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THESE

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Illustration : Claire Robert d'après John Tenniel

Pressions de sélection exercées par les résistances génétiques du melon sur les populations d'*Aphis gossypii*

Par Sophie THOMAS

Soutenue le 10 mai 2011 devant un jury composé de :

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Sommaire

Abréviations et Acronymes

CMV : *Cucumber mosaic virus*
DRO : Dérivés réactifs de l'oxygène
eIF4E : *Eukaryotic Initiation Factor 4E*
EPG : Electropénétrographie
FAO : *Food and Agriculture Organization*
HDR : Haute dose refuge
HR : Réaction d'hypersensibilité
JA : Acide jasmonique
MLG : Génotypes multilocus
NBS-LRR : *nucleotide-binding site – leucine-rich repeats*
PR : *Proteins pathogenesis-related*
PVY : *Potato virus Y*
QTL : *Quantitative Trait Loci*
RAPD : *Random Amplified Polymorphic DNA*
SA : Acide salicilique
Vat : *Virus aphid transmission*

INTRODUCTION GENERALE

Introduction générale

Les pucerons appartiennent à l'ordre des hémiptères au même titre que les cicadelles, les aleurodes ou les psylles. Ce sont des petits insectes phytophages qui grâce à leur appareil buccal de type piqueur-suceur se nourrissent de la sève phloémienne des plantes perturbant fortement la relation source-puits chez leurs hôtes (Girousse et al., 2005; Pegadaraju et al., 2005). Les pucerons ont une capacité de multiplication très élevée en condition optimale et connaissent parfois de véritables explosions démographiques. En fonction des conditions du milieu, ils ont la faculté de produire des formes résidentes aptères et des formes ailées leur permettant de disperser sur de longues distances. Toutes ces caractéristiques en font d'importants ravageurs directs de cultures, provoquant le rabougrissement des plantes et des déformations, et de redoutables vecteurs de virus de plantes, provoquant des maladies. Des moyens de lutte contre les pucerons ont été développés : lutte chimique, lutte biologique, lutte intégrée.

Si le développement de variétés à grande production, avec une qualité adéquate, reste le principal objectif du sélectionneur, l'intégration de résistance aux bioagresseurs dans les nouvelles variétés devient un objectif majeur. En effet, le Grenelle Environnement, qui réunit depuis 2007 et pour la première fois l'Etat et les représentants de la société civile, a défini une feuille de route en faveur de l'écologie, du développement et de l'aménagement durables. Dans ce contexte, le plan « Ecophyto 2018 » pour l'agriculture a pour objectifs d'interdire l'usage des pesticides les plus dangereux d'ici 2 à 4 ans à mesure de la disponibilité de solutions alternatives et de réduire fortement l'usage des pesticides à moyen terme. La France est la première consommatrice européenne de pesticides et l'objectif serait de réduire leur usage de 50% en 10 ans. Pour atteindre ces objectifs, l'utilisation de résistances génétiques apparait un moyen de lutte alternatif ou complémentaire contre les parasites et ravageurs. Durant les dernières décennies, des efforts ont été accomplis en vue de trouver et de caractériser les sources de la résistance des plantes aux insectes. De nombreuses tentatives ont été faites pour les intégrer dans de nouveaux cultivars, avec un degré variable de réussite. À la fin du 20e siècle, plus de 200 cultivars résistants aux insectes étaient cultivés dans le monde entier, parmi lesquels environ 25% résistants aux pucerons (Edwards and Singh, 2006). Plusieurs cas ont entraîné une réduction de l'usage des aphicides, équilibrée par le déploiement de variétés résistantes. Par exemple, l'utilisation d'hybrides de sorgho résistants au puceron vert (*Schizaphis graminum*), un puceron ravageur des céréales en Amérique du Nord, a permis une réduction de 90% des applications d'insecticides dans les champs de

sorgho à partir de 1972 (Dedryver et al., 2010). La dose d'insecticide (malathion, diméthoate ou lindane), nécessaire pour tuer 50% des pucerons (DL_{50}), est entre 50 et 66% plus faible sur des chrysanthèmes résistants aux pucerons que sur les variétés sensibles (Van Emden and Harrington, 2007).

Ce travail de thèse se situe dans ce contexte général et vise à favoriser une réduction des traitements aphicides des cultures maraîchères. Notre objet d'étude est le couple melon/puceron. Dans une première partie, nous présenterons les sources de résistance génétiques aux pucerons connues chez les plantes cultivées en décrivant leur déterminisme. Dans une seconde partie, nous présenterons les notions de durabilité des résistances génétiques à différentes échelles que nous resituerons dans le cadre de la résistance aux pucerons. Dans une troisième partie, nous présenterons le modèle d'étude. La problématique et la démarche de recherches de ce travail de thèse seront présentées dans une dernière partie.

CHAPITRE 1 : INTRODUCTION BIBLIOGRAPHIQUE

Chapitre 1 : Introduction bibliographique

Partie 1 : Contrôle génétique de la résistance aux pucerons

Phénotypes de résistance

Il y a trois types de résistance des plantes aux herbivores : l'antixénose, l'antibiose et la tolérance (Painter, 1951; Pilson, 2000) :

- La tolérance est l'aptitude de la plante à produire un rendement similaire en présence comme en absence du ravageur ou à supporter une forte infestation sans symptôme.
- L'antixénose ou non-acceptation est une altération du comportement de l'insecte. Ces termes sont associés aux groupes de caractères des plantes et de réponses des insectes qui conduisent les insectes à rechercher ou rejeter une plante particulière ou une variété pour pondre, se nourrir et/ou s'abriter.
- L'antibiose correspond à une modification de la physiologie de l'insecte. L'antibiose affecte le potentiel biotique des insectes ayant colonisé la plante en diminuant leur taux d'accroissement : durée de développement larvaire plus longue, fécondité et survie des adultes plus faibles.

Face aux pucerons, ces trois types de résistance sont rencontrés. L'antixénose et l'antibiose sont mesurées en termes de réponse du puceron à la plante hôte, tandis que la tolérance est mesurée par la différence de rendement entre différents cultivars infestés par des populations de pucerons de tailles égales. En condition naturelle (*in situ*), différents paramètres peuvent être mesurés sur les plantes et les pucerons, mettant en évidence à la fois de l'antixénose, de l'antibiose et de la tolérance. En condition contrôlée (*in vivo*), on peut seulement mesurer la résistance de la plante aux pucerons (antixénose et antibiose).

La tolérance, de part ses caractéristiques, ne peut être mise en évidence qu'en conditions naturelles. Le niveau de tolérance est difficilement quantifiable au niveau de la charge limite en pucerons, aussi bien en effectif qu'en durée et, par conséquent, la validation fonctionnelle de ce type de résistance est délicate. Chez les pucerons, le seul exemple pour lequel la tolérance a été clairement décrite est la tolérance du melon à *Aphis gossypii* (Bohn et al., 1973). Les feuilles de melon tolérant ne sont pas crispées malgré des fortes infestations de pucerons, et donc le rendement est *a priori* peu affecté par la présence des pucerons. La tolérance apparaît comme un mécanisme incontournable car il ne diminue pas les populations

de ravageurs (mais seulement les dégâts qu'ils occasionnent). La tolérance n'exerce donc pas de pression de sélection sur les populations. Dans la pratique, elle est très peu étudiée probablement car elle est difficile à caractériser, les variétés tolérantes produisent beaucoup d'individus qui pourront attaquer les variétés sensibles avoisinantes et finalement la tolérance pose un problème d'acceptabilité en agriculture.

L'antixénose a été étudiée chez les pucerons soit au niveau de la modification de l'acceptation de la plante, soit au niveau de la modification du comportement alimentaire. La réduction de l'acceptation (ou non préférence) est mise en évidence par des tests de choix conduits en conditions contrôlées. D'un point de vue pratique, la non-préférence est souvent étudiée sur les individus aptères. Elle a été mise en évidence chez le melon contre le puceron *A. gossypii* (Pitrat and Lecoq, 1980), chez la laitue contre le puceron *Nasanovia ribisnigri* (Liu and McCreight, 2006), chez la luzerne tronquée *Medicago truncatula* contre le puceron *Acyrthosiphon kondoi* (Klingler et al., 2005). Cependant, les études les plus pertinentes en termes d'application agronomique portent sur l'acceptation ou le rejet par les populations ailées migrantes de leur plante hôte car ce sont les premiers stades d'infestation des cultures qui sont déterminants. En fait, très peu d'études portent sur l'acceptation par les morphes ailés. Une acceptation réduite des melons résistants par *A. gossypii* a été décrite pour les morphes ailés (Kennedy and Kishaba, 1977). L'antixénose concerne aussi l'altération du comportement alimentaire. Les différentes phases aboutissant à la prise alimentaire par les pucerons ont été étudiées par des techniques analytiques ou comportementales, comme la stylectomie (coupure des stylets pour la récolte de sève) ou l'électropénétrographie (EPG) (Tjallingii, 1978). Dès l'insertion de leurs stylets dans les tissus végétaux, les pucerons effectuent des prélèvements de contenu tissulaire qui leur permettent d'identifier les propriétés physico-chimiques de la plante et d'évaluer ainsi sa compatibilité alimentaire. La première couche cellulaire est ponctionnée, ainsi que les cellules bordant le trajet vers le phloème, tout comme les fluides du compartiment intercellulaire. Les stimuli de poursuite de l'exploration alimentaire se situent à ces différents niveaux (temps de réaction allant de la minute à quelques heures). Les activités des stylets durant la recherche du phloème (salivations extra- et intracellulaires, ponctions cellulaires) peuvent induire des réactions de la plante dans les quelques minutes suivant la piqûre. La résistance perturbe très fortement le comportement alimentaire des pucerons, conduisant finalement à l'abandon de la plante avant même l'atteinte du phloème chez le melon (Chen et al., 1997; Klingler et al., 1998; Garzo et al., 2002), le pêcher (Sauge et al., 2006) ou la luzerne tronquée (Klingler et al., 2005). Sur les

tomates porteuses de la résistance à *Macrosiphum euphorbiae*, les pucerons sont capables d'atteindre le phloème mais ingèrent très peu de sève comparé aux quantités ingérées sur des plantes sensibles (Kaloshian et al., 2000).

L'antibiose, évaluée par des tests de non choix, a pour conséquence une diminution du taux intrinsèque d'accroissement des pucerons. Elle correspond souvent à une réduction de la fécondité, notamment des pucerons *Therioaphis trifolii* et *A. kondoi* (Klingler et al., 2007), du puceron du soja *Aphis glycines* (Zhang et al., 2010), du puceron du melon *A. gossypii* (Boissot et al., 2010) et du puceron du pêcher *M. persicae* (Sauge et al., 2002). L'antibiose peut aussi correspondre à une augmentation de la durée du développement larvaire notamment chez le puceron du pois *Acyrthosiphon pisum* en réponse à la résistance de la luzerne cultivée (Julier et al., 2004) ou encore à une augmentation de la mortalité comme par exemple chez le puceron lanigère du pommier *Eriosoma lanigerum* (Sandanayaka et al., 2005).

Cependant antixénose et antibiose sont des termes qui peuvent prêter à confusion. Les déterminismes génétiques qui les contrôlent peuvent être identiques. Chez le melon le gène *Vat* confère une résistance à *A. gossypii* par non acceptance et antibiose à la fois (Pitrat and Lecoq, 1982). Chez la luzerne tronquée *M. truncatula,* le gène *AKR* confère une résistance au puceron *A. kondoi* à la fois par des effets d'antixénose, d'antibiose et de tolérance. L'antixénose et l'antibiose sont des phénotypes de résistance de la plante mais exprimés par les pucerons. Ce sont des caractères quantitatifs mesurés à partir de traits d'histoire de vie des pucerons et les mécanismes qui les sous-tendent sont interconnectés. C'est le cas chez *A. gossypii,* où sur melon résistant, le stylet des pucerons n'atteint pas le phloème ou n'y reste pas. Cette modification du comportement alimentaire conduit à qualifier cette résistance d'antixénose ; cette modification du comportement alimentaire induit une forte réduction du potentiel biotique puisqu'il n'y a plus de prise alimentaire efficace. Antixénose et antibiose sont deux termes de moins en moins employés et de façon plus générale dans la littérature on trouve le terme de « résistance ». Chez les espèces céréalières, la résistance au puceron est mise en évidence par la réduction de la densité de pucerons sur la plante et par une mesure de symptômes de la plante tels que le recroquevillement ou le flétrissement des feuilles après un temps défini suite à une inoculation de pucerons (Nkongolo et al., 1989). Dans ce cas on ne peut donc parler ni d'antixénose ni d'antibiose car on considère une réponse globale de la plante face aux pucerons. De plus, les études génétiques (Tableau 1) montrent que les phénotypes de résistance peuvent avoir tout type d'hérédité (dominant, récessif, polygénique)

Tableau 1 : Principaux gènes et QTLs de résistance aux pucerons chez les plantes cultivées, phénotypes de résistance et déploiement de ces gènes. D'après Dogimont et al. (2010).

Puceron **Nom commun (*Genre espèce*)**	**Plante** **Nom commun (*Genre espèce*)**	**Gènes**	**Phénotype**	**Déploiement**
	Espèces modèles			
Puceron du pêcher *(Myzus persicae* Sulzer)	Arabette des dames *Arabidopsis thaliana*		antixénose, antibiose	-
Puceron du pois (*Acyrthosiphon pisum* Harris)	Luzerne tronquée (*Medicago truncatula)*	*RAP1*	antibiose	-
Puceron bleu de la luzerne *(Acyrthosiphon kondoi* Shinji)	Luzerne tronquée (*Medicago truncatula)*	*AKR*	antibiose	-
Puceron tacheté de la luzerne *(Therioaphis trifolii* Monell *f. maculata)*	Luzerne tronquée (*Medicago truncatula)*	*TTR*	antibiose	-
	Légumineuses			
Puceron du pois (*Acyrthosiphon pisum* Harris)	Luzerne cultivée (*Medicago sativa*)	2 QTL	antibiose	-
Puceron de la gourgane *(Aphis craccivora* Koch)	Cornille (*Vigna unguiculata*)	*Rac1, Rac2*	tolérance	-
Puceron de la gourgane *(Aphis craccivora* Koch)	Arachide (*Arachis hypogaea)*	1 gène récessif	antibiose	-
Puceron du soja *(Aphis glycines* Matsumura)	Soja (*Glycine max*)	*Rag1, Rag2, Rag3,* 2 gènes récessifs et 2 QTL épistatiques	antixénose, antibiose	-
	Arbres fruitiers			
Puceron des galles rouges *(Dysaphis devecta* Walker)	Pommier (*Malus domesticus* et *spp.*)	*Sd-1, Sd-2, Sd-3*	antibiose, tolérance	oui
Puceron cendré du pommier *(Dysaphis plantaginea* Passerini)	Pommier (*Malus domesticus* et *spp.*)	*Sm*	Hypersensibilité	oui
Puceron lanigère du pommier *(Eriosoma lanigerum* Hausmann)	Pommier (*Malus domesticus* et *spp.*)	*Er1, Er2, Er3*	antibiose, tolérance	oui

Puceron cendré du poirier (*Dysaphis pyri* Boyer de Fonscolombe)	Poirier (*Pyrus spp.*)	*Dp-1*	antibiose, tolérance	-
Puceron du pêcher *(Myzus persicae* Sulzer)	Pêcher (*Prunus persicae* et *spp.*)	*Rm1, Rm2* et 8 QTL	antixénose, antibiose	*Rm1*
Puceron du framboisier *(Amphorophora agathonica* Hottes)	Framboisier (*Rubus idaeus*)	*Ag1, Ag2, Ag3*	antixénose, antibiose	oui
Grand puceron du framboisier *(Amphorophora idaei* Börn)	Framboisier (*Rubus idaeus*)	*A1-A10, AK4a, Acor1, Acor2*	antibiose	oui
Puceron du framboisier *(Amphorophora agathonica* Hottes)	Mûrier (*Rubus occidentalis* L.)	*Ag4, Ag5*	antibiose	oui
				-
Légumes				-
Puceron de la laitue *(Nasanovia ribisnigri* Mosley)	Laitue (*Lactuca sativa* et *spp.*)	*Nr*	antibiose	oui
Puceron lanigère des racines de laitue *(Pemphigus bursarius* L.)	Laitue (*Lactuca sativa* et *spp.*)	*Ra* ou *Lra*	antibiose	-
Puceron du melon ou du cotonnier *(Aphis gossypii* Glover)	Melon (*Cucumis melo*)	*Vat,* 3 QTL et 4 QTL épistatiques	antixénose, antibiose	oui
Puceron vert et rose de la pomme de terre *(Macrosiphum euphorbiae* Thomas)	Tomate (*Solanum lycopersicum*)	*Mi-1* ou *Meu*	antibiose	-
Céréales				
Puceron russe du blé *(Diuraphis noxia* Mordvilko)	Orge (*Hordeum vulgare*)	3 QTL	antibiose	-
Puceron vert du maïs *(Rhopalosiphum maidis* Fitch)	Maïs (*Zea mays*)	*aph, aph2*	antibiose	-
Puceron vert des graminées *(Schizaphis graminum* Rondani)	Sorgho (*Sorghum bicolor*)	9 QTL	antibiose	-
Puceron russe du blé *(Diuraphis noxia* Mordvilko)	Blé (*Triticum aestivum* et *spp.*)	*Dn1, Dn2, Dn4-Dn9, Dnx, Dn2414* et *dn3*	antibiose	*Dn4*
Puceron vert des graminées *(Schizaphis graminum* Rondani)	Blé (*Triticum aestivum* et *spp.*)	*Gb2-Gb6, Gby et gb1*	antixénose, antibiose	oui

suggérant que le type de résistance (antixénose ou antibiose) ne peut pas être associé à l'hérédité de la résistance.

