC2 increase with increasing temperature and precipitation, respectively.

Adaptation cues and climatic drivers of resorption components

RR_N was negatively associated with increasing temperature of the native climate of the genotype ($r = -0.29$, $P < 0.05$) and was uncorrelated with average precipitation at the genotype collecting site (Fig. 5a,b). Resorption efficiency was not related to site temperature nor precipitation (Fig. 5c,d). The genome wide association study (GWAS) revealed 197 single nucleotide polymorphisms (SNPs) significantly associated with RR_N (Fig. 6a). Consistent with its low heritability level, we found no significant genetic association with RE_N (Fig. S6). From the genome scan for selection, none of the SNPs that contributed to the Eastern Europe-Western Europe differentiation were shared with those identified in the GWAS of RR_N. We detected 1,661 SNPs that contributed more than expected by neutral processes to the construction of the Southern Europe-Northern Europe axis (Fig. 6b). Among them, three consecutive SNPs were shared with the GWAS of RR_N. These SNPs delineated a region going from the 6,754,500 bp to the 6,775,500 bp of the fourth chromosome. Seven genes are coded in this region (see Supporting Information Table S2) including AT4G11070, a protein-coding gene called WRKY41, member of the WRKY transcription factor family.

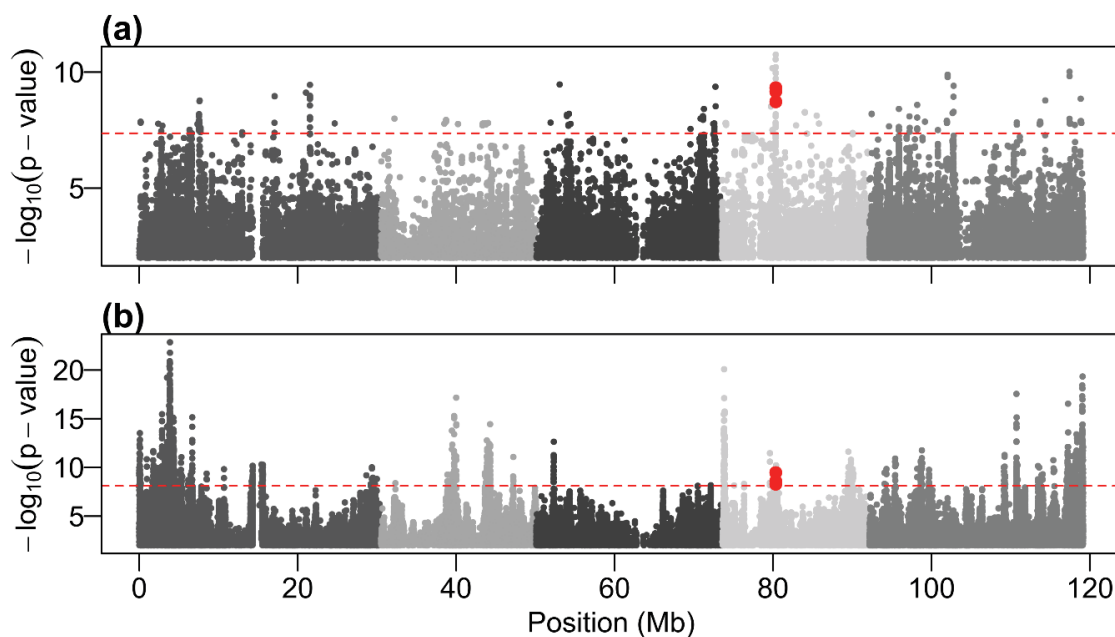


Figure 6: The polymorphism of a short sequence of *Arabidopsis thaliana* genome is associated with the resorption rate variation and is highly differentiated between populations. Panels are Manhattan plots, (a) representing the strength of the association between each single nucleotide (grey dots) polymorphism and the resorption rate variation, and (b) representing the contribution of each single nucleotide polymorphism on the genetic differentiation of *Arabidopsis thaliana* populations. Red dashed lines represent the significance threshold calculated with the Bonferroni method. Shades of grey delineate the five *A. thaliana* chromosomes.

Discussion

Historically, plant nutrient resorption is commonly evaluated through the outcome of the resorption process, i.e. resorption efficiency (Killingbeck, 1986). This approach meets a need to establish the economic record of leaves (Bloom *et al.*, 1985; Wright *et al.*, 2004). Consistent with previous interspecific comparisons, resorption efficiency in *A. thaliana* was high on average ($> 60\%$) (Kazakou *et al.*, 2007) and showed a medium value of broad-sense heritability ($< 30\%$) (e.g. Mikola *et al.*, 2018). This result suggests that, despite its theoretical importance in the context of leaf resource economics, resorption efficiency may not have the genetic basis to respond to natural selection. The main driver of nitrogen resorption is the strength of the nitrogen demand from newly formed leaves (Harper & Seltek, 1987), and, especially in annual species such as *A. thaliana*, growing flowers and seeds (Havé *et al.*, 2017). Thus, we hypothesized that selection on resorption capacity may not act on traits that maximize effectiveness at the leaf level. Instead, selection would act on traits that allow a response at the plant level to meet the nitrogen demand of surviving and growing tissues. Such a demand can be uncoupled from the individual capacity of leaves. Depending on the plant growth strategy, it might be advantageous for the plant to remobilize leaf nitrogen stocks more or less quickly. In this context, the rate of nitrogen resorption proves to be a promising candidate for describing differences in resorption strategies between genotypes. To our knowledge, this is the first time such a rate of nitrogen resorption had been reported. Using an innovative method based on the near infrared absorbance of leaf tissues (Vilmus *et al.*, 2014), we tracked daily changes in nitrogen quantity in living leaves. Resorption rate varied by a factor of 12 among genotypes and was highly heritable (broad-sense heritability = 76%). This result suggests a need for studies exploring the genetic, physiological, and morphological variations responsible for time course variations in nitrogen quantity. Additionally, we provide new insights about changes in leaf properties during leaf senescence. Previous studies reported that in *A. thaliana*, leaf senescence begins as soon as leaves reach their full adult size and maximum sugar content value (Diaz *et al.*, 2005, 2008). Diaz *et al.* observed that nitrogen, starch, protein, and chlorophyll content decrease, and leaf yellowing starts as soon as leaves reach full expansion. Our results do not support this assertion; among the 121 natural *A. thaliana* genotypes studied, leaf nitrogen concentration started to decrease on average a few days before leaves were fully expanded, suggesting that first signs of senescence occur before complete leaf maturity. This unexpected pattern may arise for two reasons: (i) the leaf N status does not reflect the photosynthetic maturity of the leaf; (ii) morphological and physiological leaf maturity are asynchronous. First,

studies showed that leaves build up N reserves at their juvenile stage and N remobilization is triggered by transition to reproductive stage (Santiago & Tegeder, 2017). Thus, the initial decrease in leaf N quantity during leaf expansion might be the result of N reallocation independent from leaf senescence. Second, leaf growth is the result of two uncoupled developmental processes: cell expansion and cell division (Tsiantis & Hay, 2003). Cell expansion is mainly constrained by water availability while cell division depends on photosynthates (Körner, 2013), suggesting that leaf growth can still occur during senescence in well-watered conditions despite the drop of carbon assimilation. Moreover, leaf dry mass still increased after the decline of leaf expansion and leaf nitrogen concentration in our dataset, consistently with the late increase in sugar concentration in several *A. thaliana* recombinant inbred lines (Diaz *et al.*, 2005). These observations challenge our conception of leaf nitrogen and carbon in- and out-fluxes during leaf ageing. Our study was limited to tracking a single leaf per plant and during the second half of its lifespan. More studies are needed to better understand how the dynamics of leaf properties are driven by plant life history events such as the production of new leaves and reproductive organs.

Given its importance for plant resource economics, nitrogen acquisition and conservation strategies have been included in a slow-fast syndrome at both plant and leaf levels (Reich, 2014). However, previous interspecific explorations of the linkage between resorption efficiency and leaf economics traits did not report clear signals (Kazakou *et al.*, 2007; Freschet *et al.*, 2010). As expected, our results showed that high resorption efficiency was associated with low specific leaf area and late maturity corresponding to a slow-leaf and slow-plant syndromes (Sartori *et al.*, 2019), respectively. Conversely, fast syndromes were characterized by low resorption efficiency. However, correlations were weak, indicating that for a given value of resorption efficiency, plants expressed a large range of specific leaf area and flowering time values.. By contrast, resorption rate correlated strongly with the plant and leaf syndromes in our dataset. The fact that slow strategies at the plant and leaf level were characterized by high leaf resorption rates contradicts our initial expectations. Leaves from fast-growing plants exhibited a slow decrease in nitrogen quantity while leaves from slow-growing plants had fast nitrogen resorption within a short time. Fast growing genotypes reach sexual maturity earlier with a lower plant biomass and leaf number than slow growing genotypes (Vasseur *et al.*, 2018a), consistent with a slow-fast syndrome (Salguero-Gómez *et al.*, 2016; Dammhahn *et al.*, 2018). Thus, the rate of leaf nitrogen resorption might be adjusted to the plant-level nitrogen demand. As reported by Diaz and colleagues (2005), the time course of leaf senescence is correlated with

the total number of leaves that compose the rosette in *A. thaliana*. We hypothesize that large plants composed of numerous leaves impose a strong nitrogen demand on senescent leaves. In addition, as we measured traits after bolting, the construction of reproductive organs may impose a supplementary nitrogen demand on leaves. Slow plant strategies are expected to produce protein-rich seeds (Westoby, 1998) that require more nitrogen than fast plant seeds. This strong source-sink relationship suggests that leaf-level resorption capacity can be uncoupled from leaf-level resource use strategies and should be considered relative to whole-plant functioning. Future experimental studies measuring nitrogen transfers between organs of the whole individuals are needed to properly test these assumptions.

Our results did not show any correlation between nitrogen resorption efficiency and temperature or precipitation, contrary to previous observations in interspecific biogeographic meta-analyses (Yuan & Chen, 2009; Drenovsky *et al.*, 2019). The most parsimonious explanation is that the effect of the environment on the resorption efficiency is mediated through phenotypic plasticity, rather than local adaptation (consistent with our lack of heritability for this trait), and growing plants in homogeneous conditions would have removed such influence of phenotypic plasticity. Since the resorption efficiency is known to vary depending on the growing conditions (Chapin, 1980), phenotypic plasticity could also be responsible for the lack of relationship in our dataset. By contrast, the rate of nitrogen resorption covaried with climate: high rates of nitrogen resorption were expressed by genotypes originating from cooler habitats. Despite the importance of microclimate for *A. thaliana* local adaptation (Brachi *et al.*, 2013), annual temperature may reflect the average growing conditions that the populations encounter. Therefore, environmental variables capturing spatially imprecise data are still considered approximate estimators of stress that plants experience (Borgy *et al.*, 2017). The relatively recent migration of *A. thaliana* toward Scandinavia was accompanied by allele fixation conferring resistance to harsh environmental conditions imposed by coldness (Krämer, 2015; Exposito-Alonso *et al.*, 2018). Such climatic constraints should have selected for trait values characteristic of a conservative syndrome (Borgy *et al.*, 2017), such as low LES scores (Sartori *et al.*, 2019), and trait values determinant for leaf senescence and lifespan such as resorption capacity. However, it will be important to account for variation in local soil resource availability to further explore the biogeographical determinants of the different facets of resorption ability (Drenovsky *et al.*, 2019). Indeed, nutrient poor environments are supposed to select for traits reducing nutrient losses (Aerts & Chapin, 1999) and should thus also regulate variation in the rate of nutrient resorption.

The high heritability of nitrogen resorption rate was reflected by the numerous genetic associations detected along the *A. thaliana* genome. To filter for relevant associations, we conducted an independent screening for loci under selection based on the genetic structure of *A. thaliana* populations. We identified major axes of genetic differentiation that correspond to the geographic differentiation of regional *A. thaliana* populations. Interestingly, one locus showed simultaneously significant association with nitrogen resorption rate and significant effect on the genetic differentiation along the latitudinal distribution of the species. The locus notably codes for a member of the WRKY transcription factor family, called WRKY41. WRKY proteins are transcription factors that play roles in diverse, important processes such as germination, senescence, and response to stresses (Rushton *et al.*, 2010). They represent the second largest family of genes expressed during leaf senescence (Guo *et al.*, 2004). Previous studies showed that deactivation and overexpression induce delayed and accelerated leaf senescence, respectively (Miao *et al.*, 2004) and affect leaf lifespan (Doll *et al.*, 2020). While, in this study, our ambition was not to identify genes that are determinant for the resorption capacity in *A. thaliana*, this result is a rare convergence between genomic and ecological findings. It demonstrates the role of nitrogen resorption in leaf senescence and lifespan, and its importance for local adaptation.

Overall, our results emphasize the ecological and evolutionary importance of a leaf nitrogen resorption component that has been poorly explored so far. Combined with genetic and climatic data, our findings reveal that high rates of nitrogen resorption, rather than high efficiency, are favored toward the northern and colder region of the *A. thaliana* distribution. We provide evidence that the resorption rate is integrated in leaf and plant level resource use strategies and growth syndrome, suggesting its importance for better plant fitness characterization. Next steps will be to explore the anatomical and molecular determinants of the resorption rate as done for nitrogen resorption efficiency, such as the leaf vein architecture (Zhang *et al.*, 2015) and the enzymes responsible for N-bearing molecules catalysis and transport (Moison *et al.*, 2018). Finally, future studies might also explore the relative role of soil nitrogen availability and plant nitrogen demand on the time-course of nitrogen at the organ and plant levels. The combined use of comparative ecology, quantitative genetics and population genetics is a promising avenue to understand the role of physiological constraints and trait syndromes in plant adaptation.

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SUPPLEMENTAL INFORMATION

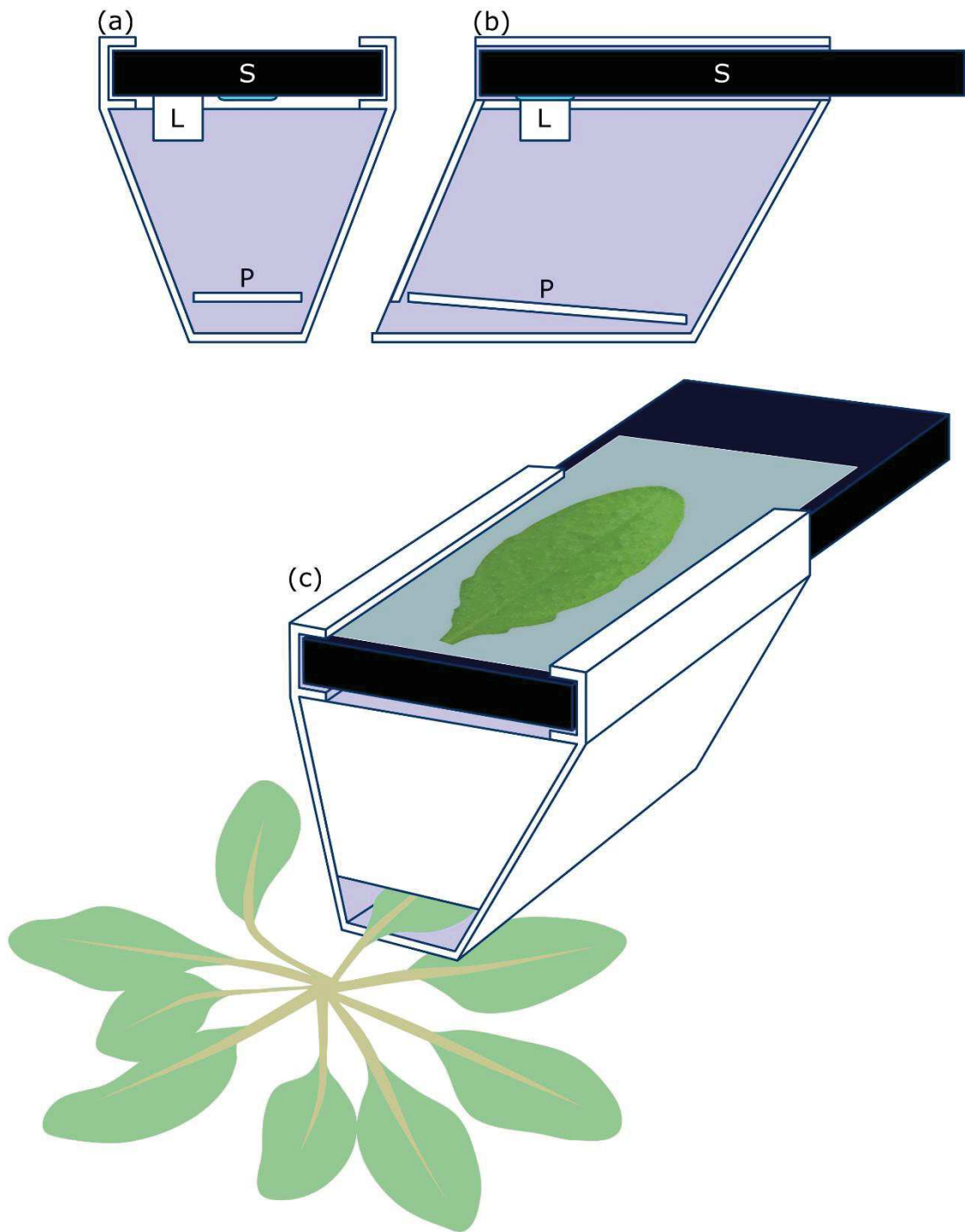


Figure S1: Diagram of the device used for measuring leaf area without cutting the leaf. (a) Frontal section, (b) sagittal section, and (c) three-dimensional view of the device. Abbreviations: (S) Smartphone, (L) light diffuser, (P) transparent piece of plastic.

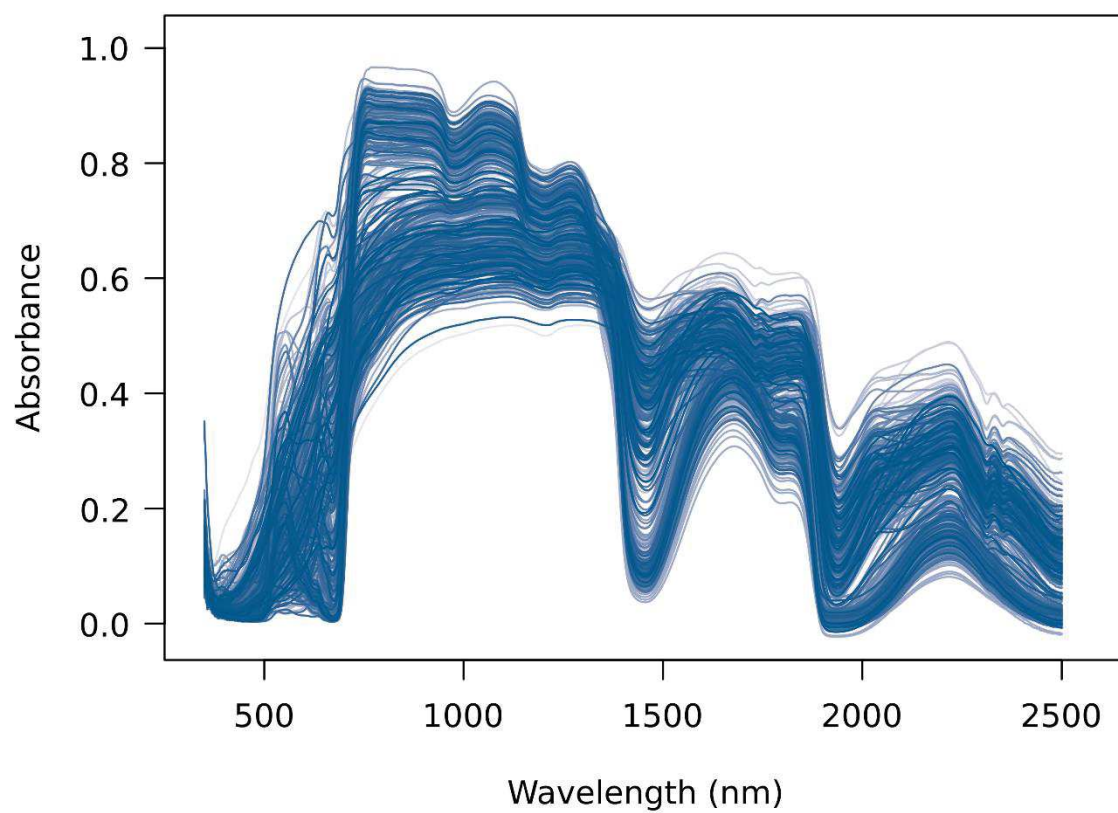


Figure S2: Absorbance spectra of fresh green to senescent leaves. Each blue line represents the averaged spectra acquired from the tip and edge of one leaf from one *A. thaliana* ecotype.

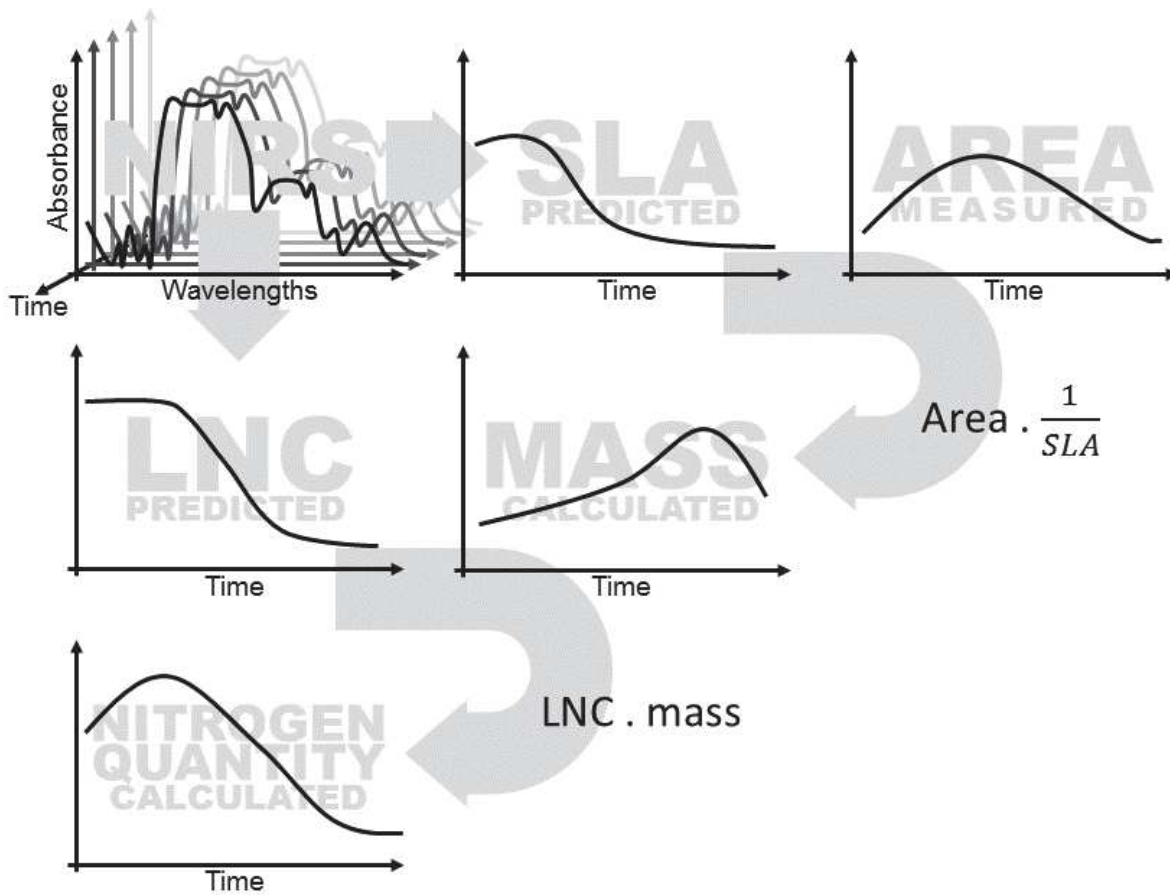


Figure S3. Procedure used to assess the dynamics of leaf nitrogen quantity. Leaf near infra-red light absorbance (NIRS) was recorded through leaf aging to estimate the specific leaf area (SLA) and leaf nitrogen concentration (LNC). Leaf area was recorded daily. Combined estimates of SLA, LNC, and area, were used to calculate the dynamics of leaf dry mass (Mass) and leaf nitrogen quantity.

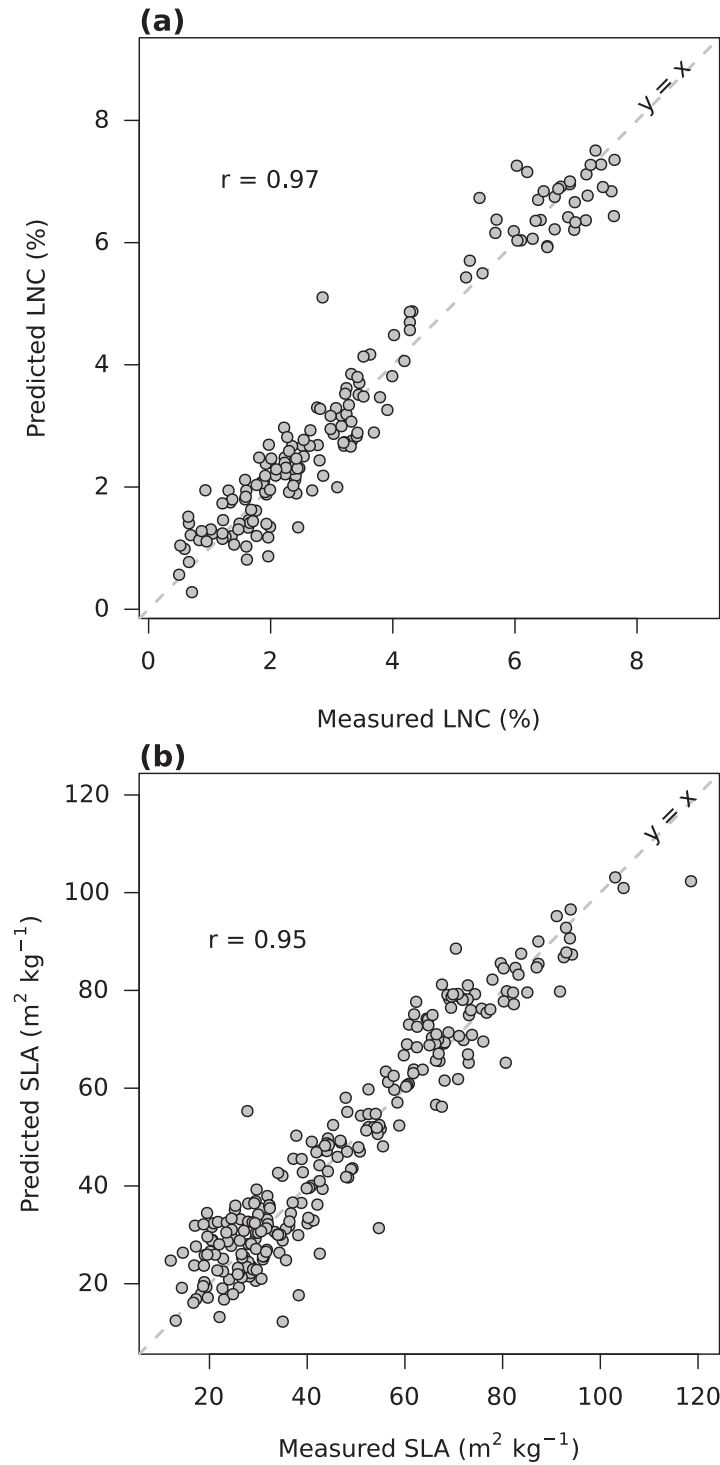


Figure S4. Performance of the predictive models for (a) leaf nitrogen concentration (LNC) and (b) specific leaf area (SLA).

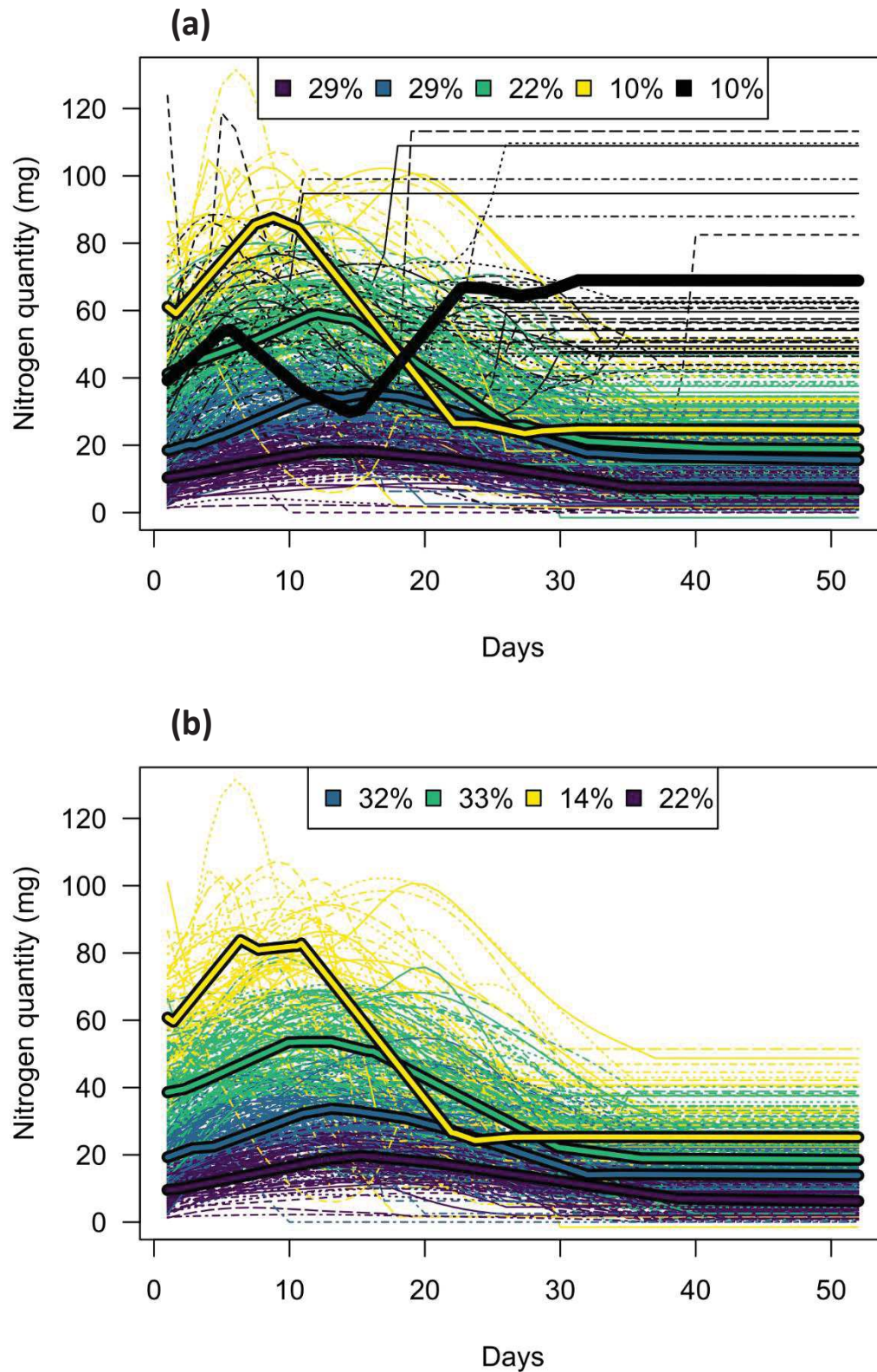


Figure S5. Result of the first step of klmShape clustering. (a) Initial clustering for the full dataset. Bold lines represent the average behavior of five different groups of nitrogen temporal variation. Dotted lines represent individual curves. Individuals from the fifth group (black line) were removed from the analysis and a second clustering were performed (b).

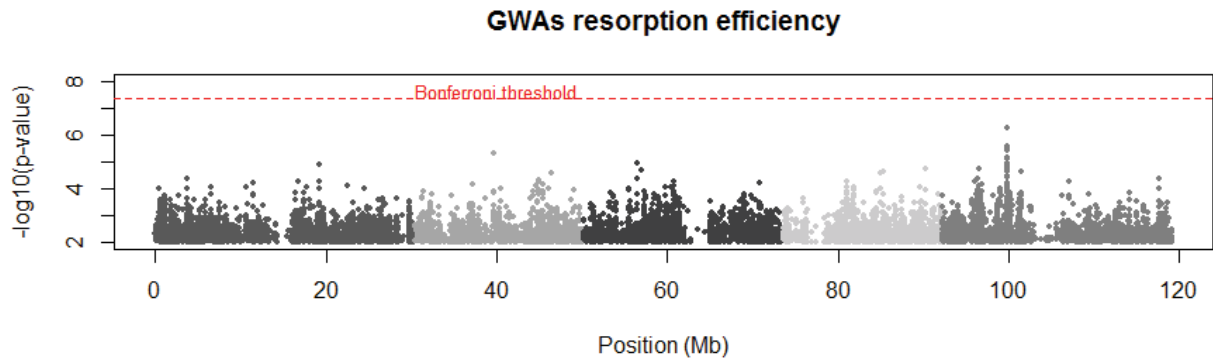


Figure S6. Genome-wide association study of the resorption efficiency. Each dot represents a single nucleotide polymorphism, its position along the x-axis reflects its position along the *Arabidopsis thaliana* genome, and its position along the y-axis gives the strength of the association. The red dashed line represents the significance threshold calculated with the Bonferroni method. Shades of grey delineate the five *A. thaliana* chromosomes.

Table S1: Accessions list. Abbreviations: FT, flowering time; BT, bolting time.

ID	FT	BT	name	CS number	country	latitude	longitude	group
10015	40,5	31,57	Ara-1	CS76382	AFG	37,29	71,3	asia
14313	56	48,5	Kos-2	CS78924	RUS	62,02	34,12	asia
159	52,75	42,17	MAR2-3	CS77070	FRA	47,35	3,93	western_europe
5151	66,75	62,4	UKSE06-325	CS78801	UK	52,2	-1,7	admixed
5165	56	48,14	UKSE06-362	CS78802	UK	51,3	0,4	admixed
5768	80,5	72,38	UKID63	CS78786	UK	54,1	-1,5	admixed
5784	67	57,14	Ty-1	CS78790	UK	56,4	-5,2	admixed
5837	45,5	34,5	Bor-1	CS76453	CZE	49,4	16,23	central_europe
6074	80,5	74,13	Or-1	CS77150	SWE	56,46	16,13	south_sweden
6076	87	84,2	Rev-2	CS77215	SWE	55,69	13,45	south_sweden
6108	54,5	44,25	T480	CS77300	SWE	55,8	13,12	western_europe
6151	73	66,5	T990	CS77328	SWE	55,65	13,22	south_sweden
6180	46,75	35,75	TaL 07	CS77339	SWE	62,63	17,69	germany
6184	67,67	58,8	TBO 01	CS77343	SWE	62,89	18,45	north_sweden
6195	58,33	49,29	TDr-9	CS77356	SWE	55,77	14,13	south_sweden
6209	67	54,29	TEDEN 02	CS77358	SWE	62,88	18,18	north_sweden
6243	44	35	Tottarp-2	CS77381	SWE	56,27	13,9	central_europe
6244	63	56,67	TRa 01	CS77384	SWE	62,92	18,47	north_sweden
6830	47,5	37,25	Kz-13	CS76994	KAZ	49,5	73,1	admixed
6897	47	38,13	Ag-0	CS76430	FRA	45	1,3	western_europe
6898	40,33	31,25	An-1	CS76435	BEL	51,22	4,4	admixed
6901	65,33	56,43	Bil-7	CS76710	SWE	63,32	18,48	north_sweden
6903	46,75	35,25	Bor-4	CS76454	CZE	49,4	16,23	central_europe
6904	48	38,75	Br-0	CS76455	CZE	49,2	16,62	western_europe
6909	47,5	39	Col-0	CS76778	USA	38,3	-92,3	germany
6911	35	24,75	Cvi-0	CS76789	CPV	15,11	-23,62	relict
6915	53,75	45,29	Ei-2	CS76478	GER	50,3	6,3	germany
6922	40,75	28,5	Gu-0	CS76498	GER	50,3	8	germany
6929	48	38,5	Kondara	CS76532	TJK	38,48	68,49	asia
6938	60	48,5	Ms-0	CS76555	RUS	55,75	37,63	asia
6945	57	49,5	Nok-3	CS76562	NED	52,24	4,45	germany
6958	41	29	Ra-0	CS76582	FRA	46	3,3	western_europe
6959	52,5	47	Rennes-1	CS77210	FRA	48,5	-1,41	western_europe
6963	69	59,38	Sorbo	CS78917	TJK	38,35	68,48	asia
6970	38,33	28,2	Ts-1	CS76615	ESP	41,72	2,93	spain
6979	35	25	Wei-0	CS76628	SUI	47,25	8,26	central_europe
6987	50,67	36,25	Ak-1	CS76431	GER	48,07	7,63	admixed
6989	47,5	38,63	Alst-1	CS76432	UK	54,8	-2,43	western_europe
7000	60	49,5	Aa-0	CS76428	GER	50,92	9,57	germany
7002	51,33	44,67	Baa-1	CS76442	NED	51,33	6,1	germany
7008	73,5	66,17	Benk-1	CS76447	NED	52	5,68	germany
7028	37,67	26,6	Bch-1	CS76444	GER	49,52	9,32	admixed
7063	50	36,63	Can-0	CS76740	ESP	29,21	-13,48	relict
7071	45,5	35,71	Chat-1	CS76463	FRA	48,07	1,34	western_europe

7077	35	24,25	Co-1	CS76468	POR	40,12	-8,25	italy_balkan_caucasus
7092	39	32	Com-1	CS76469	FRA	49,42	2,82	western_europe
7103	40,5	27,5	Dra-0	CS76476	CZE	49,42	16,27	central_europe
7111	49,25	37,75	Edi-0	CS76831	UK	55,95	-3,16	admixed
7127	45	34,6	Est	CS76485	EST	58,67	24,99	admixed
7143	49,67	37,4	Gel-1	CS76492	NED	51,02	5,87	germany
7165	52,5	40,63	Hn-0	CS76513	GER	51,35	8,29	germany
7186	45	34,25	Kn-0	CS76969	LTU	54,9	23,89	central_europe
7192	48	35,33	Kil-0	CS76526	UK	55,64	-5,66	germany
7209	45,25	34	La-0	CS76538	POL	52,73	15,23	admixed
7213	38,75	26	Ler-0	CS77020	GER	47,98	10,87	admixed
7287	52,5	40	Ove-0	CS76569	GER	53,34	8,42	germany
7288	70	60	Oy-0	CS77156	NOR	60,39	6,19	admixed
7298	56,5	45,57	Pi-0	CS76572	AUT	47,04	10,51	central_europe
7316	60,2	48,25	Rhen-1	CS78916	NED	51,97	5,57	admixed
7320	51	41	Rou-0	CS76591	FRA	49,44	1,1	western_europe
7347	43	34,5	Stw-0	CS76605	RUS	52	36	central_europe
7373	52	42,38	Tsu-0	CS77389	JPN	34,43	136,31	admixed
7382	37	27	Utrecht	CS76622	NED	52,09	5,11	admixed
7383	40,25	28,29	Van-0	CS76623	CAN	49,27	-123,21	western_europe
7394	39,67	27	Wa-1	CS76626	POL	52,3	21	admixed
7424	54	44	Jl-3	CS76519	CZE	49,2	16,62	central_europe
7461	44,25	32,14	H55	CS76897	CZE	49	15	germany
763	41	27,43	Kar-1	CS76522	KGZ	42,3	74,37	asia
8214	43,67	34,75	Gy-0	CS78901	FRA	49	2	western_europe
8231	92	79,67	Bro1-6	CS76726	SWE	56,3	16	south_sweden
8240	72,75	66,25	Kulturen-1	CS76987	SWE	55,71	13,2	south_sweden
8247	71	58,4	San-2	CS77233	SWE	56,07	13,74	south_sweden
8312	49	40	Is-0	CS78904	GER	50,5	7,5	germany
8343	46	41,63	Na-1	CS76558	FRA	47,5	1,5	admixed
8351	67,75	54,75	Ost-0	CS77154	SWE	60,25	18,37	north_sweden
8354	44,5	32,25	Per-1	CS76571	RUS	58	56,32	asia
8357	46	35,5	Pla-0	CS76573	ESP	41,5	2,25	spain
8376	69,33	57,43	Sanna-2	CS77234	SWE	62,69	18	north_sweden
8424	39,33	27	Kas-2	CS78905	IND	35	77	asia
88	40,67	30,33	CYR	CS76790	FRA	47,4	0,68	western_europe
9057	58,25	49,25	Vinslov	CS78847	SWE	56,1	13,92	south_sweden
9437	64,5	60,67	Puk-2	CS77195	SWE	56,16	14,68	south_sweden
9507	35	22	Coa-0	CS76775	POR	38,45	-7,5	spain
9518	66	60,17	Alm-0	CS76660	ESP	39,88	-0,36	spain
9524	61	47,5	Ber-0	CS78887	ESP	42,52	-0,56	spain
9535	54,5	37,29	Coc-1	CS76776	ESP	42,31	3,19	spain
9537	38,33	26,5	Cum-1	CS76787	ESP	38,07	-6,66	spain
9544	36,67	25,67	Gua-1	CS76894	ESP	39,4	-5,33	spain
9548	95	76	Hoy-0	CS76939	ESP	40,4	-5	admixed
9549	71,75	61,86	Hum-2	CS76943	ESP	42,23	-3,69	relict
9560	37	27,29	Mot-0	CS77109	ESP	38,19	-6,24	spain
9562	52	44,14	Mur-0	CS77115	ESP	41,67	2	spain
9567	82,67	72,2	Pal-0	CS77159	ESP	42,34	1,3	spain
9587	72,33	62,67	Tdc-0	CS77344	ESP	41,5	-1,88	spain

9589	66	62,6	Tor-1	CS77378	ESP	41,6	-2,83	spain
9594	78,5	69	Vdm-0	CS78837	ESP	42,04	1,01	spain
9598	65,25	54,86	Vim-0	CS78844	ESP	41,88	-6,51	relict
9606	36	25,86	Aitba-1	CS76649	MAR	31,48	-7,45	relict
9625	54	48,57	Kolyv-2	CS76977	RUS	51,31	82,59	asia
9637	50,25	40,14	Noveg-2	CS77132	RUS	51,77	80,85	asia
9640	71,5	62	Rakit-1	CS77202	RUS	51,87	80,06	asia
9649	53,5	46,4	Bivio-1	CS76713	ITA	39,13	16,17	italy_balkan_caucasus
9653	39,5	27,38	Giffo-1	CS76878	ITA	38,44	16,13	italy_balkan_caucasus
9657	35,5	25	Melic-1	CS77078	ITA	38,45	16,04	italy_balkan_caucasus
9697	53,25	40,75	Dolen-1	CS76802	BUL	41,62	23,94	italy_balkan_caucasus
9726	41	33,13	Faneronemi-3	CS76853	GRC	37,07	22,04	italy_balkan_caucasus
9737	56,75	48,75	Ulies-1	CS78815	ROU	45,95	22,62	asia
9738	50,25	43,13	Bran-1	CS76722	ROU	45,57	25,42	admixed
9741	52	46,75	Orast-1	CS77151	ROU	45,84	23,16	central_europe
9743	68	57,38	Furni-1	CS76873	ROU	45,14	25	admixed
9749	50	42,17	Knjas-1	CS76971	SRB	43,54	22,29	italy_balkan_caucasus
9758	47	38,5	Altai-5	CS76433	CHN	47,75	88,4	asia
9784	38,75	28	Erg2-6	CS76845	GER	48,5	8,8	central_europe
9927	40,25	27	ARR-17	CS76673	FRA	44,05	3,69	western_europe
9933	41,75	32	VED-10	CS78839	FRA	43,74	3,89	admixed
9941	52,67	40,33	Fei-0	CS76412	POR	40,92	-8,54	western_europe
9943	48,67	36	Cdm-0	CS76410	ESP	39,73	-5,74	spain
9944	51,33	42,83	Don-0	CS76411	ESP	36,83	-6,36	relict
9947	47	55,2	Ped-0	CS76415	ESP	40,74	-3,9	relict
9958	54,25	46,25	Shigu-1	CS76375	RUS	53,33	49,48	asia
9997	51,25	41,43	Rue3.1-31	CS76406	GER	48,56	9,16	central_europe

Table S2: Candidate gene list.

Name	Description	Position	Expressed in	Involved in
AT4G11060	Mitochondrially targeted single-stranded DNA binding protein	Chr4:6754515..6756595 (- strand)	Seed, growing tissues	Mitochondrial DNA replication, positive regulation of helicase activity
AT4G11070	WRKY family transcription factor	Chr4:6759098..6760794 (+ strand)	Senescent leaf (petiole/vein)	Regulation of transcription, DNA-templated
AT4G11080	HMG (high mobility group) box protein	Chr4:6760278..6763499 (- strand)	Inflorescences	Regulation of transcription, DNA-templated
AT4G05845	Long_noncoding_rna	Chr4:6763744..6763968 (+ strand)	Seed, growing tissues	
AT4G11090	TRICHOME BIREFRINGENCE-LIKE 23	Chr4:6764379..6766329 (- strand)	The whole plant	Synthesis and deposition of secondary wall cellulose
AT4G11100	Gelsolin protein	Chr4:6768614..6770285 (+ strand)	Inflorescences, young leaf	
AT4G11110	SPA1-related 2	Chr4:6771605..6777225 (+ strand)	Seed, flower, young leaf	Suppressing photomorphogenesis in dark- and light-grown seedlings



SYNTHÈSE ET PERSPECTIVES

Variabilité intra-spécifique des traits foliaires : amplitude, déterminisme génétique et héritabilité

Une hypothèse centrale de l'écologie comparative stipule que quel que soit le contexte environnemental de mesure d'un trait fonctionnel, le classement des espèces pour ce trait est stable. L'hypothèse sous-jacente est que la variabilité des traits fonctionnels est en général plus grande entre des individus d'espèces différentes (variabilité interspécifique) qu'entre les individus d'une même espèce (variabilité intra-spécifique) (Keddy 1992). Cette hypothèse est en général supportée par les données empiriques (Kazakou *et al.*, 2014). Au cours de la dernière décennie néanmoins, plusieurs études ont préconisé l'exploration de la variabilité intra-spécifique des traits fonctionnels en écologie, en particulier dans le cadre de l'analyse de l'assemblage des communautés (Bolnick *et al.*, 2011; Albert *et al.*, 2011; Violle *et al.*, 2012), mais aussi le fonctionnement des écosystèmes, la diversité et les flux de nutriments (Roches *et al.*, 2018), et l'adaptation des espèces à leur environnement (Donovan *et al.*, 2011). Cependant, la plupart de ces études ne permettent pas de distinguer les causes proximales (ontogénie, plasticité phénotypique, diversité génétique) de la variabilité intra-spécifique de ces traits.

En cultivant 150 à 400 génotypes du modèle *Arabidopsis thaliana* en milieu contrôlé, notre objectif était de maximiser la variance génétique d'une part et d'autre part de minimiser la variance environnementale et l'interaction génotype-environnement potentiellement responsables de la variabilité phénotypique. Comme attendu, nos données montrent que la variabilité génétique des populations d'*A. thaliana* est reflétée par une grande variabilité phénotypique. Ceci est illustré par la projection de nos données dans le spectre global des formes et fonctions des plantes, présenté en introduction (Fig. 12). Ceci est particulièrement vrai pour la concentration en azote et la vitesse de photosynthèse qui varient entre 2 et 8%, et entre 40 et 600 nmol g⁻¹ s⁻¹, respectivement, dans l'ensemble de nos données, contre 0.2% à 6.4% et 5 à 660 nmol g⁻¹ s⁻¹, respectivement, dans des comparaisons interspécifiques globales (Wright *et al.*, 2004). Ces gammes de variation chez *A. thaliana* correspondent aux gammes précédemment observées (ex. Vasseur *et al.*, 2012). L'efficacité de résorption de l'azote foliaire varie entre 30 et 90%, ce qui couvre une grande partie de la variabilité interspécifique (Yuan & Chen, 2009). En revanche, comme attendu pour une espèce annuelle, la durée de vie des feuilles est courte en moyenne et peu variable au regard de la gamme interspécifique : de 0.5 à 1.7 mois chez *A. thaliana* contre 0.9 à 288 mois à l'échelle interspécifique (Wright *et al.*, 2004). La gamme de valeurs de densité de nervures (3.4 et 8,3 mm mm⁻²) correspond à la gamme observée par le passé chez cette espèce (Rishmawi *et al.*, 2017) et se situe dans la partie basse de la

gamme interspécifique (de 0.5 à 25 mm mm⁻², Sack *et al.*, 2012). Les héritabilités relativement élevées pour la majorité des traits (Tableau 3) et les fortes associations génotype-phénotype suggèrent que les différences phénotypiques observées entre génotypes traduisent effectivement des différences génétiques, qui peuvent être sélectionnées dans des environnements différents. Ces résultats montrent qu'une certaine variance des traits fonctionnels est maintenue par la sélection naturelle entre les individus d'une même espèce, ce qui constitue un terrain d'investigation du rôle des traits fonctionnels dans la performance et l'adaptation des plantes.

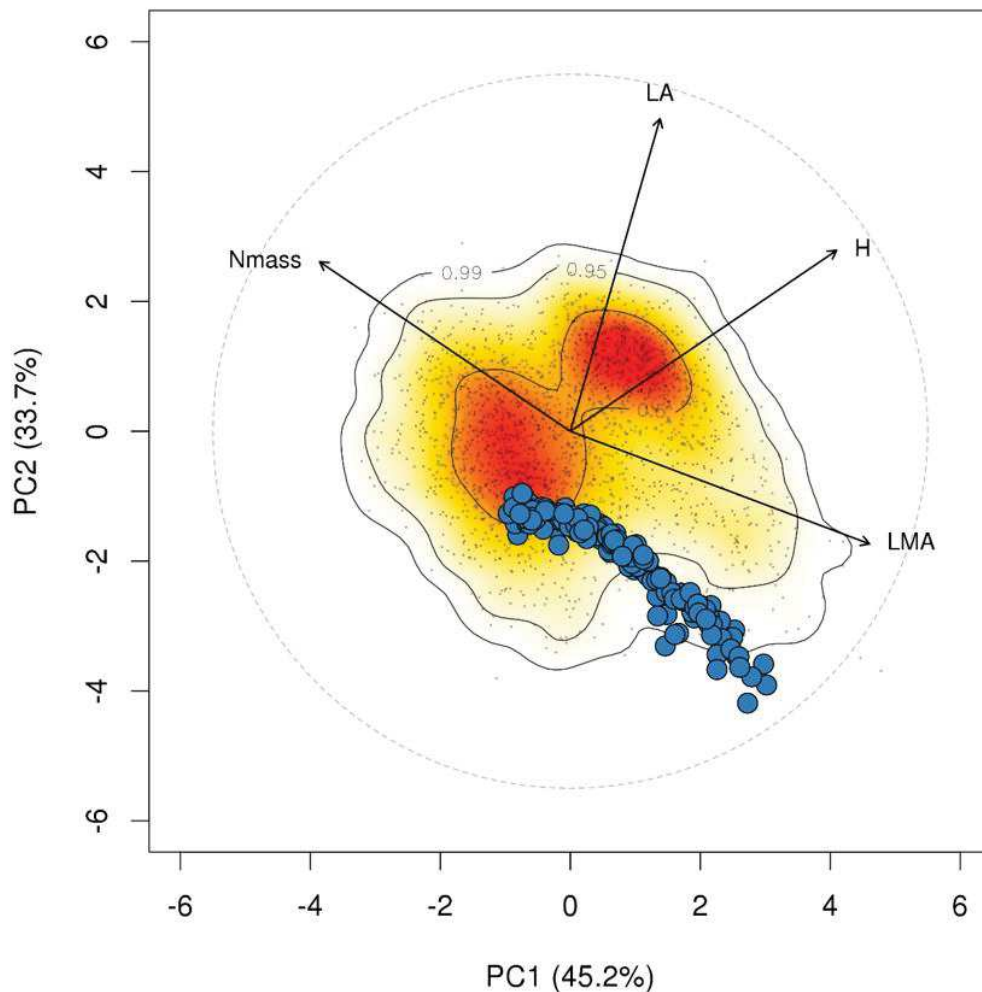


Figure 12 : Projection des valeurs de traits mesurées chez *Arabidopsis thaliana* dans le plan de l'analyse multivariée de spectre global des formes et fonction des plantes. Chaque point bleu représente un génotype de l'espèce *A. thaliana*. Chaque point gris représente une espèce de plante parmi les 2214 espèces ayant une mesure pour chacun des six traits suivants : la concentration en azote foliaire (nitrogen content per unit mass, Nmass), la surface d'une feuille (leaf area, LA), la hauteur de la plante (height, H), et l'inverse de la surface spécifique foliaire (leaf mass per area, LMA). Les axes PC1 et PC2 représentent les deux principales composantes d'une analyse multivariée réalisée sur la matrice traits-espèces. La combinaison des ellipses annotées 0.5 (0.95, 0.99) encerclent 50% (95%, 99%) des espèces du jeu de données. Les données proviennent de la base de données TRY et la figure est téléchargeable via <https://shiny.cefe.cnrs.fr/PhenoSpace/>.

Tableau 3. Héritabilité des principaux traits fonctionnels étudiés dans cette thèse.
L'héritabilité au sens strict représente la part de variance du phénotype attribuable à la variance génétique additive.

Trait	Héritabilité au sens strict
Vitesse de photosynthèse	0.70
Durée de vie foliaire	0.66
Surface spécifique foliaire	0.88
Âge à maturité	0.99
Vitesse de croissance relative	0.64
Densité de nervures foliaires	0.26
Surface foliaire	0.59
Efficacité de résorption	0.21
Vitesse de résorption	0.99

Coordination des traits foliaires et stratégies écologiques

Nous faisons l'hypothèse que le syndrome d'économie foliaire (WLES) doit s'observer à l'échelle intra-spécifique s'il est le résultat d'une contrainte physiologique ou évolutive (Donovan *et al.*, 2011). Nos résultats ont montré que chez *A. thaliana*, la vitesse maximale de photosynthèse et la durée de vie d'une feuille sont fortement négativement corrélés et la dispersion autour de la relation est faible (Sartori *et al.*, 2019). Comme attendu, la surface spécifique foliaire est un bon indicateur de la position des génotypes le long du WLES puisqu'elle est corrélée positivement au taux maximal de photosynthèse et négativement à la durée de vie. Néanmoins, les pentes des relations log-log deux à deux diffèrent des pentes observées dans des comparaisons interspécifiques (Reich *et al.*, 1999; Wright *et al.*, 2004). En dépit de la généralité apparente du WLES, les pentes des relations bivariées associées dépendent de l'échelle d'organisation considérée, que l'on compare des espèces à l'échelle globale (Wright *et al.*, 2004), les espèces d'un groupe taxonomique particulier (Anderegg *et al.*, 2018), les espèces d'un type biologique (Tucker *et al.* en révision, Annexe 1), ou les individus d'une espèce (cf. paragraphe suivant). Ce constat est probablement la conséquence de propriétés secondaires des plantes, spécifiques à certains niveaux d'organisation, qui interagissent avec les traits fondamentaux du compromis (Grubb, 2016).

Les explorations intraspécifiques des traits du WLES montrent que les combinaisons de valeurs de traits de l'ensemble des individus d'une espèce se situent dans les limites de l'« enveloppe phénotypique » définie par les comparaisons interspécifiques (ex. Niinemets,

2015; Martin *et al.*, 2017; Hayes *et al.*, 2019). Pour comprendre l'origine d'un compromis si généralisé, il faut se demander ce qui limite l'existence d'exceptions phénotypiques. Les possibilités de sortir de ce type d'enveloppe des contraintes dépendent du type de compromis considéré. Nous avons identifié quatre mécanismes pouvant être à l'origine d'un compromis entre deux traits : l'allocation de ressources limitées, la pléiotropie antagoniste, l'existence d'une contrainte physiologique et la canalisation évolutive des stratégies écologiques. Le compromis d'allocation des ressources (modèle en Y) définit une situation dans laquelle deux traits d'un organisme sont en compétition pour la même ressource (Roff & Fairbairn, 2007). Dans ce cas, il est possible d'observer des phénotypes qui maximisent simultanément les valeurs des deux traits en augmentant la réserve de ressources à allouer, soit en augmentant artificiellement les ressources du milieu, soit en améliorant la capacité des individus à les extraire. Cependant, jusqu'à présent, ni la culture des plantes en milieu maximisant les conditions de croissance (Roucou *et al.*, 2018; Sartori *et al.*, 2019), ni la sélection artificielle ou la culture de mutants (Vasseur *et al.*, 2012; Roucou *et al.*, 2018) n'ont produit de tels phénotypes. Un compromis génétique (pléiotropie antagoniste) peut exister lorsqu'un gène contrôle plus d'un trait à la fois, et que n'importe quelle version allélique de ce gène est à la fois bénéfique pour un trait et défavorable pour un autre. Chez *A. thaliana*, la mesure conjointe des traits du WLES chez des lignées recombinantes montre que la variabilité allélique de certaines portions du génome (QTL) est associée à la variabilité de la surface spécifique foliaire, la concentration en azote et la vitesse de photosynthèse (Vasseur *et al.*, 2012), suggérant l'existence de gènes pléiotropes. Cependant, la culture de génotypes mutés sur les gènes associés à ces traits n'a pas révélé l'existence d'exceptions phénotypiques (Vasseur *et al.*, 2012). Plus généralement, Donovan *et al.* (2011) ont montré que les contraintes génétiques ont un rôle moins important que les contraintes biophysiques et la sélection dans l'évolution du WLES.

La densité de nervures foliaire, une contrainte biophysique à l'origine du WLES ?

La densité de nervures du réseau vasculaire foliaire a été proposée comme une limite physiologique qui contraint à la fois la durée de vie et le taux maximal de photosynthèse des feuilles (Blonder *et al.*, 2011). La densité des nervures primaires (la ou les nervures principales qui émergent du pétiole des feuilles et les nervures qui leur sont directement connectées) est un déterminant majeur de la résistance mécanique des feuilles et, par extension, un déterminant de la durée de vie des feuilles (Hua *et al.*, 2020). De plus, la théorie métabolique de l'Écologie

postule que les taux métaboliques des organismes peuvent être prédits par l'architecture de leurs réseaux vasculaires terminaux (West, 1997). Il est notamment attendu chez les plantes que le taux maximal de photosynthèse augmente avec la densité totale du réseau de nervure foliaire (Blonder *et al.*, 2011). Le mécanisme en jeu est une réduction de la distance moyenne entre toute cellule photosynthétique et un vaisseau vecteur d'eau et de nutriments. Cependant, une telle ambivalence de l'architecture des nervures chez *A. thaliana* n'est pas supportée par nos données. Dans l'expérience décrite dans le chapitre 2, j'ai mesuré la résistance des feuilles à la coupe des 169 génotypes étudiés (Encadré 1). Une analyse préliminaire de ces données montre que la densité de nervures est significativement positivement corrélée à la résistance des feuilles à la coupure ($r = 0.22$, $P < 0.01$, Fig. 13b) (Encadré 1). Cependant, la relation entre la densité de nervures et le taux maximal de photosynthèse est inverse à l'attendu et marginalement significative ($r = -0.21$, $P = 0.04$, Fig. 13a). Des caractères propres à l'espèce peuvent être à l'origine de cette divergence entre les patrons de corrélations attendus et observés. L'architecture des nervures présente des caractères hautement spécifiques à certains genres et familles, et peut évoluer relativement rapidement entre des familles proches (Schneider *et al.*, 2018). Des comparaisons interspécifiques ont montré que la différenciation du réseau de nervures en ordres hiérarchisés permet un découplage entre la surface de la feuille et la densité de nervure (Sack *et al.*, 2012). Dans notre jeu de données, la forte relation allométrique qui existe entre la taille des feuilles et la densité de nervures (Chapitre 2) suggère qu'une distinction entre nervures primaires et terminales est peu marquée (Schneider *et al.*, 2017). Ce résultat pourrait expliquer l'absence d'un double rôle de l'architecture des nervures chez *A. thaliana*. Au moment de la rédaction de ce manuscrit, nos images de réseaux de nervations sont en cours d'analyse par l'équipe de recherche de L. Sack (University of California, Los Angeles, CA, USA) afin de mesurer les diamètres de nervures et tester cette hypothèse. L'existence du WLES chez *A. thaliana* - en dépit de ses relations attendues avec la densité des nervures - suggère que la contrainte physiologique que nous cherchons n'est pas imposée par l'architecture du réseau vasculaire des feuilles. Les divergences entre les patrons interspécifiques et intra-spécifiques sont relativement communs en écologie fonctionnelle (Price *et al.*, 2014b; Messier *et al.*, 2017; Anderegg *et al.*, 2018; Osnas *et al.*, 2018). Ils traduisent l'existence de contraintes écophysiologiques et évolutives différentes en fonction de l'échelle d'étude et/ou du niveau d'organisation.

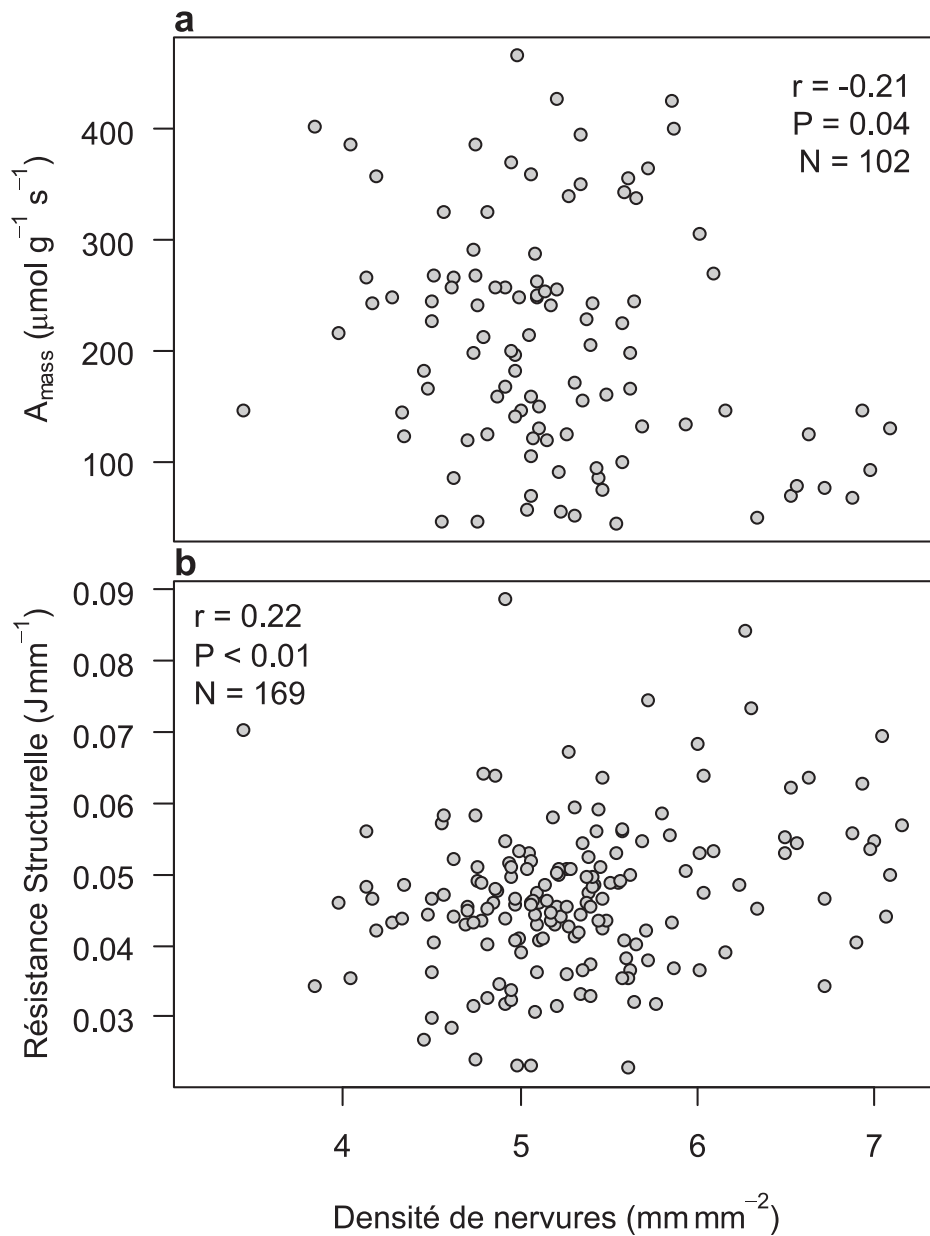


Figure 13 : Relation entre le taux maximal de photosynthèse (A_{mass}) et la densité du réseau de nervures d'une feuille (a), et relation entre la résistance structurelle et la densité du réseau de nervures d'une feuille (b). Chaque point représente un génotype.

Encadré I : La résistance mécanique foliaire

Le protocole de mesure de Ang *et al.* (2008) a été adapté à la coupe de feuilles d'*A. thaliana* en collaboration avec Nick Rowe (UMR AMAP, Montpellier).

La feuille est maintenue à plat, fixée par du ruban adhésif à deux supports espacés de 2mm (Figure 14 a et b). La fente créée entre les deux supports doit se situer au niveau de la partie du limbe la plus large. Au travers de cette fente, une lame de rasoir inclinée à 20°, coupe la feuille perpendiculairement à sa nervure principale, sur le principe d'une guillotine. La lame est fixée à un capteur de force et un capteur de déplacement qui enregistrent ces paramètres en continu (la vitesse est définie au préalable : 10mm/min). Le dispositif est relié à un ordinateur qui produit un graphique représentant la force mesurée en fonction du déplacement de la lame sur la largeur totale de feuille (Figure 14 c). Un pic est souvent observable à mi-parcours de la lame, il correspond à la nervure centrale de la feuille.

L'énergie (en Joule, J) totale nécessaire à couper la feuille est obtenue par le calcul de l'intégrale de cette courbe, soit l'énergie totale divisée par la largeur de coupe ($J.m^{-1}$) (Figure 14 d). Le début des courbes a systématiquement la même allure avec une augmentation de l'énergie de type exponentielle qui correspond à la déformation du limbe foliaire sous la pression de la lame. La première chute d'énergie correspond à la rupture du limbe foliaire sous la lame, la dernière à la fin de la coupe. Les bornes de l'intégrale sont définies par l'utilisateur en sélectionnant le premier pic et la fin de coupe.

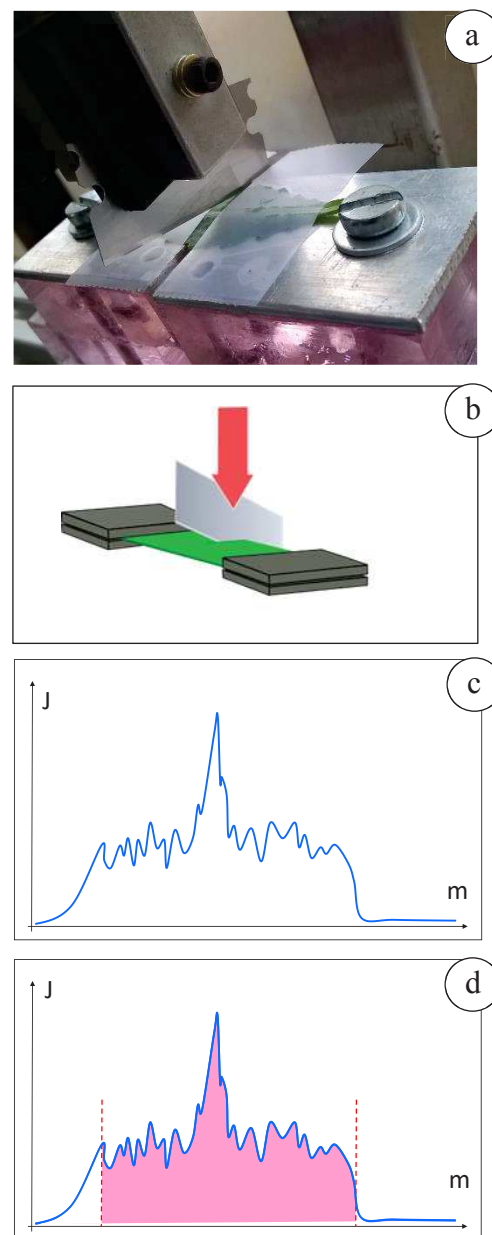


Figure 14 : Photo du dispositif de coupe utilisé pour cette expérimentation (a), schéma du dispositif de coupe (b), exemple de courbe produite par l'instrument de mesure représentant la force en Joules en fonction du déplacement en mètre (c) et délimitation de l'aire sous la courbe à mesurer (d).