Hérédité de la résistance

La résistance aux pucerons a été décrite dans un grand nombre d'espèces végétales (Dogimont et al., 2010). Le grand nombre d'accessions résistantes découvertes chez certaines espèces ne doit pas masquer le fait que la résistance aux pucerons repose généralement sur un petit nombre de gènes avec un nombre limité d'allèles de résistance. Dans la plupart des cas, les études génétiques doivent encore être réalisées afin de déterminer quelles accessions sont sources de nouveaux gènes/allèles de résistance. Chez le melon, environ 50 accessions sur 500 testées sont résistantes au puceron du melon *A. gossypii*. Parmi elles, une grande majorité porte le même allèle de résistance *Vat*, quelle que soit leur origine géographique et seulement quelques accessions portent un allèle distinct au locus *Vat* (Boissot et al., 2008; Dogimont et al., 2008).

L'hérédité de la résistance a été décrite chez 17 espèces de plantes cultivées (fruits, légumes, fourrages et céréales) répertoriées pour posséder des résistances à 19 espèces de pucerons (Tableau 1) et (Dogimont et al., 2010). Chez les espèces cultivées attaquées par plus d'une espèce de puceron, la résistance à une espèce de puceron ne confère pas de résistance à une autre espèce de puceron (Luzerne tronquée, pommier, framboisier, laitue et blé). Par exemple la résistance à *Diuraphis noxia* chez le blé et l'orge est spécifique et n'a pas d'effet sur d'autres espèces de pucerons des céréales (Messina and Bloxham, 2004). La résistance aux pucerons apparaît spécifique. Le seul exemple connu de résistance non spécifique est contrôlé par le gène *Mi* de la tomate qui non seulement contrôle *Macrosiphum euphorbiae* mais également d'autres parasites de la tomate, tels que nématodes, aleurodes et psylles (Barker et al., 2005).

En tout, 24 interactions plantes-pucerons sont répertoriées à ce jour, pour un total de 87 gènes de résistances décrits (Tableau 1). L'hérédité présente toutes les formes de déterminisme monogénique ou polygénique, gène dominant ou récessif, chaque gène ayant un effet majeur ou quantitatif. Pour 19 des interactions plantes-pucerons décrites, la résistance est conférée par un gène ou plusieurs gènes dominants agissant indépendamment (56 gènes décrits). En particulier, ces gènes dominants sont très fréquents chez le framboisier et le mûrier avec 18 gènes décrits (Sargent et al., 2007; Dossett and Finn, 2010) et chez le blé avec 16 gènes décrits (Boyko et al., 2004; Liu et al., 2005 ; Peng et al., 2007; Lu et al., 2010). Seulement 7 gènes récessifs (soit 8% des gènes décrits) conférant une résistance aux pucerons ont été identifiés. Ils sont rencontrés dans cinq des interactions décrites et constituent les seules sources de résistance à *Aphis*

craccivora chez l'arachide (Herselman et al., 2004) et à *Rhopalosiphum maidis* chez le maïs (Carena and Glogoza, 2004 ; So et al., 2010). Le soja (Mensah et al., 2008) et le blé (Nkongolo et al., 1991; Boyko et al., 2004) possèdent des résistances dominantes et récessives. Dans six interactions décrites, la résistance aux pucerons est polygénique et quantitative avec un total de 31 QTL (quantitative trait loci) identifiés (soit un tiers des résistances décrites), la majorité de ces QTL ayant été décrits chez le pêcher (8 QTL) et chez le sorgho (9 QTL). Chez la luzerne cultivée (Julier et al., 2004), l'orge (Mittal et al., 2008) et le sorgho (Agrama et al., 2002; Wu and Huang, 2008) aucune source de résistance monogénique n'a été identifiée.

Dans un certain nombre de cas, les résistances sont spécifiques de biotypes particuliers : chez le blé, différents biotypes, collectés dans différents pays, ont été caractérisés sur différentes sources de résistance et présentent des profils de virulence variables témoignant une spécificité du biotype vis-à-vis d'une résistance (Haley et al., 2004; Jyoti et al., 2006). Mais pour de nombreux cas, les études de résistance sont menées en plein champ ou avec des pucerons au génotype non contrôlé et donc, le spectre d'action d'une résistance face à la variabilité des pucerons demeure largement inconnu par rapport à ceux sur la résistance aux agents pathogènes tels que champignons, bactéries et virus. Quelques pucerons ont été décrits sur plusieurs espèces cultivées. Par exemples, *D. noxia* et *S. graminum* attaquent plusieurs espèces céréalières (blé, orge, sorgho) et des résistances génétiques spécifiques aux pucerons ont été trouvées chez ces différentes céréales. *A. gossypii* attaque de nombreuses Cucurbitacées et Malvacées; la résistance a été décrite chez le melon (Pitrat and Lecoq, 1981) ainsi que chez la pastèque (Mac Carter and Habeck, 1973) mais pas chez le concombre ou la courgette ni chez le cotonnier ou le gombo. On ne sait pas si cela est le reflet d'une absence de résistance ou d'un déficit de recherche de résistance chez ces espèces.

Bases moléculaires de la résistance

Plus de 40 gènes conférant une résistance à divers agents pathogènes, tels que les bactéries, les champignons, les nématodes et les virus ont été clonés au cours des 20 dernières années. Ces gènes ont des hérédités dominantes ou récessives. En revanche, les mécanismes moléculaires des résistances aux pucerons sont très peu connus jusqu'à présent et seulement deux gènes dominants de résistance aux pucerons ont été isolés.

Le gène *Mi-1*, qui confère la résistance à trois espèces de nématodes à galles *Meloidogyne,* a été isolé chez la tomate (Milligan et al., 1998). Le même gène et allèle confère une résistance à un biotype du puceron de la pomme de terre, *M. euphorbiae,* (Rossi et al., 1998) ainsi qu'à d'autres

insectes, les aleurodes (Nombela et al., 2003) et les psylles (Casteel et al., 2006). Le gène *Vat* qui confère une résistance au puceron du melon et du cotonnier *A. gossypii* a été isolé chez le melon (Pauquet et al., 2004). Il a la particularité de conférer aussi une résistance aux virus non-persistants quand ils sont transmis par *A. gossypii* (Pitrat and Lecoq, 1980). Les gènes *Mi-1* et *Vat* sont membres de la famille de gènes de résistance possédant des domaines nucleotide-binding (NBS) et leucine-rich repeats (LRR), famille à laquelle appartient la majorité des gènes de résistance aux agents pathogènes isolés à ce jour (Dangl and Jones, 2001). Plusieurs gènes de résistance aux pucerons, pas encore isolés, ont été localisés dans des clusters de gènes de type CC-NBS-LRR, TIR-NBS-LRR, RGC2 NBS-LRR et codent potentiellement pour des protéines de type NBS-LRR (Dogimont et al., 2010) : chez la luzerne tronquée le gène *AKR* conférant la résistance à *A. kondoi* (Klingler et al., 2005), chez le soja le gène *Rag1* conférant la résistance à *A. glycines* (Kim et al., 2010), chez la laitue le gène *Ra* conférant la résistance à *Pemphigus bursarius* (Wroblewski et al., 2007) et chez l'orge et le maïs un QTL de résistance à *Rhopalosiphum maidis* (Seah et al., 1998).

Les gènes *Vat* et *Mi-1* présentent des similarités structurales. Les deux gènes sont exprimés à de faibles niveaux et codent pour des protéines localisées dans le cytoplasme (Dogimont et al., 2010). Leurs protéines prédites appartiennent à la sous-famille de protéines de résistance coiled-coil (CC)-NBS-LRR qui possèdent un domaine coiled-coil à l'extrémité N terminale de la région NBS. Elles diffèrent cependant dans plusieurs caractères spécifiques. Le gène *Mi-1* possède une longue extrémité N-terminale codant pour une région riche en leucine d'environ 200 acides aminés et jouant un rôle dans la régulation de la transduction du signal et la mort cellulaire (Hwang and Williamson, 2003). En revanche, la région N-terminale du gène *Vat* est courte. La région C-terminale du gène *Vat* comprend quatre répétitions quasi-identiques codant pour 65 acides aminés, ces quatre répétitions sont encadrées par des copies imparfaites du motif LRR, absentes chez *Mi-1*. Ces caractéristiques suggèrent que la résistance des plantes aux pucerons est sous contrôle de la reconnaissance spécifique des effecteurs protéiques hôte-puceron qui déclenche des cascades de signalisation activant rapidement les défenses des plantes contre les pucerons. Ce système a largement été décrit dans la plupart des interactions plantes-pathogènes.

L'interaction qui s'établit entre la plante et le puceron déclenche, comme lors de l'attaque par des agents pathogènes, des réactions parfois appelées défenses basales. Ces réactions activent les voies de signalisation de l'acide salicylique (SA), de l'éthylène et/ou de l'acide jasmonique (JA) (Moran and Thompson, 2001; Ellis et al., 2002; de Ilarduya et al., 2003; Gao et al., 2005;

Kaloshian and Walling, 2005) et entraîne une augmentation des protéines pathogenesis-related (PR) (Walling, 2000). L'altération de ces stratégies de défense, via des éliciteurs introduits dans la plante, est largement employée par les pathogènes pour faciliter leur développement. Les pucerons, comme les autres insectes piqueurs suceurs, introduisent différents composants dans la plante via leur salive, et ces composants vont comme les éliciteurs des agents pathogènes, altérer la mise en place des défenses basales (Walling, 2008). Lors des piqûres, les pucerons mettent rapidement en place une gaine salivaire qui constitue 'un tunnel' vers le phloème et permet d'éviter le contact avec les défenses apoplastiques (Miles, 1999). Mutti et al. (2008) ont montré qu'au moins une protéine salivaire (C002) est indispensable pour la prise de nourriture d'*A. pisum* sur la fève. Arrivés au phloème, les pucerons vont cimenter la gaine salivaire aux tissus du phloème en modifiant les réponses de callose de la plante (Walling, 2008). D'autres gènes activés montrent une réponse systémique, spécifique au compartiment phloémien de la plante (Divol et al., 2005).

L'ensemble de ces phénomènes est observé dans la réaction compatible, par contre le dialogue moléculaire activé par les gènes de résistance aux pucerons est peu connu. Les données disponibles à ce jour indiquent que ce dialogue moléculaire chevauche partiellement celui activé par les interactions plante-pathogène (Kaloshian and Walling, 2005). Il s'agit d'abord de la mise en place de nécroses locales par production de polyphénols répulsifs ou de dérivés réactifs de l'oxygène (DRO) qui s'accompagne dans certains contextes génétiques de réactions d'hypersensibilité (HR), incluant un syndrome de mort cellulaire programmée (qui pourrait limiter l'extension de l'infection dans le cas des agents pathogènes). La réponse HR a été observée chez certaines variétés résistantes d'orge lors de l'attaque par *D. noxia* (Belefantmiller et al., 1994) et chez le pêcher lors de l'attaque par *Myzus persicae* (Massonie and Maison, 1979). Chez ces variétés, en cas d'inoculations successives par deux biotypes, la réponse HR n'est exprimée que lors de l'inoculation par le premier biotype même si les variétés sont résistantes aux deux biotypes (Zaayman et al., 2009). Chez la tomate, lors de l'interaction *M. euphorbiae* / *Mi-1* aucune lésion nécrotique n'a été observée alors que des réactions d'hypersensibilité sont observées lors de l'interaction nématodes / *Mi-1* (de Ilarduya et al., 2003). Chez la luzerne tronquée infestée par *Acyrthosiphon pisum,* la réponse HR n'est pas nécessaire pour induire la résistance (Stewart et al., 2009). Enfin chez des variétés de melon résistantes, les plantes expriment une réponse HR microscopique sans symptôme nécrotique visible à l'œil nu (Villada et al., 2009).

Chez la tomate, la résistance à *M. euphorbiae,* conférée par le gène *Mi,* n'implique que la voie de l'acide salicylique (de Ilarduya et al., 2003). Chez *M. truncatula*, la résistance à *A. kondoi,*

conférée par le gène *AKR,* met en jeu la voie de signalisation des octadécanoïdes, et de l'acide jasmonique, cette dernière étant plus connue pour son implication dans la résistance aux insectes phytophages de type broyeur. Chez *Arabidopsis thaliana*, une résistance à *M. persicae*, puceron généraliste, est conférée par le gène *PAD4* (PhytoAlexin Deficient4), connu pour son implication dans la sénescence. Ainsi, malgré la similitude avec la réponse aux agents pathogènes, l'agression aphidienne semble induire chez la plante une réponse spécifique qui reste à déchiffrer.