Le WLES, un syndrome général d'utilisation des ressources avant et pendant la sénescence foliaire ?

Le WLES a été interprété comme un syndrome de traits plus général, qui définit des stratégies écologiques allant de feuilles dites conservatrices à des feuilles dites acquisitives des ressources. Une plante possédant des attributs foliaires qui confèrent une capacité d'acquisition rapide du carbone doivent avoir des capacités d'acquisition rapides pour d'autres ressources essentielles, telles que l'eau et les nutriments (Reich, 2014). En effet, une vitesse élevée de photosynthèse nécessite une conductance élevée pour l'eau et une concentration élevée en Rubisco. A l'opposée du syndrome, une durée de vie longue doit être associée à une stratégie de rétention des nutriments. Dans ce contexte, nous nous attendions à observer une corrélation positive entre les traits foliaires associés à la conservation du carbone (longue durée de vie et faible de taux photosynthétique) et les traits liés à la rétention des nutriments tels que l'efficacité de résorption de l'azote. Cependant, les comparaisons interspécifiques qui ont testé cette hypothèse (Kazakou *et al.*, 2007; Freschet *et al.*, 2010), ainsi que nos données (chapitre 3) ne révèlent pas de signal clair. Nos résultats n'ont pas mis en évidence une coordination des stratégies écologiques liées à l'acquisition et la conservation du carbone et de l'azote. Nous avons identifié trois éléments qui permettent de comprendre l'origine éventuelle de ce découplage. Premièrement, les traits liés à la composition chimique des feuilles présentent une grande sensibilité aux conditions de croissance, ce qui peut réduire la force de la corrélation avec les traits du WLES dans les comparaisons intra-spécifiques (Kazakou *et al.*, 2014; Siefert *et al.*, 2015). Dans notre jeu de données, la corrélation entre la concentration en azote et la surface spécifique foliaire n'était pas significative dans un milieu de culture relativement pauvre en azote (chapitre 3). La variation de la concentration en azote est orthogonale à la variation de la vitesse de photosynthèse et de la surface spécifique foliaire chez le blé en condition de haute fertilisation (Roucou *et al.*, 2018). Deuxièmement, la présence d'épines ou de trichomes, la présence de fibres, de cellules de stockage et l'augmentation de l'épaisseur de la feuille entraînent une diminution de la surface spécifique foliaire pour une concentration d'azote donnée (Grubb, 2016). Ces attributs sont également susceptibles d'augmenter la durée de vie des feuilles en réduisant leur appétence pour les herbivores. Ils sont également responsables d'une réduction de la vitesse de photosynthèse par une augmentation de la proportion de carbone investie dans la structure de la feuille (Grubb, 2016). Enfin, la discussion du chapitre 3 rappelle qu'une proportion non négligeable de l'azote foliaire n'est pas impliquée dans la machinerie photosynthétique. Elle peut représenter une réserve temporaire d'azote qui réduit la

corrélation entre concentration en azote et capacité photosynthétique. Le WLES décrit donc principalement un compromis d'économie du carbone impliquant une relation négative entre la vitesse de photosynthèse et la durée de vie foliaire, tel qu'il avait été formulé par Bloom, Chapin et Money (1985) et révélé par l'écologie comparative.

Le maintien de la variabilité au sein de l'enveloppe phénotypique du WLES

Même si cette thèse n'est pas en mesure d'apporter une réponse claire, l'hypothèse généralement admise de l'origine du WLES est celle d'une contrainte physiologique, et la position des espèces ou des génotypes le long de ce syndrome est imposée par la sélection naturelle (Wright *et al.*, 2004; Shipley *et al.*, 2006; Blonder *et al.*, 2011; Donovan *et al.*, 2011; Reich, 2014; Onoda *et al.*, 2017). La variabilité le long de l'axe du WLES est supposée être maintenue dans la nature car toutes les combinaisons de traits observées sont équivalentes en termes de productivité et favorisées dans des combinaisons particulières de facteurs environnementaux. Le produit du taux d'assimilation de carbone par la durée de vie d'une feuille donne une approximation de la quantité de carbone assimilé au cours de la vie de cette feuille (le revenu total de carbone, *lifetime carbon gain*). Pour que les stratégies rapides et lentes du WLES soient maintenues, elles doivent fournir un revenu de carbone équivalent. Dans le cas contraire, la sélection naturelle favoriserait la stratégie la plus rentable. Pour tester cette hypothèse, Westoby *et al.* (2000) ont étudié les relations interspécifiques entre la surface spécifique foliaire et la durée de vie des feuilles. Les auteurs montrent que la stratégie lente, c'est-à-dire une longue durée de vie pour une faible capacité d'interception de la lumière, permet en théorie un meilleur revenu de carbone. Les auteurs proposent que les capacités d'assimilation de carbone se détériorent avec l'âge des feuilles, ce qui équilibre le revenu des stratégies lentes et rapides. Nous avons estimé le revenu de carbone d'une feuille par le produit de la vitesse de photosynthèse et de la durée de vie dans notre jeu de données. L'analyse montre que le revenu de carbone est négativement corrélé à la durée de vie chez *A. thaliana* (Fig. 15). Contrairement aux études interspécifiques, une stratégie caractérisée par une durée de vie courte et des vitesses de photosynthèse élevées permettrait un meilleur revenu de carbone chez *A. thaliana*. Dans ce contexte, nous pouvons nous demander comment les stratégies lentes du WLES sont maintenues. Kikuzawa et Lechowicz (2006) rappellent que la vitesse d'assimilation de carbone n'est jamais maintenue à son maximum dans la nature et en conditions expérimentales. En prenant en compte des données environnementales telles que la longueur de la période favorable à la croissance, les auteurs montrent que le revenu net de carbone a une

valeur identique de 4 g g^{-1} pour toutes les espèces étudiées. Ce résultat signifie que quelle que soit la stratégie économique déployée par une feuille, pour un gramme de carbone investi dans sa construction, elle assimilera quatre grammes de carbone au cours de sa vie. Notons qu'en prenant en compte l'alternance de 12 h jour/ 12 h nuit dans notre expérience, le revenu moyen d'une feuille d'*A. thaliana* est proche mais en moyenne significativement inférieur à l'attendu théorique ($\mu = 2.88 \pm 1.25$, $P_{\text{T test}, \mu = 4} < 0.01$, Fig. 15). De futures expérimentations de terrain devraient approfondir ce type d'analyse chez *A. thaliana*, et d'une manière générale dans des études intra-spécifiques pour mieux comprendre le rôle du WLES dans l'adaptation des plantes à l'environnement. En résumé, la contrainte à l'origine du WLES limite le revenu de carbone de toute une vie de feuille, quels que soient les attributs des feuilles. La variabilité rencontrée le long de ce syndrome reflète les différents moyens d'atteindre un revenu de carbone équivalent dans des contextes environnementaux variés (Kikuzawa & Lechowicz, 2006).

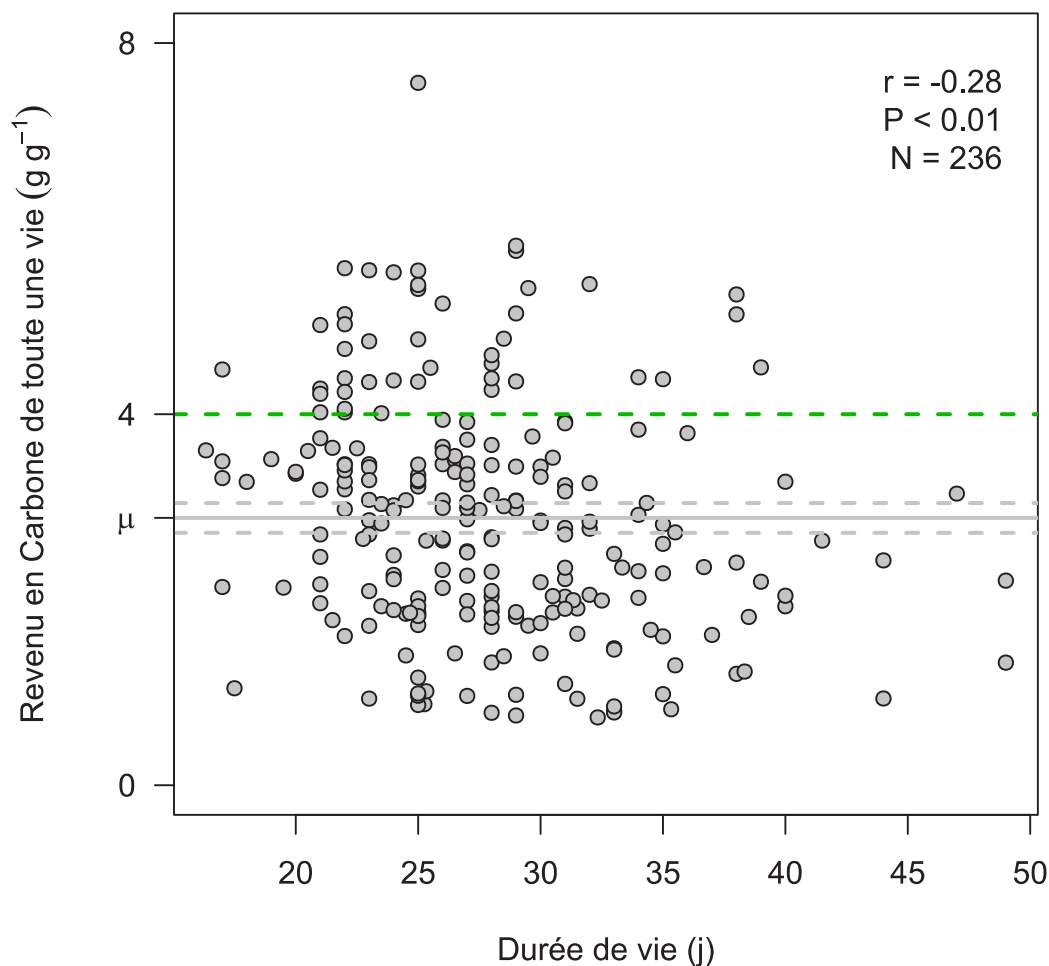


Figure 15 : Revenu en carbone de toute une vie de feuille en fonction de la durée de vie de la feuille. Le revenu moyen en carbone d'une feuille d'*Arabidopsis thaliana* (μ , droite grise continue, plus ou moins l'erreur standard en pointillés gris) est proche mais significativement inférieur au revenu théorique moyen (4 g g^{-1} , droite en pointillés verte).

La coordination des syndromes foliaires avec les syndromes de la plante entière

Le rôle du syndrome d'économie foliaire dans la performance des plantes a été suggéré dès les premières démonstrations du compromis (Reich *et al.*, 1999; Wright *et al.*, 2004). Il est attendu que les attributs qui confèrent à la feuille une capacité d'acquisition rapide du carbone influencent les traits d'histoire de vie, la croissance et la survie à l'échelle de la plante entière (Reich, 2014). Le résultat de cette coordination feuille-plante est un syndrome d'économie général à l'échelle de la plante. Il simplifie et synthétise les autres schémas généraux de fonctionnement des plantes en écologie comparative. L'axe de variation allant de stratégies d'acquisition (productivité) à des stratégies de conservation (persistance) du WLES résume l'axe Compétition-Stress tolérance du triangle C-S-R de Grime (1974), et l'axe « feuille » du schéma LHS de Westoby (1998) et du schéma général des formes et fonctions de plantes de Díaz *et al.* (2016). La mesure conjointe des traits associés au compromis - durée de vie / taux métabolique - à l'échelle de la feuille (le WLES) et à l'échelle de la plante (le syndrome du rythme de vie) révèle qu'ils sont coordonnés chez *A. thaliana* (Vasseur *et al.*, 2012; Sartori *et al.*, 2019). Les stratégies rapides à l'échelle de la feuille (vitesse maximale de photosynthèse élevé et durée de vie courte) sont associées à des stratégies rapides à l'échelle de la plante (vitesse maximale de photosynthèse élevé, taux de croissance élevé, maturité sexuelle précoce), et inversement (chapitre 1). D'un point de vue physiologique et évolutif, une absence de corrélation voire une corrélation négative sont difficilement envisageables. Produire des feuilles à faible taux de photosynthèse n'est pas avantageux pour une plante à durée de vie courte. De même, un remplacement très rapide de feuilles à durée de vie courte n'est pas soutenable pour une plante ayant une longue durée de vie. Néanmoins, nous observons un certain découplage entre les compromis mesurés à l'échelle de la feuille et à l'échelle de la plante entière. Nous avons estimé la position des génotypes le long de l'axe du WLES et le long de l'axe du compromis croissance-âge à maturité. La dispersion autour de la relation entre ces deux axes est relativement grande ($r = 0.65$, $P < 0.001$, chapitre 1). De plus, la capacité photosynthétique maximale des feuilles augmente plus vite que le taux de croissance des plantes. En d'autres mots, pour une valeur de taux de croissance donnée, les génotypes expriment une gamme étendue de vitesses de photosynthèse. Ce résultat suggère que les variations des traits à l'échelle de la feuille et de la plante sont soumises à des déterminants différents.

La vitesse de photosynthèse foliaire et la croissance de la plante peuvent être découplées si les déterminants écologiques et évolutifs de ces traits diffèrent. Marks (2007) a modélisé la croissance et la survie des jeunes arbres dans différents environnements pour évaluer les

déterminants écologiques et évolutifs des traits associés. L'étude montre que la variation des traits à l'échelle de la plante est plutôt soumise aux pressions de sélection environnementale, tandis que la variation des traits à l'échelle des organes est plus contrainte par la phylogénie. Le découplage entre le taux de photosynthèse foliaire et la croissance de la plante est également attendu dans une certaine mesure car les stratégies d'utilisation des ressources à l'échelle de la feuille ne constituent pas les seuls déterminants de la croissance. Poorter *et al.* (2014) décomposent la croissance relative (quantité de carbone assimilé par la plante par unité de temps et standardisé par la quantité de carbone de la plante) en trois éléments : l'assimilation journalière de carbone par unité de masse d'une feuille, la part de masse sèche foliaire de la masse sèche totale de la plante et l'efficacité d'utilisation du carbone. Les deux derniers éléments permettent de prendre en compte la part de carbone assimilé qui n'est pas directement allouée à la croissance. Par exemple, la maintenance des tissus vivants consomme du carbone par photo-respiration et les racines consomment du carbone en produisant les exsudats nécessaires à l'absorption des nutriments. Les stratégies d'acquisition et de conservation des nutriments par les racines elles-mêmes peuvent être découplées des stratégies des feuilles (Isaac *et al.*, 2017). Par conséquent, plusieurs stratégies d'acquisition du carbone à l'échelle foliaire peuvent conduire à une même stratégie de croissance, moyennant les ajustements liés aux capacités des autres organes et des fonctions qui ont un rôle dans la croissance. Comme l'ont montré plusieurs études en biologie évolutive, il peut y avoir plusieurs solutions fonctionnelles pour atteindre la même stratégie évolutive (Bock, 1959; Lewontin, 1978; Korner, 1991; Marks & Lechowicz, 2006).

Rôle des traits fonctionnels dans la performance des plantes

Outre leur fonction pour la croissance, les traits du WLES peuvent avoir un rôle dans la valeur sélective des plantes au travers de deux autres traits de performance, la fécondité et la survie (Violle *et al.*, 2007). La fécondité est fonction de la masse d'une graine, du nombre de graine et du taux de germination des graines. Les comparaisons interspécifiques font état d'un compromis entre l'investissement par la plante dans la survie individuelle d'une graine (masse d'une graine élevée) et la probabilité de survie de la descendance (nombre de graines élevées) (Westoby, 1998). Des études montrent également l'existence d'un compromis entre le rendement et la concentration en protéines et en azote des graines chez des espèces cultivées et chez *A. thaliana* (Beninati & Busch, 1992; Masclaux-Daubresse & Chardon, 2011). Ainsi, l'investissement plus important en ressources et en nutriments dans de grosses graines

améliorerait la survie pendant les premiers stades de développement des jeunes plantes en milieu pauvre en ressources (Westoby, 1998). Dans notre jeu de données, la comparaison des stratégies de reproduction chez les génotypes d'*A. thaliana* suggère que la masse et le nombre de graines produites sont soumis à ce compromis ($r = -0.42$, $P < 0.01$, Fig. 16a). La surface spécifique foliaire, indicatrice de la position des génotypes le long du WLES, est négativement corrélée à la masse d'une graine ($r = -0.21$, $P < 0.01$). Cependant, la croissance relative n'est pas significativement corrélée à la masse d'une graine ($P = 0.5$). Il existe donc un lien entre les stratégies du WLES et la performance des plantes, indépendamment de la croissance chez *A. thaliana*. Les plantes qui présentent des feuilles longévives et caractérisées par un taux de photosynthèse bas, produisent en moyenne des graines plus grosses et moins nombreuses. La vitesse de résorption de l'azote foliaire est également positivement corrélée à la masse d'une graine ($r = 0.33$, $P = 0.02$, Fig. 16b). Les mesures de résorption ayant été effectuées après l'apparition du bourgeon floral, la demande en azote provenant des organes de reproduction pourrait être un facteur déterminant de la vitesse de résorption de l'azote foliaire. De la même manière, Masclaux-Daubresse & Chardon (2011) ont montré que la concentration en azote des graines est fonction de l'efficacité de résorption de l'azote foliaire chez *A. thaliana*. En résumé, les génotypes caractérisés par des feuilles ayant une capacité élevée de fixation de carbone et une vitesse lente de résorption de l'azote produisent de nombreuses petites graines, pour lesquelles on peut faire l'hypothèse d'une faible concentration en azote et en protéines. À l'inverse, les génotypes caractérisés par des feuilles capables d'une résorption rapide de l'azote et une faible capacité de fixation du carbone produisent peu de graines potentiellement riches en azote et en protéines.

La survie peut être estimée par une variable binaire : un individu, un génotype ou une espèce est vivante ou morte dans un environnement donné (Violle *et al.*, 2007). Ce qui peut se traduire par une probabilité de survie (continue) à l'échelle de la population. J'ai participé à l'encadrement de Aurélien Estarague, étudiant en Master BEE à Montpellier, dont l'objectif était de caractériser la tolérance à l'aridité de trente génotypes d'*A. thaliana* issus d'un gradient latitudinal allant de la côte scandinave à la côte méditerranéenne. Plusieurs répétitions par génotype ont été cultivées dans cinq conditions : une condition contrôle irriguée (15°C jour/nuit), un environnement froid irrigué (15°C jour/-6°C nuit), un environnement chaud irrigué (35°C jour/15°C nuit), un environnement froid non-irrigué (15°C jour/-6°C nuit), et un environnement chaud non-irrigué (35°C jour/15°C nuit). Dans une analyse préliminaire, nous avons calculé la moyenne génotypique de survie tout environnements confondus. L'analyse

montre que la survie des plantes est positivement corrélée à la densité de nervures foliaires ($r = 0.65$, $P < 0.01$), à la vitesse de résorption ($r = 0.77$, $P < 0.001$) et à l'âge à maturité ($r = 0.7$, $P < 0.001$), et négativement corrélée à la surface spécifique foliaire ($r = -0.63$, $P < 0.001$) (Fig. 17). Reich (2014) montre que la stratégie de croissance lente, associée plus haut à une maturité tardive, une faible surface spécifique foliaire, et vitesse de résorption élevée, est associée d'une manière générale à une meilleure tolérance aux milieux pauvres en ressources. L'auteur souligne en particulier le rôle de l'ensemble des propriétés hydrauliques des feuilles (et des autres organes) dans la tolérance au manque d'eau. Comme le suggèrent nos résultats, la densité du réseau de nervures joue un rôle dans la tolérance au stress hydrique qui peut survenir dans des environnements peu irrigués, mais aussi sous des températures extrêmes réduisant la disponibilité de l'eau. La diminution de la disponibilité en eau peut provoquer une rupture de la continuité hydraulique du système circulatoire des plantes par des phénomènes d'embolie des vaisseaux (apparition de bulles d'air). Dans les feuilles, un réseau dense de nervures interconnectées fournit des routes alternatives au transport de l'eau en cas d'obstruction de vaisseaux par des bulles d'air (Brodribb *et al.*, 2016). Ces résultats suggèrent que certains attributs de traits, et des combinaisons d'attributs de traits puissent être sélectionnés dans des environnements naturels particuliers.

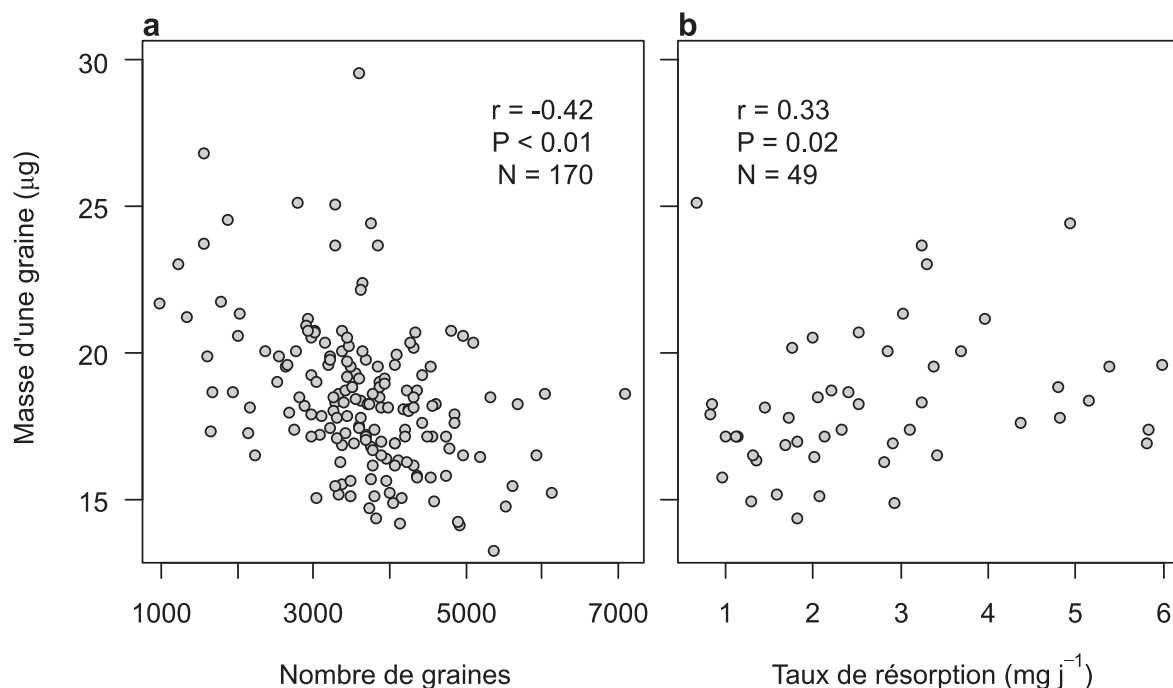


Figure 16 : Relation entre la masse d'une graine et le nombre de graines produites par un individu (a). Relation entre la masse d'une graine produite par un individu et la vitesse de résorption de l'azote foliaire (b). Chaque point gris représente un génotype d'*Arabidopsis thaliana*.

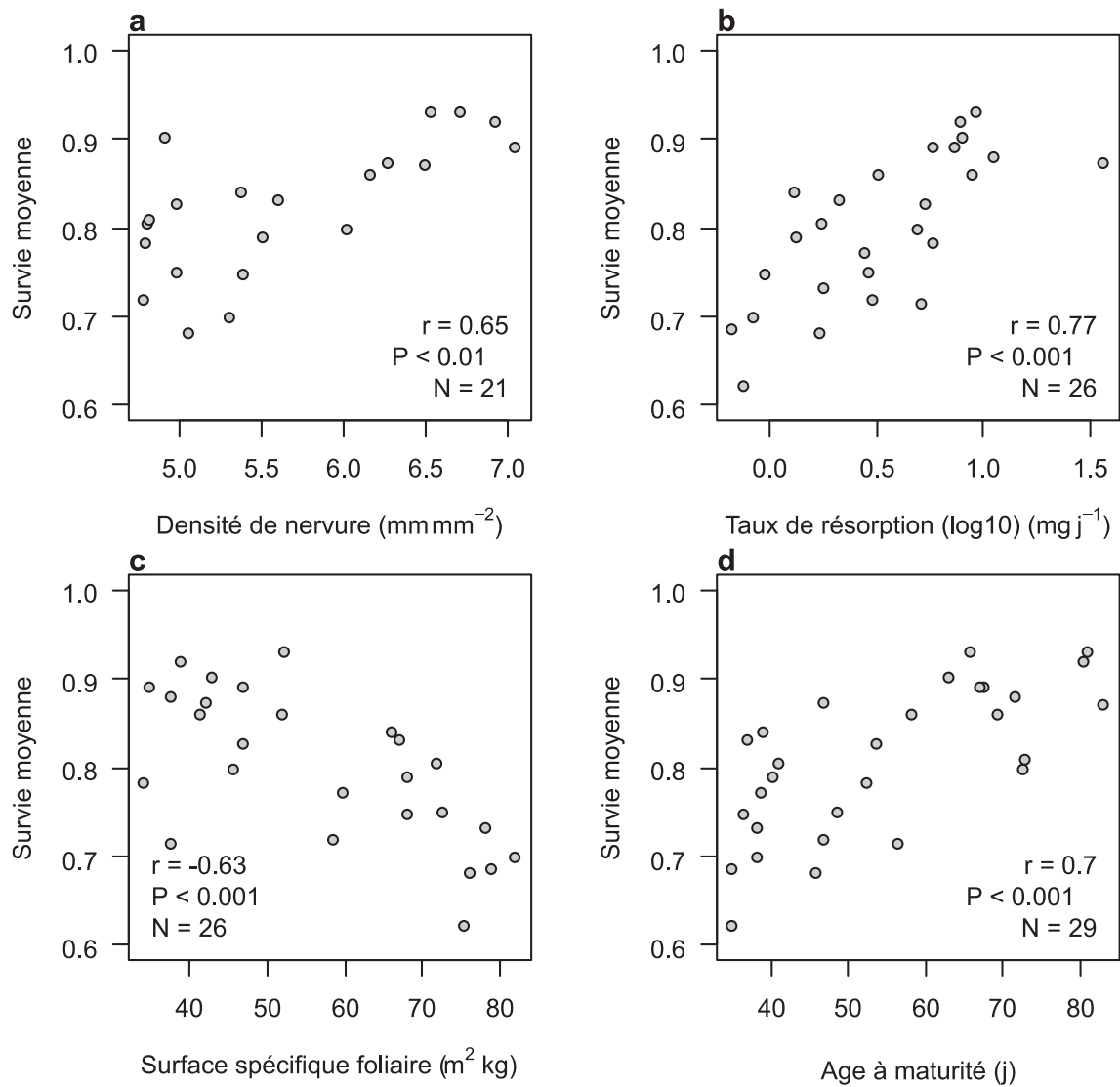


Figure 17 : Relation entre la survie moyenne de génotypes d'*A. thaliana* cultivés dans différentes conditions de stress environnementaux et la densité de nervure (a), le taux de résorption (b), la surface spécifique foliaire (c) et l'âge à maturité (d). Chaque point gris représente un génotype d'*Arabidopsis thaliana*.

Rôle des traits fonctionnels dans l'adaptation des plantes à l'environnement

Les relations trait-environnement sont utilisées pour interpréter la signification évolutive de la variabilité des traits. Dans des comparaisons interspécifiques, elles ont permis notamment d'observer des tendances générales et d'émettre certaines hypothèses quant aux mécanismes évolutifs impliqués. Par exemple, la température moyenne annuelle et les précipitations moyennes annuelles sont corrélées positivement à la taille des feuilles chez plus de 7500 espèces échantillonnées tout autour du globe (Wright *et al.*, 2017). Les auteurs expliquent qu'une grande surface d'échanges permet des variations de température plus rapides, ce qui a des conséquences différentes en fonction des conditions du milieu. Une grande surface est avantageuse dans des milieux favorables (chauds et humides) en permettant à la feuille d'atteindre rapidement des températures élevées favorables à l'activité enzymatique photosynthétique tout en évitant la « surchauffe » grâce à la transpiration. Une petite surface est avantageuse dans des milieux défavorables froids et/ou arides, respectivement en diminuant les pertes de chaleur et la dessiccation par transpiration. Cependant, une étude à cette échelle peut souffrir de biais phylogénétiques si les communautés de plantes vivant dans les tropiques, les zones arides et les zones tempérées ont divergé depuis longtemps. L'étude de la différenciation phénotypique de populations d'une seule espèce le long de gradients environnementaux permet d'apporter des preuves de phénomènes d'adaptation locale sous-jacents à ces schémas de relations trait-environnement interspécifiques (Weber & Agrawal, 2012). Chez *A. thaliana*, nos données montrent que le gradient latitudinal allant du pourtour méditerranéen à la Scandinavie est le principal axe de différenciation phénotypique des populations. L'espèce se rencontre essentiellement dans des milieux tempérés humides et variant surtout sur un axe Sud-Nord de températures croissantes (chapitre 1). La relation positive entre la taille des feuilles et la température dans notre jeu de données confirme les résultats observés à l'échelle interspécifique (Chapitre 2, Fig. 3). Même si les corrélations sont en général faibles, nous avons montré au fil des chapitres que les populations vivant dans les milieux froids du nord de l'Europe se distinguent des populations d'Europe centrale par une vitesse de croissance plus lente, une densité de nervures foliaires plus élevées et des vitesses de résorption de l'azote plus élevées (Fig. 18). Cependant, nos données ne révèlent pas de lien entre les variables climatiques et les traits du WLES. Ce résultat est en accord avec l'exploration des déterminants environnementaux du WLES à l'échelle interspécifique (Wright *et al.*, 2004). Les auteurs expliquent que les traits mesurés varient davantage entre les espèces d'une même communauté qu'entre les communautés, suggérant une réponse à des variations plus locales du climat.

Les variables climatiques qui décrivent la température et l'humidité des sites d'études utilisées à la fois dans les comparaisons interspécifiques à grandes échelles et dans nos travaux sont le résultat de modélisations climatiques à grandes échelles (Karger *et al.*, 2017), ce qui limite la caractérisation fine des milieux étudiés. Une caractérisation plus précise à une échelle locale des paramètres du milieu pourrait permettre de mieux évaluer le caractère adaptatif du WLES chez *A. thaliana*. A l'échelle de la région Midi-Pyrénées, Frachon *et al.* (2018) ont observé une forte variabilité génétique associée à une grande variabilité climatique entre des populations locales d'*A. thaliana*. Ce type d'étude couplée à une caractérisation phénotypique des populations permettrait de tester plus précisément le rôle des traits du WLES dans l'adaptation locale. Une expérience de transplantation réciproque constitue également une piste à privilégier pour tester l'adaptation locale (Kawecki & Ebert, 2004). Ce type d'expérience consiste d'abord à sélectionner plusieurs populations qui se développent dans des environnements différents ou le long d'un gradient environnemental. La performance de l'ensemble des populations est mesurée dans l'ensemble des environnements d'origine. L'observation d'une meilleure performance de chaque population dans leur environnement d'origine constitue une preuve d'adaptation locale. Il est également possible de réaliser cette expérience en conditions expérimentales pour tester l'effet d'une variable environnementale particulière. Nous avons par exemple cultivé des populations d'*A. thaliana* issues du pourtour méditerranéen, d'Europe centrale et d'Europe du Nord en croisant deux niveaux de température et deux niveaux d'irrigation en conditions expérimentales (données non publiées). L'analyse préliminaire, présentée dans la section précédente, montre que la faible surface spécifique foliaire des populations nordiques est un attribut qui explique la meilleure performance de ces populations en conditions de sécheresse et de froid prolongés (Fig. 18). Enfin, les caractéristiques du milieu à une résolution encore plus fine, le microclimat, pourrait être responsable de l'hétérogénéité des stratégies d'utilisation des ressources des espèces d'une même communauté (Stark *et al.*, 2017). Par exemple, la disponibilité de la lumière est un facteur essentiel à prendre en compte dans l'exploration des déterminants de stratégies d'économie du carbone. Les espèces végétales vivant en sous-bois sont caractérisées par des surfaces spécifiques foliaires plus élevées que les arbres qui leur font de l'ombre (Poorter *et al.*, 2009). De la même façon, au cours d'une succession post-culturale, les espèces herbacées à surfaces spécifiques foliaires élevées se font remplacer au cours du temps par des espèces à surfaces spécifiques foliaires plus basses (Kazakou *et al.*, 2006; Vile *et al.*, 2006). Ainsi, la variabilité des traits WLES n'est pas sous l'effet de la sélection par les gradients climatiques à grande échelle mais pourrait répondre à des facteurs de sélection à une échelle plus locale, liés en particulier à l'interaction entre les espèces telle que la compétition.