Si les mécanismes sous-jacents aux résistances monogéniques dominantes aux pucerons émergent, les bases moléculaires des résistances récessives n'ont pas été explorées à ce jour. Près de la moitié des résistances monogéniques contre les virus sont récessives (Diaz-Pendon et al., 2004; Kang et al., 2005) et pour ces résistances Fraser (1990) a suggéré que l'allèle dominant de sensibilité code pour une protéine nécessaire à l'accomplissement du cycle du pathogène dans la plante, les virus ne pouvant pas se multiplier de manière indépendante. C'est le cas du premier gène de résistance récessif cloné, *pvr2* chez le piment, impliqué dans la résistance au PVY (*Potato virus Y*) et au TEV (*Tobacco etch virus*). Le gène *Pvr2* code pour le facteur d'initiation de la traduction eIF4E (eukaryotic Initiation Factor 4E) et les allèles sensibles à ce locus sont utilisés par ces potyvirus pour leur traduction/réplication. Ce sont les mutations dans la protéine eIF4E qui abolissent l'affinité avec le virus et induisent la résistance (Ruffel et al., 2002). Parmi les résistances récessives élucidées pour d'autres pathogènes, la résistance aux oïdiums, contrôlée par les gènes de la famille *Mlo*, est la mieux connue. Les oïdiums se nourrissent aux dépends de la plante hôte en développant des suçoirs à l'intérieur des cellules végétales. Le gène *Mlo* chez l'orge régule vraisemblablement plusieurs mécanismes impliqués dans la résistance à la pénétration de l'oïdium, et les mutations *mlo* provoqueraient des dysfonctionnements de la protéine Mlo. Les plantes *mlo* présentent une mort cellulaire spontanée au niveau des feuilles, précédée par l'apparition d'appositions de la paroi cellulaire caractéristiques lors de la pénétration des suçoirs (Buschges et al., 1997). Chez les pucerons, aucun gène de résistance récessive n'a été cloné à ce jour, mais on peut imaginer une implication dans la complémentation de certaines voix de signalisation chez la plante. Par exemple, chez *A. thaliana*, il a été démontré qu'une mutation (récessive) du gène *SSI2* (Suppressor of Salicylic acid Insensitivity2) conduit à une hyper-résistance par antibiose au puceron *M. persicae* en réduisant sa fertilité ; l'effet de ce gène est dépendant d'un autre gène de résistance par antixénose *PAD4* au puceron du pêcher (Louis et al., 2010).

Enfin, il a été démontré d'une part que certaines bactéries symbiotiques étaient impliquées dans la spécialisation aux différentes plantes hôtes, en particulier chez *Acyrthosiphon pisum* (Ferrari et al., 2007), et d'autre part que ces symbiotes pouvaient contribuer à l'adaptation des pucerons aux plantes résistantes notamment chez *Macrosiphum euphorbiae* (Francis et al., 2010). Ces endosymbiontes produiraient des composés tels des enzymes qui seraient impliquées dans les voies de signalisation de l'interaction plante-puceron.

Partie 2 : Durabilité de la résistance

L'une des limites à une plus large utilisation des résistances génétiques pour la lutte contre les agents pathogènes et ravageurs des cultures est due à la capacité des bioagresseurs à s'adapter aux résistances nouvellement déployées. Dès la fin des années 70, la durabilité des résistances est sujette à discussion dans la communauté scientifique (Nelson, 1978) et en 1981 Johnson (1981) la définit de la manière suivante : la résistance à un pathogène est durable si elle reste efficace lorsque qu'une variété est déployée en culture pendant une grande échelle de temps et dans des conditions favorables à la maladie ou au ravageur. Le risque de contournement peut s'exprimer en termes de probabilité d'occurrence et d'importance des dégâts mais il ne peut être évalué qu'*a posteriori*. La définition de Johnson n'implique ni le contrôle génétique de la résistance, ni son mécanisme, ni son degré d'expression. Pour dépasser cette définition, la réflexion va se déplacer vers l'évaluation de la durabilité *a priori* et vers la gestion des résistances. La gestion des résistances est envisagée à deux niveaux: i) la gestion des gènes *in planta* c'est-à-dire introgresser des gènes ou des combinaisons de gènes qui seront *a priori* durables et ii) la gestion des gènes *in situ* c'est-à-dire déployer différents cultivars avec des combinaisons de gènes de résistance/sensibilité. La réflexion peut alors s'engager à trois échelles : à l'échelle du gène, à l'échelle de l'individu et à l'échelle de la population.

A l'échelle du gène

Il s'agit d'utiliser les connaissances de l'interaction hôte-parasite pour choisir un gène *a priori* durable, en prenant en compte soit la fonction du gène de résistance, soit la modalité et les conséquences du passage de l'état avirulent à l'état virulent du bioagresseur.

Le choix d'un gène de résistance qui soit un gène indispensable au développement du bioagresseur -allèle de sensibilité dominant- et dont une ou des mutations confère la résistance -allèle de résistance récessif- devrait assurer une très grande durabilité à la résistance (Diaz-Pendon et al., 2004). L'exemple type est le cas de l'orge chez qui la résistance à l'oïdium est conférée par l'allèle récessif *mlo*. Aucune souche d'oïdium virulente vis à vis de *mlo* n'a été observée à ce jour. Les mécanismes histologiques, phytopathologiques, moléculaires et génétiques de cette résistance sont comparables à ceux exprimés dans le cas d'une résistance non-hôte (Humphry et al., 2006). Un contre exemple est celui du gène *pvr2* chez le piment, conférant une résistance au virus PVY. L'allèle de sensibilité est impliqué dans la réplication du PVY mais des études en serre ont montré que la résistance conférée par *pvr2* était

fréquemment contournée avec 37% de plantes sensibles dès la première inoculation (Ayme et al., 2006).

La relation gène d'avirulence/gène de résistance a pour la première fois été mis en évidence chez le champignon *Melampsora lini* responsable de la rouille du lin *Linum usitatissimum*, par Flor dès 1956 (Flor, 1956 ; 1971) qui a montré que résistance et virulence étaient déterminées génétiquement : l'hôte et le pathogène ont développé au cours de l'évolution des systèmes génétiques complémentaires tels qu'à chaque gène de résistance (dominant) chez le lin correspond un gène spécifique de virulence (récessif) chez le champignon, qui permet à ce dernier de contourner la résistance de l'hôte. Dans cette interaction gène pour gène, les gènes de résistance du lin appartiennent à la famille NBS-LRR, plus de 30 allèles de résistance ont été identifiés au niveau de 5 loci (Ellis et al., 2007) et plusieurs gènes d'avirulence de la rouille ont été clonés (Dodds et al., 2006). Une seule mutation au niveau du gène d'avirulence suffit pour induire une perte de reconnaissance avec le gène de résistance et l'accumulation de mutations n'entraîne pas ou peu de perte de valeur sélective (Dodds et al., 2006). Chez le piment, il a été démontré que la résistance conférée par *pvr2* pouvait être très durable ou au contraire très facilement contournée selon les allèles à ce locus et le nombre de mutations requises par le virus pour le contournement. Trois résistances récessives au PVY sont conférées au même locus *pvr2* par les allèles $pvr2^1$, $pvr2^2$ et $pvr2^3$. Cinq mutations différentes et indépendantes dans la protéine VPg sont responsables de la virulence du PVY face à l'allèle $pvr2^3$ du piment (Ayme et al., 2006). La majorité des mutations induisant de la virulence face à l'allèle $pvr2^3$ induit de l'avirulence face à l'allèle $pvr2^2$ alors qu'une seule induit de l'avirulence face à l'allèle $pvr2^1$. En outre, l'addition d'une ou deux substitutions révèle des épistasies antagonistes entraînant une perte de virulence de certains mutants (Ayme et al., 2007).

D'un point de vue évolutif, le maintien des gènes/allèles d'avirulence ne paraît pas être une bonne stratégie pour l'agent pathogène mais il peut expliquer la durabilité des gènes de résistance qui leur correspond (Leach et al., 2001). Le maintien des gènes/allèles d'avirulence peut être lié à une perte de valeur sélective lors du passage d'un variant avirulent à un variant virulent. Dans le pathosystème bactérie *Xanthomonas oryzae*/riz, répertorié dans les interactions de type gène-pour-gène, les études *in situ* suggèrent que la durabilité du gène de résistance *Xa7* du riz est largement basée sur la réduction de valeur sélective de la bactérie associée à la mutation virulente (Vera Cruz et al., 2000). Les études menées sur le pathosystème Colza *Brassica napus*/*Phoma* (*Leptosphaeria maculans*) ont montré que la perte du caractère d'avirulence était associée à une diminution des symptômes ainsi qu'à une valeur

sélective des souches virulentes plus faible que celle des souches avirulentes (Huang et al., 2006; Huang et al., 2010).

Cependant, ce n'est pas parce qu'un génotype virulent apparaît avec une faible valeur sélective qu'il ne posera pas de problème à terme. Au sein d'une nouvelle population virulente soumise à la sélection, les individus les plus compétitifs peuvent répondre en rétablissant leur valeur adaptative, comblant ainsi leur retard par rapport aux autres populations avirulentes. Ce type d'observation existe par exemple pour des résistances aux antibiotiques. Van Valen (1973) a donné à cette définition de la coévolution le nom de « modèle de la Reine Rouge » en référence aux personnages du roman de Lewis Carroll, « De l'autre côté du miroir ». Alice, entraînée par la Reine Rouge, doit courir très vite pour rester sur place. De la même façon, les espèces animales et végétales doivent évoluer à la même vitesse que leur environnement et que les autres espèces interagissant avec elles, pour rester adaptées. Il est donc pertinent de dépasser le concept de base du coût des virulences pour progresser sur l'évaluation de la durabilité *a priori* et vers la gestion des résistances. Une nouvelle approche permettant de prédire la durabilité des gènes de résistances a été proposée par Janzac et al. (2010), elle repose sur l'analyse de la contrainte évolutive exercée sur les gènes d'avirulence. Chez le piment, le gène *Pvr4* confère une résistance au virus PVY (Janzac et al., 2009). L'analyse moléculaire d'isolats de PVY vis-à-vis de *Pvr4* a mis en évidence que la substitution non-synonyme d'un seul nucléotide (changement de l'acide aminé correspondant) était responsable du passage de l'état avirulent à virulent et était associé à une baisse de valeur sélective des variants virulents. La seule possibilité pour les variants virulents, de rétablir leur valeur sélective au niveau de celle de variants avirulents, est une réversion de la mutation, suggérant le fort potentiel du gène *Pvr4* en termes de durabilité.

A l'échelle de l'individu

A l'échelle de l'individu, la combinaison de gènes de résistance augmente théoriquement leur durabilité (Nelson, 1978). Ceci repose sur différentes hypothèses selon que l'on considère des gènes à effets majeurs ou des QTL

i) plus il y a de facteurs de résistance à contourner, plus il faut de mutations dans le génome des agents pathogènes ce qui rend l'émergence d'un variant virulent peu probable ; une hypothèse associée est qu'il n'existerait pas d'allèles conférant une adaptation croisée à plusieurs gènes de résistance (Stukenbrock and McDonald, 2008).

ii) les QTL diminuent la fitness des génotypes adaptés à la résistance contrôlée par un gène majeur dans la combinaison gène majeur-QTL (Pietravalle et al., 2006).

iii) la pression de sélection exercée par la résistance quantitative, contrôlée par des QTL, est inférieure à celle exercée par un gène à effet majeur et ne favorise pas l'émergence de mutants virulents dans la population pathogène (Parlevliet, 2002).

Les bénéfices attendus de l'association de gènes (majeurs ou QTL) ont rarement été confirmés expérimentalement. Le principe sous-jacent à la première hypothèse est que si les allèles conférant une adaptation au gène de résistance ont une faible fréquence dans la population pathogène ou de ravageur (au moment où le gène est déployé), alors les individus qui portent les allèles conférant une adaptation à deux ou plusieurs gènes de résistance sont extrêmement rares. Les modèles théoriques développés pour le couple blé/mouche de Hesse (Cox and Hatchett, 1986; Gould, 1986) ou Maïs-*Bt* (Gould, 1998, 2003) sur la base de simulation suggèrent aussi que la combinaison de gènes de résistance retarde l'évolution vers la résistance au *Bt* des populations d'insectes par rapport à l'utilisation de résistances monogéniques développées séquentiellement ; la durabilité serait accrue s'il y a une forte épistasie entre les gènes de résistance de la plante. Deux études *in situ* ont montré que l'association de QTL à un gène majeur permettait de retarder ou d'empêcher l'apparition de variants virulents. Des inoculations de PVY en laboratoire mais aussi en culture (vecteur *M. persicae*) ont montré, chez le piment, que le gène *pvr2*^{3} était très rapidement contourné lorsqu'il était introgressé dans une variété sensible, alors qu'il ne l'était pas dans un fond génétique partiellement résistant. (Palloix et al., 2009). De même, une étude en plein champ sur des inoculations artificielles des isolats de phoma sur Colza a montré que le gène majeur était contourné à la 3ème saison de culture alors que le gène majeur associé à une résistance quantitative n'était toujours pas contourné après 5 années de sélection (Brun et al., 2010).

La combinaison de différents gènes de résistances naturelles peut être difficile, voire impossible, si on ne dispose pas de marqueurs moléculaires de ces gènes ou de souches de bioagresseurs différentielles pour mettre en œuvre une sélection assistée par marqueurs (SAM) (Yencho et al., 2000). La combinaison de transgènes a été développée pour les gènes *Bt* en particulier chez le coton et le maïs. Sur le modèle chou-*Plutella xylostella*-*Bt*, Zhao et al. (2003) ont montré expérimentalement que la combinaison de transgènes de résistance (différents gènes *Bt*) retarde l'évolution vers la résistance aux gènes *Bt* des populations d'insectes par rapport à l'utilisation de résistances monogéniques en séquentiel (ou en mosaïque). L'impact de cette

stratégie peut être fortement amoindri si, dans le même temps, un des gènes de résistance est déployé seul dans un cultivar (Zhao et al., 2005). De même chez les virus, l'utilisation tout d'abord de la résistance monogénique, en favorisant la sélection de bioagresseurs virulents, peut ensuite servir de tremplin évolutif pour l'évolution vers la résistance polygénique (Palloix et al., 2009).

A l'échelle de la population

A cette échelle, les espèces d'agents pathogènes ont des potentiels évolutifs très contrastés. Ce potentiel évolutif peut être prédit par l'analyse de la structure génétique des populations d'agents pathogènes. La variabilité existant au sein des populations de ravageurs peut être générée, maintenue ou modifiée par des forces évolutives qui s'exercent sur une population et qui modifient sa structure allélique au cours des générations. Il s'agit d'une part de forces évolutives biologiques propres à ces organismes, telles que la reproduction sexuée et la mutation (d'une manière générale, on admet que le taux de mutation moyen chez les eucaryotes est de l'ordre de 10^{-6}), qui sont à l'origine de nouvelles combinaisons alléliques. Il s'agit, d'autre part, de pressions de sélection extérieures, induites par la plante-hôte, par le milieu, ou résultant de flux de gènes entre populations parasites dépendant de leur capacité à migrer, qui modifient la structure des populations en sélectionnant les individus selon leurs aptitudes à se maintenir dans des conditions déterminées. Les espèces d'agents pathogènes avec un fort potentiel évolutif ont plus de chance de contourner les résistances génétiques que les espèces avec un faible potentiel évolutif. Les pathogènes présentant le plus grand risque de contournement des gènes de résistance ont un système de reproduction mixte, *i.e.* alternance d'un cycle de reproduction sexuée et de plusieurs cycles de reproduction clonale, un fort potentiel de flux génétique, des tailles effectives de population grandes et des taux de mutations élevés (McDonald and Linde, 2002).

Du point de vue de l'hôte, la population locale de plantes cultivées est composée d'un nombre de génotypes variable, plus ou moins proches génétiquement. Une variété nouvellement introduite partagera ou non des facteurs de résistance avec les variétés déjà cultivées. Les succès commerciaux de la variété introduite et donc la surface qu'elle occupe est variable et influence la pression de sélection exercée sur la population pathogène. La performance d'une variété au moment de son inscription ne préjuge pas de sa stabilité au cours du temps. Chez la pomme de terre, les variétés ayant jusqu'à présent obtenu les notes les plus élevées dans les

épreuves d'inscription, du fait de la présence d'un ou de plusieurs gènes majeurs de résistance, ont vu leur résistance rapidement surmontée lors de leur déploiement à grande échelle (Montarry et al., 2006). A l'échelle de la population hôte, la durabilité est donc liée à la stratégie d'utilisation des résistances dans l'espace et le temps. Des stratégies sont développées pour protéger les résistances introduites dans les variétés, qu'elles soient supposées *a priori* durables ou non afin d'améliorer leur durabilité effective une fois que les variétés sont cultivées à grande échelle. En théorie elle repose sur le coût associé aux virulences inutiles dans une population pathogène.