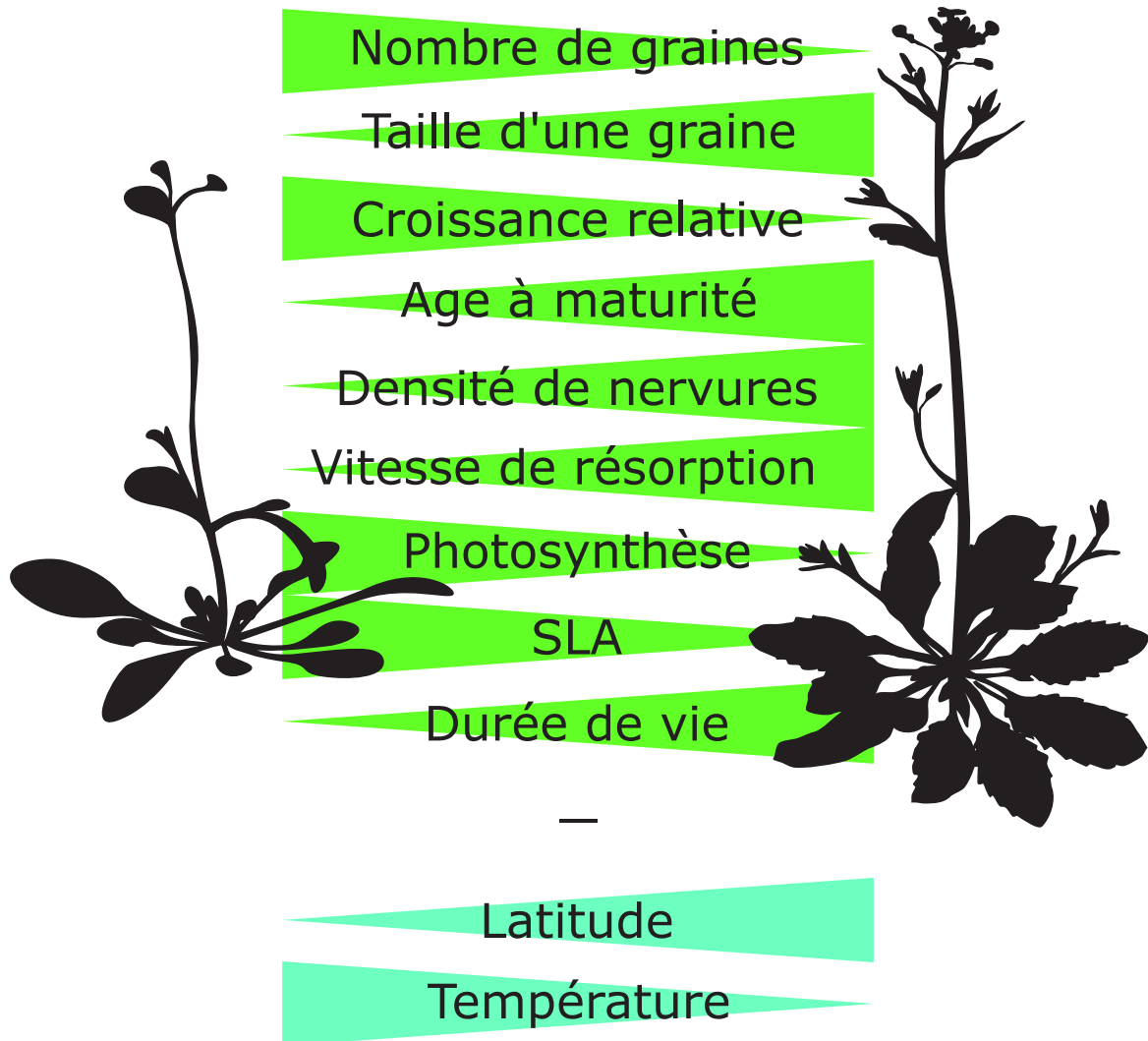


Figure 18 : Résumé simplifié des attributs de traits associés aux génotypes qui présentent une stratégie rudérale (à gauche) et aux génotypes qui présentent une stratégie stress tolérante (à droite). L'extrémité large (versus la pointe) des triangles représente des valeurs élevées (versus basses) de traits fonctionnels en vert et de variables environnementales en bleu.

Marqueurs génétiques de la valeur sélective des traits fonctionnels

L'utilisation du modèle *A. thaliana* permet d'aller plus loin dans l'exploration de la dimension adaptative des traits fonctionnels grâce aux données génétiques disponibles. Les déterminants génétiques et les processus moléculaires impliqués dans la photosynthèse et l'âge à maturité sont relativement bien connus car ils sont étudiés depuis longtemps chez *A. thaliana* (Krämer, 2015). Vasseur *et al.* (2012) ont montré que certaines portions du génome sont associées à la fois à la vitesse de photosynthèse et à l'âge à maturité chez cette espèce. Les gènes impliqués dans la formation du réseau de nervures sont également relativement bien étudiés chez *A. thaliana* (ex. Ceserani *et al.*, 2009). En effet, le réseau de nervures constitue un objet d'étude intéressant pour la compréhension du développement de structures complexes (Alonso-Peral, 2006). Les déterminants de la capacité de résorption sont également étudiés chez *A. thaliana* dans la perspective de mieux comprendre les déterminants de la production agricole (Havé *et al.*, 2017). Aujourd'hui, les données génétiques disponibles pour plus de 1300 génotypes originaires d'une grande partie de la distribution de l'espèce (Alonso-Blanco *et al.*, 2016) permettent de développer de nouvelles questions de recherche. Ces données permettent notamment de rechercher les gènes impliqués dans la variabilité naturelle des traits fonctionnels et d'explorer leurs rôles dans l'adaptation des plantes à des environnements variés. Par une méthode de génétique d'association, nous avons notamment identifié des gènes associés à la variabilité naturelle de la densité de nervures et des processus de sénescence foliaire (chapitres 2 et 3). Certains de ces gènes avaient déjà été identifiés par le passé par des méthodes de désactivation de gènes (ex. WRKY, chapitre 3) et des méthodes de mesure d'activité protéinique (ex. VH1/BRL2, chapitre 2). Il s'agit d'une rare convergence entre des études purement génétiques et une caractérisation de la variabilité naturelle génétique et phénotypique. De plus, des mesures de différenciation génétique et phénotypique des populations ont révélé que les versions alléliques de ces gènes, qui confèrent une forte densité de nervure et une vitesse élevée de résorption de l'azote, ont été sélectionnées dans les populations du nord de la distribution d'*A. thaliana*. En revanche, nous n'avons pas observé de différenciation significative des populations à grande échelle pour les traits du WLES. Ce résultat est d'autant plus surprenant que nous avons établi des liens entre la SLA et les trois composantes de la valeur sélective des plantes : la croissance, la survie et la reproduction. La caractérisation génétique et phénotypique de populations à une échelle plus locale (ex. Frachon *et al.*, 2018) est une piste prometteuse pour expliquer l'importante diversité des stratégies du WLES rencontrées à cette échelle.

*Implication de nos résultats pour l'écologie comparative en général et l'écologie d'*Arabidopsis thaliana* en particulier*

Les traits fonctionnels les plus variables de nos jeux de données (vitesse de photosynthèse, SLA) n'expliquent pas les patrons de différenciation à l'échelle biogéographique. Ce constat remet en question le choix de la méthodologie en écologie comparative. En effet, la recherche des patrons généraux de fonctionnement des plantes dans les grandes méta-analyses s'appuie en général sur des méthodes d'analyses multivariées (Díaz *et al.*, 2016). Ces méthodes recherchent les dimensions de plus grande variabilité dans l'enveloppe phénotypique observée. Elles sont également limitées par le choix des traits utilisés dans la construction de cette enveloppe phénotypique. En effet, il s'agit en général des traits fonctionnels qui présentent le plus grand nombre d'occurrences de mesure dans les bases de données. Ces traits sont souvent intégrateurs de plusieurs fonctions des plantes et sont donc soumis à des pressions de sélection variées, ce qui explique leur grande variabilité et la difficulté d'établir des relations claires avec des gradients environnementaux. La surface spécifique foliaire a notamment fait l'objet d'une revue de littérature des causes et conséquences écologiques et évolutives de sa variabilité (Poorter *et al.*, 2009). Pour mieux comprendre les contraintes et les déterminants physiologiques et évolutifs de la diversification des plantes, des auteurs suggèrent de généraliser la mesure de traits « mécanistes » (Brodribb, 2017) plutôt que des traits fonctionnels plus intégrateurs. Par exemple, la vulnérabilité du xylème aux phénomènes d'embolie a permis de comprendre les limites de distribution d'espèces du genre *Callitris*, qui comprend les espèces d'arbres les plus résistantes à la sécheresse (Larter *et al.*, 2017). Alors que *A. thaliana* est considérée comme une espèce plutôt rudérale, nous avons montré que la résistance à la sécheresse est également une dimension principale de sa variabilité phénotypique et génétique. La différenciation des populations scandinaves est associée à une densité de nervures plus élevée, et à de nombreux gènes ayant un rôle déterminé dans la résistance à la sécheresse et aux stress environnementaux en général (chapitre 2).

La coordination des traits foliaires avec les traits de la plante et leur performance dans des environnements où les ressources varient est attendue en écologie comparative car certains traits du WLES définissent également l'axe Compétiteur-Stress tolérant du triangle C-S-R (Reich, 2014). Les données phénotypiques présentées dans ces pages ont permis d'estimer la position des génotypes d'*A. thaliana* dans le triangle des stratégies C-S-R par la méthode développée par Pierce *et al.* (2013). Trois traits foliaires, la taille de la feuille, la surface spécifique foliaire et la teneur en matière sèche foliaire, permettent d'évaluer de manière

relativement précise la position d'une espèce dans le triangle des stratégies C-S-R. L'analyse montre que la grande variabilité phénotypique exprimée par *A. thaliana* est le résultat d'une diversification de stratégies écologiques qui s'étend le long d'un axe Stress tolérante – Rudérale (Vasseur *et al.*, 2018, Annexe 2). L'analyse génétique des populations montre que cet axe de différenciation phénotypique est adaptatif le long de la distribution latitudinale de l'espèce, conformément aux résultats de cette thèse. Dans ce contexte, il est cohérent d'observer que les traits associés à la résistance au stress hydrique (VD) et à la rétention des nutriments (vitesse de résorption) sont adaptatifs chez cette espèce. Il est également cohérent d'observer que les traits du WLES, plutôt considérés comme constituant un axe d'aptitude à la compétition, sont peu différenciés entre les populations d'*A. thaliana*. L'une des critiques principales du triangle C-S-R est de ne pas suffisamment expliciter la notion de stress alors que différentes ressources peuvent constituer autant d'axes de différenciation phénotypiques (Westoby, 1998). Or il apparaît que des traits associés à des stress variés (la faible disponibilité de l'azote ou de l'eau, les températures froides) sont généralement coordonnés (Grubb, 2016). En effet, en synthétisant nos résultats nous observons que les géotypes stress-tolérants du nord de l'Europe sont à la fois caractérisés par des stratégies de croissance lente, des traits conférant un recyclage rapide de l'azote, des traits conférant une meilleure résistance à la cavitation et une meilleure résistance à la sécheresse et au froid (Fig. 18). La même conclusion a été tirée de la comparaison de 17 espèces d'arbres de la famille des Dipterocarpaceae (Zhang *et al.*, 2015). Les espèces ayant une stratégie de croissance relative lente sont caractérisées par une meilleure efficacité de résorption et des densités de nervures plus importantes, indépendamment des variations de la SLA. Chez *A. thaliana* les protéines qui catabolisent les protéines riches en azote au moment de la senescence et de la résorption sont localisées spécifiquement dans les cellules des tissus conducteurs (Moison *et al.*, 2018). C'est un mécanisme qui peut expliquer l'augmentation de l'efficacité et de la vitesse du processus de résorption de l'azote avec l'augmentation de la densité du réseau de nervures. Il apparaît important à l'avenir de caractériser les traits et les mécanismes impliqués dans la tolérance à des stress variés, qui sont responsables de l'intégration du phénotype à l'échelle des stratégies de la plante.

Intérêt du modèle Arabidopsis thaliana en écologie comparative

L'espèce *A. thaliana* se caractérise par une petite taille et un cycle de vie très court, et des interactions biotiques réduites : elle ne développe pas de symbioses mycorhiziennes pour l'absorption des nutriments et ne dépend pas non plus d'insectes pollinisateurs pour sa

reproduction. Ces propriétés, associées à un génome de très petite taille, en ont fait un modèle de choix pour des expérimentations en milieu contrôlé, en physiologie et en génétique. Ce sont également ces propriétés qui en ont fait une espèce peu étudiée en écologie fonctionnelle. Les spécificités de son histoire naturelle et de sa physiologie feraient d'elle une espèce particulière située à un extrême des schémas généraux de forme et de fonction des plantes (Díaz *et al.*, 2016). Néanmoins, cette espèce représente un nœud central de l'interdisciplinarité en biologie des plantes. Elle a permis de nombreux progrès fondamentaux dans la compréhension du développement, de la biologie cellulaire, du métabolisme et de la physiologie, de la génétique et de l'épigénétique (Krämer, 2015). Son utilisation s'est étendue au domaine de l'évolution, notamment, car elle appartient à un genre contenant neuf espèces. Cela permet d'étudier et de dater des événements de spéciation et d'estimer la variabilité du taux de mutation d'une lignée et la fréquence d'apparition de nouveaux génotypes (Weigel, 2012). Son utilisation s'est également étendue au domaine de l'écologie des interactions car elle possède des caractères qui diminuent l'appétence des feuilles pour les herbivores, comme la présence de trichomes foliaires et la production de glucosinolates (Mauricio & Rausher, 1997). Certains gènes impliqués dans la production de ces défenses ainsi que les chaînes métaboliques impliquées ont été identifiés (Blau *et al.*, 1978; Hauser *et al.*, 2001). La fabrication de ces moyens de défense a un coût pour la plante et en particulier dans le bilan économique des feuilles. A ce titre, les recherches en écologie comparative ont un intérêt particulier à se connecter aux autres disciplines de l'écologie au travers du modèle *A. thaliana*. Nous avons réalisé ce type d'exercice interdisciplinaire pour explorer l'effet des traits fonctionnels dans les cycles des nutriments à l'échelle de l'écosystème (Kazakou *et al.*, 2019, Annexe 3). En effet, la morphologie et la composition chimique des feuilles ont une incidence après la vie des feuilles, pendant le processus de décomposition (Cornwell *et al.*, 2008). Le pourcentage de décomposition varie d'un facteur 2,7 entre les génotypes d'*A. thaliana* et l'héritabilité de ce trait était d'environ 25%. Les données montrent que le pourcentage de décomposition est associé négativement à la présence de métabolites secondaires tels que les glucosinolates mais ne covarie pas avec les traits du WLES. Il s'agit d'une rare démonstration de comment la variabilité génétique de traits fonctionnels (les glucosinolates) sous pression de la sélection naturelle (ex. l'herbivorie) peut modifier les cycles biogéochimiques (Harguindeguy & Gorné, 2019). L'étude d'un organisme modèle tel que *A. thaliana* permet de croiser des approches méthodologiques et des cadres conceptuels et permettra d'améliorer notre compréhension des patrons écologiques et évolutifs généraux du fonctionnement des plantes dans leur environnement.

Un autre regard sur le WLES et l'importance évolutive de la durée de vie foliaire

L'exploration des processus de senescence associés à la variation de la durée de vie est un sujet de recherche actif dans la littérature scientifique. La senescence est la détérioration des fonctions et des performances d'un organisme, qui apparaît au cours du vieillissement. Ce processus était connu exclusivement chez les humains, les plantes et animaux captifs car dans la nature la prédation, la compétition et le parasitisme sont les principales causes de mortalité (Maklakov & Chapman, 2019). Les organismes expriment alors un éventail de stratégies allant de « vivre rapidement et mourir jeune » à « vivre lentement et mourir vieux » en fonction de leurs traits d'interactions avec l'environnement biotique et abiotique. Le syndrome d'économie foliaire est également interprété comme un compromis lié à l'investissement dans les capacités de défense contre l'herbivorie. Une teneur en masse sèche foliaire élevée traduit un investissement important dans des traits de résistance face aux dommages biotiques et abiotiques en général, mais limiterait la vitesse de photosynthèse (Grubb, 2016). Cependant, une récente revue de littérature montre que la senescence est un processus commun qui affecte la plupart des organismes dans leur milieu naturel (Nussey *et al.*, 2013). Nous observons également une variabilité importante de la durée de vie chez des plantes cultivées en conditions de croissance favorables (chapitre 1), suggérant l'existence d'un compromis intrinsèque aux tissus foliaires, indépendant des conditions du milieu. L'hypothèse selon laquelle l'épaisseur des parois cellulaires végétales est la contrainte principale du WLES (Shipley *et al.*, 2006; Onoda *et al.*, 2017) permet d'expliquer comment des parois cellulaires épaisses réduisent la photosynthèse *via* une réduction de la conductance de CO₂ et augmente la durée de vie *via* une meilleure résistance mécanique. En revanche, elle ne permet pas d'expliquer pourquoi des feuilles qui investissent peu dans leur défense vieillissent et meurent rapidement y compris en milieu contrôlé. En biologie animale, les évolutionnistes et gérontologistes disposent d'une littérature plus abondante. Il existe deux théories non exclusives permettant d'expliquer les variations naturelles de vieillissement et de durée de vie, et leurs causes directes et évolutives. La théorie de l'accumulation de dommages (AD) prédit que l'investissement dans la maintenance des tissus et la réparation de l'ADN réduit les dommages cellulaires causés par l'accumulation avec le temps de protéines mal repliées et d'erreurs de réplication de l'ADN (Clark, 2004). Dans une feuille, un fort investissement dans la machinerie photosynthétique pourrait causer l'accumulation de ces dommages cellulaires. De plus, étant un organe exposé en permanence à la lumière, la résistance des feuilles aux mutations causées par les rayons ultraviolets (Tanada & Hendricks, 1953) pourrait être directement liée à l'épaisseur des parois

cellulaires. La théorie de la pléiotropie antagoniste (PA) stipule que des gènes pléiotropes peuvent avoir un effet favorable sur le début de la vie et un effet délétère sur la fin de vie (Williams, 1957). Ces gènes seraient quand même favorisés car la sélection est moins forte chez les organismes âgés qui se sont déjà reproduits. Il existe alors un compromis entre une croissance rapide et une durée de vie courte si ces gènes sont hautement exprimés, et une croissance lente et une durée de vie longue si ces gènes sont moins exprimés. La théorie PA peut être appliquée à l'organe foliaire en reconsidérant un cadre conceptuel développé par John Harper et peu cité jusqu'à très récemment (Hodgson *et al.*, 2017). Une plante peut être considérée comme une population de feuilles (ou de rameaux tiges-feuilles) qui se comporte comme un modèle d'étude de la démographie ou de la biologie des populations (Harper & Seltek, 1987). Chaque feuille se comporte comme un individu qui se développe suivant des phases successives pendant lesquelles il est un puit de ressources (émergence, croissance), puis une source de ressources (maturité, senescence). Les ressources accumulées par une feuille sont utilisées pour la construction de nouvelles feuilles, et la croissance d'une plante est le taux de naissance des feuilles. Des gènes pléiotropes peuvent avoir des actions à la fois bénéfiques et délétères pour la feuille en fonction de son âge et ainsi imposer un compromis entre croissance et durée de vie. L'étude des mécanismes évolutifs et physiologiques qui façonnent la senescence, jusqu'à présent étudiés chez la levure, les nématodes, la souris et la mouche du vinaigre, pourrait être étendue à l'étude des plantes (Maklakov & Chapman, 2019). L'étude de la feuille comme unité biologique pourrait également améliorer notre compréhension de la généralité de ces compromis à différentes échelles. Enfin, l'écologie comparative bénéficiera de ces cadres conceptuels développés en évolution et gérontologie pour comprendre les mécanismes impliqués dans les compromis fonctionnels.

Perspectives

Cette thèse contribue à l'exploration des mécanismes évolutifs et physiologiques sous-jacents à la diversification des plantes le long des axes de différenciation phénotypique, identifiés en écologie comparative. Une attention particulière a été apportée au syndrome d'économie foliaire qui représente essentiellement un compromis entre le taux d'assimilation photosynthétique et la durée de vie des feuilles. Il s'agit d'un compromis général du fonctionnement des plantes car il est observé d'une manière générale dans les comparaisons interspécifiques et intraspécifiques. Néanmoins, les pentes et la dispersion autour des relations entre traits ne sont pas universelles. À l'issue de cette thèse, une question reste en suspens : quelles sont les propriétés taxon-spécifiques qui font varier la pente de ce compromis ? Les différents processus écophysiologiques et évolutifs qui déterminent la senescence et la durée de vie ont des importances relatives différentes en fonction des groupes taxonomiques (Maklakov & Chapman, 2019). Par conséquent, il n'existe pas de consensus sur la primauté d'un processus ou d'un autre pour expliquer les compromis évolutifs. De plus, dans le cadre d'un compromis entre des fonctions A et B, les groupes taxonomiques peuvent présenter des relations variables entre les traits A et B car ils diffèrent très probablement dans des propriétés C, D, E, etc. (Grubb, 2016). La compréhension des compromis identifiés en écologie comparative pourrait bénéficier de l'étude de ces causes directes et ultimes à différentes échelles taxonomiques. Nos résultats montrent que la pente du WLES chez *A. thaliana* est différente de celle observée à l'échelle interspécifique. Pour comprendre les changements qui opèrent entre l'échelle intra-spécifique et l'échelle interspécifique, il faut explorer les niveaux taxonomiques intermédiaires. Nous avons regroupé les valeurs de traits du WLES et du schéma général de forme et fonction des plantes mesurés chez 212 espèces annuelles à partir de bases de données publiques (DivGrass et TRY), et chez 145 des génotypes étudiés dans cette thèse (Tucker et al., en révision, Annexe 1). L'analyse révèle que les structures de covariances des traits et les taux relatifs de divergence des traits diffèrent de manière relativement importante entre les deux échelles considérées. Ce résultat montre que même si le WLES est causé par une contrainte physiologique universelle, les forces évolutives responsables des structures de covariations diffèrent entre les échelles taxonomiques. Pour compléter cet exercice d'exploration du WLES au travers de différents niveaux taxonomiques, une analyse intra-genre est nécessaire. Elle permettrait de mesurer les taux de divergence entre espèces et l'apparition de mutations liées aux traits du WLES et ainsi de mieux distinguer l'effet des propriétés physiologiques spécifiques de l'effet de l'évolution sur la variation de la pente du WLES. Le genre *Arabidopsis* est un modèle de choix pour ce

type d'étude. En effet, il est composé de seulement neuf espèces, variables d'un point de vue de la phénologie, de la morphologie et de leurs préférences d'habitats. Il existe un gradient de pérennité, avec l'annuelle *A. thaliana*, la biannuelle *A. arenosa*, à la pérenne *A. cebennensis*. Différentes formes de reproduction existent également, de la stricte autoféconde *A. thaliana* à la stricte alloféconde *A. lyrata*. L'espèce *A. suecica* est étudiée pour le caractère tétraploïde de son génome (Novikova *et al.*, 2017). L'espèce *A. halleri* est étudiée pour sa qualité d'hyper-accumulatrice des métaux du sol (Zhao *et al.*, 2000). Ces différentes propriétés ont probablement une incidence sur l'importance des différentes pressions de sélection pour chaque espèce et pourraient moduler l'expression des compromis écophysiologiques. De futures études devraient bénéficier des connaissances acquises chez les membres du genre *Arabidopsis*, en combinant les approches fonctionnelles et évolutives pour mieux comprendre les lois générales de la diversification des plantes.

Conclusion

L'approche comparative appliquée à de nombreux écotypes de l'espèce modèle *Arabidopsis thaliana* a permis de tester des hypothèses relatives au rôle adaptatif des contraintes du fonctionnement des plantes. La combinaison des méthodes de mesures des traits phénotypiques à haut débit et de génétique quantitative a permis d'identifier les bases génétiques de syndromes de traits identifiés en écologie fonctionnelle. Le syndrome d'économie foliaire représente un axe de variation phénotypique potentiellement impliqué dans la différenciation de l'espèce à l'échelle locale, tandis qu'une différenciation des stratégies de tolérance aux stress climatiques a été observée à l'échelle de sa distribution géographique. L'utilisation du modèle *A. thaliana* permet d'établir des liens entre l'écophysiologie et la génétique des populations, et ouvre des perspectives pour permettre de mieux comprendre et prédire l'évolution des espèces et leur distribution géographique face aux modifications de leur environnement.

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ANNEXES

**Annexe 1 - The scaling of plant variation:
comparing evolutionary constraints on trait relationships across taxonomic scales.**

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Abstract. Globally, plants span great variation in form and function, but traits provide an important tool for describing and understanding plant ecology across space and taxonomy. It has been suggested that despite this great variation, trait space is highly constrained and different traits covary predictably. However, little is known about the evolutionary processes underlying these trait relationships, and whether they are truly general across taxonomic scales, or experience different patterns of selection and constraint. This has been stymied by the lack of paired genetic and trait data at multiple taxonomic scales. We grew and phenotyped a collection of pan-European *Arabidopsis thaliana* accessions for six ecologically relevant traits, and obtained extensive genetic data. We selected 212 European annual plant species with matching trait data and a phylogeny. Across these two taxonomic scales, we evaluated the congruency of the observed trait covariance structure, the effect of the underlying phylogenetic structure on those covariances, and the associated rates of trait divergence. There was only a modest and non-significant correlation between intra- and inter-specific trait covariance structures that further weakened after controlling for the underlying phylogenetic relationships. Instead, specific traits differed meaningfully between scales – particularly leaf nitrogen content – as did the relative rates of trait divergence. This implies that differences in the trait covariance structure can arise due to differences in evolutionary drivers at the different scales. Further, comparisons of trait values must consider the potential that there are different ecological and evolutionary constraints structuring trait variation at different scales.

Significance statement. Recent work has highlighted that there are global patterns of trait structure reflecting key life history strategies in plant species. However, the question of whether these trait relationships are general across taxonomic scales of organization is unknown, but important for understanding the evolutionary processes producing ecological differences. Using large sets of intra- and inter-specific trait and phylogenetic data, we found that patterns of trait structure differ greatly between scales, driven by underlying differences in relative rates of trait divergence. This implies that constraints on traits may also differ across taxonomic scales, which may impede generalization regarding constraints on plant ecologies.

Introduction. Plant life on Earth spans a great variety of form and function. These phenotypic differences between plant species—molecular, physiological, phenological, and morphological—are key to understanding their ecological differences (1-3). If differences in ecologically relevant traits capture differences in growth, reproduction, or/and mortality in a given environment (3, 4), they may be useful predictors of plant performance. Though individual traits can be informative predictors (2, 5, 6), multiple traits considered in concert may best capture multi-dimensional niche differences (7-9). Recent studies have shown the importance of considering the covariation between traits for describing the patterns of plant form and ecological function (9-12). For example, across a global dataset of more than 40,000 plant species, Diaz et al. (2016) presented evidence suggesting that the structure of covariance among 6 functional traits was highly constrained globally. The relationships between these traits were associated with several general ecological strategies across this wide set of species, particularly the scaling relationships between full plant size and seed mass (13, 14) and a slow-fast tradeoff in resource acquisition and resource conservation [i.e. the leaf economic spectrum, LES (15-17)]. Because of the consistent covariation between traits, a small number of traits were sufficient to provide insight into global ecological strategies, highlighting the potential of trait-based approaches for ecological understanding and prediction.

Diaz et al. (10) did not explore the evolutionary background of the trait covariance patterns they identified, however such patterns can reflect selection or constraints at multiple taxonomic scales. Evolutionary biologists have explored the evolution of patterns of complex trait relationships (referred to that literature as 'phenotypic integration') (9, 18, 19), but this body of work rarely considers how such

patterns relate to ecological strategies. To understand both the evolutionary drivers and the ecological consequences of phenotypic integration, it is valuable to consider how ecologically relevant patterns of trait covariance evolve. It is useful to consider whether patterns of trait covariance are congruent across multiple taxonomic scales (e.g. between versus within-species). Past studies of phenotypic integration in evolutionary biology suggest that it tends to be conserved at higher scales of organization and more labile below the level of genus (20), reflecting different constraints and selective regimes. In ecological studies, comparisons of the trait relationships have been restricted to small numbers of traits: for example, the trait covariance structure producing the LES is typically but not consistently identified as being similar within-species and between-species (21-25).

A proposed advantage to trait-based approaches in ecology is that they facilitate comparisons across inter- and intraspecific data sets (24, 26, 27), implying that traits have similar ecological meaning at multiple scales. If true, this implies that trait covariance structure is conserved, as the result of similar developmental, physiological, ecological, and/or genetic processes promoting trait evolution across scales (28). Alternatively, structures may diverge if the selective pressures and constraints present over the short time scales differentiating populations differ from those occurring between species (29), or if pressures and constraints are similar but undergo drift (30). Comparing trait covariance structure across taxonomic scales, as well as the associated genetic differences, provides a way to understand the generality of trait relationships and ecological strategies in plants.

Disentangling the evolutionary causes and ecological consequences of trait

covariance structure is difficult (31) in part because data suitable for comparisons across multiple taxonomic scales using multiple traits and associated genetic variation remains rare (11). A conservative comparison between inter- and intraspecific data would require a large interspecific data set, in addition to data for a large number of genetically- and ecologically-distinct natural populations (with appropriate trait and genetic data), and preferably similar climatic and geographic distributions and similar life histories for both the within- and between-species data sets. The model plant species *Arabidopsis thaliana* provides such a system, in which it is possible to describe genetic and phenotypic variation across a large number of distinct, natural-collected populations ('accessions') (32-34). Phenotypic variation is large enough across accessions to meaningfully estimate intraspecific covariance structure (Figure S1). Recent efforts have been made for in-depth characterization of the genetic diversity of this species (35), and have resulted in 1,307 natural accessions genotyped with a 250K SNPs chip (36), and 1,135 accessions have been fully sequenced (32). Accessions of *A. thaliana* are widely distributed throughout Eurasia, are selfing, and so exhibit strong genetic structure, allelic homozygosity, and high effective population sizes (32, 37).

We explored how the phenotypic space in Diaz et al. (10)—defined by six traits relevant to plant growth, survival, and reproduction—might vary between inter- and intra-specific scales. For 145 accessions, we grew and then measured all of adult plant height (PH, m), stem specific density (SSD, mg mm^{-3}), leaf area (LA, mm^2), leaf mass per area (LMA, g m^{-2}), leaf nitrogen content per unit mass (LNC, mg g^{-1}), and seed mass (SM, mg) (see Methods, and Table S1 for summary details). We collected matching trait data for common European herbaceous annual plant species (38) from a global database

(39) for plant trait data, identifying a total of 212 such species with complete trait coverage (Table S3 for species; Table S4 for TRY attribution). The annual plant data set included independent trait measurements for *A. thaliana*. We compare the patterns of trait covariance between these two trait data sets by comparing trait matrices using the Mantel test and Selection Response Decomposition (SRD) (40). To partition the relative role of drift in driving patterns of trait covariance structure, we contrast these results with those from data that are corrected for phylogeny (30) (see Methods).

Results. For the annual plant species, the pattern of covariance between the 6 traits are broadly similar to those previously identified for global non-woody plant species in (10) (Figure 1). Similar tradeoffs and trait loadings structure the first two PC axes – plant height and seed mass load together, highlighting plant size scaling relationships. Further, the resource economic spectrum is reflected by a negative relationship between LNC and LMA values. The mean position of *Arabidopsis thaliana* (Figure 1, green point) based on the annuals data falls within the cloud of annual plant species, and is also positioned centrally relative to the intraspecific values (dark blue). However, the distribution of *A. thaliana* accessions—when projected into the annuals' ordination space—is strikingly different than the distribution of the interspecific points. Accessions have disproportionately large variation in LMA and LNC relative to their variation in PH and SM. Many accessions occupy trait combinations more extreme than those associated with average species-level positions.

It is often hypothesized that interspecific variation should be greater than intraspecific variation (27); however we are primarily interested in whether the

underlying trait structure—not total trait variance—differs between the annuals and accessions. If the patterns of phenotypic integration are similar at both inter- and intraspecific scales, the underlying magnitude and direction of trait correlations should be similar for both data sets. We calculated trait correlation matrices for annual species and accessions, and found a moderate, non-significant Mantel correlation ($r=0.42$, $p=0.08$), between the pattern of annual plants and *A. thaliana* accession trait relationships. Using a random skewers based analysis (SRD, 40), we could also determine which traits drove the differences between trait structures at the inter- and intraspecific scales, and found that leaf nitrogen content (LNC) was significantly different between the two covariance matrices (Supplementary Figure 2).

Phylogenetic relationships may affect these results: differences between the trait covariance structures can result from drift, while similarities may reflect non-independence of residuals due to underlying patterns of ancestry (31). For this reason, we applied phylogenetic independent corrections (PIC) (41, 42) separately to both trait data sets. After calculating the phylogenetically-independent variance covariance and correlation trait matrices (following 30), we repeated the previous matrix comparisons. We found that the modest Mantel correlation between the European annuals and *A. thaliana* accessions was further weakened ($r=0.27$, $p=0.14$). Two traits were responsible for this divergence: LNC and SSD values differed significantly between the groups (SRD, see Supplementary Figure 2B)(Figure 2 highlights the distribution of traits on the two phylogenies).

The SRD results suggest that certain traits (especially LNC) will drive different responses to selection between the European annuals and *A. thaliana* accessions. We

confirmed that not only are the observed trait covariances quite different, but found that the relative rates of trait evolution (estimated using a multivariate Brownian motion model of evolution (σ^2)) differed between the European annuals and *A. thaliana* accessions (Figure 3). The estimated multivariate evolutionary rates (Supplementary Table 5) showed that the rates at which traits are diverging covary in different fashions within the annuals as compared to the accessions. In some cases, the direction of covariance is notably different (e.g. leaf area and leaf nitrogen content). The relative magnitude of covariance is also often different: in *A. thaliana* the rate of divergence in seed mass has almost no variation and so does not covary with other traits, while in the annuals there is meaningful variation between seed mass and leaf area. Only in a few cases are similar patterns of rate covariance observed (e.g. plant height and leaf area).

Discussion. Trait covariance structure is fundamental to understanding how variation in form and function among plants contributes to ecological strategies. The ecological consequences of this trait structure can be better understood in light of the evolutionary processes that produce it. We provide here a test of the generality of patterns of trait covariance structure across a large number of species and multiple genotypes within a species, and highlight that great divergence in trait covariance can occur between inter- and intraspecific taxonomic scales. This may reflect differences in the processes that link trait values and challenges our ability to use patterns of trait covariation to infer process across these scales (31).

To our knowledge, this is the most comprehensive test of plant trait covariance structure for ecologically relevant traits, across inter- and intraspecific scales of

organization. Ecological analyses typically focus on whether trait relationships reflect ecological strategies, and whether such relationships are observed at different taxonomic scales (e.g. 5, 25). Previous comparisons across such taxonomic scales have been limited to only a subset of the traits considered here (e.g. 26), and vary in their conclusions about generality in trait relationships (24, 26). Such studies have generally considered local populations, where ecological sorting between genotypes may obscure patterns. The benefit of using *A. thaliana* accessions is that they represent natural inbred populations for which a comprehensive genetic structure is known, and thus ecological sorting can be excluded as a cause of differences in trait relationships. Similarly, other potential drivers of trait covariation such as gene flow and genetic drift are unlikely to be important (31), as accessions are large, isolated natural populations (37). *A. thaliana* accessions were chosen for comparison with the European annual species for their comparably large phenotypic variation (Figure S1) and similar climatic range. Even so, we did not find a significant correlation between inter- and intraspecific trait structure.