Les producteurs de plantes *Bt* ont adopté la stratégie dite de haute dose refuge (HDR) qui implique la culture de plantes hôtes sensibles à proximité de plantes résistantes (Gould, 1998). Cette stratégie a pour objectifs de réduire la pression de sélection exercée par la résistance, de diluer les allèles de résistance aux toxines par brassage génétique avec des individus sensibles (se développant sur les plantes refuges), et d'augmenter la récessivité des gènes de contournements de la toxine. Cette stratégie concerne i) des espèces pathogènes diploïdes à reproduction sexuée pour lesquelles le contournement de la résistance de la plante est contrôlé par un allèle récessif et la fréquence de cet allèle récessif doit être initialement rare ii) des espèces pour lesquelles il y a des flux entre des plantes résistantes et sensibles dites plantes refuges et un accouplement aléatoire entre les pathogènes contournants et non contournants, ce qui requiert une phénologie comparable sur les plantes sensibles et résistantes. Les conditions de l'HDR ne sont donc pas remplies pour de nombreux pathogènes et ravageurs et les études expérimentales sur l'efficacité de la stratégie HDR sont rares. La réduction de la proportion d'insectes (*Plutella xylostella*) résistants à la toxine *Bt* quand la proportion de plantes (chou brocoli) non toxiques est élevée a été vérifiée (Tang et al., 2001). De nombreuses cultures résistantes *Bt* aux insectes ont été mises en place depuis 1996 (maïs, coton…) et d'après les observations menées aux Etats-Unis par l'USDA, les insectes cibles ont développé peu voire pas de résistance à la toxine (Fox, 2003). Cette observation suggère que la stratégie HDR a été payante puisqu'elle a été uniformément adoptée depuis 2000. Il a été exigé des agriculteurs la mise en place des zones refuges représentant 5 à 50% des surfaces cultivées en fonction de la culture et de l'agrosystème. La mise en place de la stratégie HDR nécessite donc une participation active des agriculteurs, voire une gestion communautaire de l'HDR puisqu'il a été montré qu'à l'échelle de dispersion du ravageur, l'agrégation des parcelles refuges est moins favorable à l'évolution de la résistance qu'une disposition aléatoire ou uniforme, l'agrégation des parcelles refuges pouvant même favoriser l'évitement des cultures *Bt* par les ravageurs.

Aux Etats-Unis, c'est aux distributeurs de semences qu'il revient de s'assurer que les agriculteurs respectent les recommandations (et de surveiller l'évolution de la résistance).

Plus généralement, les associations de variétés sont proposées pour diversifier et valoriser les gènes de résistances. C'est pour les céréales que l'utilisation des associations variétales est la plus répandue. Une expérience à grande échelle a été menée dans le Yunnan (Chine) pour la résistance à la pyriculariose du riz. La culture en association de variétés de riz gluant, de haute valeur gustative mais très sensibles à la pyriculariose, avec des variétés hybrides résistantes à la pyriculariose (Zhu et al., 2000) a permis de réduire de 3 à 7 les traitements fongicides, qui étaient en culture monovariétale, jusqu'à un seul traitement la première année d'expérimentation dans les associations variétales et de les supprimer complètement dès la seconde année. La sévérité de la maladie, qui était de 20 % en culture monovariétale de riz gluant, n'est que de 1 % dans les associations. Cependant, si l'efficacité des associations variétales pour diminuer les traitements contre les maladies fongiques a été mise en évidence sur le riz comme sur d'autres céréales, l'orge de printemps par exemple (Wolfe et al., 1992), l'effet sur l'émergence de variants virulents reste à caractériser.

Durabilité des résistances aux pucerons

- A l'échelle du gène : La relation gène pour gène (Résistance/Avirulence) n'a pas été formellement caractérisée à ce jour pour la résistance des plantes aux pucerons. La virulence dans le cadre des pucerons peut être définie comme la capacité à coloniser une plante. L'acquisition de la virulence peut se faire par deux mécanismes indépendants : i) soit la virulence est acquise par détoxification des effecteurs de la résistance comme c'est le cas dans l'adaptation aux insecticides (Walling, 2008) ; ii) soit la virulence est acquise par la perte de la reconnaissance entre le gène de résistance et le gène d'avirulence. A ce jour, aucun gène de virulence aux pucerons ni à d'autres hémiptères n'a été caractérisé. Le cadre de l'interaction gène pour gène chez les hémiptères a conduit à l'hypothèse que le produit du gène d'avirulence doit se trouver dans les composants de la salive (Kaloshian et al., 2000).
- A l'échelle de l'individu : L'effet de la combinaison de gènes de résistances (majeurs ou QTL) aux pucerons sur les populations de pucerons n'est pas documenté à ce jour.
- A l'échelle de la population : en termes de potentiel évolutif, les pucerons constituent des espèces de ravageurs présentant le plus grand risque de contournement des gènes de résistance. D'après McDonald and Linde (2002), le flux de gène des populations pathogènes est le facteur influençant le plus la durabilité des résistances, il est dépendant du mode de reproduction, des

capacités migratoires et de la taille effective des populations dans ces processus. Les pucerons ont un mode de reproduction mixte *i.e.* alternance d'un cycle de reproduction sexuée permettant de générer de nouveaux génotypes et de plusieurs cycles de reproduction clonale permettant aux génotypes localement adaptés d'augmenter considérablement en fréquence et de contribuer ainsi significativement au pool de gènes lors de la prochaine phase sexuée. La perte de la génération sexuée, chez certaines espèces ou populations de pucerons, constitue un processus évolutif dynamique qui peut être sélectionné dans des environnements stables et prédictibles. Les différentes stratégies de reproduction des pucerons peuvent être considérées comme adaptatives, en réponse aux variations environnementales (Hales et al., 1997; Rispe et al., 1998; Halkett et al., 2005). De plus, toujours en fonction des conditions environnementales (qualité de la plante, densité de populations), les pucerons ont la capacité de produire des individus ailés, morphes de dispersion (Muller et al., 2001). Ces populations ailées sont connues pour leur capacité à effectuer des mouvements à longue-distance, actifs ou passifs, lors de la migration saisonnière ou lors de phénomènes de dispersion, qui sont initiés suite à des forces intrinsèques et extrinsèques : qualité de la plante hôte, disponibilité des ressources, densité des populations, présence d'ennemis naturels, conditions atmosphériques (Van Emden and Harrington, 2007). Les pucerons connaissent des tailles efficaces de population très variables en réponse aux variations environnementales, notamment en fonction du climat. En région tempérée, pendant l'hiver, les effectifs de populations de pucerons sont fortement diminués et un goulet d'étranglement s'exerce sur les populations.

Sur les 87 gènes de résistance aux pucerons décrits chez les plantes cultivées une trentaine a été déployée chez 10 modèles plantes/puceron (Tableau 1). Un des déploiements les plus anciens, depuis 1930, concerne la résistance du framboisier à *Amphorophora agathonica* Hottes. Aujourd'hui toutes les variétés américaines de framboisier sont résistantes. Les premiers clones contournant le gène de résistance *Ag1* ont été observés dans les années 1980, soit une efficacité du gène pendant 50 ans (Keep, 1989). Chez le melon, des foyers d'*A. gossypii* capables de se développer sur les plantes résistantes ont été repérés. Cependant la résistance *Vat* reste toujours efficace en culture (Lombaert et al., 2009). Chez le blé deux biotypes de *D. noxia* contournent la résistance conférée par le gène *Dn4* (Shufran and Payton, 2009) et des biotypes de *S. graminum* contournent les résistances conférées par les gènes *Gb2* et *Gb3* (Puterka and Peters, 1995). Chez le pommier, deux gènes de résistance, *Er1* et *Er3*, sont contournés par *E. lanigerum* (Sandanayaka et al., 2005). Chez la laitue, le gène de résistance, *Nr*, est contourné par *N. ribisnigri* (données non publiées). Les gènes dominants de résistance aux pucerons ont

donc une efficacité limitée dans le temps. Aucun gène récessif de résistance aux pucerons n'a été déployé à notre connaissance et leur durabilité reste à démontrer. L'exception de résistance déployée à grande échelle et à long terme, mais non contournée à ce jour, est représentée par la résistance au phylloxera. L'hérédité de cette résistance n'est pas documentée. Le phylloxéra, *Dactylosphaera vitifoliae* (Fitch), est un puceron ravageur de la vigne décrit en 1868 et originaire de l'est des Etats-Unis, qui provoqua une grave crise du vignoble européen à partir de 1863. Il a en effet fallu plus de trente ans pour protéger le vignoble européen, en utilisant des porte-greffe issus de plants américains résistants au phylloxéra (Boubals, 1966). Quelques biotypes développant de petits foyers sur certains porte-greffe résistants ont été décrits. Les tests *in vivo* ont montré leur capacité à se développer sur ces accessions. Cependant aucun dommage sur des vignobles pourvus de porte-greffe résistants n'a été enregistré jusqu'à présent (Granett et al., 2007).

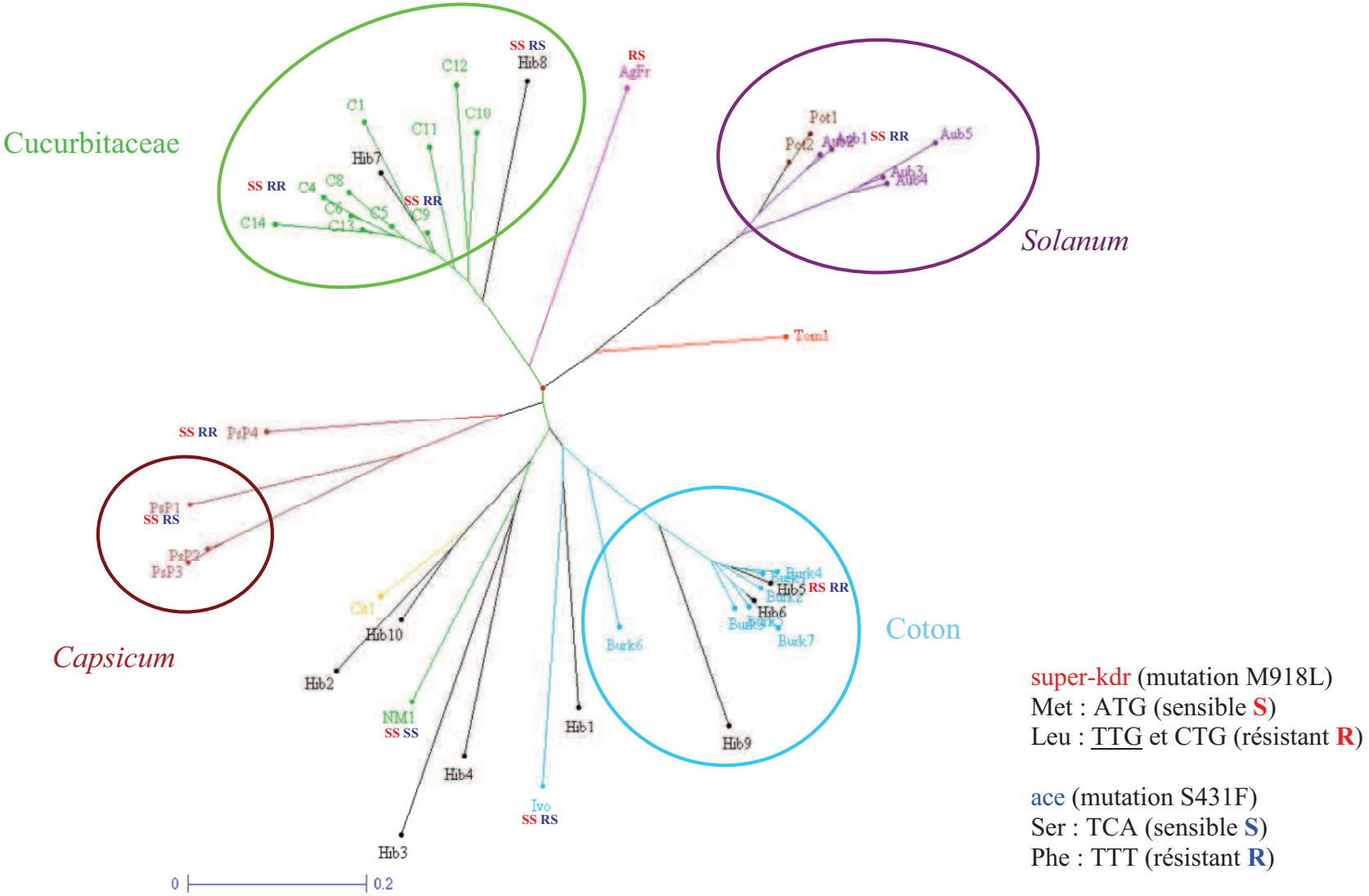
Partie 3 : Le modèle *Aphis gossypii* / *Cucumis melo*

Aphis gossypii

A. gossypii (Sternorrhyncha, Aphididae) est un puceron cosmopolite, très polyphage (il se nourrit sur 912 espèces de plantes appartenant à 116 familles (Inaizumi, 1980). Il s'attaque à de très nombreuses plantes spontanées ou ornementales mais il a une préférence pour les Cucurbitacées (melon, concombre, courgette), les Malvacées (cotonnier, Hibiscus) et les Rutacées (Citrus). On le rencontre dans les deux hémisphères, dans toutes les régions tempérées, subtropicales et tropicales à l'exception des zones désertiques et de l'Asie centrale. En Europe, son aire de répartition s'étend jusqu'au sud de la Scandinavie. Il est très dommageable en agriculture surtout dans les régions tempérées. L'espèce a été décrite à partir d'individus récoltés sur le cotonnier (*Gossypium sp.*), d'où son nom. Les Anglosaxons l'appellent donc cotton aphid mais aussi melon aphid car il fait, aux États-Unis, d'importants dégâts sur la pastèque et le melon.

Spécialisation et Adaptation

L'étude du génome mitochondrial a permis de différencier *A. gossypii* d'une espèce voisine, *Aphis frangulae* morphologiquement proche, en mettant en évidence la variabilité du gène codant pour le *cytochrome b*. Une variabilité de la taille d'amplification a également été observée au sein de l'espèce *A. gossypii*, avec trois haplotypes mis en évidence parmi des individus collectés en Europe, en Afrique, en Australie, en Amérique du Sud, à Madagascar et en Guadeloupe. Un haplotype regroupe les individus collectés sur piment, un deuxième haplotype regroupe les individus collectés sur Cucurbitacées et enfin un troisième haplotype regroupe les individus collectés sur d'autres hôtes (cotonnier, pomme de terre, aubergine et fraisier) (Carletto et al., 2009). Des marqueurs nucléaires de type RAPD avaient déjà mis en évidence l'existence d'une structuration par la plante hôte Cucurbitaceae (Vanlerberghe-Masutti and Chavigny, 1998). Plus récemment, sur la base de 8 marqueurs microsatellites, 11 MLG ont été identifiés parmi 1176 individus prélevés sur différentes espèces de plantes cultivées au Cameroun (Brevault et al., 2008) et parmi 559 individus en Tunisie (Charaabi et al., 2008). En utilisant le même set de marqueurs microsatellites, 44 génotypes multilocus (MLG) ont été décrits parmi 3611 individus échantillonnés sur tous les continents et sur différentes espèces cultivées de Cucurbitaceae, coton, piment, tomate, pomme de terre, citron,



super-kdr (mutation M918L)
Met : ATG (sensible **S**)
Leu : TTG et CTG (résistant **R**)

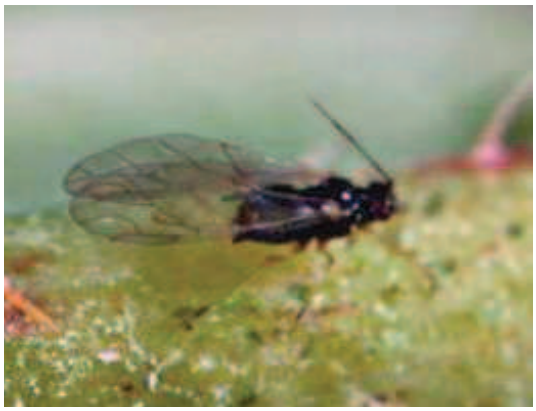
ace (mutation S431F)
Ser : TCA (sensible **S**)
Phe : TTT (résistant **R**)

Figure 1 : Arbre de Neighbor-Joining basé sur la distance allélique partagée par 8 marqueurs microsatellites entre les 44 MLG d'*A. gossypii* décrits par Carletto et al. (2009) en fonction des plantes hôtes où ils ont été collectés et de leur profils de résistance aux insecticides.

fraise ainsi que sur hibiscus (Carletto et al., 2009). Les 44 MLG se structurent en 6 clusters dont 4 races d'hôtes : Cucurbitacées, coton, piment et Solanum et 2 groupes non définis (Figure 1). Dans chacun de ces 6 clusters, on retrouve des génotypes décrits sur hibiscus. Douze MLG d'*A. gossypii* colonisant les Cucurbitacées ont été décrits, ils se répartissent dans deux clusters phylogénétiquement aussi éloignés entre eux qu'ils le sont des clusters de clones spécialisés sur d'autres plantes hôtes tels le coton ou la pomme de terre. Le MLG NM1, retrouvé sur Cucurbitacées uniquement dans le Sud-Est de la France, se trouve génétiquement isolé des autres MLG observés sur Cucurbitacées. Ceux-ci se regroupent dans un cluster clairement déterminé ; dans ce cluster, on trouve le MLG C9 présent dans toute l'aire de répartition d'*A. gossypii* (88% des clones observés dans le monde sur Cucurbitacées). En utilisant un set de 4 marqueurs microsatellites parmi les 8 utilisés par les auteurs précédents, 118 MLG ont été identifiés en Iran parmi 245 individus collectés sur différentes espèces de plantes cultivées et hibiscus (Razmjou et al., 2010). Toutes ces études montrent une structuration de l'espèce *A. gossypii* par la plante hôte, mise en évidence aussi bien à partir de l'ADN nucléaire que de l'ADN mitochondrial du puceron. En particulier, la spécialisation sur Cucurbitacées et sur piment révélée par les séquences mitochondriales suggèrent une spécialisation plus ancienne sur ces hôtes. La structuration génétique est en accord avec des observations biologiques obtenues en conditions contrôlées : lors d'expériences de transfert sur plantes hôtes différentes de leur hôte d'origine, les clones montrent une très forte diminution de leur potentiel biotique mais tous les clones se développent normalement sur *Hibiscus syriacus* (Liu et al., 2008; Carletto et al., 2009).

En plus de cette adaptation à différentes espèces cultivées, qui n'est actuellement pas datée, *A. gossypii* s'est adapté aux insecticides utilisés pour le contrer. La lutte chimique a été largement utilisée contre *A. gossypii* qui est devenu résistant aux organophosphates, au carbamate, à l'organochlorine et aux pyréthrinoïdes dans différents endroits du monde sur coton mais aussi sur cucurbitacées cultivées en champ et en serre. Les mécanismes de base de la résistance d'*A. gossypii* aux insecticides comprennent principalement une détoxification métabolique augmentée par des taux de carboxylesterases élevés (Delorme et al., 1997) due à des mutations dans les gènes structuraux du système nerveux central. L'acétylcholinestérase modifiée (gène *ace-1* ou *p-Ace*) confère une insensibilité au pirimicarbe (carbamate) et à l'ométhoate (organophosphorés) (Andrews et al., 2004; Toda et al., 2004). La modification du canal sodium voltage-dépendant sur le gène *para* (mutation super-kdr) induit une résistance aux pyréthrinoïdes. Carletto et al. (2010) ont étudié 6 clones d'*A. gossypii* sélectionnés à partir de leur spécialisation à différentes races d'hôte (Figure 1). Ces clones sont sensibles à

a. morphe aptère



b. morphe ailé

c. colonie de puceron, une femelle aptère et sa descendance larvaire

d. Rabougrissement des feuilles de melon causé par *A. gossypii*

e. Foyer de puceron

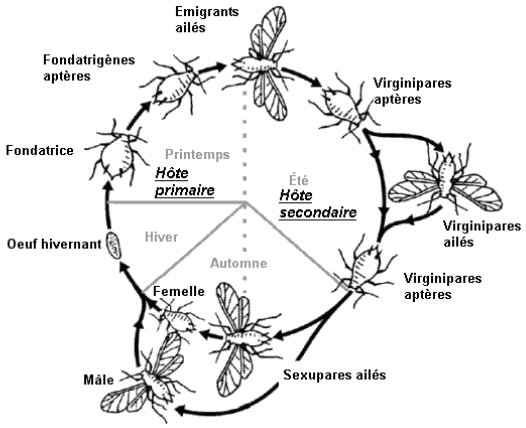
Figure 2 : Photographies du puceron du melon : morphes d'*A. gossypii* et symptômes sur plantes de melon.

l'acétamipride (neocotinoid) et au carbosulfan (carbamate) et résistants au diméthoate (organophosphate). Seulement deux de ces clones sont résistants à la cyperméthrine (pyréthrinoïde). En absence de résistance aux insecticides, le contrôle des pucerons est facile. Les plus efficaces, tels que les néonicotinoïdes (imidaclopride, thiamethoxam et dinotefuran utilisés aux Etats-Unis) et le pymétrozyne, donnent de bons résultats depuis des années. Aucun cas de résistance stable à l'imidaclopride n'a été trouvé et aucune information n'existe pour la résistance au pymétrozyne.

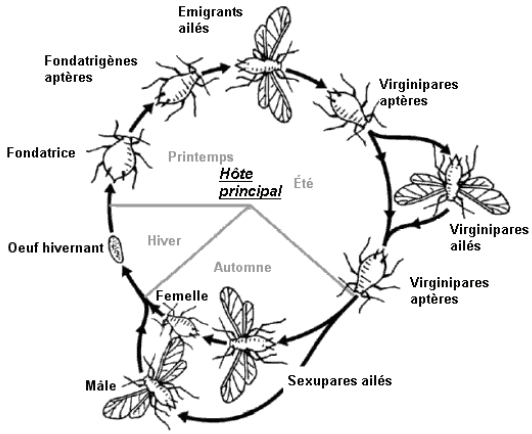
A. gossypii ravageur et vecteur de virus du melon

Les femelles ailées (Figure 2b) d'*A. gossypii* venant du milieu environnant colonisent les plantules de melon dès leur plantation. Elles s'installent sur les feuilles cotylédonaires et sur les premières feuilles et donnent naissance par viviparité à des larves qui évoluent en adultes aptères (Figure 2a). Les pucerons envahissent progressivement les feuilles de la plante au fur et à mesure de la croissance de la population. Une feuille peut héberger sur une face une colonie de plus de 150 individus (Figure 2e). Des femelles ailées apparaissent dans les colonies au cours des générations suivantes. L'apparition des femelles ailées est liée soit à un effet de groupe, soit à une diminution de la qualité nutritive de la plante hôte. Durant la période végétative du melon, les feuilles très turgescentes sont favorables au développement des pucerons. Après sa floraison, le melon se dessèche progressivement ; les femelles ailées deviennent alors de plus en plus nombreuses et quittent la plante pour en rechercher d'autres, plus favorables à leur développement. Ces femelles vivipares sont très mobiles et vont de plante à plante déposer leur progéniture. Pendant toute cette phase la reproduction est parthénogénétique.

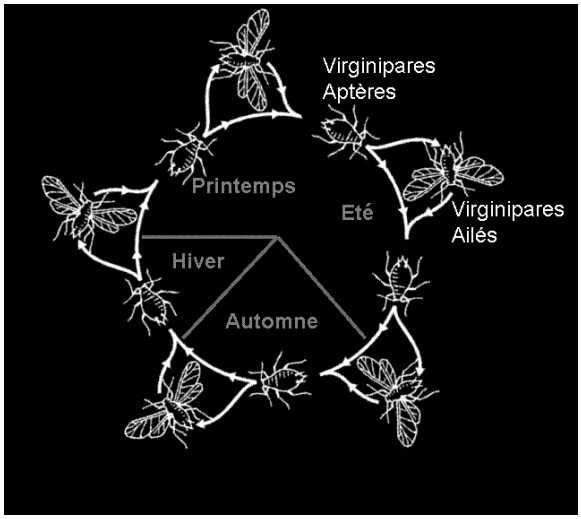
Les piqûres nutritionnelles des pucerons (larves et imagos) provoquent sur le melon des dégâts directs qui se traduisent par des déformations des feuilles en début de végétation (Figure 2d), un ralentissement de la croissance (Miles and Peng, 1989) et une diminution de la production de fruits. Les produits de la digestion, qui ne sont ni assimilés ni transformés, sont rejetés sous forme de miellat. Celui-ci est non toxique mais il peut directement occulter les stomates ou provoquer un effet osmotique à la surface des feuilles favorisant l'évaporation. De plus, il constitue un milieu de culture très favorable au développement de champignons saprophytes qui recouvrent les feuilles d'un feutrage noirâtre, la fumagine. Celle-ci entrave la respiration et l'assimilation chlorophyllienne et souille les parties consommables alors impropres à la commercialisation. Au laboratoire, à une température moyenne de 24-25°C (photopériode 18:6



a. Holocyclie hétéroécique



b. Holocyclie monoécique



c. Anholocyclie

Figure 3 : Cycles biologiques des pucerons.

heures), le développement larvaire d'*A. gossypii* dure environ six jours sur melon. Trois à quatre heures après sa mue imaginale, la femelle vivipare donne directement naissance à de jeunes larves capables aussitôt de se déplacer et de s'alimenter. Dans les élevages, on obtient en moyenne 26 descendants par femelle en 10 jours, et pour certains individus, la période de reproduction peut atteindre 17 jours. La fécondité des pucerons est probablement plus importante sur le melon en plein champ car ils disposent alors d'une meilleure alimentation et d'un espace plus grand. Leurs populations sont cependant limitées par différents antagonistes, prédateurs, parasites ou pathogènes, ainsi que par l'action du vent et de la pluie souvent associés en orages. Près de 20 à 40% d'une colonie d'*A. gossypii* peut ainsi être détruite par le passage d'un orage.

Le cycle d'*A. gossypii* diffère selon les latitudes, on peut trouver différents cycles intermédiaires entre l'holocyclie stricte et l'anholocyclie (Figure 3). En région tropicale *A. gossypii* peut se maintenir toute l'année sur plusieurs espèces de Cucurbitacées sans reproduction sexuée ; en région tempérée à hiver doux, il peut hiverner sur des Cucurbitacées dans les zones protégées telles que les serres; il a alors un comportement anholocyclique (Van Emden and Harrington, 2007). Dans les régions plus septentrionales, une génération sexuée alterne avec plusieurs générations parthénogénétiques : *A. gossypii* présente alors un développement holocyclique. Le déroulement de l'holocycle est le suivant : à l'automne apparaissent des femelles parthénogénétiques appelées sexupares qui donnent naissance soit à des mâles (généralement ailés) soit à des femelles ovipares (le plus souvent aptères) soit à des individus des deux sexes (Takada, 1988; Ferrari and Nicoli, 1994). Les femelles fécondées pondent quelques œufs d'hiver sur la plante hôte primaire qui appartient au genre *Hibiscus*. Chaque œuf d'hiver demeure en diapause jusqu'au printemps ; il donne alors naissance à une femelle parthénogénétique, généralement aptère, appelée fondatrice. La fondatrice engendre une ou plusieurs générations de femelles parthénogénétiques, les fondatrigènes ; celles de la première génération sont presque toujours aptères, puis la proportion d'ailées augmente dans les générations suivantes. Les fondatrigènes ailées quittent la plante hôte primaire pour coloniser d'autres plantes de la même espèce ou d'espèces différentes où elles donnent naissance à plusieurs générations de femelles parthénogénétiques aptères ou ailées, les virginogènes ou virginipares.

A. gossypii est un redoutable vecteur de virus chez les Cucurbitacées et le melon en particulier (Tableau 2). C'est par les micro-blessures produites lors de piqûres d'essai ou d'alimentation

Tableau 2 : Principaux virus transmis par *A. gossypii* infectant les Cucurbitacées cultivées : distribution géographique et gamme d'hôtes. D'après Lecoq et al. (1998).

Genre et virus	Mode de transmission	Premier rapport	Distribution	Gamme d'hôtes	
				Cucurbitacées	Non-Cucurbitacées
Cucumovirus					
Cucumber mosaic virus, CMV	non-persistant	1916	mondial	large	large
Luteovirus					
Cucurbit aphid borne yellows virus, CABYV	persistant	1992	mondial	large	intermédiaire
Potyvirus					
Clover yellow vein virus, CIYVV	non-persistant	1965	mondial	étroite	large
Melon vein-banding mosaic virus, MVBMV	non-persistant	1993	Taïwan	large	intermédiaire
Papaya ringspot virus, PRSV	non-persistant	1949	mondial, tropical et subtropical	large	étroite
Telfairia mosaic virus, TeMV	non-persistant	1975	Nigéria	large	large
Morocco Watermelon mosaic virus, MWMV	non-persistant	1974	Afrique et bassin méditerranéen	large	étroite
Watermelon mosaic virus, WMV	non-persistant	1954	mondial, tempéré et méditerranéen	large	large
Zucchini yellow fleck virus, ZYFV	non-persistant	1981	Bassin méditerranéen	large	étroite
Zucchini yellow mosaic virus, ZYMV	non-persistant	1981	mondial	large	intermédiaire

que les virus pourront être prélevés ou introduits dans les plantes. La majorité des virus attaquant le melon sont transmis selon le mode non persistant : ils sont ingérés en quelques secondes à quelques minutes par le puceron au moment des piqûres d'essai. Les particules virales sont retenues sur la cuticule tapissant les canaux du stylet ou la partie antérieure du tube digestif durant quelques minutes. La phase d'inoculation se ferait par égestion et/ou salivation sur une plante saine. Moury et al. (2007) ont montré qu'un puceron transmettait seulement 0.5 à 3.2 particules virales en moyenne ce qui est extrêmement faible comparé à la population virale présente dans une plante infestée. Ces virus transmis sur le mode non-persistant sont plusieurs potyvirus et le *Cucumber mosaic virus*, CMV (Tableau 3). Un seul virus, le *Cucurbit aphid borne yellows virus*, un luteovirus, est transmis au melon selon le mode persistant par *A. gossypii*. Il infecte principalement les tissus du phloème et l'acquisition par les pucerons s'opère pendant les phases prolongées d'absorption de sève élaborée au niveau du phloème. Les particules virales effectuent un circuit complexe dans le corps du puceron et franchissent différentes couches de cellules.

Tableau 3 : Principales caractéristiques des différents modes de transmission des virus par les pucerons. D'après Leclant (1982).