Evolutionary biologists have also examined whether patterns of phenotypic integration are congruent across taxonomic scales (e.g. 29, 30, 43), particularly with reference to the implications for phenotypic diversification. There are some key differences between their approach and that of ecological studies, particularly that 'ecological' traits tend to be more complex, integrating multiple components (such as leaf mass and leaf area) to yield integrative 'functional' measures (44), while evolutionary studies often focus on morphological markers. Incorporating the evolutionary background of ecological strategies proved insightful: the congruency in trait covariance structure decreased after correcting for phylogeny, highlighting that observed trait relationships are

in part conserved deep in the tree of life. The importance of correcting for the constraints related to ancestral trait relationships is not surprising, given that particular families of plants are well known to represent particular subsets of trait values (45). Trait covariance structure is often found to be conserved at higher taxonomic levels in the phenotypic integration literature, while intraspecific measures frequently differ from those observed at higher taxonomic scales (e.g. 20). The differences in trait covariance structure were driven at least in part by the differences in the relative rates of trait evolution: the rates that co-varied most strongly at one scale rarely matched those at the other scale. Rates of trait divergence vary both through time and space, and between different clades of the phylogeny (46), such that it is not surprising to find that rates in a particular clade of the tree over a particular time period may differ significantly from those estimated for the entire annuals tree. However, it is precisely this fact – reflecting context-dependent differences in selective environments and genetic constraints over different time frames – that generates the incongruencies between *A. thaliana* accessions and European annuals.

The underlying mechanisms driving the observed relationships between plant traits, including developmental tradeoffs, adaptive evolution, genetic drift, and phylogenetic history, are likely to vary in importance with scale (13, 24). For example, leaf nitrogen content appeared less constrained compared to at the inter-specific scale (47, 48). Large and genetically determined variation in leaf nitrogen has been previously in *A. thaliana* accessions, and was tied to variation in nitrogen uptake and nitrogen usage efficiency. The underlying cause of this natural variation is not yet known, but experimental work suggests that dramatic changes in covariance structure can arise rapidly (e.g. 49). As a consequence, it may be questionable to assume generality in terms

of the ecological meaning of a trait. Even if traits are found to covary at several scales (e.g. the leaf economic spectrum), the relationship might differ in strength, orientation and/or total variation. Of course, we present here a single example, capturing one extreme of population structure: in other contexts where drift, founder effects, and/or gene flow are high and selection is weakened, we might predict greater congruency of trait covariance structure across scales (e.g. if selection in populations is inefficient or varied). Future research would be aided by additional data, particularly the availability of additional ecologically-relevant traits and genetic data collected for multiple populations and species. In addition, it is unclear how the use of data collected from a controlled, common environment versus from existing databases where data may come from multiple populations in their home environment may alter expectations. The causes and consequences of trait covariance have received great attention in terms of phenotypic evolution (18), but the question of how these interactions feedback to inform ecological strategies is only beginning to receive focused attention (e.g. 10, 11, 50). Integrating these ecological and evolutionary approaches promises to provide appropriate tests of evolutionary hypotheses while allowing the formulation of new hypotheses regarding the relevance of trait covariance to trait ecology.

Materials & Methods.

All analyses were performed in the R 3.3.0 (51) and all R code is available on request.

***Arabidopsis thaliana* accessions - Intraspecific data.**

Accessions traits. We measured all six traits used in Díaz, *et al.* (10) on 145 accessions (Table S2) selected from a random sampling from the worldwide lines of the RegMap

population (36). We grew four replicate individuals in a greenhouse on organic soil. Seeds were sown and stratified at 4 °C for four days. At the emergence of the first two true leaves, plants were transplanted in 300 mL individual pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost (Neuhaus N2). To reduce environmental heterogeneity in the greenhouse, walls were painted in white and a semi-transparent curtain was installed below ceiling. Photoperiod and temperature were kept constant at 12 h day length, and 18/16 °C day/night, respectively with light supplemented to *ca.* 65 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD at plant height.

At the visible emergence of flower bud (bolting stage), one adult, fully-exposed and non-senescent leaf was measured for leaf nitrogen content (LNC, %) with Near Infra-Red Spectrometry (NIRS) following previous studies (52, 53). The same leaf was harvested after NIRS measurement, water-saturated in deionised water at 4 °C for 24 h and weighed. Leaves were scanned for area (LA, mm^2) determination with ImageJ, then dried at 65 °C for 72 h, and weighed. Leaf dry matter content (LDMC, mg g^{-1}) and specific leaf area (SLA, $\text{mm}^2 \text{mg}^{-1}$) were calculated as the ratio of lamina dry and water-saturated weights, and the ratio of lamina dry mass to lamina area, respectively. Plants were harvested after fruiting and complete senescence. The length of the main flowering stem (PH, cm) was measured, and seeds were harvested. Seed mass (SM, μg) was measured by weighing 20 seeds per plant individual.

All traits were natural log-transformed and converted where necessary into appropriate units for further analysis (Table S1), following procedures in (9). LDMC values were converted to SSD following their Supplementary Methods. Trait covariation and correlation matrices were calculated using the mean trait values per accession.

Accession data is available from the authors on request.

Accessions phylogeny.

We obtained Single Nucleotide Polymorphisms (SNPs) data from the 250K SNPs chip generated on 1307 natural accessions from the RegMap worldwide panel (36), and trimmed the low-quality polymorphisms, leaving 214,050 SNPs. A single genotype was available for each accessions and we used Tassel (54) to build a neighbor-joining tree across all 1307, based on the modified Euclidean distance. This tree was rooted by identifying Accession 6911 as the outgroup - this accession has been identified as a 'relict' or ancestral population of *A. thaliana* by (32), the only such accession in the RegMap panel. The tree was then made ultrametric using *chronopl* in the R package *ape* (55), and the branch lengths rescaled to match the age of the *A. thaliana* node (~4.2 mya) on the annual plant species phylogeny. This produced a tree with comparable branch lengths to the annuals phylogeny. This node-age is relatively conservative, in that if *A. thaliana* accessions are younger than this estimate (as they likely are (32)), rates of trait divergence will simply be even higher. We also transformed this tree to reflect a much younger age (100,000 ya) and compared the phylogenetically-corrected trait structures that would result from these two trees. The Mantel correlation between the two sets of PICs was ~1. We pruned this tree to the 145 focal accessions for which we had matching trait data.

European annual species - Interspecific data.

Annuals traits. Interspecific trait data were obtained from the Divgrass consortium (38), which integrates trait values originally obtained from the TRY database (39), and averaged per species where replicates were available, retaining only those species with

full coverage for all six traits used in Díaz, *et al.* (10) (Table S1 for trait summaries; Table S3 for full species list and details). We restricted the analysis of species with available trait values to annual, herbaceous species occurring in Europe using a recent comprehensive botanical database of Western Europe (38), and identified a total of 212 such species. Traits were natural log-transformed and converted where necessary into appropriate units for further analysis (Table S1). Leaf dry matter content (LDMC) values were converted to specific stem density (SSD) using the equations provided in Díaz, *et al.* (10). Trait covariation and correlation matrices were calculated using the mean species' trait values. While plasticity and genetic variation both may contribute to trait variation, the mean or aggregate trait values have been shown previously to be sufficient to identify meaningful trait relationships (e.g. 10, 15, 56). The specific TRY contributors are listed in Table S4.

Annuals phylogeny. A phylogenetic tree was constructed using the comprehensive Angiosperm species-level phylogeny from Zanne, *et al.* (57), as updated by Qian and Jin (58). This time-calibrated tree includes nearly all families of extant seed plants: species placement relied on seven gene regions, with orders and families constrained by the APG III. We used S.PhyloMaker (58) to generate a phylogeny containing our 212 annual plant species. Where species or genera were not represented in the larger tree, they were added as uninformative (e.g. basal) polytomies, however, position did not affect final results. The Mantel correlation between the phylogenetically-corrected trait structure we present in the text, and between values calculated using alternate positions for missing species were between 0.99 and 1. The final tree was ultrametric with time-calibrated branches.

Phylogenetic correction. We corrected the data for phylogeny using the approach

described in Revell, Harmon, Langerhans and Kolbe (30). We calculate phylogenetic independent corrections (PICs) (41) on each individual trait within a data set (*pic* function in the R package *ape*) and then calculated new covariance or correlation matrices using these corrected values. For this approach, the mean rate of divergence for a given trait can be estimated as the diagonal of the corrected covariance matrix.

An independent contrasts approach assumes that Brownian motion (BM) is the model of trait evolution – under BM, the expectation for trait variance is determined by branch length (time) and the mean rate of trait evolution. This model is appropriate for populations as well, under assumptions of minimal gene flow and high effective population sizes, such as observed among the *A. thaliana* accessions (32, 37). Thus a Brownian motion model for PIC is reasonable for correcting both the annuals and accessions trait values (30).

Analyses.

Principal component analysis. We performed a principal component analysis (PCA) on the annuals plant data, using the *prcomp* function in R, with data both centred and scaled. *A. thaliana* accessions were projected onto this ordination spaces using the *predict* function for plotting purposes.

Mantel test. Tests of covariance matrices (e.g. Procrustes) can confound differences in shape and orientation with differences in total variation between matrices. Thus we first used analyses of correlation matrices—which are standardized—to isolate differences in the shape and orientation between the annuals and accessions trait matrices. Correlation matrices for both annuals and accessions were compared using the Mantel test, with 10 000 randomizations to determine significance.

Selection Response Decomposition. SRD allows one to determine which traits specifically drive incongruencies between trait covariance matrices (40). SRD is an extension of Random Skewers that determines the global average value of each trait across the two compared matrices. It then compares each trait/matrix combination to the global average and determines which trait/matrix combinations differ in their mean position and/or in their associated variance. Random permutations are used to calculate the significance of any divergence in variance and mean value.

Estimating rates of trait divergence. Variance and covariance in rates of trait divergence (σ^2) were estimated for both phylogenies using the *ratematrix* function under the assumptions of multivariate Brownian Motion [using *ape* (55)]. Ellipses were plotted by calculating the first two eigenvectors and eigenvalues for each pairwise set of rates.

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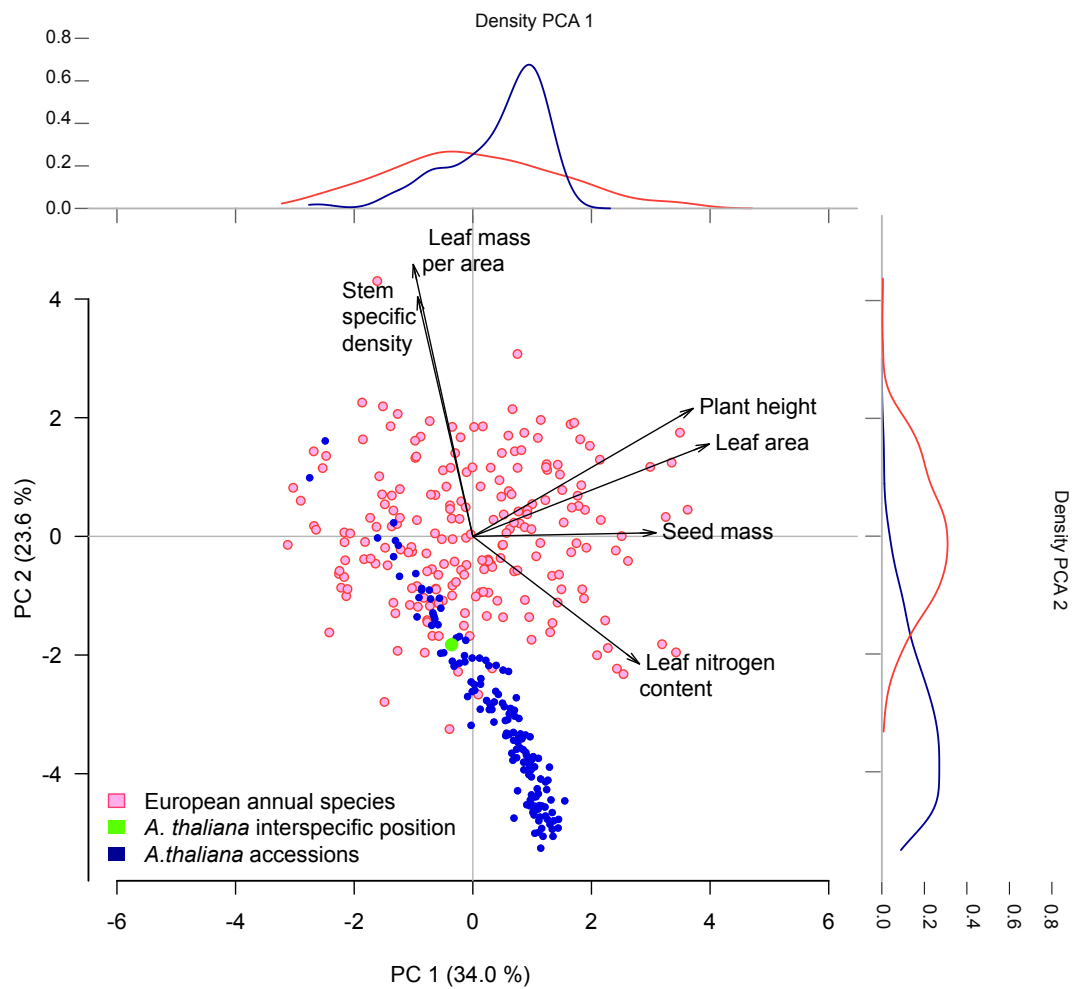
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Figure 1. Annuals (interspecific) trait covariance structure as reflected by the first 2 PC axes. The species-level expectation for 212 annual plant species is shown (pink circles) Black arrows indicate the direction and strength of trait vectors for the 6 plant traits (LMA, PH, SSD, LA, LNC, SM). The position of *Arabidopsis thaliana*, as determined using the interspecific data, is highlighted in green. Projected onto the annual plant space are the positions of each of the 145 accessions of *A. thaliana* in dark blue. Above and to the right are plots showing the density of points for the annuals and accessions along the 2 PC axes.

Figure 2. Phylogenies for *Arabidopsis thaliana* accessions (left panel) and annual plant species (right panel) with colour-coded trait values (log-transformed) represented below: LMA - leaf mass per area, PH - plant height, SSD - stem specific density, LA - leaf area, LNC - leaf nitrogen content per unit mass, SM - seed mass. Trait values are range-scaled across both pooled annuals and accessions trait values, such that the colour values scale from dark purple (minimum trait value) to yellow (maximal trait value). The red branch and asterisk shows the position of *A. thaliana* on the annuals tree.

Figure 3. A comparison of the relative variance and covariance in rates of trait divergence (σ^2) estimated under Brownian motion (see Methods), for the annual species (pink) and the *A. thaliana* accessions (dark blue). Ellipse orientation shows the primary eigenvector for each bivariate relationship, and ellipse length and width is proportional to the first and second eigenvalues. We show pairwise relationships to visualize differences in the relative covariance in rates, but these values come from a multivariate estimate of the evolutionary rate matrices.

551 **Figure 1.**



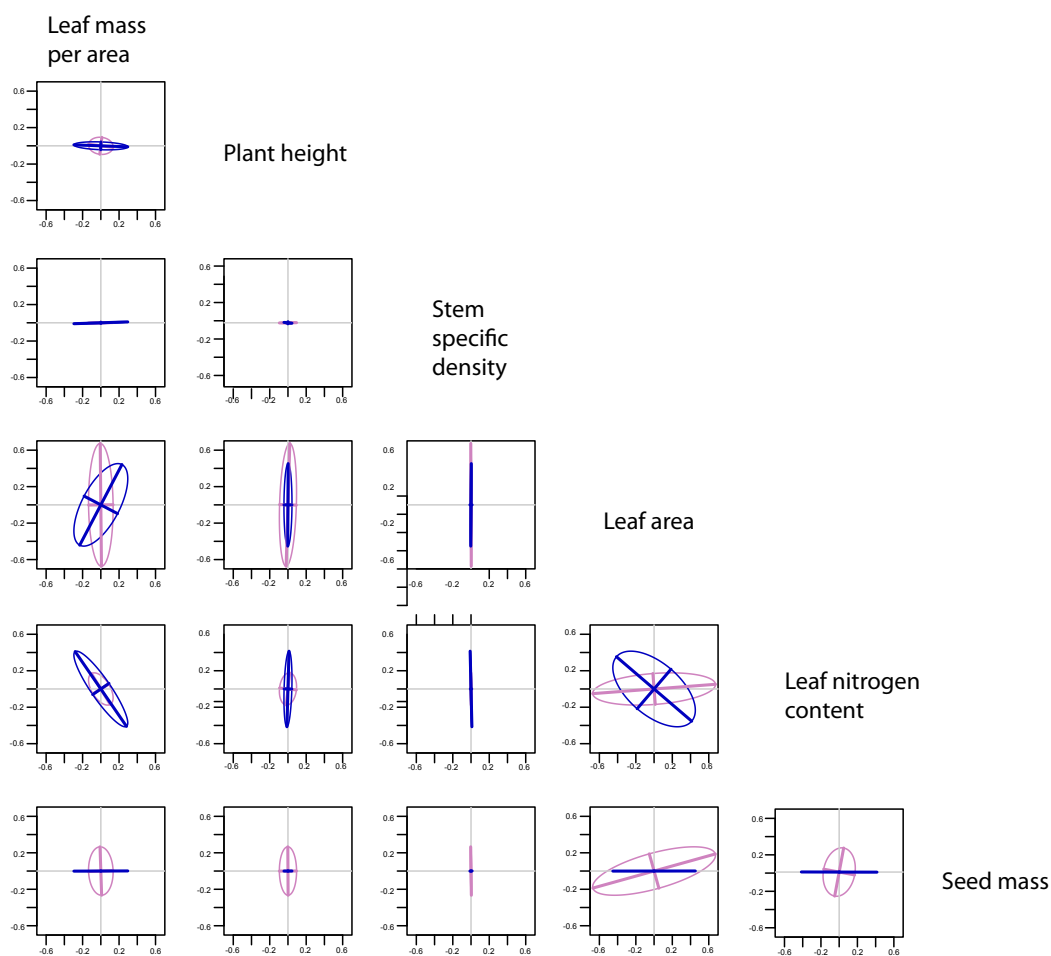
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556 **Figure 3.**

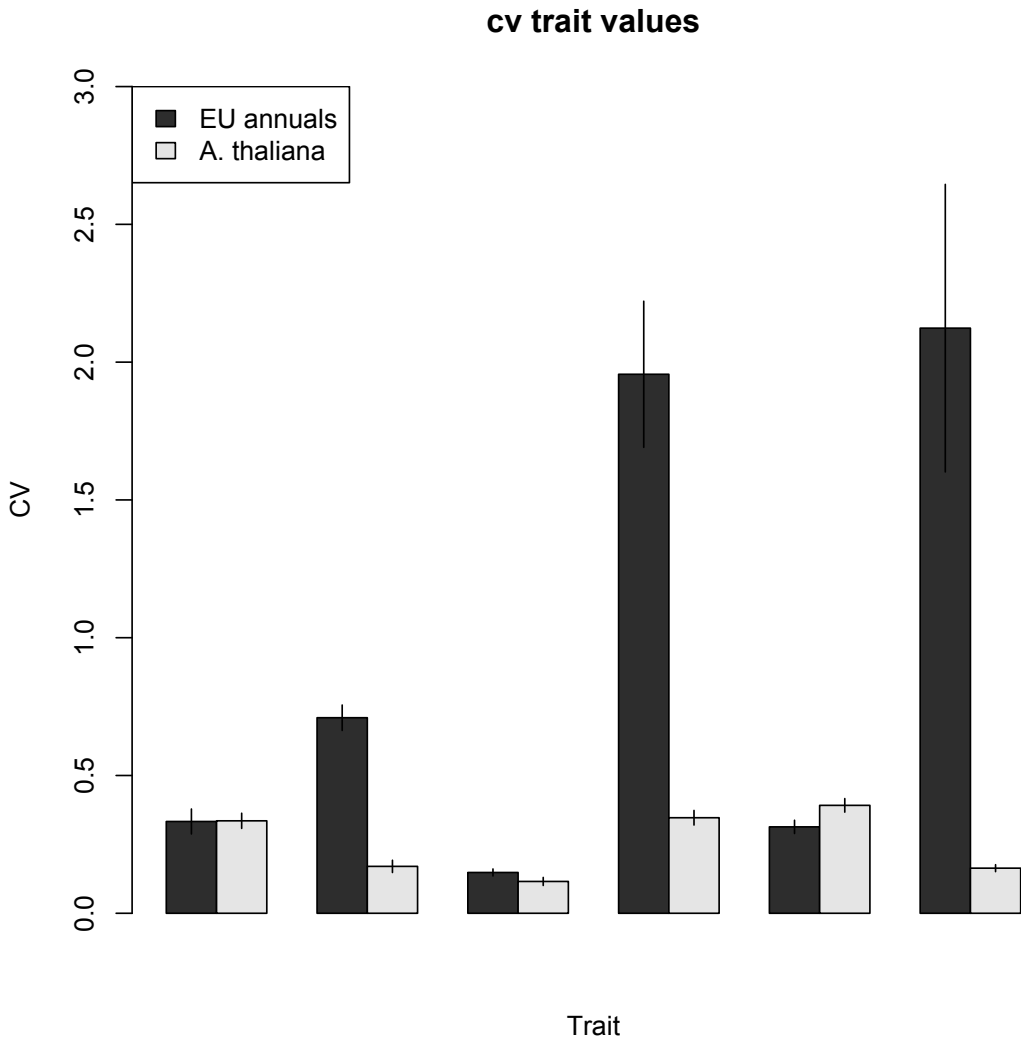


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Supplementary Information.

Figure S1. Coefficients of variation across the six functional trait values, compared for the 212 annual plant species and the 145 *A. thaliana* accessions. Error bars represent standard errors estimated using bootstrapping, for 1000 replicate subsets of 100 species/populations randomly selected from the larger dataset.

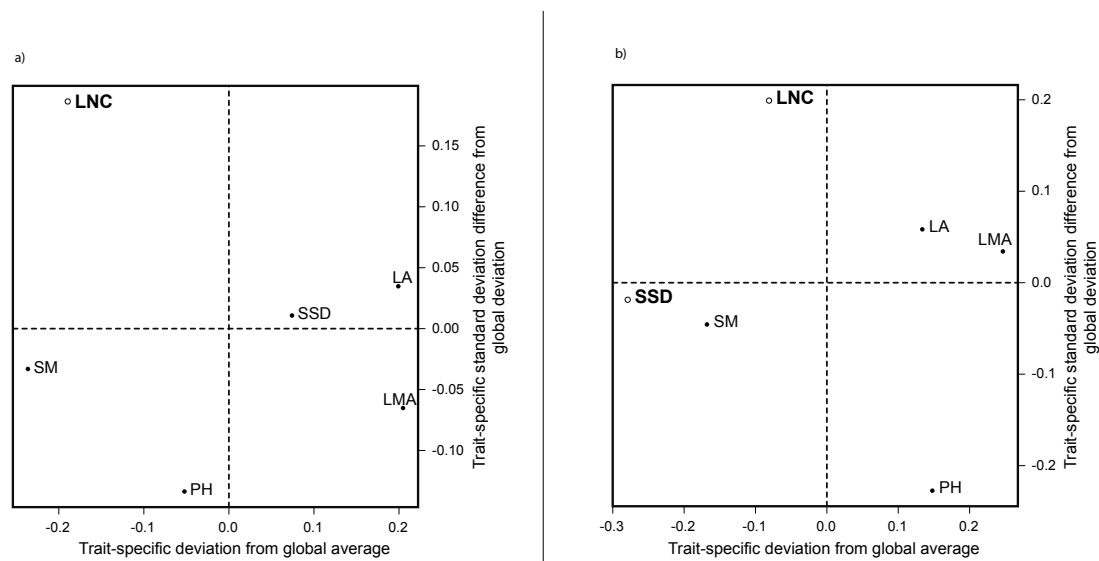


567

568 **Figure S2.** Comparison between *A. thaliana* accessions and annuals trait matrices using
569 Selection Response Decomposition (SRD). The x-axis indicates, for each individual trait,
570 the difference in the accessions average value compared to the global average across both
571 data sets. The y-axis indicates, for each trait, the difference in the accessions' standard
572 deviation as compared to the global standard deviation. Significantly different traits are
573 bolded and indicated using open circles. (a) Provides results for the comparison between
574 raw covariance matrices; (b) provides results for the comparison between the phylogeny-
575 corrected matrices. PH - plant height, SSD - stem specific density, LA - leaf area, LMA -
576 leaf mass per area, LNC - leaf nitrogen content per unit mass, SM - seed mass.

577

578



579

Trait	unit	Mean annuals (log)	SD annuals (log)	Mean <i>A. thaliana</i> (log)	SD <i>A. thaliana</i> (log)
Height	m	0.356	0.204	0.300	0.046
Stem specific density	mg mm ⁻³	0.178	0.024	0.142	0.015
Leaf area	mm ²	6.541	1.914	6.008	0.425
Leaf mass per area	g m ⁻²	3.761	0.303	3.026	0.302
Leaf nitrogen content	mg g ⁻¹	3.249	0.295	3.730	0.441
Seed mass	mg	0.833	0.772	0.017	0.003

580

581 **Table S1.** Summary data for the 212 annual plant species and the 145 *A. thaliana*
582 accessions.

583

584

Accession IDs

6897	8297	194	7317	100000
6988	6921	203	269	6980
6898	8214	219	7320	6982
6992	7461	8337	7514	6984
6994	8304	252	8378	6985
6899	8235	6937	8412	
6709	6039	6938	6961	
7015	6923	6939	6962	
7034	6924	6940	6963	
8266	8310	185	6966	
5837	8236	8420	6967	
6903	8311	8343	6108	
6904	8312	318	6180	
5719	7192	6942	6184	
6906	7186	357	6195	
69	6040	6944	6209	
8274	6928	7270	6242	
8275	8239	6945	6243	
7071	6929	8348	275	
6907	9412	6064	296	
6730	8240	7519	297	
7081	6930	6946	368	
7092	6931	258	377	
86	94	8354	6970	
6911	8323	7296	6971	
88	104	7507	7373	
89	146	7508	9470	
8233	157	7307	8426	
8284	8325	7523	6973	
6008	8222	8213	6975	
6914	6933	295	6976	
6916	7520	6958	7382	
8215	259	8365	374	
6919	6936	6959	9057	
8296	159	6960	396	

Table S2. List of *Arabidopsis thaliana* accessions used in the analyses. Naming follows (32).

Annual plant species

Achillea millefolium	Asarum europaeum	Cirsium dissectum	Galeopsis tetrahit	Impatiens capensis	Polygonum aviculare	Vicia tetrasperma
Adonis aestivalis	Aster amellus	Cirsium oleraceum	Galium aparine	Impatiens noli-tangere	Prunella vulgaris	Viola tricolor
Adoxa moschatellina	Astrantia major	Cirsium palustre	Galium boreale	Inula conyza	Ranunculus sardous	
Aegopodium podagraria	Bellis perennis	Cirsium vulgare	Galium mollugo	Kickxia spuria	Rhinanthus minor	
Agrimonia eupatoria	Beta vulgaris	Clinopodium vulgare	Galium odoratum	Leontodon saxatilis	Rudbeckia hirta	
Ajuga reptans	Blackstonia perfoliata	Colchicum autumnale	Galium saxatile	Lepidium densiflorum	Rumex acetosella	
Alchemilla glabra	Campanula glomerata	Conium maculatum	Galium uliginosum	Lepidium ruderales	Rumex crispus	
Alchemilla glaucescens	Campanula persicifolia	Convallaria majalis	Galium verum	Linum catharticum	Rumex obtusifolius	
Alchemilla monticola	Campanula rotundifolia	Convolvulus arvensis	Gentiana verna	Lotus corniculatus	Sagina procumbens	
Alchemilla vulgaris	Capsella bursa-pastoris	Crepis biennis	Geranium dissectum	Medicago lupulina	Scabiosa atropurpurea	
Alchemilla xanthochlora	Cardamine hirsuta	Crepis capillaris	Geranium molle	Medicago minima	Scabiosa columbaria	
Alliaria petiolata	Carduus acanthoides	Crepis foetida	Geranium pratense	Melampyrum pratense	Scleranthus annuus	
Allium carinatum	Carduus nutans	Crepis paludosa	Geranium robertianum	Melampyrum sylvaticum	Senecio vulgaris	
Allium ursinum	Carlina acaulis	Crepis vesicaria	Geranium rotundifolium	Mellilotus albus	Sherardia arvensis	
Alyssum alyssoides	Carlina vulgaris	Cruciata laevipes	Geranium sanguineum	Myosotis discolor	Silene dioica	
Ambrosia trifida	Carum carvi	Daucus carota	Geranium sylvaticum	Myosotis ramosissima	Silene gallica	
Anagallis arvensis	Centaurea jacea	Descurainia sophia	Geum rivale	Myosotis sylvatica	Silene vulgaris	
Androsace septentrionalis	Centaurea nigra	Digitalis purpurea	Geum urbanum	Odontites vernus	Sonchus oleraceus	
Anemone nemorosa	Centaurea scabiosa	Dipsacus fullonum	Glechoma hederacea	Origanum vulgare	Spergula arvensis	
Anemone ranunculoides	Centaureum erythraea	Drosera rotundifolia	Gymnadenia conopsea	Orlaya grandiflora	Spergularia rubra	
Angelica archangelica	Cerastium arvense	Echium vulgare	Helichrysum arenarium	Oxalis acetosella	Stellaria media	
Angelica sylvestris	Cerastium fontanum	Epilobium hirsutum	Helleborus foetidus	Paris quadrifolia	Tanacetum vulgare	
Antennaria dioica	Cerastium glomeratum	Epilobium palustre	Heracleum sphondylium	Parnassia palustris	Thlaspi arvense	
Anthemis arvensis	Cerastium pumilum	Erodium cicutarium	Herniaria glabra	Pedicularis palustris	Tordylium maximum	
Anthemis cotula	Chaerophyllum hirsutum	Eryngium campestre	Hieracium laevigatum	Persicaria hydropiper	Torilis japonica	
Anthriscus sylvestris	Chenopodium album	Eupatorium cannabinum	Hieracium murorum	Persicaria lapathifolia	Tragopogon dubius	
Anthyllis vulneraria	Chenopodium bonus-henricus	Euphorbia cyparissias	Hieracium pilosella	Persicaria maculosa	Tragopogon pratensis	
Aquilegia vulgaris	Chenopodium glaucum	Euphorbia exigua	Hieracium umbellatum	Picris hieracioides	Trifolium arvense	
Arabidopsis thaliana	Chondrilla juncea	Euphorbia helioscopia	Hippuris vulgaris	Pimpinella major	Trifolium campestre	
Arabis hirsuta	Chrysosplenium alternifolium	Fallopia convolvulus	Hyacinthoides non-scripta	Pimpinella saxifraga	Trifolium dubium	
Arenaria serpyllifolia	Chrysosplenium oppositifolium	Filago pyramidata	Hydrocotyle vulgaris	Pinguicula vulgaris	Trifolium repens	
Arnica montana	Cichorium intybus	Filipendula ulmaria	Hyoscyamus niger	Plantago coronopus	Verbascum thapsus	
Artemisia campestris	Circaea alpina	Filipendula vulgaris	Hypericum perforatum	Plantago lanceolata	Veronica arvensis	
Artemisia vulgaris	Circaea lutetiana	Fragaria vesca	Hypochaeris maculata	Plantago major	Veronica serpyllifolia	
Arum maculatum	Cirsium arvense	Fumaria officinalis	Hypochoeris radicata	Plantago media	Vicia sativa	

587

588 Table S3. List of the annual plant species used in the analyses.

589

590

A. thaliana	LMA	PH	SSD	LA	LNC	SM
LMA	8.73E-02	-3.15E-03	2.96E-03	8.47E-02	-1.10E-01	3.38E-04
PH	-3.15E-03	1.94E-03	-1.78E-04	9.51E-04	4.75E-03	1.06E-05
SSD	2.96E-03	-1.78E-04	1.50E-04	1.38E-03	-4.01E-03	1.06E-05
LA	8.47E-02	9.51E-04	1.38E-03	2.05E-01	-1.06E-01	2.51E-04
LNC	-1.10E-01	4.75E-03	-4.01E-03	-1.06E-01	1.72E-01	-4.34E-04
SM	3.38E-04	1.06E-05	1.06E-05	2.51E-04	-4.34E-04	9.73E-06

Annuals	LMA	PH	SSD	LA	LNC	SM
LMA	1.82E-02	-1.17E-03	3.22E-04	-4.52E-03	-1.30E-02	-1.75E-03
PH	-1.17E-03	8.88E-03	1.33E-04	1.33E-02	2.42E-03	-3.77E-04
SSD	3.22E-04	1.33E-04	5.88E-05	-1.71E-03	-3.47E-04	-9.53E-04
LA	-4.52E-03	1.33E-02	-1.71E-03	4.53E-01	3.23E-02	1.16E-01
LNC	-1.30E-02	2.42E-03	-3.47E-04	3.23E-02	3.13E-02	7.60E-03
SM	-1.75E-03	-3.77E-04	-9.53E-04	1.16E-01	7.60E-03	7.06E-02

591

592 Table S5. The estimated evolutionary variance-covariance matrix of the six traits under a
593 multivariate Brownian motion model. Diagonal elements represent rate estimates for
594 individual characters, while off-diagonal elements represent the estimated pairwise
595 covariances.

596

Annexe 2 - Climate as a driver of adaptive variations in ecological strategies in *Arabidopsis thaliana*

François Vasseur, Kevin Sartori, Etienne Baron, Florian Fort, Elena Kazakou, Jules Segrestin,

Eric Garnier, Denis Vile and Cyrille Violle.

Published

RESEARCH IN CONTEXT

Climate as a driver of adaptive variations in ecological strategies in *Arabidopsis thaliana*

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• **Background and aims** The CSR classification categorizes plants as stress tolerators (S), ruderals (R) and competitors (C). Initially proposed as a general framework to describe ecological strategies across species, this scheme has recently been used to investigate the variation of strategies within species. For instance, ample variation along the S–R axis was found in *Arabidopsis thaliana*, with stress-tolerator accessions predominating in hot and dry regions, which was interpreted as a sign of functional adaptation to climate within the species.

• **Methods** In this study the range of CSR strategies within *A. thaliana* was evaluated across 426 accessions originating from North Africa to Scandinavia. A position in the CSR strategy space was allocated for every accession based on three functional traits: leaf area, leaf dry matter content (LDMC) and specific leaf area (SLA). Results were related to climate at origin and compared with a previous study performed on the same species. Furthermore, the role of natural selection in phenotypic differentiation between lineages was investigated with Q_{ST} – F_{ST} comparisons, using the large amount of genetic information available for this species.

• **Key Results** Substantial variation in ecological strategies along the S–R axis was found in *A. thaliana*. By contrast with previous findings, stress-tolerator accessions predominated in cold climates, notably Scandinavia, where late flowering was associated with traits related to resource conservation, such as high LDMC and low SLA. Because of trait plasticity, variations in CSR classification in relation to growth conditions were also observed for the same genotypes.

• **Conclusions** There is a latitudinal gradient of ecological strategies in *A. thaliana* as a result of within-species adaptation to climate. Our study also underlines the importance of growth conditions and of the methodology used for trait measurement, notably age versus stage measurement, to infer the strength and direction of trait–environment relationships. This highlights the potential and limitations of the CSR classification in explaining functional adaptation to the environment.

Key words: *Arabidopsis thaliana*, adaptive differentiation, climate, CSR classification, ecological strategy, functional trait, genetic diversity, Grime triangle, latitudinal gradient, Q_{ST} – F_{ST} , trait–environment relationships.

INTRODUCTION

Screening approaches allow species comparison on the basis of key functional traits, i.e. traits representative of major functions, such as growth, stress resistance, defence and reproduction (Keddy, 1992; Violle *et al.*, 2007). Trait-based approaches in plant ecology have a long history of classifying plant species into functional groups according to the combination of phenotypic traits they exhibit (Garnier *et al.*, 2016). Such approaches have been mainly applied for comparative analyses at the inter-specific level to identify general patterns of trait variation and covariation. However, recent comparative analyses argue for a better integration of intraspecific variability for understanding the role of trait covariation in plant adaptation, ecosystem functioning and community assembly (Albert *et al.*, 2010, 2011; Violle *et al.*, 2012; Siefert *et al.*, 2015).

Amongst the prominent examples of plant species classification, Grime (1977) defined ecological strategies based on

the idea that there are two main ecological drivers of plant diversification: (1) the effect of stress related to the shortage of resources (e.g. nutrient, water and light); and (2) the effect of disturbance. Stress is viewed in this context as any environmental factors or combination of factors that reduce plant growth, although the shortage of nutrients, water or light can each affect specific traits (Grime and Hunt, 1975; Grime, 1977; Hodgson *et al.*, 1999). By contrast, disturbance is viewed as factors that cause the partial or total destruction of plant biomass, which include grazing, trampling and mowing, but also extreme climatic events such as severe drought, frost and fire (Grime and Hunt, 1975). Differences in disturbance and stress intensity are expected to result in quantitative variation in three ecological strategies: (1) stress tolerators (S) in stressed, resource-poor habitats with low disturbance, which invest resources to protect tissue from stress damages; (2) ruderals (R) in resource-rich environments associated with repeated disturbance, which

invest resources in rapid reproduction and propagule dispersal; and (3) competitors (C) in highly productive habitats with low stress intensity and disturbance, which invest resources in the rapid growth of large organs to outcompete neighbours. The S–R axis is traditionally viewed as an axis of resource-use variations at the leaf level (Pierce *et al.*, 2013), where ruderality is associated with acquisitive resource use (characterized by short-lived, flimsy leaves with high nutrient concentration and high net photosynthetic rate), and stress tolerance is associated with conservative resource use (characterized by long-lived, tough leaves with low nutrient concentration and low net photosynthetic rate). By contrast, variation in competitive ability along the C axis is thought to reflect variation in plant and organ size, and it is expected to operate where the impacts of stress and disturbance are low (Grime, 1977; Hodgson *et al.*, 1999).

Originally designed in the context of temperate herbaceous vegetation, the CSR scheme has been extended to other types of vegetation (Caccianiga *et al.*, 2006; Navas *et al.*, 2010; Schmidtlein *et al.*, 2012), including a recent worldwide application (Pierce *et al.*, 2017). An algorithm has recently been developed to quantify the CSR scores of diverse plant species based on the measurement of three leaf traits: leaf area (LA); specific leaf area (SLA); and leaf dry matter content (LDMC) (Pierce *et al.*, 2013, 2017). Albeit less precise than methods that consider whole-plant traits, which are more closely associated with stress response, competitive ability and ruderality (Hodgson *et al.*, 1999), classification tools based on a few leaf traits have the advantage that many measurements can be performed with minimal effort. This makes it possible to compare very ecologically disparate species (Pierce *et al.*, 2017), or many genotypes and populations within species (May *et al.*, 2017).

Arabidopsis thaliana is a small, rosette-shaped species that is widely used in molecular biology and quantitative genetics. It has recently gained renewed interest in evolutionary ecology due to the large collection of natural accessions collected from various climates and genotyped at high density (Weigel, 2012). Furthermore, *A. thaliana* has been shown to exhibit a significant range of phenotypic variation in relation to climate, making it possible to investigate the genetic and evolutionary drivers of functional diversification (Vasseur *et al.*, 2018). For instance, Q_{ST} – F_{ST} analysis has been proposed as a powerful way to discriminate adaptive and non-adaptive processes at the origin of phenotypic differentiation between genetic groups, populations or lineages (Leinonen *et al.*, 2013). Indeed, this method allows one to compare the level of phenotypic differentiation (Q_{ST}) with the genetic differentiation (F_{ST}) expected under the neutral model of population divergence. In plants, this has been used to investigate the role of selection at the origin of between-population phenotypic differences related to resource-use traits (Brouillette *et al.*, 2014), drought resistance (Ramírez-Valiente *et al.*, 2018), life history traits (Moyers and Rieseberg, 2016) and functional adaptation to an elevation gradient (Luo *et al.*, 2015).

Arabidopsis thaliana is generally described as a ruderal species that, like most annual plants, reproduces quickly and preferentially invests resources in the production and dispersal of propagules (Díaz *et al.*, 2016; Pierce *et al.*, 2017). In a recent paper, May *et al.* (2017) used the CSR framework to investigate intraspecific variation in ecological strategies within this

species. Using 16 accessions originating from contrasted climates in Europe, they found that *A. thaliana* actually exhibits a wide range of variation from ruderals to stress tolerators, with most accessions being classified as intermediate (SR) and none as a competitor. Interestingly, May *et al.* (2017) also found that ruderality was negatively correlated with the temperature at the site where the accession originated. For instance, stress tolerators originated predominantly from sites in hot climates (Libya, Sicily and Cape Verde Islands). However, May *et al.* (2017) used a relatively low number of accessions, which prevents examination of the evolutionary and adaptive bases of CSR variations with the environment.

In the present study, we analysed CSR variations in a set of 426 *A. thaliana* accessions originating from contrasting climates in Europe, North Africa and East Asia. Using the classification method based on three leaf traits (LA, SLA and LDMC) (Pierce *et al.*, 2017), we tested the range of ecological strategies exhibited by these accessions. We investigated whether the variation in strategies can be attributed to adaptive processes, using the genetic data available in this species to perform Q_{ST} – F_{ST} analysis. We also examined how CSR strategies measured with leaf traits correlated with whole-plant traits related to competitive ability (rosette size) and propagule dispersal (fruit number). Finally, we compared our results with the findings of May *et al.* (2017), and discuss the possible causes of differences between studies, such as the direction of trait–environment relationships.

MATERIALS AND METHODS

Plant material

Two experiments were performed in this study: the first one in the PHENOPSIS automaton (see below) and the second one in a greenhouse. In the first experiment we used a total of 400 natural accessions of *Arabidopsis thaliana* representative of a geographical sampling from the worldwide lines of the RegMap population (Horton *et al.*, 2012) ($n = 214$) and from French local populations (Brachi *et al.*, 2013) ($n = 186$). In the second experiment, we used a total of 200 accessions from a random sampling from the worldwide lines of the RegMap population. Overall, 426 accessions ranging latitudinally from North Africa to Scandinavia were phenotypically characterized, 172 of which were common to the two experiments (Supplementary Data Tables S1 and S2).

Experimental design

In Experiment 1 (PHENOPSIS), plants were grown in the high-throughput phenotyping platform PHENOPSIS (Granier *et al.*, 2006) in 2014, using one replicate plant per accession, except for Col-0 for which there were ten replicates. Seeds were stratified in the dark at 4 °C for at least 1 week before sowing to ensure homogeneous germination among genotypes. Four to six seeds were sown at the soil surface in 225-mL pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost. Prior to sowing, the soil surface was moistened with one-tenth strength Hoagland solution, and pots were kept in the dark for

48 h under controlled environmental conditions (20 °C, air relative humidity 70 %). Pots were then placed in the PHENOPSIS automaton growth chamber at 20 °C, daylength 12 h, relative humidity 70 % and photosynthetic photon flux density (PPFD) 175 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Pots were sprayed with deionized water three times per day until germination, and soil water content was then adjusted to 0.35 g $\text{H}_2\text{O g}^{-1}$ dry soil (soil water potential -0.07 MPa) to ensure optimal growth (Aguirrezábal *et al.*, 2006; Vile *et al.*, 2012; Vasseur *et al.*, 2014). After emergence of the fourth leaf, seedlings were thinned to keep only one plant in each pot.

In Experiment 2 (greenhouse), plants were grown in four replicates per accession in a greenhouse between December 2015 and May 2016. Seeds were sown on organic soil and stratified at 4 °C for 4 d. At the emergence of the first two true leaves, plants were transplanted into 300-mL individual pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost. Pots were randomly distributed among four blocks that were rotated every day in the greenhouse. All pots were watered twice a week. To reduce environmental heterogeneity in the greenhouse, walls were painted white and a semi-transparent curtain was installed below the glass roof. Additional light was provided to reach $\sim 65 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. Photoperiod and temperature were kept constant at day length 12 h and day/night temperatures of 18/16 °C, respectively.

Trait measurement

In both experiments, traits were measured following standardized protocols (Perez-Harguindeguy *et al.*, 2013) at a fixed phenological stage when flower buds were macroscopically visible [i.e. bolting stage, used as measurement of flowering time (FT)]. The lamina of a fully expanded, adult, non-senescent leaf exposed to light was detached from the rosette, kept in deionized water at 4 °C for 24 h for water saturation, and then weighed (mg). After determination of water-saturated mass, individual leaves were scanned for determination of LA (mm^2) using ImageJ (<https://imagej.nih.gov/ij/>). Dry mass of the leaf lamina was obtained after drying for 72 h at 65 °C. We calculated LDMC (mg g^{-1}) and SLA ($\text{mm}^2 \text{mg}^{-1}$) as the ratio of lamina dry and water-saturated masses and the ratio of lamina area to lamina dry mass, respectively (Perez-Harguindeguy *et al.*, 2013). In the PHENOPSIS, plants were harvested at first opened flower and rosette fresh mass (mg) was measured. In the greenhouse, plants were harvested after full senescence and the total number of fruits was manually counted on the inflorescence. Overall, out of the 400 and 200 accessions in PHENOPSIS and the greenhouse, respectively, 357 and 198 accessions were completely phenotyped for all traits (Supplementary Data Tables S1 and S2), with 152 accessions common to both experiments.

We calculated CSR scores (i.e. percentages along the C, S and R axes) for all accessions in the PHENOPSIS and the greenhouse based on the three traits LA, LDMC and SLA, using the recent method developed by Pierce *et al.* (2017). The method is based on an algorithm that combines data for three leaf traits (LA, SLA and LDMC) that were shown to reliably position the species in the CSR scheme. We calculated CSR scores for each

accession using the average trait value per experiment using the calculation table provided in the supplementary information of Pierce *et al.* (2017).

Re-analysis of published data

In our study there were several accessions in common with a previously published analysis of CSR variations in *A. thaliana* (May *et al.*, 2017). Ten accessions were common between May *et al.* and the PHENOPSIS experiment and six accessions in common with the greenhouse experiment. In May *et al.*, CSR scores were calculated based on six traits with a method previously proposed by Hodgson *et al.* (1999). To compare the two datasets, we first recalculated CSR scores from data given by May *et al.* with Pierce's method, using LA, LDMC and SLA provided for their 16 accessions (May *et al.*, 2017), and compared them with the CSR scores they measured with Hodgson's method.

Genetic analysis and Q_{ST} - F_{ST} comparisons

Genetic groups in *A. thaliana* were determined by clustering of 395 accessions for the PHENOPSIS dataset and 198 accessions for the greenhouse dataset, both using the 250 K single nucleotide polymorphism (SNP) data available in Horton *et al.* (2012). Clustering was performed with ADMIXTURE (Alexander *et al.*, 2009) after linkage disequilibrium pruning ($r^2 < 0.1$ in a 50-kb window with a step size of 50 SNPs) with PLINK (Purcell *et al.*, 2007), resulting in 24 562 independent SNPs. We assigned each genotype to a group if $>60\%$ of its genome derived from the corresponding cluster. The accessions not matching this criterion were labelled 'admixed' and were not used for the F_{ST} and Q_{ST} calculation. Cross-validation for different numbers of genetic clusters revealed that the PHENOPSIS dataset was composed of six genetic groups (group 1, 74 accessions; group 2, 48; group 3, 18; group 4, 55; group 5, 5; group 6, 71; admixed, 123), while the greenhouse dataset was composed of four genetic groups (group 1, 38 accessions; group 2, 16; group 3, 83; group 4, 7; admixed, 54). Consistent with the hypothesis of genetic divergence because of isolation by distance, these genetic groups were geographically clustered (Supplementary Data Fig. S1). We calculated Weir and Cockerham F_{ST} value for all 24 562 SNPs, and Q_{ST} as the between-group phenotypic variance divided by the total phenotypic variance, using mixed-effect models with group as random factor. We used a parametric bootstrap method to generate 95 % confidence intervals (CIs) around Q_{ST} values with the package MCMCglmm in R (R Core Team, 2014) (100 000 iterations).

Statistical analyses

Genotypic means in the greenhouse experiment were estimated as the fitted genotypic values from the linear models, using the lsmeans function. The genotype effect on trait variation and broad-sense heritability (H^2) were assessed using

individual data from the greenhouse experiment (Supplementary Data Table S3). The genotype effect was tested with one-way ANOVA following linear modelling, using genotype and block as explanatory variables. We measured H^2 as the ratio of phenotypic variance attributable to genotypic effect to total phenotypic variance, using mixed-effect models with block as fixed factor and genotype as random factor, using the package nlme in R.

Climate variables at the collection points of each accession were extracted from the Worldclim database (<http://www.worldclim.org/bioclim>), with resolution 2.5 arc-min. Trait–trait, trait–environment and trait–CSR relationships were examined with Spearman's rank coefficients of correlation (ρ) and associated P -values, using the function cor.test (Supplementary Data Table S4). Pearson coefficients of correlation (r) between traits and climatic variables were also calculated (Supplementary Data Table S5). Regression lines were drawn from Standard Major Axis (SMA), using the package smatr. All analyses were performed in R 3.2.3 (R Core Team, 2014).

RESULTS

Trait variation and covariation

All traits varied significantly among accessions (all $P < 0.001$; Supplementary Data Table S3). We found that FT ranged between 30 and 101 d (57 d on average) in the PHENOPSIS and between 25 and 115 d (61 d on average) in the greenhouse. Trait variation was mainly due to genetic variability among accessions, as measured by the high amount of phenotypic variance accounted for by the genotype effect (H^2 ranged between 0.58 for LA and 0.73 for SLA, 0.88 for FT; Supplementary Data Table S3). Most traits were correlated with each other (Supplementary Data Fig. S2, Tables S4 and S5): SLA and LDMC were negatively correlated (Spearman's $\rho = -0.94$ and -0.88 in the PHENOPSIS and the greenhouse, respectively; both $P < 0.001$) (Supplementary Data Fig. S2F), and FT was positively correlated with LDMC ($\rho = 0.63$ and 0.86 ; $P < 0.001$) (Supplementary Data Fig. S2B)

and negatively with SLA ($\rho = -0.73$ and -0.92 ; both $P < 0.001$) (Supplementary Data Fig. S2D).

CSR classification

Arabidopsis thaliana accessions mainly varied along the S–R axis, between purely ruderals (R) and moderate stress tolerators (S/SR) (Fig. 1). We found only three accessions (together $<1\%$) classified as CS, CR or CSR. The accessions were mainly R-oriented: R, R/CR, R/CSR and R/SR represented 84 and 91 % of all accessions in the PHENOPSIS and the greenhouse, respectively (Table 1). Although we calculated CSR scores with only three leaf traits using Pierce's method, whole-plant traits were consistent with our classification. For instance, the C-axis is expected to be related to plant size and height, while the R-axis is expected to be related to FT and seed dispersal (Grime, 1977; Hodgson et al., 1999). Accordingly, we found that the C- and R-axes were positively but poorly correlated with rosette fresh mass and the total number of fruits, respectively ($\rho < 0.50$, $P < 0.05$; Supplementary Data Fig. S3).

The CSR scores were significantly correlated between the PHENOPSIS and greenhouse experiments, as measured across the 152 accessions common to both experiments ($\rho = 0.34$, 0.41 and 0.54 for C, S and R, respectively, all $P < 0.001$; Supplementary Data Fig. S4). However, they were also significantly different between the two experiments ($P < 0.01$ for all the three scores). Accordingly, 78 accessions (51 %) were classified in different CSR groups between the two experiments ('plastic' accessions hereafter). Globally, plastic accessions shifted towards more ruderal strategies in the greenhouse compared with the PHENOPSIS, as reflected by the differences in S and R scores between experiments (Fig. 2). Twenty-two percent of the plastic accessions were classified as R in the PHENOPSIS and R/CR in the greenhouse (inversely, 18 % were classified as R/CR in the PHENOPSIS and R in the greenhouse). Comparatively, C scores did not differ a lot between the two experiments (Fig. 2B).

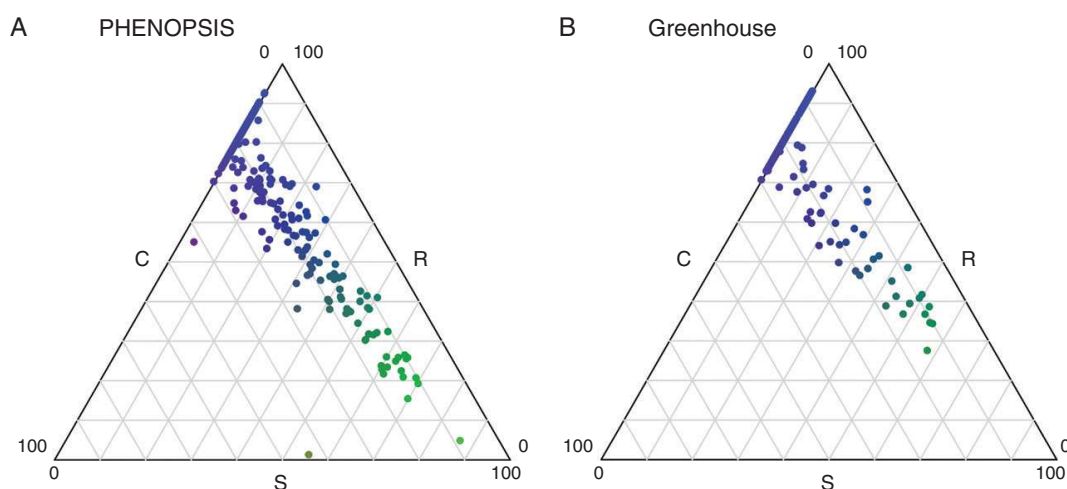


FIG. 1. CSR variation (%) in *A. thaliana*. (A) CSR representation of the 357 accessions from the PHENOPSIS. (B) CSR representation of the 198 accessions from the greenhouse. Dots are coloured according to CSR score following the colour code provided in Pierce et al. (2017).

TABLE 1. Proportion (%) of ecological strategies among *A. thaliana* accessions.

	PHENOPSIS	Greenhouse	Original scores from May et al. (2017)	Recalculated scores with data from May et al. (2017)
R	25.5	24.2		31.3
R/CR	47.6	58.6	12.5	
R/CSR	8.7	7.1		
R/SR	2.2	1.5	6.2	6.2
SR/CSR	8.4	4.5		12.5
SR	0.6	3.5	56.3	25.0
S	0.2			
S/CSR	3.1	0.6		18.7
S/SC			25.0	
S/SR	2.8			6.3
CSR	0.3			
CS	0.3			
CR	0.3			

Relationships between CSR scores, flowering time and climate

Ruderality was positively correlated with SLA and mean annual temperature (MAT, °C) at the collection point of the accessions, but negatively with FT and LDMC (Fig. 3; Supplementary Data Tables S4 and S5). Thus, our results suggest that ruderality is typical of early-flowering plants with leaf traits representative of fast resource acquisition, as reflected by low LDMC and high SLA values (Wright et al., 2004; Shipley et al., 2006). Inversely, stress tolerators were characterized by late flowering, with resource-conservative trait values such as high LDMC and low SLA, which were negatively correlated with MAT (Supplementary Data Fig. S5). Consistently, S and R strategies were positively and negatively correlated with latitude, respectively (Supplementary Data Table S4).

The Q_{ST} - F_{ST} analysis suggested that the latitudinal variations in CSR strategies resulted from adaptive processes such

as natural selection acting on leaf traits. Indeed, a value of Q_{ST} significantly higher than F_{ST} at neutral loci is generally considered a signature of diversifying selection on the underlying traits (Leinonen et al., 2013). Here, we used the 95th quantile of the F_{ST} distribution genome-wide as a threshold of significance for phenotypic differentiation above neutral expectation. In the greenhouse, both S and R scores were significantly above neutral F_{ST} ($Q_{ST} = 0.95$, 95 % CI 0.72–1.00 for S; $Q_{ST} = 0.82$, 95 % CI 0.62–1.00 for R; mean $F_{ST} = 0.09$ and F_{ST} 95th quantile = 0.35; Fig. 4A). In the PHENOPSIS, only R scores were above, but not significantly above, neutral F_{ST} ($Q_{ST} = 0.37$ versus F_{ST} 95th quantile = 0.33). The S scores were slightly, and non-significantly, below the neutral expectation ($Q_{ST} = 0.29$, 95 % CI 0.10–0.80; Fig. 4C). By contrast, in both the greenhouse and the PHENOPSIS, Q_{ST} values of C scores were close to 0, suggesting that this axis of plant strategies did not vary under the influence of adaptive processes in *A. thaliana*. The lower Q_{ST} values reported for the PHENOPSIS experiment can be explained by the absence of individual replicates in this experiment. By contrast, using the genotypic mean in the greenhouse across four replicates made it possible to reduce intra-genotypic variance and thus total phenotypic variance compared with phenotypic variance between genetic groups. Consistent with these results, plotting the distribution of *A. thaliana* ecological strategies across Europe (Fig. 4B, D) revealed that accessions with S-oriented strategies (S, SR, SR/CSR, S/CSR, S/SC, SC and SC/CSR) originated from northern regions, Sweden in particular.

Comparison with observations from May et al. (2017)

In contrast with our results, the 16 accessions in the study published by May et al. were mainly categorized as S-oriented: S/SC, S/SR, S/CSR, SR and SR/CSR (Table 1; Supplementary Data Fig. S6). May et al. used Hodgson's method to calculate

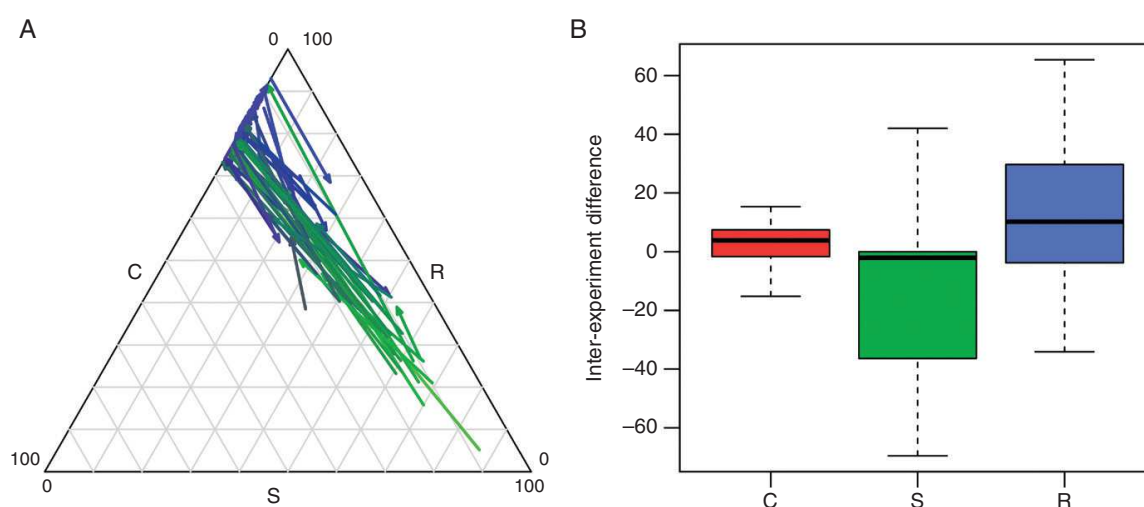


FIG. 2. Plasticity of CSR classification in *A. thaliana*. (A) The 78 plastic accessions that have a different CSR classification (%) between the PHENOPSIS and greenhouse experiments are plotted. Arrows start at the greenhouse position and end at the PHENOPSIS position, and are coloured according to CSR scores in the PHENOPSIS, following the colour code provided in Pierce et al. (2017). (B) Boxplot representing the difference in CSR scores (%) between experiments (greenhouse values minus PHENOPSIS values).

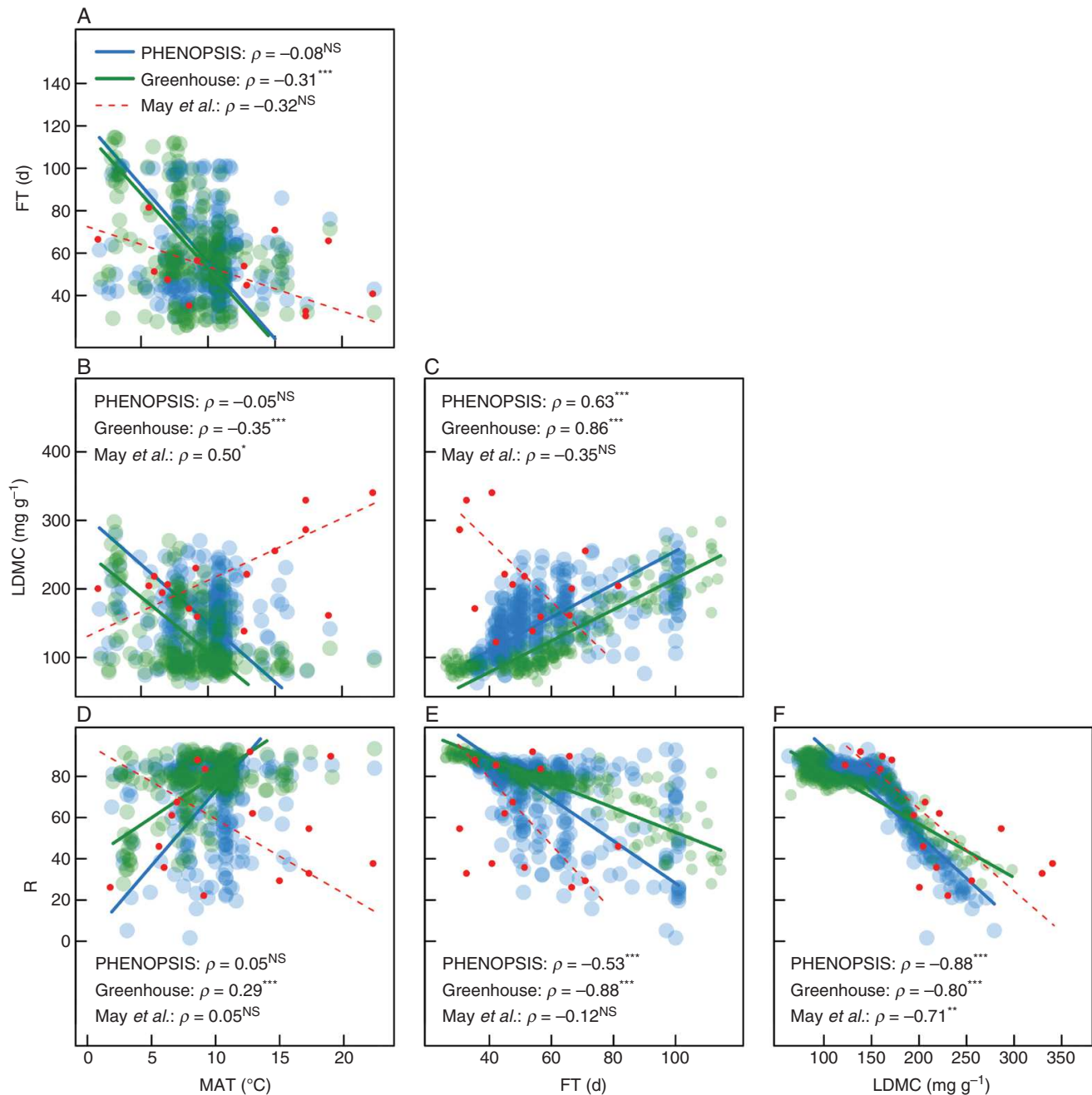


FIG. 3. Relationships between ruderality, traits and environment in *A. thaliana*. Leaf trait and flowering time data were obtained from May et al. (2017) (red dots, $n = 16$), the PHENOPSIS (green dots, $n = 357$) and the greenhouse (blue dots, $n = 198$). Ruderality (R) was calculated with Pierce's method (2017) for all data. Mean annual temperature (MAT, °C) was extracted at the collection point of the accessions using Worldclim. NS, $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Regression lines were drawn using standard major axis (SMA).

CSR scores with seven traits, including FT and duration, two important components of ruderality (Hodgson et al., 1999). To compare the two classification methods, we used the trait values for LA, SLA and LDMC provided by May et al. to calculate CSR scores with Pierce's method and compared them with those calculated with Hodgson's method. The CSR scores calculated with the two methods were positively correlated

($\rho = 0.77$, 0.79 and 0.73 for C, S and R, respectively; all $P < 0.01$; Supplementary Data Fig. S7), suggesting that the two methods return similar categorizations (Table 1). However, and despite the significant correlations, CSR scores varied substantially between the two methods (Supplementary Data Fig. S7). This showed that the traits related to ruderality (FT and duration) and competition (plant height and lateral spread) used in

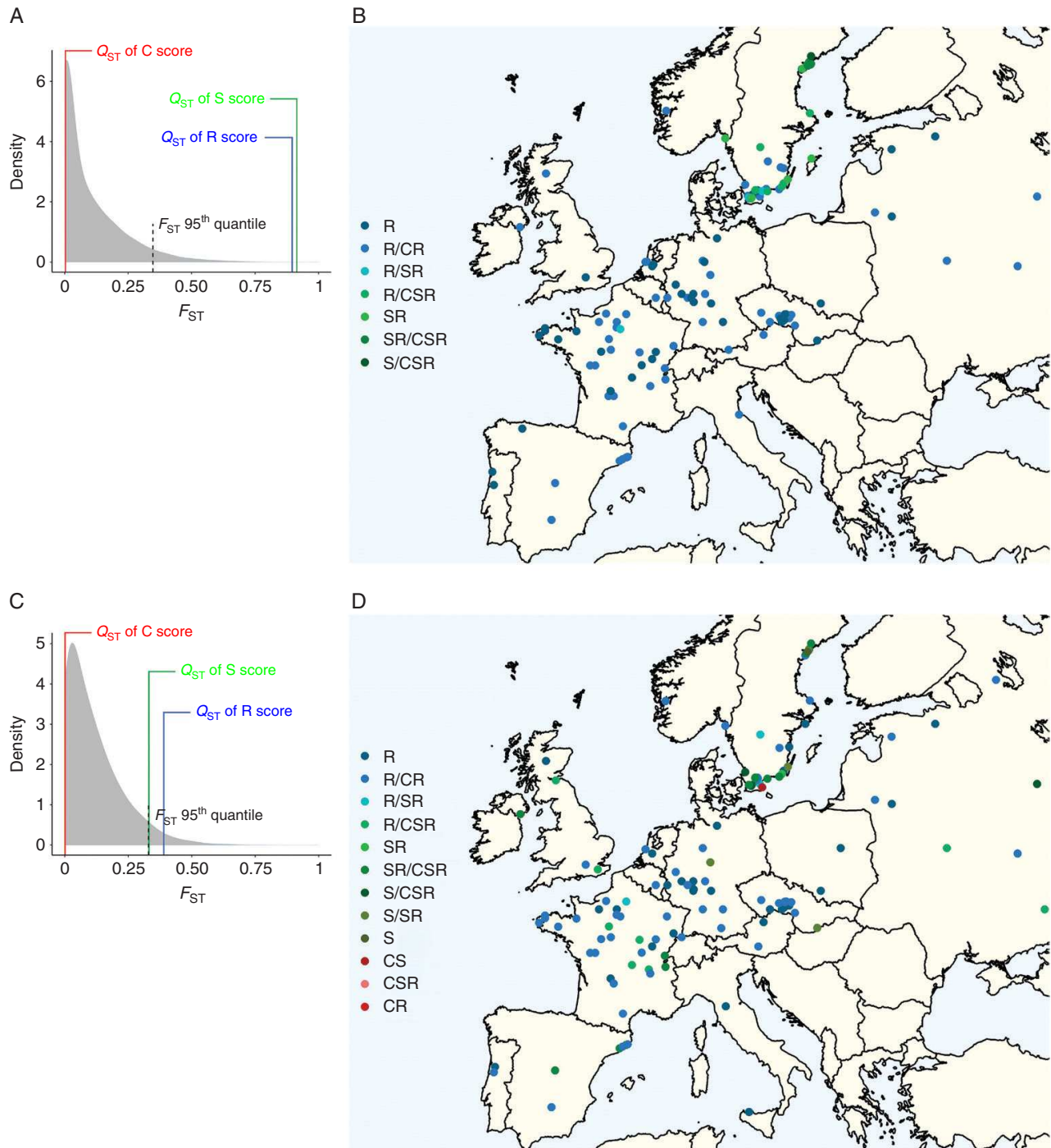


FIG. 4. Q_{ST} - F_{ST} analysis and geographical location of CSR strategies in *A. thaliana*. Distribution (in grey) of F_{ST} values across the 24 562 SNPs with the 95th quantile threshold of non-neutral expectation (dashed line), and Q_{ST} values for the C, S and R scores measured as the ratio of phenotypic variance between genetic groups over total phenotypic variance. (A, C) Analysis was performed independently on the greenhouse dataset (A) and the PHENOPSIS dataset (C). (B, D) Geographical location of CSR strategies with the greenhouse dataset (B, $n = 198$) and PHENOPSIS dataset (D, $n = 357$). Note that not all CSR strategies are found equally across Europe (for frequencies see Table 1).