	Virus non circulants		**Virus circulants**	
	virus non persistants virus de stylets	**virus semi-persistants**	**virus persistants non multipliants**	**multipliants**
Acquisition	très brève (secondes) (piqûres d'essais)	brève (minutes)	longue (heures)	longue (heures)
Latence	non	non	oui	oui
Rétention	courte (quelques heures)	assez longue (plusieurs heures à quelques jours)	longue (plusieurs jours à toute la vie)	longue (toute la vie)
Conservation après une mue	non	non	oui	oui
Transmission à la descendance	non	non	non	non/oui
Spécificité de transmission	faible	étroite	étroite	étroite

En conclusion, les principaux caractères de la biologie d'*A. gossypii* montrent l'extraordinaire potentiel adaptatif de cette espèce au milieu :

- adaptation à l'exploitation rapide de la plante hôte du fait de la viviparité, de la parthénogenèse et du chevauchement de générations,

Figure 4 : Diversité de fruits de melon (photos INRA)

Tableau 4 : Campagne de production de melon 2010 en France (janvier à décembre).

Unités : surface : ha, production : t

Estimations au 01-Sep-2010 (2007 pour la Guadeloupe)		**Centre Ouest**	**Sud Ouest**	**Sud Est**	**Autres bassins**	**France**	**Guadeloupe**
Surface	Serre	2	28	677	21	**728**	///
	Abri bas	3 067	751	3 587	8	**7 413**	///
	Plein air	2 264	2 563	1 774	7	**6 608**	///
	Total surfaces	5 333	3 342	6 038	36	**14 749**	515
	Evol 1 an	*2%*	*-1%*	*0%*	///	***1%***	-2%
	Evol 5 ans	*-1%*	*-4%*	*3%*	///	***0%***	36%
Production	Total production	95 376	63 435	125 656	775	**285 242**	9 061
	Evol 1 an	*0%*	*-6%*	*0%*	///	***-1%***	10%
	Evol 5 ans	*8%*	*2%*	*1%*	///	***3%***	23%

Source : Agreste

- adaptation à la colonisation de nouvelles plantes hôtes, après la détérioration du milieu, par la production de morphes ailés,
- adaptation à une très large gamme de climat et aux conditions climatiques locales qui se traduisent tantôt par la reproduction sexuée obligatoire et la ponte d'œufs d'hiver diapausant tantôt par la persistance de générations parthénogénétiques,
- adaptation aux contraintes anthropiques avec un statut de ravageur sur de nombreuses espèces cultivées et adaptation aux insecticides largement utilisés en agriculture.

Melon

Importance de la culture

Ce légume présente une diversité considérable, beaucoup plus large que celle limitée des variétés de type Charentais cultivées en France (Figure 4). Ce fruit charnu appartient à la famille des Cucurbitacées au même titre que le concombre, la pastèque ou les courges. Il est originaire d'Afrique intertropical (Renner et al., 2007) sous la forme sauvage et s'est diversifiée depuis l'Asie, de la méditerranée à l'Extrême-Orient (Renner et al., 2007). Les premières traces de domestication remontent à 2000-2700 avant JC en Egypte, en Mésopotamie et dans l'est de l'Iran et l'on retrouve des traces de la présence d'espèces cultivées vers 1000 avant JC en Inde puis au 1er siècle après JC en Grèce et en Italie (Pitrat et al., 2000). En France, les premières traces de melon remontent au capitulaire de Charlemagne vers 795 puis il a largement été cultivé à partir du XVIe siècle. De part sa grande diversité, il est destiné à des usages très variés que ce soit au niveau de la consommation, de l'intérêt aromatique ou de l'ornementation. Sa sélection porte essentiellement sur la taille, l'épaisseur de la chair, l'arôme et le caractère sucré des fruits. Ainsi le melon est cultivé dans environ 80 pays. Il se place au 7ème rang parmi les légumes avec plus de 27 millions de tonnes produites en 2008 dont environ 300000 tonnes en France (données FAO, 2008) et (Tableau 4). La France se situe au 11ème rang mondial de la production de melon, la Chine étant le premier producteur, fournissant 38% de la production. Pour l'Union Européenne, c'est l'Espagne qui est le premier producteur de melon. En France, le melon est essentiellement consommé en tant que fruit sucré et le type Charentais est le plus cultivé depuis une cinquantaine d'années.

La résistance à *A. gossypii* chez le melon

En 1967, l'équipe de Kishaba et Bohn a engagé une étude systématique de la résistance aux pucerons chez le melon. Ils retiennent la lignée LJ 90634, appelée plus tard PI 414723, peu attaquée au champ. Cette accession exprime de l'antibiose contrôlée par un gène majeur et des gènes mineurs (Kishaba et al., 1971, 1976), de l'antixénose (Kennedy and Kishaba, 1977) et de la tolérance (Bohn et al., 1973) contrôlée par une gène dominant *Ag*. Plus tard, Pitrat et Lecoq étudient la lignée coréenne PI 161375 qui possède le gène *Ag* (Bohn et al., 1973). PI 161375 exprime de l'antixénose, observée sur aptères (Pitrat and Lecoq, 1980) et est complètement résistante au CMV quand il est transmis par *A. gossypii* (Lecoq et al., 1979). Ces deux caractères co-ségrégent et sont contrôlés par un gène dominant dénommé *Vat* (Pitrat and Lecoq, 1980). PI 414723 exprime aussi de l'antixénose sur aptères, et est résistante au CMV quand il est transmis par *A. gossypii* (Pitrat and Lecoq, 1980). Antibiose et antixénose, qui co-ségrégent dans des descendances de PI 414723 sont contrôlées par un gène dominant (Pitrat and Lecoq, 1982). De plus, dans un programme de back-cross de l'antixénose de PI 161375 dans le type Charentais, comme dans un programme de back cross de la résistance aux pucerons de PI 414723 dans le type Honeydew, les nouvelles lignées créées sont résistantes au CMV après transmission par *A. gossypii*. Considérant l'ensemble de ces résultats Pitrat and Lecoq (1982) font l'hypothèse que l'antibiose, l'antixénose et la résistance aux virus transmis par *A. gossypii*, appelée résistance à la transmission, sont contrôlées par un même locus, le locus *Vat* (pour *Virus aphid transmission* resistance). La résistance aux virus après transmission par puceron n'est exprimée que lorsque le vecteur est *A. gossypii* et elle est efficace contre le CMV, le Watermelon mosaic virus (WMV), le Papaya ringspot virus (PRSV anciennement WMV1) (Lecoq et al., 1979; Lecoq et al., 1980) et le Zucchini yellow mosaic virus (ZYMV anciennement MYSV) (Risser et al., 1981), tous transmis selon le mode non persistant. De nombreuses autres accessions se sont révélées résistantes à *A. gossypii* et résistantes aux virus transmis selon le mode non persistant par *A. gossypii*. Ces accessions sont originaires aussi bien du Moyen-Orient que d'Europe ou d'Afrique (MacCarter and Habeck, 1974; Pitrat and Lecoq, 1980, 1988; Soria et al., 2000; Boissot et al., 2008). L'hérédité de la résistance n'est pas établie pour toutes les accessions et en fait peu d'informations sont disponibles pour évaluer la variabilité de la résistance. Mais dès 1971, Kishaba et al. notaient que la résistance au biotype d'*A. gossypii* présent dans le Sud-Est des Etats-Unis était inefficace contre le biotype présent dans le Sud-Ouest ; MacCarter and Habeck (1974) ont observé le cas contraire. L'accession PI 161375 fortement résistante à un clone de génotype NM1 (observé jusqu'à maintenant seulement en France) et aux virus quand ils sont transmis par ce clone apparait peu résistante à

un clone espagnol (non identifié) et un clone de génotype C9, cependant la résistance aux virus reste entière quand ils sont transmis par ces clones (Soria et al., 2000; Boissot et al., 2008).
Le locus *Vat* a été positionné sur le groupe de liaison V en position sub-télomériс (Périn et al., 2002) puis cloné dans l'accession PI 161375 (Dogimont et al., 2008). L'alignement des cartes obtenues à partir de PI 161375 et PI 414723 a confirmé que le même locus, ou deux loci très proches, contrôlait la résistance dans les deux accessions (Périn et al., 2002). Ce gène appartient à la famille de gène de résistance de type NBS-LRR (voir la structure détaillée dans la partie 1.3). Plusieurs QTL à effets additifs ou épistatiques impliqués dans l'antixénose et l'antibiose ont été détectés et localisés sur le génome (Boissot et al., 2008). Les bases moléculaires de la résistance du gène *Vat* sont décrites dans la partie 1.3.

Les melons exprimant le gène de résistance *Vat* ont une double résistance : i) à *A. gossypii*, ii) aux virus non-persistants lorsqu'ils sont transmis par *A. gossypii*. Nous tenterons ici de faire une synthèse des réactions de la plante porteuse du gène *Vat* et du puceron qui tente de la coloniser en rapportant les faits spécifiquement observés dans l'interaction plante *Vat*/*A. gossypii* et les faits extrapolés (qui seront présentés en italique) à partir des connaissances générales de l'interaction plante/puceron ou des réactions dues à l'interaction gène de résistance de type NBS-LRR/ facteur d'avirulence.
Dès son arrivée sur la plante, le puceron effectue des piqûres d'essais ayant lieu les 10 à 20 premières minutes de contact plante-puceron et pendant lesquels il y a des phases d'ingestion du contenu cellulaire ou extracellulaire et d'égestion de salive (Martin et al., 1997). Les virus non-persistants, qui infectent l'ensemble des tissus de leur hôte végétal, sont ingérés par le puceron en quelques secondes à quelques minutes au moment des piqûres d'épreuves aussi bien sur melon sensible que sur melon résistant (préalablement inoculé mécaniquement) (Lecoq et al., 1980). Dès ces piqûres d'essai, *si le puceron est porteur dans ses stylets d'un virus, celui-ci sera transmis dès ces premières phases. Le puceron va injecter dans la plante divers composés contenus dans la salive qui auront pour mission de détourner les défenses basales. Au cours de cette phase, le puceron va injecter le facteur d'avirulence correspondant au gène* Vat*. L'interaction du facteur d'avirulence avec la protéine Vat (interaction directe ou indirecte) peut avoir lieu dès ces phases précoces, elle déclenchera la mise en place chez la plante des effecteurs de la résistance.* Les plantes expriment une réponse d'hypersensibilité microscopique sans symptôme nécrotique visible (Villada et al., 2009). *La multiplication virale est bloquée. Face aux effecteurs, A. gossypii* présente différents types de réponses :

- des réponses comportementales : sur Margot (dérivant de PI 161375 chez qui *Vat* a été cloné) lors des piqûres d'essai et la recherche du phloème, la durée et la fréquence des ponctions intracellulaires dans des tissus non-nourriciers sont réduites (Chen et al., 1997). Cette phase conduit finalement à l'abandon de la plante avant même l'atteinte du phloème. De même sur PI 161375, PI 414723 et AR 5 (dérivant de PI 414723) dès les 3 premières heures, l'alimentation phloémienne est entravée : l'activité de salivation est augmentée et la phase d'ingestion est réduite voir inexistante (Klingler et al., 1998; Garzo et al., 2002). Sur l'accession 90625 (porteur d'un allèle de *Vat* différent de celui de PI 161375) le puceron s'alimente uniquement dans le xylème sans jamais atteindre le phloème (Boissot et al., 2000).
- des réponses biologiques : Certains individus peuvent se maintenir sur la plante mais atteignent peu le phloème et limitent leurs apports en sucres. Sur AR 5, cette faible alimentation cause un retard de 5h dans l'excrétion des pucerons qui produisent ensuite très peu de miellat (Klingler et al., 1998). Sur une plus longue période allant de quelques jours à quelques semaines la durée de développement larvaire est augmenté (d=6,2 sur Védrantais et d=7,5 sur Margot), la période de fertilité est diminuée ainsi que leur longévité et leur potentiel biotique sans formation de colonies (r_m=0,44 sur Védrantais et r_m=0,27 sur Margot).

Problématique

Notre modèle d'étude est l'interaction melon-puceron : Chez le melon, la résistance à *A. gossypii* conférée par le gène *Vat* induit un double phénotype de résistance à *A. gossypii* et aux virus quand ils sont transmis par *A. gossypii*. De plus ce gène a été cloné et plusieurs allèles de résistance au locus *Vat* sont aujourd'hui connus. Chez *A. gossypii*, il existe des marqueurs moléculaires permettant d'explorer la diversité génétique de l'espèce. Des génotypes de pucerons de référence, NM1 et C9, isolés dans le sud-est de la France ont été caractérisés pour leur performance sur des accessions de melons sensibles et résistants.

Chez le melon, la résistance au puceron *A. gossypii* contrôlée par le gène *Vat* est présente dans une grande partie des variétés de melon cultivées dans le Sud-Est de la France depuis le début des années 1990. La moitié des nouvelles variétés de type Charentais inscrites chaque année au Catalogue français des Obtentions Variétales et 80% des hybrides cultivés sous serre possèdent la résistance. Les traitements aphicides des cultures de melons résistants sont quasiment supprimés dans le Sud-Est de la France. La résistance constitue désormais une pression de sélection à l'échelle de la région PACA sur les populations de pucerons, et on voit ainsi apparaître, de manière localisée, depuis quelques années, des foyers de pucerons capables de contourner la résistance de la plante. Les seuls contournants observés, sur melons résistants, jusqu'à présent, sont de génotype C9 (Lombaert et al., 2009).

Cependant la durabilité d'une résistance est *a priori* inconnue. La durabilité d'une résistance peut être définie comme la persistance de son efficacité dans le temps et dans l'espace. Elle dépend donc directement de la capacité des populations du parasite à s'adapter aux variétés résistantes, lorsque celles-ci sont déployées en culture, et de la pression de sélection exercée par les résistances sur les populations parasites. L'adaptation est le résultat de l'ensemble des forces évolutives s'exerçant sur les populations (sélection, flux de gènes, mutation, recombinaison et dérive génétique). Dans un premier chapitre, nous étudierons la diversité existant au sein des populations naturelles d'*A. gossypii* représentées par les populations infestantes de printemps ainsi que leur potentiel adaptatif (sélection par la plante hôte, mode de reproduction) et la variabilité génétique des clones d'*A. gossypii* sur melon.

Afin d'assurer la durabilité des résistances, l'enjeu est de gérer les pressions de sélection exercées par les gènes de résistance ou combinaisons de gènes de résistance sur le bio-agresseur et de proposer des éléments stratégiques aux semenciers pour qu'ils développent des variétés avec des résistances durables. Dans un second chapitre, nous présenterons les combinaisons de résistances utilisées dans ce travail (*Vat* et QTL). Ces résistances ont été confrontées aux

populations naturelles décrites dans le premier chapitre afin d'estimer leur efficacité, l'influence de *Vat* et des QTL sur la densité des populations et sur la structuration génétique des populations d'*A. gossypii.*

Dans un troisième chapitre, la caractérisation de clones de référence sur un set d'accessions et de clones contournant la résistance (valeur sélective des biotypes et la virulence, structure génétique des populations, fréquence de contournement) permettront de mettre en évidence le mode de contournement.