Hodgson's method impacted the inference of plant ecological strategies compared with leaf traits alone.

Values of FT measured in this study were strongly positively correlated with those measured by May *et al.* under controlled

conditions ($n = 10$ and 6 in the PHENOPSIS and the greenhouse, respectively, both $r = 0.96$, $P < 0.01$; Supplementary Data Fig. S8A). By contrast, LDMC measured by May *et al.* was negatively correlated with our measurements (Supplementary

Data Fig. S8D), possibly because of three individuals with early FT and extremely high LDMC values ($>250 \text{ mg g}^{-1}$) compared with our measurement ($<110 \text{ mg g}^{-1}$) on the same accessions (red dots in Fig. 3C). As a result, FT and LDMC were negatively, albeit non-significantly, correlated in the May *et al.* study ($\rho = -0.35$; Fig. 3C). Furthermore, there was a positive correlation between LDMC and MAT in May *et al.* ($P < 0.05$), while we found the opposite in both the PHENOPSIS and the greenhouse (Fig. 3B). By construction of the CSR classification method, LDMC participates strongly in the S and R axes (Fig. 3F; Supplementary Data Fig. S5). Consequently, the positive correlation between LDMC and MAT found in May *et al.* was associated with a positive correlation between S and MAT (Supplementary Data Fig. S5D), and inversely a negative correlation between R and MAT (Fig. 3D), although these two relationships were not significant with the 16 accessions from May *et al.* when using Pierce's method of CSR classification.

DISCUSSION

Functional adaptation to climate in *A. thaliana*

The relationship between CSR and climate at the interspecific level is still not well established (Pierce *et al.*, 2017). More broadly, trait–environment relationships remain a central question in functional ecology and functional biogeography (Poorter *et al.*, 2009; Violle *et al.*, 2014; Borgy *et al.*, 2017; Butler *et al.*, 2017; Šímová *et al.*, 2018). By contrast, adaptation to climate has been widely studied within species, notably genetic adaptation along latitudinal or altitudinal gradients in annual plants, and in *A. thaliana* in particular (Johanson *et al.*, 2000; Picó *et al.*, 2008; Banta *et al.*, 2012; Guo *et al.*, 2012; Brachi *et al.*, 2013; Wolfe and Tonsor, 2014; Bloomer and Dean, 2017; Tabas-Madrid *et al.*, 2018). Indeed, *A. thaliana* has been the model species in molecular biology, plant genetics and evolution in recent decades (Bergelson and Roux, 2010; Weigel, 2012). It is widely distributed in various climates, but is generally considered as a ruderal species that grows fast, reproduces early and dies right after seed dispersal (Pierce *et al.*, 2017). As expected, we found in this study that *A. thaliana* was predominantly ruderal, secondly a stress tolerator and poor competitor. However, we showed an important range of CSR variation among *A. thaliana* accessions along the S–R axis and associated with FT variation.

Consistent with previous studies, FT was positively correlated with latitude (Caicedo *et al.*, 2004; Lempe *et al.*, 2005; Banta *et al.*, 2012). For instance, northern accessions exhibit late flowering and a long life cycle even when they are grown under controlled conditions in a growth chamber or greenhouse (Vasseur *et al.*, 2018). Our results showed that FT was positively correlated with LDMC, and that values for the two traits were higher in accessions originating from higher latitudes and lower temperatures. Thus, northern accessions exhibit a suite of traits associated with resource conservation and longevity, such as late flowering, high LDMC and low SLA (Wright *et al.*, 2004; Shipley *et al.*, 2006; Vasseur *et al.*, 2012). The $Q_{ST}-F_{ST}$ analysis revealed that these latitudinal variations result from the adaptive diversification of leaf traits. These adaptive shifts can be explained because, in cold regions, biomass production during

the growing season is limited by various stresses. Low temperatures directly limit plant growth rate by slowing metabolic processes. Furthermore, cold indirectly limits plant growth rate because of the reduction in the availability of water and nutrients. In these conditions, a slow-growing genotype with a long life cycle, associated with high LDMC, low SLA and low metabolic activities, can be an efficient strategy. Interestingly, stress tolerance has been shown to be selected at both ends of the geographical range of *A. thaliana*, but is expressed under different temperature conditions (Exposito-Alonso *et al.*, 2018; Vasseur *et al.*, 2018).

Conversely, ruderal strategies were more abundant in temperate and hot environments. Ruderal plants are typically associated with a short life cycle, low LDMC and high SLA, and presumably high metabolic rate and low tissue protection (Grime, 1977). In temperate climates with a relatively long growing season and high resource availability, these characteristics may allow *A. thaliana* individuals to complete their growth cycle early and avoid competition with taller species. Furthermore, in hot and dry climates with a shorter growing period (e.g. the Mediterranean climate), fast-growing strategies may allow *A. thaliana* individuals to complete their growth cycle and disperse before the onset of drought, which operates as a disturbance rather than a stress, and should therefore be more favourable to ruderality (Madon and Médail, 1997; Volaire, 2018). This result is consistent with interspecific studies at global scale that reported a positive relationship between SLA and temperature in herbaceous species (Borgy *et al.*, 2017; Šímová *et al.*, 2018). This can be interpreted as a sign of selection for fast-growth ruderal strategies in hot and stressing environments at both intra- and interspecific levels (Anderegg *et al.*, 2018).

The lack of adaptive differentiation between genetic groups along the C axis, as reflected by the low Q_{ST} values compared with neutral F_{ST} , can be explained by the low variation in competitive ability among *A. thaliana* accessions. Additionally, it could suggest that competitive environments can be found in various climates as long as stress does not dominate vegetation processes. This would also explain the lack of a clear geographical pattern and latitudinal gradient of competitive ability across plant populations and species (Damgaard and Weiner, 2017).

Influence of classification methodologies, trait measurement and growth conditions on trait–environment relationships

Trait–trait, trait–CSR and trait–environment relationships were sometimes opposite between May *et al.* (2017) and our study. For instance, May *et al.* reported a positive correlation between stress tolerance and mean temperature, while we found the opposite. A first explanation of these differences is the methods used to calculate CSR scores among accessions. Although Pierce's and Hodgson's scores were all positively correlated when performed on the same set of traits and accessions, scores obtained from the two methods varied substantially. For instance, an accession had an S score of 35 % with Hodgson's method but 0 % with Pierce's method (Supplementary Data Fig. S7B). The re-analysis of the data of May *et al.* (2017) made

by the authors of that paper (A. Wingler, University College Cork, Ireland, pers. comm.) indicated that the three accessions with very high values for S identified using Hodgson's method (Mt-0, Cvi-0 and Ct-1) were no longer in the top three ranked accessions for S when using Pierce's method, which led to a lack of correlation of S and R with temperature when using this method. This can be explained because life history traits at whole-plant level, notably FT and plant size, are important components of ruderality and competitive ability in herbaceous species (Violle *et al.*, 2009; Hodgson *et al.*, 2017), but they are not included in Pierce's method of CSR classification. Here, we found that C and R axes calculated with leaf traits were positively, but poorly, correlated with rosette fresh mass and fruit number, respectively. Additionally, many early-flowering accessions were similarly classified as purely ruderal (R = 100 %), although they displayed variations in leaf traits and FT, and consequently in their level of ruderality. This was translated into no or small differences in CSR strategies between accessions from temperate and Mediterranean climates (Fig. 4), although Mediterranean accessions can be very short-lived and thus more ruderal than accessions from less stressing environments (Vasseur *et al.*, 2018). Together, this suggests that classification methods based on leaf traits can be a powerful means of screening large databases or performing many measurements at global scale, but might be of limited value in the examination of subtle variations within species and/or in specific taxa. For instance, including other, easily measurable traits might be necessary to better describe ruderality in annual plants, such as phytomer miniaturization and the number of juvenile phytomers, because each promotes early maturity (Hodgson *et al.*, 2017).

A second explanation of the opposite trait–environment relationships found between this study and May *et al.* (2017) is the difference in the protocols used for trait measurement. In our experiments, we followed the recommended procedures to phenotypic traits of all individuals at the same ontogenetic stage (Reich *et al.*, 1999; Perez-Harguindeguy *et al.*, 2013). Specifically, LDMC and SLA were measured at the transition to flowering (i.e. bolting stage). By contrast, leaf traits were measured in a growth chamber at the same age by May *et al.* (61 d for LDMC), although FT in the growth chamber varied from 30 to 82 d (and some accessions did not flower at all), and although it is widely recognized that leaf traits strongly vary during plant ontogeny (Walters *et al.*, 1993; Hérault *et al.*, 2011; Pantin *et al.*, 2012). In other words, LDMC was measured 30 d after flowering for the earliest accessions and before flowering for the latest ones. With such a procedure, the leaves compared might have been in contrasted physiological stages. In particular, leaves measured on the early-flowering accessions might have been – at least in part – senescing, which may result in much higher LDMC values – and lower SLA values – in these accessions (Fig. 3C). In agreement with this hypothesis, the LDMC values measured on the early flowering accessions in our experiment were approximately half of the values estimated by May *et al.* As LDMC strongly participates in the S–R axis, this could explain the opposite correlations between CSR and environment between the two studies. Furthermore, we found that FT was positively correlated with LDMC, consistently with previous studies in a smaller set of accessions (Vile *et al.*, 2012), as well as in recombinant inbred lines (El-Lithy *et al.*,

2010; Vasseur *et al.*, 2012, 2014). Previous studies have notably reported that early-flowering genotypes have resource-acquisitive strategies, characterized by high SLA but low LDMC and short lifespan (El-Lithy *et al.*, 2010; Vasseur *et al.*, 2012, 2014, 2018; Blonder *et al.*, 2015).

Finally, opposite correlations between studies might also result partly from trait plasticity to growth conditions. In *A. thaliana* FT is expected to vary with light conditions and temperature (Mouradov *et al.*, 2002). For instance, *A. thaliana* does not generally flower under short-day conditions. In our study, traits were measured in controlled and constant conditions, on plants grown in a 12-h photoperiod and without cold exposure (i.e. vernalization). However, we could expect FT and leaf traits, and thus CSR–environment relationships, to be different when measured on plants grown outside, as in May *et al.* (2017), after vernalization or in short- or long-day conditions. Consistent with this idea, we found that half the accessions common to the PHENOPSIS and the greenhouse did not have the same position in the CSR space: plants grown in the greenhouse were generally shifted towards the R end of the spectrum compared with plants grown in the PHENOPSIS. This can be explained by the relative low light intensity provided by artificial lamps in the greenhouse compared with the phenotyping platform (65 versus 175 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD). In addition, plants were grown in the greenhouse at higher density than in the PHENOPSIS, which could have increased competition for light between plants. The shade-avoidance syndrome has been described as a suite of leaf trait responses to low light and competition (Kim *et al.*, 2005; Mullen *et al.*, 2006). This includes an increase in leaf angle and SLA, associated with a reduction in LDMC and FT (Kim *et al.*, 2005; Vasseur *et al.*, 2011). This is consistent with a shift towards resource-acquisitive strategies in the greenhouse. Importantly and more broadly, controlled conditions are very different from the natural conditions that plants experience in the wild, and where plants should ideally be measured to properly infer their ecological strategies. However, it remains difficult to take into account genotype $\times$ environment interactions when screening genotypes in natural conditions. Consequently, trait-based approaches for the functional classification of plants were initially proposed as a tool to infer the adaptive significance of traits in controlled conditions (Grime and Hunt, 1975).

Conclusions

Intraspecific variation in functional strategies varied substantially along the S–R axis in *A. thaliana*. Tolerance to stress seems to be favoured in cold environments at higher latitudes while ruderality is predominant in temperate and hot climates. However, CSR categorization within species, specifically in a herbaceous species like *A. thaliana*, is sensitive to several parameters, such as the type of traits used to classify accessions and the protocols used for trait measurement. Furthermore, our results suggest that phenotypic plasticity to growth conditions can significantly impact trait values and thus the determination of plant ecological strategies. This suggests that the use of trait databases for local or global analyses of trait–environment relationships at species level might suffer from biases due to both phenotypic plasticity and intraspecific trait variation. In a

recent analysis, ruderality has been demonstrated to correlate positively with the probability of naturalization of alien species (Guo *et al.*, 2018). In future studies it will be interesting to examine in more detail the response of traits, trait combinations and strategies to environmental conditions. For instance, analysing the plasticity of CSR strategies to different temperatures and water stresses could reveal whether S-related strategies are constitutive or stress-induced, and whether invasive species show greater plasticity in ecological strategies than other species.

SUPPLEMENTARY DATA

Supplementary Data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: phenotypic traits measured in the PHENOPSIS experiment. Table S2: phenotypic traits measured in the greenhouse experiment. Table S3: heritability and genetic effects on traits measured in the greenhouse experiment. Table S4: Spearman's pairwise correlations between traits and environments. Table S5: Pearson's pairwise correlations between traits and environments. Fig. S1: geographical location of the genetic groups defined by SNP clustering. Fig. S2: trait–trait relationships in *A. thaliana*. Fig. S3: correlations between C and R axes, plant biomass and fruit number. Fig. S4: correlations between CSR scores in the PHENOPSIS and greenhouse experiments. Fig. S5: CSR–trait and CSR–environment relationships in *A. thaliana*. Fig. S6: CSR representation of the 16 accessions from May *et al.* (2017). Fig. S7: correlation between Hodgson's and Pierce's methods for quantifying CSR. Fig. S8: correlations between traits measured by May *et al.* (2017) and the present study.

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Annexe 3 -

Secondary metabolites have more influence than morphophysiological traits on litter decomposability across genotypes of *Arabidopsis thaliana*

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Published.

Secondary metabolites have more influence than morphophysiological traits on litter decomposability across genotypes of *Arabidopsis thaliana*

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Summary

- Although interspecific variation in plant phenotype is recognised to impact afterlife processes such as litter decomposability, it is still unclear which traits and selection pressures explain these relationships. Examining intraspecific variation is crucial to identify and compare trait effects on decomposability, and investigate the potential role of natural selection.
- We studied the genetic variability and relationships between decomposability, plant traits typically related to decomposability at species level (morphophysiological traits), and leaf metabolites among a set of genotypes of *Arabidopsis thaliana* grown under controlled conditions. We also investigated correlations between decomposability and environmental variables at genotypes collection site. We investigated the genetic architecture of decomposability with genome-wide association studies (GWAS).
- There was large genetic variability in decomposability that was correlated with precipitation. Morphophysiological traits had a minor effect, while secondary metabolites, especially glucosinolates, were correlated with decomposability. Consistently, GWAS suggested that genes and metabolites related to the composition of cell membranes and envelopes control the variation of decomposability across genotypes.
- Our study suggests that decomposability varies within species as a result of metabolic adaptation to climate. Our findings highlight that subtle variations of defence-related metabolites like glucosinolates may strongly influence after-life processes such as decomposability.

Introduction

Tremendous efforts in trait-based ecology have helped us elucidate the drivers of key ecological processes involved in carbon and nutrient cycling, including litter decomposability (Chapin, 1980; Coûteaux *et al.*, 1995; Swift *et al.*, 1998; Cornwell *et al.*, 2008; Garnier *et al.*, 2016). Phenotypic drivers of litter decomposability have been mainly studied by comparative approaches at the interspecific level, which allow generalisations and predictions (Shipley, 2007; Garnier *et al.*, 2016), but remain limited in explanatory power. It is crucial to take into account intraspecific variation to understand the rules that determine community assembly and ecosystem functioning (Crutsinger *et al.*, 2009; Des Roches *et al.*, 2017). Recent studies have showed that there are similar levels of intraspecific and interspecific trait variations (Albert *et al.*, 2010). Moreover, it has been suggested that genetic diversity within plant populations could alter key ecological processes such as litter decomposability (Wright *et al.*, 2016).

Exploring the effect of genetic variability on the traits underlying these processes can be a way to reconcile ecological and evolutionary drivers (e.g. Donovan *et al.*, 2011). Here, using a large set of *Arabidopsis thaliana* natural genotypes, we evaluated how much variation in litter decomposability was correlated with genetic variation in morphophysiological traits, including leaf functional traits and plant life history traits, and leaf metabolism such as the abundance of secondary metabolites.

Among the different factors expected to impact litter decomposability, that is litter mass loss measured in standardised conditions, interspecific variation in leaf structure and physiology has important 'after-life effects' on litter quality (Cornelissen & Thompson, 1997; Cornelissen *et al.*, 1999; Santiago, 2007; Parsons & Congdon, 2008). In particular, the trade-off between carbon acquisition and nutrient retention at the leaf level (the so-called 'leaf economics spectrum', LES, Wright *et al.*, 2004) involves a suite of leaf functional traits that explain variation in leaf decomposability across species (Cornwell *et al.*, 2008). For instance, specific leaf area (SLA; leaf area divided by leaf dry mass) tends to be negatively associated with high foliar

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concentrations of structural (e.g. lignin) or chemical compounds that promote protection against herbivores or the abiotic environment (Choong *et al.*, 1992; Grime *et al.*, 1997). SLA is related to leaf thickness and tissue density, and is positively correlated with litter decomposability across species (e.g. Cornelissen *et al.*, 1999; Vaieretti *et al.*, 2005; Santiago, 2007; Kurokawa & Nakashizuka, 2008). By contrast, leaf dry matter content (LDMC; ratio leaf dry: fresh mass; (Kazakou *et al.*, 2006; Cortez *et al.*, 2007; Kurokawa & Nakashizuka, 2008) and leaf toughness (Cornelissen & Thompson, 1997; Cornelissen *et al.*, 1999; Kazakou *et al.*, 2009) have a negative effect on litter decomposability. Finally, physical antiherbivore defence, such as trichome density at the leaf surface, can also affect decomposition and nutrient release (Gallardo & Merino, 1993; Cornelissen & Thompson, 1997). Species with long-lived leaves (high leaf lifespan, LLS) are often characterised by higher concentrations of lignin but lower concentrations of N and P (Coley, 1988). Evidence that the litter decomposability depends on the lignin : N ratio as well as on leaf N and C concentrations (Aerts, 1997; Cornelissen & Thompson, 1997) suggests that there is a link between plant physical defence and litter decomposability (Grime *et al.*, 1996; Cornelissen *et al.*, 1999).

As suggested in the review of Chomel *et al.* (2016), the relationships between morphophysiological traits and litter decomposability are often idiosyncratic, which can be explained by the key role played by secondary metabolites. Several studies have demonstrated that certain secondary metabolites, especially polyphenolics, can limit growth and activity of decomposers, from microorganisms to macroarthropods (Hättenschwiler & Vitousek, 2000; Loranger *et al.*, 2002; Chomel *et al.*, 2016). Litter decomposability is generally negatively correlated with the concentration of lignin, a group of complex aromatic polymers present in plant cell walls that is recalcitrant to enzymatic degradation and serves as a structural barrier impeding microbial access to labile carbon compounds (Austin & Vitousek, 2000). Most studies linking secondary metabolites and decomposability have focused only on lignin, polyphenols and tannins. However, as suggested by Chomel *et al.* (2016), the secondary metabolites present in the leaf and subsequently in the litter should be considered as a key factor influencing decomposer activity. For instance, variation in the abundance and diversity of defence metabolites could also have after-life effects on tissue degradation by decomposers. Nonetheless, no intraspecific study has investigated and compared the respective role of plant morphophysiological traits, including leaf traits typically related to decomposability at species level, to a large number of secondary metabolites potentially involved in litter decomposability.

A critical step in trait-based ecology is to evaluate the extent of genetic variation within species. Recent research has emphasised the importance of intraspecific variation on ecosystem functioning, including primary productivity (Zhu *et al.*, 2000; Crutsinger *et al.*, 2006), ecosystem stability (Hughes & Stachowicz, 2004; Reusch *et al.*, 2005) and community structure (Violle *et al.*, 2012). Like plant species, different plant genotypes can vary considerably in the quantity and quality of leaf litter produced, creating genotype-specific differences in rates of decomposition and

nutrient release (Driebe & Whitham, 2000; Treseder & Vitousek, 2001; Madritch & Hunter, 2004; Madritch & Hunter, 2005; Schweitzer *et al.*, 2005; Silfver *et al.*, 2007). Most studies on intraspecific variation in decomposability have focused on tree species (for example Silfver *et al.*, 2007 for *Quercus* and *Alnus* species and Silfver *et al.*, 2015 for *Betula pendula* species) or aquatic species (Lecerf & Chauvet, 2008). Plants have been compared between few genetically distinct genotypes (Treseder & Vitousek, 2001; Jackson *et al.*, 2013), or from hybrids and their parental species (Driebe & Whitham, 2000; Schweitzer *et al.*, 2004). For instance, Silfver *et al.* (2007) found that 19 genotypes of *Betula pendula* had substantial genotypic variation in leaf litter mass loss at the early stages of the decomposition process, and that this variation was associated with genotypic variation in herbivore resistance and leaf concentrations of soluble proteins and total nitrogen (N). Hines *et al.* (2013) found that variation in litter chemistry among 12 genotypes of *P. australis* can be as great as, or greater than, variation reported among species (Cornwell *et al.*, 2008) or in response to environmental variation (Reich & Oleksyn, 2004; Wright *et al.*, 2004; Santiago, 2007). Finally, Lecerf & Chauvet (2008) showed that intraspecific variation in decomposability of senescent leaves of alder (*Alnus glutinosa*) was within a range similar to that reported for interspecific variation among co-occurring riparian plant species in Europe. By contrast, Crutsinger *et al.* (2009) found that *Solidago altissima* genotypic effects being much weaker than species-level effects as variation among other goldenrod species in decomposition rate was more than twice that of genetic variation within *S. altissima*. Therefore, the significance of within-species variation is still poorly known (Silfver *et al.*, 2009, 2015), although it is essential to perform intraspecific analysis where phylogenetic correlation do not obscure causation. For instance, using a large number of genotypes covering a wide geographical range would enable quantitative genetic analyses – such as genome-wide association studies (GWAS) – and population genetics analyses, a prerequisite for inferring evolution and adaptation. To date, the genes and molecular pathways that influence litter decomposability still remain unknown.

The model species *A. thaliana* is promising to examine the phenotypic and genetic drivers of litter decomposability. Indeed, *A. thaliana* has been widely studied in molecular biology, cell biology and quantitative genetics because of its small stature and short life cycle (e.g. Clark *et al.*, 2007; Platt *et al.*, 2010; Horton *et al.*, 2012; Alonso-Blanco *et al.*, 2016). The genomic sequencing of natural populations in this species allows performing GWAS to examine the genetic architecture of litter decomposability and identify candidate genes. Combined with its wide distribution across contrasted climates and its important phenotypic variability, *A. thaliana* is also becoming a species of interest for trait-based ecology. For instance, recent studies have shown the same patterns of trait covariations in *A. thaliana* and across species, such as relationships between SLA, LNC, leaf lifespan and photosynthetic rate (Vasseur *et al.*, 2012, 2018b). In addition, high-throughput metabolomics has been successfully performed across hundreds of *A. thaliana* genotypes (Wu *et al.*, 2018). This generated massive and unprecedented data to

investigate the role that leaf metabolites could have on specific ecological processes. For instance, glucosinolates are important secondary metabolites involved in plant defence among species of the Brassicaceae family. In *A. thaliana*, they are notably involved in plant defence against herbivores, bacteria, and fungi (Kliebenstein *et al.*, 2002; Reichelt *et al.*, 2002). However, their potential role in litter decomposability remains to be elucidated.

In the present study, we hypothesised that litter decomposability is related to the heritable variation of underlying traits, and therefore genes. We tested this hypothesis using 211 genotypes of *A. thaliana* and addressed four questions. First, does genetic variation for litter decomposability occur within a global set of genotypes of *A. thaliana*? Second, at the within-species level, is litter decomposability better explained by morphophysiological traits or by secondary metabolites? Third, is intraspecific variation in litter decomposability correlated with climate at the genotypes' collection sites? Finally, is litter decomposability heritable and what are the genes involved in this after-life process?

Materials and Methods

Plant material and experimental design

We randomly sampled 211 genotypes from the RegMap collection (Fig. 1; Supporting Information Table S1) (Horton *et al.*, 2012). Seeds used in the present study were provided by Fabrice Roux (Laboratoire des interactions plantes microorganismes (LIPM), Auzeville, France). Plants were grown in three replicates in a semi-controlled glasshouse environment. Seeds were sown on organic soil and stratified at 4°C for 4 d. At the emergence of the first two true leaves, plants were transplanted in 300 ml individual pots filled with a 1:1 (v:v) mixture of loamy soil and organic compost (Neuhaus N2). Pots were randomly distributed among three blocks (one replicate per block) that were rotated every day. All pots were watered twice a week. Additional light provided *c.* 65 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD) at plant height. Photoperiod and temperature were kept constant at 12 h 18°C: 12 h 16°C, day: night, respectively.

Litter collection and decomposition assay

Plant litter was collected at the moment of maximum leaf senescence for each genotype. As *A. thaliana* retain dead leaves on the rosette, we cut off leaves that were dead to prevent decomposition by fungi when litters were in contact with the soil. Litters were carefully cleaned, then air dried and stored in the laboratory. The decomposability of each litter sample was assessed using a standard laboratory bioassay in microcosms as described by Wardle *et al.* (2002) (for a detailed description see Methods S1).

The percentage of the initial litter mass remaining after incubation (LMR, %) was calculated as: $\text{LMR} = (\text{Litter mass}_{\text{initial}} - \text{Litter mass}_{\text{final}}) / \text{Litter mass}_{\text{initial}} \times 100$, where $\text{Litter mass}_{\text{initial}}$ is the dry litter leaf mass at the beginning of incubation and $\text{Litter mass}_{\text{final}}$ the dry litter leaf mass after the 60 d of incubation in the microcosms.

Measurement of plant morphophysiological traits

Plant morphophysiological traits, including leaf functional traits and plant life history traits, were measured using standardised procedures in three replicates per genotype (Pérez-Harguindeguy *et al.*, 2013). For each plant, leaf lifespan (LLS) was estimated as the average number of days from leaf emergence to full senescence using daily pictures of one plant replicate per genotype where three consecutive leaves were tracked from emergence to full senescence. One adult, fully exposed and nonsenescent leaf was harvested from each individual ($n=3$ per genotype) at bolting stage. Harvested leaves were water-saturated in deionised water at 4°C for 24 h, scanned for leaf area, measurement (LA, mm^2) and weighed. Specific leaf area (SLA, $\text{m}^2 \text{kg}^{-1}$) and leaf dry matter content (LDMC, mg g^{-1}) were calculated as the ratio between leaf area and leaf dry mass, and between leaf dry mass and saturated fresh mass, respectively. Average trichome density (cm^{-2}) was estimated from trichome counts on adaxial and abaxial leaf sides of two 28 mm^2 zones of the harvested leaf. We determined the leaf nitrogen content (LNC, %) on one leaf replicate per genotype with an elemental analyser (model EA 1108; Carlo Erba Instruments, Milan, Italy). According to Onoda *et al.* (2011) an approximation of the structural resistance to shearing (LRS, J m^{-1}) was measured using a protocol similar to that of Ang *et al.* (2008) using a single blade cutting device mounted on a portable Instron inspec 2200, instrumented device that measured the force (N) and distance of the downward cutting blade, (Instron Engineering Corp., Canton, MA, USA). Leaves were cut in the middle position of the leaf length, from the leaf margin up to but not including the midrib. The horizontal distance cut was calculated via the vertical displacement and the angle of the blade set to 30° from the horizontal. Flowering time (FT) was measured at bolting stage (first emergence of the floral bud) and was included because it represents a major trait associated with plant life history and resource-use strategies (Vasseur *et al.*, 2012, 2018a,b).

Leaf metabolites dataset

Quantitative variation in untargeted metabolites – that is all measurable metabolites – has been measured recently across 309 genotypes by Wu *et al.* (2018). Wu and colleagues reported variation for 4182 metabolites in nonstressing conditions. Ninety one genotypes were common to Wu *et al.* (2018) and the present study. Due to missing data, only 2132 metabolites were retained for subsequent analyses here. Four genotypes among the 91 had missing data for one or several metabolites, which were inputted with the 'MISSMDA' package in R. We first investigated whether there were correlations between decomposition rate and metabolites over all metabolites measured in Wu *et al.* (Fig. 3c,d, see in the next sentence). Then, we went further by looking more specifically to a subset of 103 metabolites for which functions were identified in Wu *et al.* (2018) (Fig. 4, see in the next sentence). In doing so, our approach was voluntarily not oriented toward a specific class of metabolites, but rather to test if there is an effect of metabolites on decomposability over the broadest range of metabolites that we could use. To

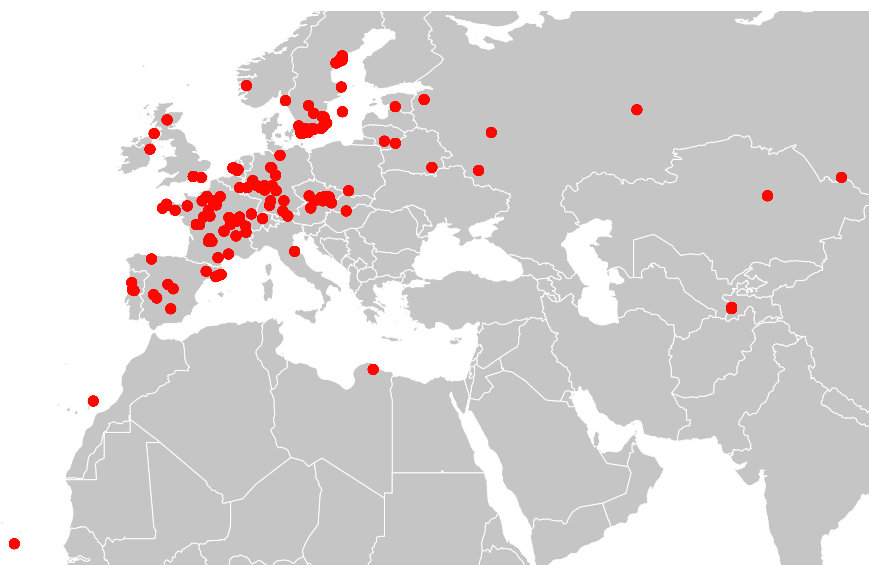


Fig. 1 Geographic origin of the 211 genotypes of *Arabidopsis thaliana* used in this study.

compare the role of different classes of metabolites, we used the set of 103 identified metabolites from Wu *et al.* (2018), and investigated the correlations between each metabolite and LMR. Spearman's coefficients of correlation were reported, after grouping by metabolite class (amino acid – amine, coenzyme, flavonoid, glucoside – sugar, glucosinolate, nucleoside – nucleotide and their derivative, organic acid, and phenylpropanoid). For convenience, amines and amino acids were treated together, as well as sugars and glucoside, and nucleotide, nucleoside and their derivatives. Only the classes containing at least three identified metabolites were kept for the analysis.

Statistical analyses

For each trait, genotypic means (trait mean by genotype) were estimated from linear models (package 'LSMEANS'). Principal component analyses (PCA) were performed separately on morphophysiological traits (morphophysiological PCA), metabolites (metabolomic PCA) and climate variables (climatic PCA) using the 'ADE4' package. Morphophysiological PCA was performed across the 91 genotypes with both morphophysiological traits and metabolites, using leaf area, leaf resistance to shearing (LRS), SLA, LDMC, leaf lifespan (LLS), bolting time (FT), nitrogen concentration (LNC) and trichome density. Metabolomic PCA was also performed on the same 91 genotypes, using the 2132 untargeted metabolites measured by Wu *et al.* (2018). Climatic PCA was also performed on the 91 accessions, using the climate variables at the collection points of the genotypes extracted from WorldClim database (<http://www.worldclim.org/bioclim>). For each PCA, we retained all principal components (PCs) that explain >5% of total genetic variability (Fig. S2) for subsequent analyses.

To quantify the respective role of functional traits and metabolites on litter decomposability, we performed a multivariate random forest model, using the 'RANDOMFOREST' package in R, which allows model classification based on Breiman's random forest

algorithm (Breiman, 2001). All PCs were scaled (standard deviation = 1) and centred (mean = 0) before analysis. In addition, and following previous approaches (e.g. Barnagaud *et al.*, 2013; Leonard *et al.*, 2014; Shantz & Burkepile, 2014; Welte & Joern, 2015), we fitted with linear regressions all possible combinations of explanatory variables (i.e. morphophysiological PCs and metabolomic PCs) nested within the full model, using the *dredge* function in the 'MuMIN' package (Barton, 2016). Then, we plotted the averaged coefficients fitted for each explanatory variable across all possible models. This approach is powerful, as it helps to minimise loss of information contained in the data (Schielzeth, 2010). Moreover, as predictor variables (i.e. PCs) were rescaled before analysis, the distribution of these fixed effects can be interpreted as effect sizes. That is, a coefficient with value + 1 indicates that a 1-SD increase in the predictor variables (i.e. PCs) directly causes a 1-SD increase in the response variable (i.e. LMR), after accounting for any other predictor variables (Blonder *et al.*, 2018). We followed the same procedure for climate PCs. All analyses were performed using R 3.15 (R Core Team, 2019, v.3.5.3).

Quantitative genetics analysis

Classical ('monogenic') GWAS were performed on 214 050 biallelic single-nucleotide polymorphisms (SNPs) from the publicly available RegMap genetic panel (Horton *et al.*, 2012). GWAS exploits natural genotypic variation and enables the analysis of significant associations between hundreds of thousands of SNPs and specific phenotypes (Horton *et al.*, 2012). GWAS was carried out using EMMAX (Kang *et al.*, 2010) implemented in the online platform easyGWAS (<https://easygwas.ethz.ch/>; Grimm *et al.*, 2017), with minimum allele frequency at each SNP above 5%, and which including the two-first axes of a PCA performed on SNPs as covariates to take into account population structure in *A. thaliana*. Significance threshold that considers multiple testing correction was determined using the Bonferroni method ($\alpha = 0.05$ or 0.1).

Polygenic GWAS were performed with Bayesian Sparse Linear Mixed model (BSLMM) implemented in EMMA (Zhou *et al.*, 2013), which accommodates both poly- and oligogenic architectures. Chip heritability (h^2) was calculated from BSLMM models as the sum of phenotypic variance explained by all SNPs, assuming genetic additivity. Given the importance of metabolome PC3, we performed BSLMM and Gene Ontology (GO) analysis to examine the biological functions associated with this axis. By contrast with monogenic GWAS, BSLMM models two effect hyperparameters, a basal effect, alpha, that captures the fact that many SNPs contribute to the phenotype, and an extra effect, beta, which captures the stronger effect of only a subset of SNPs. We summed alpha and beta to estimate total SNP effect and investigate the correlation between SNP effects on LMR and metabolome PC3, respectively. We then used the 1% top-SNPs (i.e. 0.5% at the lower tail and 0.5% at the upper tail) of the distribution of SNP effect to identify the genes that contributed most to the phenotypic effect. Gene names were extracted from SNPs position using gene annotation along the *A. thaliana* genome from the TAIR10 GFF3 file. This resulted in 1910 top-genes in total, for LMR, and 3398 top-genes for metabolome third PC axis. We then performed GO analysis using the AgriGO (Du *et al.*, 2010) and REVIGO online tools to visualise significant enrichment in GO categories (Supek *et al.*, 2011).

Results

Genetic variability in litter decomposability and morphophysiological traits

We found a marked variation in litter decomposability among the 211 genotypes of *A. thaliana* (Fig. 2): litter mass remaining (LMR) ranged from 21% to 57%. Significant variability was also found in morphophysiological traits (Fig. S1): trichome density was the most variable trait (25-fold variation; from 0 to 24 trichomes per cm², Fig. S1h). LES traits were also very variable, notably leaf dry matter content (LDMC) (5.4-fold variation

between 65 and 351 mg g⁻¹, Fig. S1c) and leaf nitrogen content (LNC) (20.8-fold variation between 0.4% and 8%, Fig. S1f), while specific leaf area (SLA) and leaf life span (LLS) varied 5 and 3.5 times (from 12.3 to 91.3 m² kg⁻¹ and from 15 to 53.5 d, respectively) (Fig. S1d,g). In addition, flowering time and leaf area varied between 25 and 113 d, and between 104 and 811 mm², respectively (Fig. S1a,b).

Comparing the effect of plant morphophysiological traits and metabolites on litter decomposability

The two-first PCs of the morphophysiological PCA explained together 80% of the multivariate genetic variability (62% and 18% for PC1 and PC2, respectively; Fig. 3a), while PC3 and PC4 explained 10% and 5%, respectively, of total genetic variability. PC1 represented the leaf economics spectrum: positive PC1 values were associated with acquisitive strategies characterised by high specific leaf area (SLA) and high leaf nitrogen content (LNC), but short lifespan, low leaf dry matter content (LDMC) and low structural resistance. Inversely, negative PC1 values were associated with conservative strategies characterised by opposite leaf trait values. PC2 was mainly explained by variation in leaf area (Fig. 3a). The four-first PCs of the metabolomic PCA together explained 36% of metabolomic variability (PC1: 13%, PC2: 11%, PC3: 7%, PC4: 5%; Fig. 3b).

We then used random forest models and multiple model evaluation to compare the respective role of morphophysiological trait variation and metabolomic variation on litter mass remaining (LMR). Models were performed on the 91 genotypes subset, using the first five PCs of both morphophysiological traits and metabolomic PCA. Random forest model indicated that PC3 of the metabolomic PCA was the main contributor of litter mass remaining (LMR), followed by the second PCA axis of morphophysiological traits (Fig. 3c). By comparison, intraspecific variations related to the leaf economics spectrum and structural resistance (morphophysiological PC1) had only small effects on LMR variability among genotypes, even less than morphophysiological PC4 and metabolomic PC2 (Fig. 3c). Consistently,

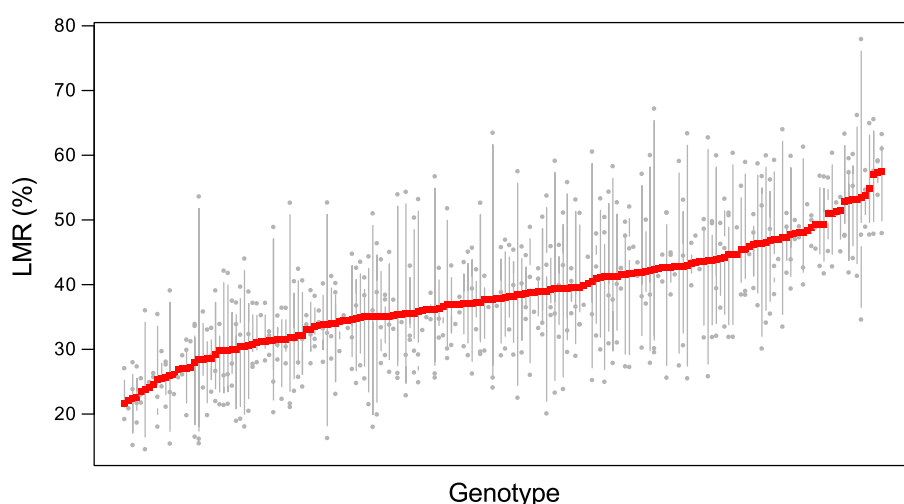


Fig. 2 Variation in litter mass remaining (LMR, %) across *Arabidopsis thaliana* genotypes. For each accession, linked grey dots represent individual values ($n = 3$) and red dots represent mean genotypic value. Genotypes are ordered by increasing mean LMR values.

multiple model evaluation suggested that metabolomic PC3 had the strongest effect (+40%) on LMR, while morphophysiological PC1 had no significant effect (Fig. 3d). Multiple model evaluation also suggested that morphophysiological PC2 had a significant negative effect on litter mass remaining (LMR), suggesting that bigger leaves had higher decomposability (i.e. lower LMR).

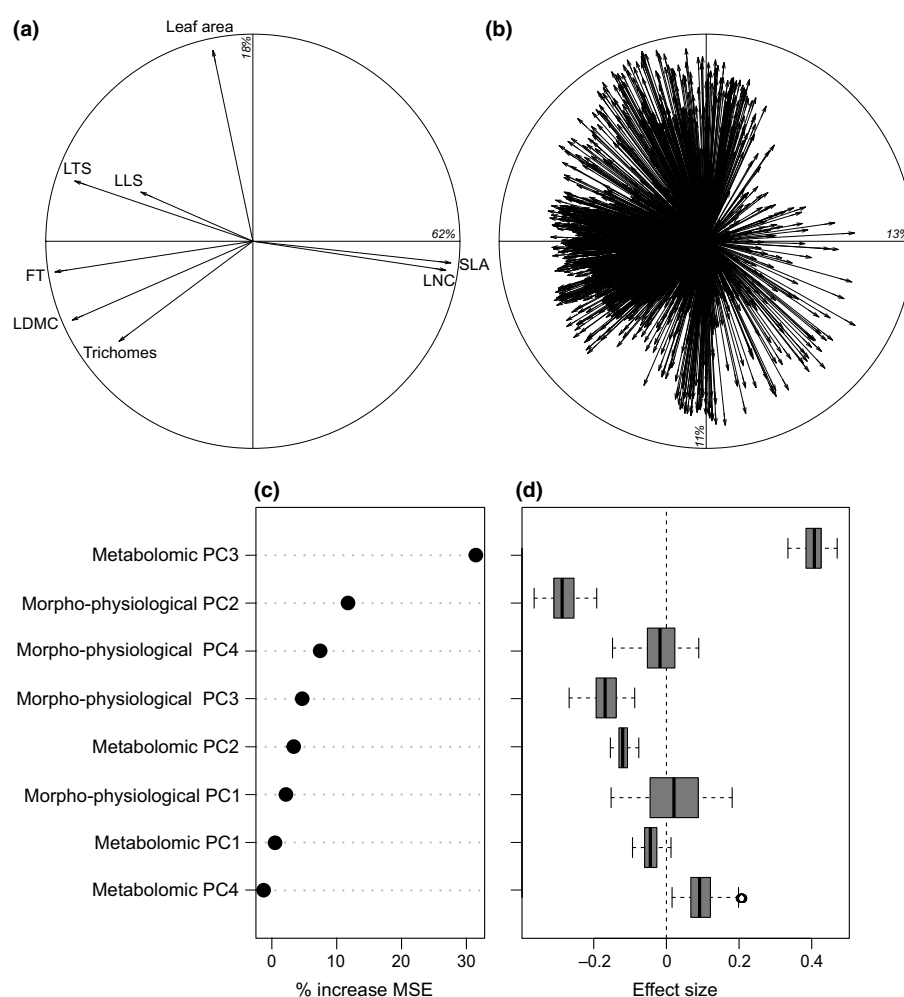
Using a set of 103 metabolites identified from Wu *et al.* (2018), we explored the role of different classes of metabolites on leaf decomposability (Fig. 2; Table S2). Results suggest that glucosinolates, a group of secondary metabolites involved in plant defence in Brassicaceae like *A. thaliana*, also had globally a negative effect on decomposability, as illustrated by the positive correlations between individual glucosinolates and LMR (14 glucosinolates out of 32 were significantly and positively correlated with LMR, $P < 0.05$; Table S2). In addition, amino acids composition also had a notable effect on decomposability, with some amino acids being negatively correlated with LMR (e.g. L-glutamic acid, indole-3-lactic acid, homoserine; Table S2) and others being positively correlated (e.g. L-tryptophan, methionine, valine; Table S2). The same result was found for flavonoids, nucleotides/nucleosides and organic acids (Fig. 4). Finally, phenylpropanoids were on average negatively correlated with

LMR, although none of the correlation with the underlying metabolites was significant ($P > 0.05$; Table S2).

Effect of climate of origin on litter decomposability

PCA on the 19 climate variables that describe the environment of origin of each genotype (Fig. 5a) showed that PC1 was mainly associated to temperature variables (with those related to winter temperature as top contributors), while with PC2 mainly accounted for precipitation variables (with those related to summer precipitation as top contributors). Together, PC1 and PC2 accounted for 62% of variability (PC1 = 35%, PC2 = 27%). Rainfall-related variables at collection sites (PC2) had a small but significant effect on litter mass remaining (LMR (Fig. 5b), suggesting that adaptation to water availability leads to variation in leaf properties that can alter decomposability. However, the lack of correlation between climatic PC2 and metabolomic PC3 ($r^2 = 0.01$, $P = 0.30$) suggests that the changes in leaf properties associated with precipitation are independent from the metabolites identified here as associated with litter decomposability (metabolomic PC3) (Table S3). Finally, morphophysiological PC1 was negatively correlated to climatic PC1, relating species

Fig. 3 Relationships between morphophysiological traits, metabolites and litter decomposability of *Arabidopsis thaliana* (a) 'Morphophysiological PCA' performed on seven traits: leaf area, specific leaf area (SLA), leaf nitrogen content (LNC), leaf lifespan (LLS), leaf tensile strength (LTS), leaf dry matter content (LDMC) and trichome density (Trichomes); as well as flowering time (FT). The two-first PCs are represented. (b) 'Metabolomic PCA' performed on 2132 metabolites extracted from Wu *et al.* (2018). Both PCAs were performed on the 91 genotypes with morphophysiological and metabolomic data. For convenience, metabolites names were not represented. Morphophysiological traits and metabolites were all scaled before PCAs. (c) Random forest analysis of the contribution of metabolomic and morphophysiological PCs on LMR ($n = 91$). Only PCs that explained >5% of total phenotypic variation (i.e. PCs 1 to 4 for each PCA) were retained for further analyses ($\pm$ SD). Stronger increase in mean square error (MSE) reflects more important contribution to LMR. (d) Results of multiple regressions to compare effect size and significance of metabolomic and morphophysiological PCs on LMR ($n = 91$). All PCs were scaled before analysis.



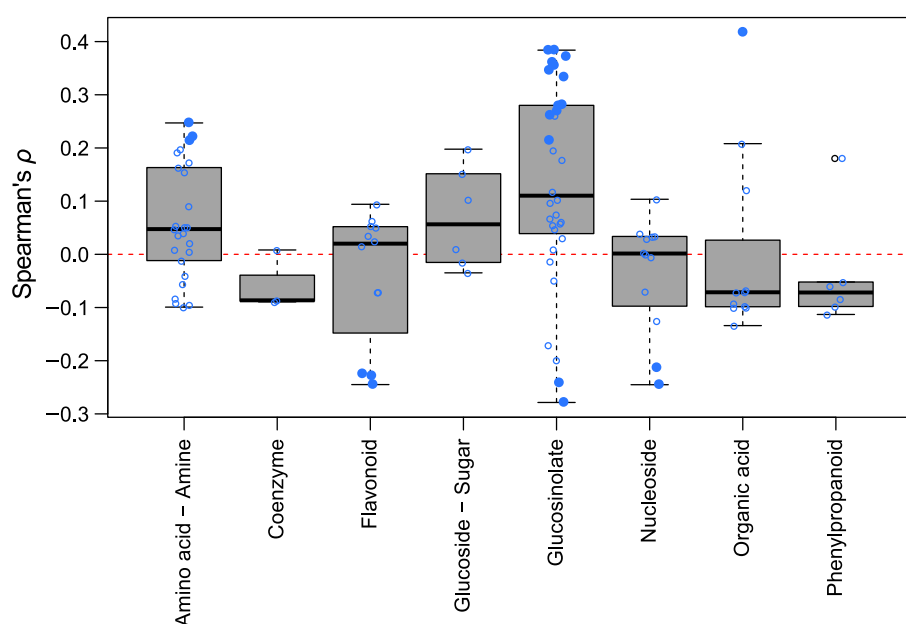


Fig. 4 Correlations between litter decomposability and specific metabolites. Metabolites classes were extracted from Wu *et al.* (2018) study. Correlations were estimated with Spearman's rank coefficient of correlation (ρ) between each individual metabolite and LMR. For convenience, amines and amino acids were treated together, as well as glucosides and sugars, and nucleoside, nucleotide and their derivatives ('Nucleoside' in the figure). Only the classes with at least three individual metabolites were analysed. Blue points represent the coefficients of correlation between individual metabolites and LMR. Empty circles represent nonsignificant correlations ($P > 0.05$), and solid circles represent significant correlations ($P < 0.05$). Red dashed line represents zero axis ($\pm$ SD).

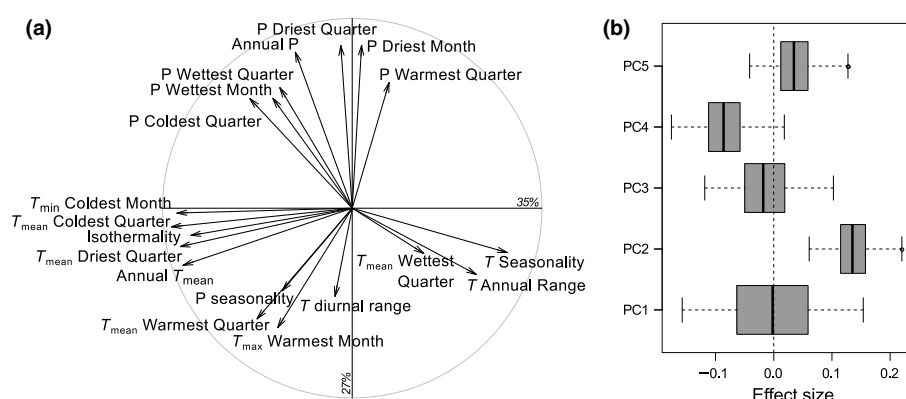


Fig. 5 Effect of climate of origin on litter decomposability. (a) 'Climatic PCA' performed on 19 variables extracted from the Worldclim database at the collection points of 211 *Arabidopsis thaliana* genotypes. T, temperature ($^{\circ}$ C); P, precipitation (mm). (b) Results of chain regressions to compare effect size and significance of climate PCs on LMR ($n = 211$). All PCs were scaled before analysis ($\pm$ SD).

with high acquisitive strategies to warmer temperatures than species with conservative strategies (Table S4).

Genetic bases of litter decomposability

Chip heritability of litter mass remaining, a measure of narrow-sense heritability (h^2), was estimated to 0.25 with BSLMM. This indicated a substantial genetic control of litter decomposability, presumably because the underlying determinants of litter decomposability (e.g. leaf metabolome) have a strong genetic basis. No SNP was significantly associated with LMR along the genome after multiple testing correction (Fig. 6a), which can be explained by a large number of genes contributing to the phenotype, each with small effect – that is a 'polygenic' architecture – rather than few genes with strong effect on the phenotype, as classically tested under the monogenic GWAS. We found that the 1910 top-genes involved in LMR were indeed significantly enriched for specific cellular components such as proteins localised in plasma membrane and chloroplast envelope (Fig. 6b). In addition, we also found a significant enrichment in the biological processes related

to postembryonic development, posttranslational protein modification and transport (Fig. S3c). Finally, the molecular functions of genes involved in litter decomposability were significantly enriched for functions related to ATP binding and ATPase activity, as well as transmembrane transporter activity (Fig. S3e).

Given the importance of metabolomic PC3, we performed BSLMM and GO analysis to examine the biological functions associated with this axis. Interestingly, GO analysis of the 1% top-genes revealed a significant enrichment for proteins localised in plasma membrane and chloroplast envelope (Fig. 6c), similar to that found for LMR (Fig. 6b). Metabolomic PC3 was also significantly enriched for 1,3- β -D-glucan, a key polymer of cell wall composition. Moreover, we estimated with BSLMM the SNP effects on each individual variable (i.e. metabolomic PC3 and LMR), and inspected their correlation (Fig. 6d). As expected many SNPs had a null effect on each variable, generating a cross along the zero axes when plotting the relationship between SNP effects on each variable in Fig. 6(d). However, we found that SNP effects on metabolomic PC3 were significantly and positively correlated with SNP effects on LMR ($r = 0.05$; $P < 0.001$;

Fig. 6 Genetic analysis of litter decomposability. (a) Monogenic genome-wide association studies (GWAS) performed with EMMAX on LMR across 199 genotypes with genetic markers (214K SNPs). Dot colors represent chromosomes. Red lines represent significance thresholds (Bonferroni correction at $\alpha = 0.05$ and $\alpha = 0.1$ for solid and dashed lines, respectively). (b) Gene ontology (GO) enrichment analysis for LMR, performed with REVIGO online tool. Box size proportional to significance ($\log_{10}(P\text{-value})$) of the GO category. Only the cellular component class is represented. (c) GO enrichment analysis for metabolomic PC3, performed with REVIGO online tool. Box size proportional to significance ($\log_{10}(P\text{-value})$). Only the cellular component class is represented. (d) Correlation between SNP effect on metabolomic PC3 and SNP effect on LMR. SNP effect estimated with BSLMM. r is Pearson's coefficient of correlation. Dashed line represents linear regression.

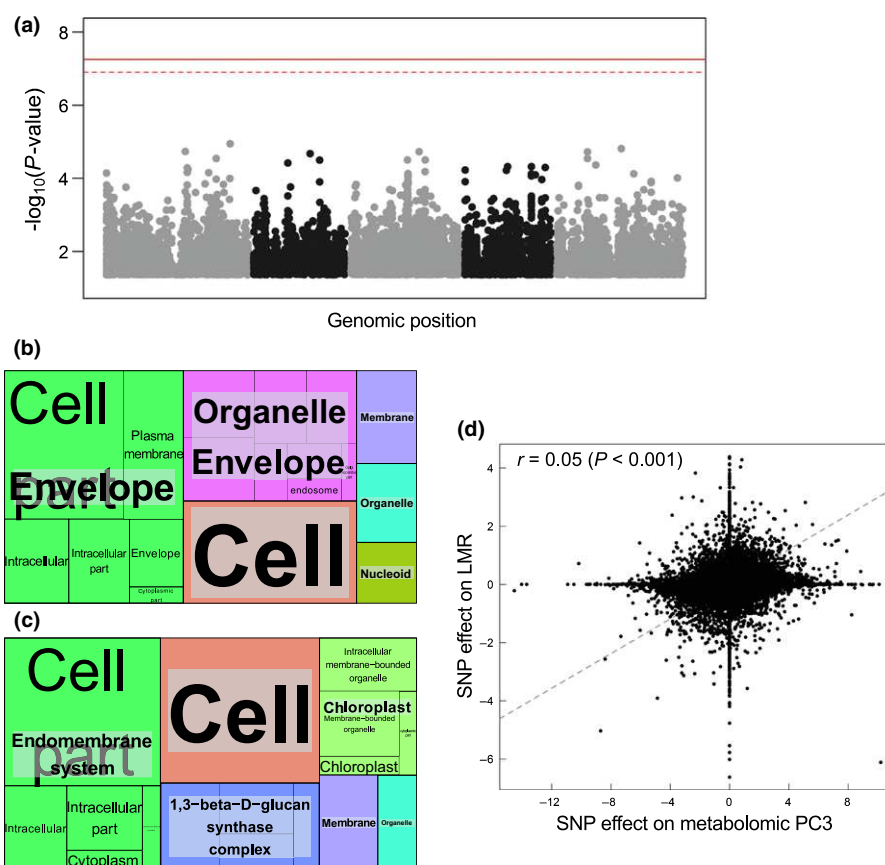


Fig. 6d), which suggests that differences in litter decomposability across *A. thaliana* genotypes are at least in part explained by variation in metabolomic PC3, such as the chemical properties of plasma membranes and cell wall.

Discussion

In this study, we examined the drivers of litter decomposability within species by comparing the role of plant morphophysiological traits and leaf metabolites among *A. thaliana* genotypes. Our results showed that litter decomposability vary by 2.7-fold and is partly heritable ($h^2 = 0.25$). This heritable variation of litter decomposability can be attributed to adaptation to different precipitations across the distribution range of *A. thaliana* and to genetic variations in leaf metabolites but not to genetic variation across the LES.

The intraspecific range of variation in litter decomposability observed in this study is comparable with the variability found in several interspecific analyses. For example, LMR from several herbaceous species measured in similar conditions (leaf litter incubated in microcosms for 90 d with standardised soil and controlled temperature and soil humidity) ranged from 30% for *Crepis foetida* to 92% for *Carex humilis* (three-fold) (data from Kazakou *et al.*, 2006 and Bumb *et al.*, 2018). Previous studies at the interspecific level have demonstrated that litter decomposability is partly controlled by tissue nutrient concentration and by the density of structural material in the leaf (Cornelissen *et al.*,

1999; Wardle *et al.*, 2002; Cornwell *et al.*, 2008). For instance, nutrient concentration in green leaves, such as nitrogen, carbon or phosphorus content, are likely to be linked to litter nitrogen content or with the litter lignin : nitrogen ratio (Wright *et al.*, 2004). Traits such as leaf dry matter content (LDMC) might also reflect structural support and defence against herbivores, which makes them good predictors of litter quality and therefore litter decomposability across species (Cornelissen & Thompson, 1997; Kazakou *et al.*, 2006). Indeed, positive correlations were observed, always at the interspecific level, between leaf dry matter content (LDMC) and lignin content (Cornelissen *et al.*, 2004; Kazakou *et al.*, 2006; Quedstedt *et al.*, 2007; Fortunel *et al.*, 2009). Together, these traits are part of the leaf economics spectrum that describes resource-use strategies of living leaves (Wright *et al.*, 2004). We therefore tested the hypothesis that, within species, litter decomposability can be predicted by a set of leaf traits in a manner similar to what has been observed across species, such as traits related to the leaf economics spectrum. In particular, we expected a positive relationship between resource-acquisitive strategies and litter decomposability. Our results showed that the leaf economics trade-off was indeed reflected by leaf trait covariations across the set of genotypes studied, in a similar way as at the interspecific level. This variation was captured by morphophysiological PC1: rapid acquisition of resources is generally correlated with high specific leaf area (SLA) and leaf nitrogen content (LNC), while high leaf dry matter content (LDMC), high leaf resistance to shearing (LRS) and long leaf life span (LLS) reflect a

resource conservation strategy. Interestingly, both flowering time and trichome density were negatively correlated with these traits, suggesting that late-flowering plants have conservative strategies with abundant trichomes at the leaf surface. This relationship is consistent with the analysis of ecological strategies among *A. thaliana* genotypes (Vasseur *et al.*, 2018a,b). However, despite the large range of variation in functional leaf traits, our initial hypothesis was not supported by our analyses. Neither particular leaf traits nor morphophysiological PC1 explained the intraspecific variation in litter decomposability in *A. thaliana*.

Secondary metabolites could also impact litter decomposability and therefore nutrient turnover. For example, numerous studies have pointed out the role of secondary metabolites (lignin, cellulose, hemicellulose) as regulating factors of litter decomposability (Berg, 2000), but the role of secondary metabolites still remain largely underexplored. Our results showed that a large number of metabolites, together grouped along metabolomic PC3, was the main contributor of the variation of LMR in *A. thaliana*. Furthermore, genetic analysis showed that the top-genes associated with LMR variation between genotypes are mainly localised in cell and organelle envelopes. Interestingly, the same enrichment was observed for the genes associated with metabolomic PC3, and SNP effects measured for LMR and metabolomic PC3 were significantly correlated. Therefore, litter decomposition in *A. thaliana* seems to be mainly driven by the abundance and diversity of metabolites, notably those related to the chemical composition of cell and organelle envelopes. In addition, glucosinolates were also negatively with the rate of leaf decomposition across genotypes pointing the role of defence metabolites in after-life effects. However, we have to notice that our results were based on the secondary metabolites identified by Wu *et al.* (2018) and other unidentified metabolites may play an important role to the decomposition process. The remarkable diversity of secondary compounds that have been quantified from the model plant *A. thaliana*, present tremendous challenges for the study and interpretation of secondary metabolism.

We found that intraspecific variation in litter decomposability is shaped by numerous genes along the genome, each with relatively weak effect (i.e. a polygenic architecture). Monogenic GWAS did not reveal any significant association, presumably because no gene had an effect strong enough to reach significance threshold after multiple testing correction. This polygenic architecture of leaf decomposability is consistent with a prominent role of secondary metabolites, which are numerous and diverse, and encoded by many genes. We also found a positive correlation between climatic PC2 and LMR. Hence, drier environments might have favoured genotypes with lower LMR (i.e. higher decomposability). This relationship between precipitation and litter decomposability corroborates with the findings of Austin & Vitousek (2000) suggesting that litter quality and especially lignin concentrations in litter increased significantly with increasing precipitation. A complicating factor is that the temperature sensitivity of litter decomposability is also dependent on the quality of the carbon compounds in litter: litter enriched in C-based secondary compounds (i.e. low quality) is more sensitive to temperature and change than

litter with high amounts of-quality C compounds (Fierer *et al.*, 2005). However, our analysis suggests that variation in LMR associated with adaptation to water availability is independent of the class of metabolites involved in decomposition, as climatic PC2 and metabolomic PC3 were not correlated. A possible explanation is that adaptation to water availability relies on diverse metabolites that were not associated with a specific metabolomic PC, and not with the axis related to cell and organelle envelopes (PC3) in particular. This relation is consistent with recent findings that showed the polygenic architecture of drought adaptation in *A. thaliana* (Exposito-Alonso *et al.*, 2018). Together, our results suggested that selection for resistance to specific stresses during plant life can have after-life effects on important ecological processes such as microbial activity and nutrient cycling. Further studies will be required to identify precisely the metabolites having an after-life effect.

Conclusion

We find a large genetic variability in litter decomposability as well as in morphophysiological traits, but these did not correlate with each other. By contrast, metabolites located in cell membranes and envelopes played a significant role in litter decomposability. In addition, metabolites related to plant defence, such as glucosinolates, were also negatively correlated with decomposability. Therefore, our results illustrated the prominent effect of secondary metabolites on after-life processes at the intraspecific level. Further explorations of the associations between decomposability and secondary metabolites (and especially glucosinolates) are needed, as well as the genes and physiological mechanisms that explain the variation in litter decomposability along environmental gradients. In this perspective, manipulating specific genes related to glucosinolates biosynthesis, for instance using mutants and/or introgression lines, offers a promising avenue to confirm the role of these metabolites in litter decomposability. Moreover, using reciprocal transplants of different genotypes along specific environmental gradients (related to temperature, precipitation and/or nutrient availability), to measure changes in metabolomic profiles and decomposition rate, would allow teasing apart the effects of different environmental conditions on litter decomposability and its metabolic drivers.

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Author contributions

EK, FV, KS, EB and DV planned and designed the research. EK, FV, KS, EB, NR performed experiments, conducted fieldwork,

analysed data. EK and FV wrote the manuscript. EK and FV contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Variation in morphophysiological traits measured on the 211 genotypes.

Fig. S2 Part of variance (%) explained by the main principle components.

Fig. S3 Gene ontology enrichment analysis for litter decomposability and metabolomic PC3.

Methods S1 Description of the decomposition assay in the laboratory.

Table S1 List of accessions with measured phenotypic traits.

Table S2 Correlation of LMR with leaf traits, leaf-traits PCs and metabolomic PCs.

Table S3 Sixty top contributors of metabolomic PC3.

Table S4 Correlations between PCA axes.

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See also the Commentary on this article by Pérez Harguindeguy & Gorné, **224**: 1409–1411.

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j'espère qu'un jour on montera ce laboratoire mobile de phénotypage in situ des populations d'arabette !

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Je termine par quelque chose que je ne savais ni où ni comment mettre. Je me suis laissé accaparer l'esprit par la science et certaines contraintes liées à la thèse en oubliant parfois de prêter attention à toute la dimension artistique que pouvait offrir l'étude de l'architecture des nervures foliaires. C'est pourtant la plus belle découverte de cette thèse, j'ai adoré explorer la « Vein Density » et tout ce qui gravite autour, pour toute les facettes quelle combine, pour tout ce qu'elle a à nous apprendre évidemment, mais aussi pour sa dimension esthétique et artistique unique.

« Any sufficiently advanced technology is indistinguishable from magic »

Toute technologie suffisamment avancée est indiscernable de la magie.

Troisième loi de Clarke, Arthur Charles Clarke, Profiles of the Future, 1973.

Résumé

L'écologie comparative, grâce à des campagnes de mesures de caractères morpho-physiologiques à grande échelle, a fait état de contraintes dans la diversification des végétaux. Ces contraintes se manifestent par des covariations généralisées de traits dit « fonctionnels ». Ces observations mettent en évidence l'existence de stratégies écologiques des végétaux favorisées par certaines combinaisons de traits. À l'échelle de la feuille, la relation négative entre la durée de vie et la vitesse de photosynthèse représente un compromis majeur entre des capacités d'acquisition et d'utilisation des ressources, commun à l'ensemble des végétaux. De toute évidence, ce syndrome d'économie des ressources implique d'autres caractères liés à la conservation et la distribution de l'eau et des nutriments. Notamment, l'architecture des nervures foliaires est supposée être un acteur central dans le contrôle des compromis associés à l'assimilation du carbone, l'utilisation de l'eau et la longévité des feuilles. Ces compromis sont supposés avoir une influence jusqu'à la fin de la vie de la feuille, pendant la résorption de l'azote, et après la mort de la feuille, pendant la phase de décomposition. Cependant, jusqu'à présent les mécanismes écologiques et évolutifs de ces compromis restent mal connus. Une des raisons est le fait que la grande majorité des études s'appuie sur des comparaisons interspécifiques. Ainsi, les travaux développés dans cette thèse ont pour objectif d'analyser les déterminismes génétiques et physiologiques de ce syndrome d'utilisation des ressources, avant et pendant la senescence pour améliorer notre compréhension des mécanismes écologiques et évolutifs mis en jeu. Pour cela, nous avons appliqué une approche comparative à l'échelle intraspécifique en bénéficiant du matériel et des méthodes génétiques disponibles chez l'espèce modèle *Arabidopsis thaliana*. Nous avons analysé les covariations de traits liées à la conservation de l'azote et les caractéristiques du réseau de nervures des feuilles sur un grand nombre de génotypes en conditions semi-contrôlées. Ce phénotypage à haut-débit nous a permis d'identifier des déterminismes génétiques des traits pouvant être impliqués dans l'émergence des compromis identifiés à l'échelle de la feuille mais aussi de la plante entière. Une approche de génétique populationnelle a également permis d'ouvrir des perspectives prometteuses quant au rôle sélectif de l'environnement sur les populations locales.

Summary

Comparative ecology, through large-scale screening of plant morphophysiological traits, has brought evidence that plant diversity is constrained. Several covariations between functional traits appear to be pervasive across species and ecosystems. This has been interpreted as differing resource use ecological strategies. At the leaf scale, the negative correlation between leaf lifespan and leaf photosynthetic rate represents a major tradeoff of carbon economy common to all plants. This tradeoff is expected to involve other leaf properties related to water and nutrient acquisition and conservation within an integrated resource economics syndrome. Notably, the leaf vein architecture is supposed to constraint simultaneously the carbon assimilation, the water use and leaf robustness and lifespan. This trade-off is supposed to influence leaf properties during leaf senescence through nitrogen resorption and after leaf death through decomposition. However, up to now the evolutionary and physiologic bases of this syndrome are poorly understood. This thesis aims to fill this gap by exploring the genetic and physiologic mechanisms involved. We translated the interspecific comparative approach to the study of large sets of *Arabidopsis thaliana* genotypes. We analyzed the covariation between traits related to carbon economy and nitrogen conservation at the leaf and plant scales, and explored the role of leaf vein architecture in the resource economics syndrome. Taking advantage from the extensive genetic data and the genetic tools available for the species, we detected significant associations between genes and functional traits involved in this syndrome. Further, a population genetic approach revealed evidence of selection associated with natural variation of plant level ecological strategies, leaf vein architecture and nitrogen conservation.