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CHAPITRE 2 : DIVERSITE GENETIQUE DU PUCERON DU MELON, *APHIS GOSSYPII*, DANS DIFFERENTS BASSINS DE PRODUCTION DE MELON FRANÇAIS

Chapitre 2 : Diversité génétique du puceron du melon, *A. gossypii,* dans différents bassins de production de melon français

On sait qu'il existe des biotypes au sein de l'espèce *A. gossypii*, associés à une baisse de fécondité d'un clone placé sur une plante hôte appartenant à une autre famille botanique que celle sur laquelle il a été prélevé *in natura* (Inaizumi, 1981; Takada, 1988; Guldemond et al., 1994; Wool et al., 1995). Plus récemment, le développement des marqueurs moléculaires a permis de montrer que ces biotypes correspondaient en réalité à des génotypes différents et, une première structure génétique dans l'espèce *A. gossypii*, correspondant à une famille de plantes hôtes a pu être identifiée, la race d'hôte « Cucurbitacées » (détaillé dans la partie 3.1.1 de l'introduction bibliographique) (Vanlerberghe-Masutti and Chavigny, 1998; Fuller et al., 1999). L'originalité de l'étude présentée ici réside dans l'échantillonnage et l'analyse génétique, à l'aide de marqueurs microsatellites, de pucerons ailés puis de pucerons aptères prélevés sur des plantes de melon sensibles d'une même culture. L'objectif est de connaître la diversité d'*A. gossypii* atterrissant sur une culture de melon afin de répondre aux différentes questions : Est-ce que la diversité observée chez les ailés est comparable à celle observée chez les aptères ayant développés des colonies sur les plantes ? Dans ce cas aucun filtre sélectif dû à la plante hôte n'agirait sur les populations ailées. Ou au contraire est-ce que la diversité observée chez les ailés est plus grande que celle observée chez les aptères ? Dans ce cas la plante hôte exercerait une pression sur les populations ailés. La connaissance du rapport entre la diversité disponible et la diversité efficace d'*A. gossypii* sur les cultures de melon nous permettrait d'émettre des hypothèses sur les méthodes de gestion des résistances à mettre en place. En outre, une étude de génétique des populations d'*A. gossypii* permettrait d'évaluer le potentiel adaptatif de l'espèce en réponse à son environnement afin de prédire le risque d'extension du contournement des résistances.

En Europe, l'espèce *A. gossypii* est très voisine et pratiquement indiscernable sur des critères morphologiques de l'espèce *Aphis frangulae* Kaltenbach. Elles constituent avec les espèces *Aphis capsella* Kaltenbach, *Aphis beccabungae* Kock un complexe de sous-espèces défini comme le complexe *frangulae*. L'unique différence morphologique entre les espèces *A. gossypii* et *A. frangulae* réside dans la différence du rapport entre la longueur du processus terminal et la longueur de la partie basale de l'article antennaire VI (ou V). Ce ratio est égal à

3.0 chez les aptères de l'espèce *gossypii* contre 2.9 pour ceux de l'espèce *frangulae*. D'un point de vue moléculaire, l'étude de la variabilité nucléotidique dans une séquence du gène du cytochrome b a révélé l'existence d'une différence de 2% entre les deux espèces et l'étude du produit d'amplification de l'intron du gène paralogue a révélé une différence de taille de 400 paires de bases (Carletto et al., 2009). Chez *A. Frangulae*, une autre différence se situe au niveau du marqueur microsatelllite ago 53 qui est monomorphe à 110 paires de bases à ce locus (Vanlerberghe, communication personnelle). Afin de s'affranchir des échantillons susceptibles d'être des *A. frangulae*, j'ai éliminé tous les individus monomorphes au locus ago 53 (110 pb). Puis j'ai testé 10 échantillons, en particulier des échantillons prélevés à Angers et dont le MLG est assigné au cluster A (voir article 1), avec le marqueur spécifique de l'intron dans le gène codant pour le cytochrome b, décrit ci-dessus, afin de s'assurer que nos échantillons étaient bien des *A. gossypii*.

Les échantillonnages ont été réalisés dans 16 essais plein champ. J'ai supervisé les 10 essais de 2008 et 2009 et pris en charge les échantillonages avec l'équipe de l'UR GAFL-Avignon et les équipes expérimentales de l'INRA de Guadeloupe, de l'INRA d'Angers et du CEFEL de Montauban. Les 6 essais réalisés avant 2008 ont été supervisés par l'équipe de l'UR GAFL-Avignon qui a aussi réalisé les échantillonages. J'ai récupéré les données de génotypage de 2004 et 2006 (835 échantillons) obtenues par Jérome Carletto et j'ai réalisé le génotypage des échantillons de 2007, 2008 et 2009. En 2007, 2008 et 2009, sur 8128 échantillons prélevés et analysés à l'aide des 8 marqueurs microsatellites, j'en ai retenu 5950 répondant au moins à 7/8 marqueurs. Parmi ces 5950, j'en ai éliminé 149 qui étaient monomorphes au locus ago 53, tous ces individus avaient été échantillonnés à Angers.

Ce travail est présenté sous forme d'un article, appelé dans la suite du manuscrit article 1, qui sera soumis à BMC Evolutionary Biology.

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Article 1 : What do spring migrants reveal about sex and host choice in the melon aphid ?

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Abstract

Aphis gossypii is a major pest of Cucurbitaceae and cotton worldwide. We investigated the genetic diversity and population structure of *A. gossypii*, in melon agrosystems in relation to events of sexual reproduction. Alate and apterous aphids were sampled on melon crops in four areas of France. Aphids were characterized using 8 microsatellite markers. We identified 596 multilocus genotypes (MLG), 82% of the individuals have a MLG observed common to both alate and apterous samples. The clonal richness is lower in apterous samples than in alate samples suggesting a strong selective pressure on the alate populations exerted by melon crops. The genetic diversity of the whole sample was structured by region. About a quarter of the MLGs clustered with MLGs previously assigned to the Cucurbitaceae host race; more than 70% of the individuals had one of these MLGs. The large diversity observed is puzzling given the strict asexual reproduction of *A. gossypii*, then we hypothesized events of sexual reproduction Because sexual reproduction probably occurred the previous autumn, we looked for genetic fingerprints in alate populations infesting crops in Spring. No trace of sexual reproduction was identified among the cucurbit cluster except in western France. Traces of sexual reproduction were identified among the spring migrants not assigned to the cucurbit cluster; these populations could originate from *Hibiscus siriacus* which have been shown to be host of *A. gossypii* sexual morphs. Therefore the effective diversity for Cucurbitacae crop agrosystems appeared restricted to a part of the whole *A. gossypii* diversity and this effective diversity was not significantly altered by sexual reproduction.

Introduction

Predictability and abundance of host resource is often assumed to favour ecological specialization in populations of parasites having a large spectrum of hosts, through disruptive selection on host preference, leading gradually to reproductive isolation and speciation if assortative mating occurs (Jaenike, 1990; Via, 2001; Berlocher and Feder, 2002; Dres and Mallet, 2002; Fry, 2003; Rundle and Nosil, 2005). In plant-feeding insects, these host-adapted populations are called host races (Peccoud et al., 2010). Aphids are small, soft-bodied insects that feed specifically from the sieve element of plants and cause damage by draining plant nutrients. Like many parasites, aphids have evolved complex life cycles with successive generations of individuals often being specialized to cope with different ecological conditions, but they are among the few organisms showing plasticity in their reproductive mode as an

adaptive response to seasonal changes (Simon et al., 2010). The typical aphid reproductive cycle, called holocycly, involves a succession of parthenogenetic generations (clonal or asexual reproduction), followed by a single sexual reproduction event within the annual life cycle. About 90 % of aphids are monoecious (Eastop, 1986), *i.e*. they remain on the same host species or closely related plant species throughout the life cycle. In this case, host plants exert considerable selective pressures as plants constitute their feeding, mating and ovipositing sites. On the contrary, heteroecious aphids alternate between the primary host – usually a woody plant – on which they migrate in autumn for sexual reproduction, and secondary hosts – usually herbaceous plants – on which they migrate in spring and develop numerous parthenogenetic colonies. In addition, 30-40% of the aphid species show a variation in reproductive mode between and even within populations (Moran, 1992).

Most of the approximately 4700 species of aphid are specialists as they feed on only one or a few species of plants, and closely related aphid species tend to feed on related species of plants. Only 5% of aphid species are polyphagous that frequently encompass host races or host-specialized populations with a narrow host range (Peccoud et al., 2010). Such patterns have been described in the pea aphid *Acyrthosiphon pisum* (Via, 1991; Simon et al., 2003; Frantz et al., 2006), in the grain aphid *Sitobion avenae* (Lushai et al., 2002; Vialatte et al., 2005), in the peach-potato aphid *Myzus persicae* (Blackman, 1987; Margaritopoulos et al., 2000; Zamoum et al., 2005) and in the cotton-melon aphid *Aphis gossypii* (Carletto et al., 2009). The species *A. pisum* and *S. avenae* are monoecious and their host plant range is restricted to Fabaceae and Poaceae respectively. On the contrary, *M. persicae* and *A. gossypii,* that are known as heteroecious species, are specialized on the primary host and polyphagous on secondary hosts which belong to numerous plant families (Van Emden and Harrington, 2007). Both species developed parthenogenetically on secondary hosts represented by cultivated crops.

A. gossypii distributed worldwide and was found on numerous host plants. At the world level, Inaizumi (1980) listed 912 plant species belonging to 116 families. *A. gossypii* is a pest on cultivated Cucurbitaceae (melon, marrow, zucchini, watermelon), Malvaceae (cotton, okra, ornamental hibiscus), Solanaceae (potato, chili pepper, sweet pepper, eggplant) and it is also found in abundance in citrus orchards and on many ornamental plants including chrysanthemums and *Hibiscus* (Ebert and Cartwright, 1997). Despite a distribution over a wide area, the genetic diversity of *A. gossypii* on several cultivated host plants has appeared very low. Genetic studies of *A. gossypii* populations collected from all over the world in

heavy infested crops (large colonies of apterous) revealed a strong structuration according to host plants. Indeed, the analysis of more than 3000 aphids using eight microsatellite loci revealed only 44 distinct multilocus genotypes (MLGs) that clustered into six genetic groups. Four of these groups corresponded to host races respectively specialized on Cucurbitaceae, cotton, *Solanum* and pepper (Carletto et al., 2009). These host races were dominated by asexual clones. In warm areas of the world, *A. gossypii* reproduces throughout the year by obligate parthenogenesis. In Europe, *A. gossypii* is also considered a clonal species, overwintering in glasshouses (Blackman and Eastop, 2000). However, few cases of sexual reproduction events have been reported on *Hibiscus syriacus* (Ferrari and Nicoli, 1994). Sexual reproduction leads to disruption of co-adapted genes complexes responsible for the high fitness on the previous crop (Hales et al., 1997).

Ultimately, up to now the investigation of *A. gossypii* diversity has been restricted to a small number of plants among the *A. gossypii* hosts. We have got a poor view of the species genetic diversity. Possible ways of investigating genetic diversity of *A. gossypii* were i) to sample individuals among numerous plant families distributed worldwide and belonging to the wild compartment or ii) to study the spring migrant populations putatively coming from various hosts. In this study, our objectives were to investigate the genetic diversity at the agrosystem level and the reproductive mode in France and, to answer the question of the host recognition by alate morphs. We investigated the genetic diversity of *A. gossypii* populations in agrosystems where Cucurbitaceae are heavily grown and *H. syriacus* is found as ornamental perennial plants located near crops. Cucurbitaceae are the main vegetables cultivated in France according to surfaces used for production, about 20,000 ha in mainland France and 4,000 ha in the French West Indies. About 70% of this surface is dedicated to melon crops, which are mainly produced in southeast, southwest, and west of France and in Guadeloupe and Martinique. To investigate the reproductive mode, we conducted a genetic analysis of the alate spring migrant populations. They either derive from sexual reproduction events (which could have taken place on *Hibiscus syriacus* plants) or result strictly from asexual reproduction through the survival of parthenogenetic females during winter on wild herbaceous plants or in protected situations such as glasshouses. To answer the question of host recognition by alate morphs, we compared the genetic structure and the diversity of alate and apterous populations on melon crops. The settlement of a new colony challenges alate aphids since they have to make long flights to find a suitable host plant (Powell et al., 2006). Alate aphids were shown using visual and olfactory cues to alight (Hardie, 1989; Nottingham

and Hardie, 1993; Hardie et al., 1994). The ability of alate morphs to recognize a suitable host plant at distance would selectively advantageous, nevertheless this ability has never been shown *in natura*.

Materials and methods

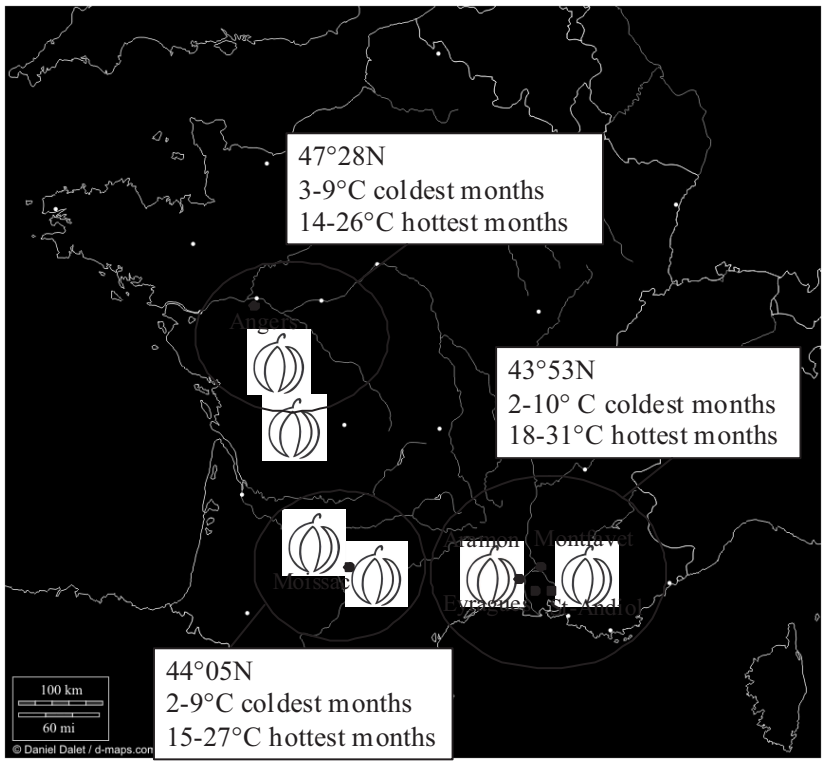
Melon growing areas description and sampling locations

Four melon growing areas were considered in this study, southeast, southwest, west of France and Guadeloupe in the French West Indies (Figure 1). In South-eastern (SE), aphids were sampled at Eyragues, Montfavet, Saint-Andiol and Aramon. This area is characterized by a Mediterranean climate with a wet winter/dry summer seasonality of precipitation. South-western (SW) and Western (W) France bathed in a more temperate climate under the influence of the Gulf Stream. Aphids were sampled at Moissac (SW) that has early springs, long warm summers and mild winters and in Angers (W) that is located in the northern part of the melon growing area, with a mild and humid weather all year round. In the French West Indies (FWI), where sugarcane and banana are the major crops, aphids were sampled in the melon crop area at Petit-Canal on Grande-Terre Island (Guadeloupe). This limestone plateau regularly experiences severe droughts, with almost no temperature variation along year.

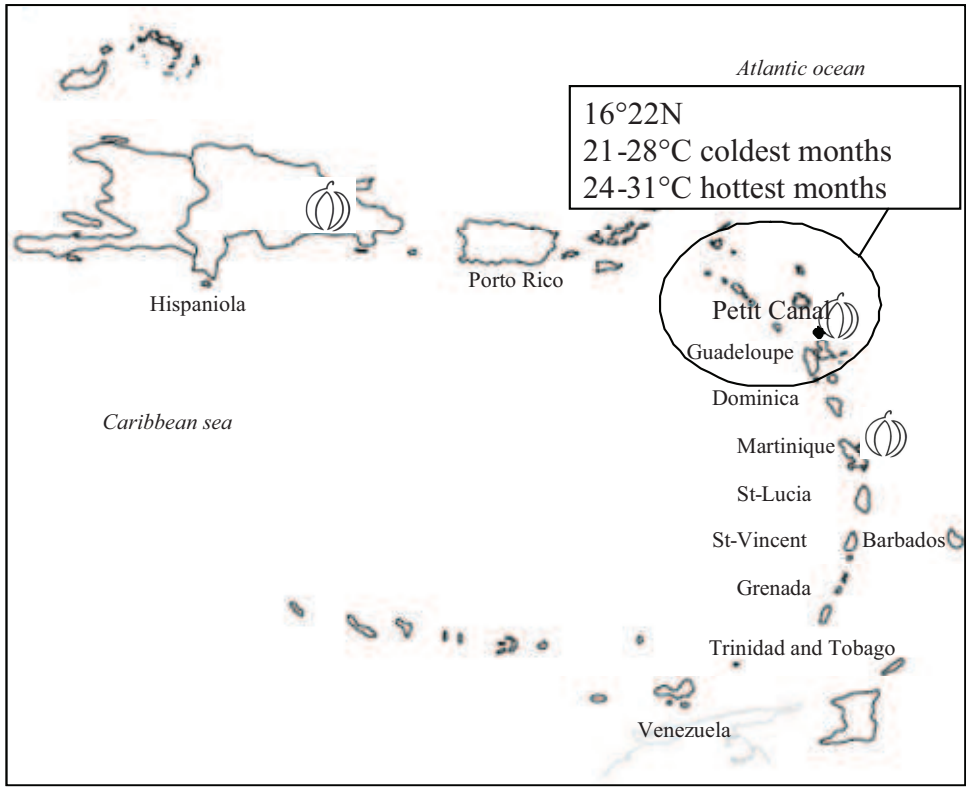
Sampling aphids and DNA analysis

Aphids were sampled over 5 years (2004, 2006, 2007, 2008 and 2009) in seven melon fields (plants without genetic resistance to aphids). One population is defined as a sample collected in a field a given year and with a given morph – alate or apterous. We collected 15 to 228 alate morphs per field during the first three weeks after plantation (in early April in the FWI, in May in SE, SW and W of France); they were collected on melon plants and constituted a sample of the spring migrants that alighted on melon crops. Then, we collected 80 to 491 apterous morphs – being the alate females offspring – per field. Each apterous was collected either as an isolated individual or as one individual representing the colony produced by a single asexual founding mother. Each colony sampled was tagged to avoid any re-sampling.

DNA of 4792 individual aphids was extracted using a 5% (w/v) Chelex resin solution, as described in Fuller *et al.* (1999). DNA amplifications at eight microsatellite loci specific to the *A. gossypii* genome (Vanlerberghe-Masutti et al., 1999) were performed in two PCR



a.



b.

Figure 1: Melon growing areas and aphid sampling sites in a. France and b. the Lesser Antilles.

reactions as described in Carletto et al. (2009). The allele size at each locus was identified by comparison with molecular size standard using the software GeneMapper v3.7; a multilocus genotype (MLG) was subsequently assigned to each aphid.

Data analyses

Genetic diversity

Three indices were computed for each population as proposed by Arnaud-Haond et al. (2007), by considering alate and apterous samples separately, to estimate the genetic diversity and the level of aggregation of identical genotypes. The index of clonal richness (*R*) was computed as $R = \frac{G_r - 1}{N - 1}$, where G_r is the number of distinct genotypes in the population and N is the total number of sampled individuals from the population. The unbiased Simpson's complement (*D**) was calculated as $D^* = 1 - \sum_{i}^{G_r} \frac{n_i(n_i - 1)}{N(N-1)}$, where n_i is the number of genotype *i* and *N* the total number of sampled individuals from the population. This index can be interpreted as the probability that two individuals chosen at random have different genotypes and can thus be considered as an exact measure of clonal heterogeneity. The Simpson evenness index (*V*) was computed as $V = \frac{(D^* - D_{\min})}{(D_{\max} - D_{\min})}$ where $D_{\min} = \left[\frac{(2N - G_r) \times (G_r - 1)}{N^2}\right] \times \frac{N}{(N-1)}$ and $D_{\max} = \frac{(G_r - 1)}{G_r} \times \frac{N}{(N-1)}$. *V* is an equitability index which describes the repartition and relative amount of clones. *R* and *D** are from 0 if all individuals from the population are clones (G_r = 1) to 1 when each sampled individual in the population bears a distinct genotype (G_r = *N*). Simpson evenness index (*V*) varies between 0 and 1 which represent extreme skew and evenness, respectively. We used the Jackknife procedure to estimate the standard error of *R*, *D** and *V:* for each sample set, we recomputed the 3 indices leaving out one observation at a time from the sample set. From this new set of observations, an estimate for the variance of the indices was calculated and thereafter their interval of confidence at 5% (Palm, 2002).

Genetic structure

We used hierarchical analyses of molecular variance, AMOVA, in Arlequin version 3.1 to test for genetic structure due to aphid morph, growing area, sampling year or locality. Significance was tested by 100000 permutations.

Table 1: Number of individuals, N, number of multilocus genotypes, G, number of multilocus genotypes when frequencies were higher than 5%, g_1, when frequencies were lower than 5%, g_2, and number of single MLG, g_3, in alate (Al) ans apterous (Ap) *A. gossypii* sampled in France and the FWI.

	Total	Total Al	Total Ap	Southeast of France		Southwest of France		West of France		French West Indies	
				Al	Ap	Al	Ap	Al	Ap	Al	Ap
N individuals	4792	1868	2924	1148	1540	200	402	173	260	347	722
N alleles	226	203	165	119	108	117	72	140	134	69	20
G	596	413	280	189	136	84	41	136	128	18	3
g_1 (>5%)	4	5	6	4	4	4	4	1	3	1	1
g_2 (<5%)	162	93	84	61	59	8	16	20	18	6	1
g_3 (single)	430	315	190	124	73	72	21	115	107	11	1

Table 2: AMOVA results for microsatellite data analysis of *A. gossypii* grouped by aphid morph, year of sampling, growing area or SE locality.

Source of variation		d.f.	SS	Variance components	Percentage of variation	*P*
Aphid morph	Among groups	1	80.58	-0.02	-0.90	0.75
	Among populations	27	3895.73	0.44	18.79	$<10^{-5}$
	Within populations	9555	18379.12	1.92	82.11	$<10^{-5}$
Year of sampling	Among groups	4	824.57	0.03	1.24	0.32
	Among populations	24	3151.74	0.41	17.29	$<10^{-5}$
	Within populations	9555	18379.12	1.92	81.47	$<10^{-5}$
Growing area	Among groups	3	3117.03	0.51	20.05	$<10^{-5}$
	Among populations	25	859.27	0.10	4.07	$<10^{-5}$
	Within populations	9555	18379.12	1.92	75.88	$<10^{-5}$
SE Locality	Among groups	3	156.11	0.02	0.73	0.14
	Among populations	13	363.75	0.09	4.15	$<10^{-5}$
	Within populations	5359	10680.51	1.99	95.12	$<10^{-5}$

Assignment to host races

In our assignment procedure we will refer to the four host races that have been unambiguously identified (Cucurbitaceae, cotton, *Solanum* and pepper) among numerous samples of *A. gossypii* collected over several years at a large geographical scale on annual crops from different plant families (Carletto et al., 2009). We assigned, or not, each MLG observed in our samples to the cucurbit cluster as follows. The MLGs identified in the different populations were analyzed together with the 44 MLGs previously assigned by Carletto et al. (2009) using the Bayesian program STRUCTURE (Pritchard et al., 2000). We used the admixture model with a burn-in of 500000 and a subsequent Markov Chain of 250000 iterations. Ten replicate runs for each value of putative number of clusters *K* (varying from 1 to 30) were compared to check the consistency of estimates and to determine the likeliest number of genetic clusters. We paid particular attention to those MLGs which clustered with the 12 cucurbit reference MLGs defined by Carletto et al. (2009).

Tracking genetic fingerprints of sexual reproduction

Genetic fingerprints of sexual reproduction were looked for in spring migrant populations *i.e.* alate samples collected in the early season in melon fields. We computed three parameters: departures from Hardy-Weinberg equilibrium (HWE) for each locus, linkage disequilibrium (LD) for each pair of loci and F_{IS} using exact tests (p=0.05) available in ARLEQUIN version 3.1 considering repeated genotypes (Smith et al., 1993). MLGs only observed in apterous populations were added as these MLGs were necessarily present in the alate mother population. F_{IS} can be thought of as a measure of the identity of alleles within individuals relative to the identity of alleles randomly drawn from two different individuals issued from the same subpopulation. F_{IS} is interpreted in terms of deviation from random mating, caused by the breeding system of the organism under study: for a strictly clonal population, $F_{IS} = -1$, for a strictly sexual population, $F_{IS} = 0$ and for a strictly inbreeding population, $F_{IS} = +1$. To reduce the Wahlund effect, samples collected in each growing area were split according to the genetic clusters evidenced by STRUCTURE. Calculations of HWE, LD and F_{IS} were conducted on these sub-samples.

Results

Genetic diversity of *Aphis gossypii* populations in melon crops

To investigate the genetic diversity of *A. gossypii,* we sampled alate and apterous aphids from 2004 to 2009 in four locations in the south-east (SE) part of France, one location in the

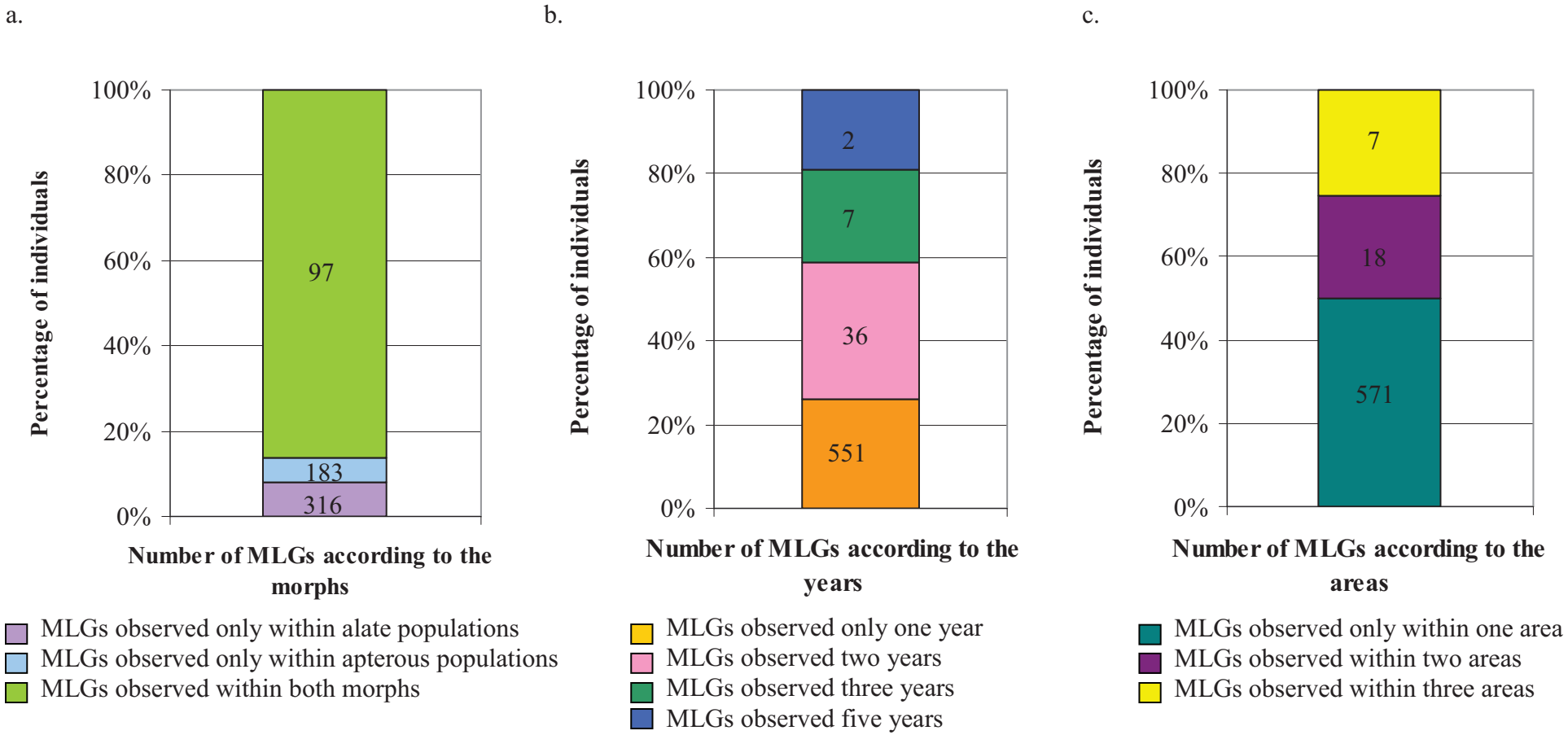


Figure 2: Occurrence of MLGs of *A. gossypii* populations sampled in France and FWI represented by the numbers of MLGs and the percentage of individuals with a MLG observed a. within aphid morphs, b. within years and c. within areas.

southwest (SW) and west (W) part of France and the last one in the French West Indies (FWI) (Figure 1). They were genotyped using eight microsatellite markers.

Among the 4792 aphids analysed, we discriminated 596 MLGs. The great majority of MLGs was present as a single copy and only four MLGs had a frequency higher than 5% of individuals (Table 1). We discriminated 413 MLGs among the 1868 alate aphids; 18 to 189 MLGs were characterised in the populations from different growing areas. In the alate aphid samples, allelic diversity ranged from 9 to 49 alleles per locus with 203 alleles identified across all loci. We discriminated 280 MLGs among the 2924 apterous aphids; three to 136 MLGs were characterised in the populations from different growing areas. In the apterous aphid samples, allelic diversity ranged from 9 to 44 alleles per locus with 165 alleles identified across all loci.

Among the 596 MLGs discriminated, 316 were only observed within the alate samples, 183 were observed only within the apterous samples and 97 were observed within both morphs (Figure 2a). The distribution was different when we considered the numbers of individuals: 8% of individuals had a MLG observed only within alate samples, 6% individuals had a MLG observed only within apterous samples and 86% of individuals had a MLG observed within both alate and apterous samples. We observed 551 MLGs only one year but more than 70% of the individuals had a MLG observed over two years or more (Figure 2b). We observed 571 MLGs in only one area but half of the individuals had a MLG observed in two areas or more (Figure 2c). None of the MLGs observed in the FWI were observed in the others areas.

Genotypic diversity of alate populations vs apterous populations

Among each morph, the genotypic diversity of the samples was investigated at three levels: within an area, between years and between areas. Three diversity indexes were considered, reflecting either the clonal richness, *R* (number of clones relative to the sampling size), the clonal diversity *D** and their evenness, *V*.

The four SE localities were similar for these indexes considering either alate populations or apterous populations (supplementary data, Figure A). For example, in alate populations sampled in 2004 the Simpson evenness index (*V*) was 0.86±0.05 in St-Andiol, 0.83±0.03 in Montfavet and 0.81±0.01 in Eyragues (interval of confidence at 5%). No strong differences were observed in these three indexes over different year in each locality. The comparison between alate and apterous populations showed a significant decrease of the clonal richness, whatever the locality and the area (supplementary data, Figure A). We decided to pool the

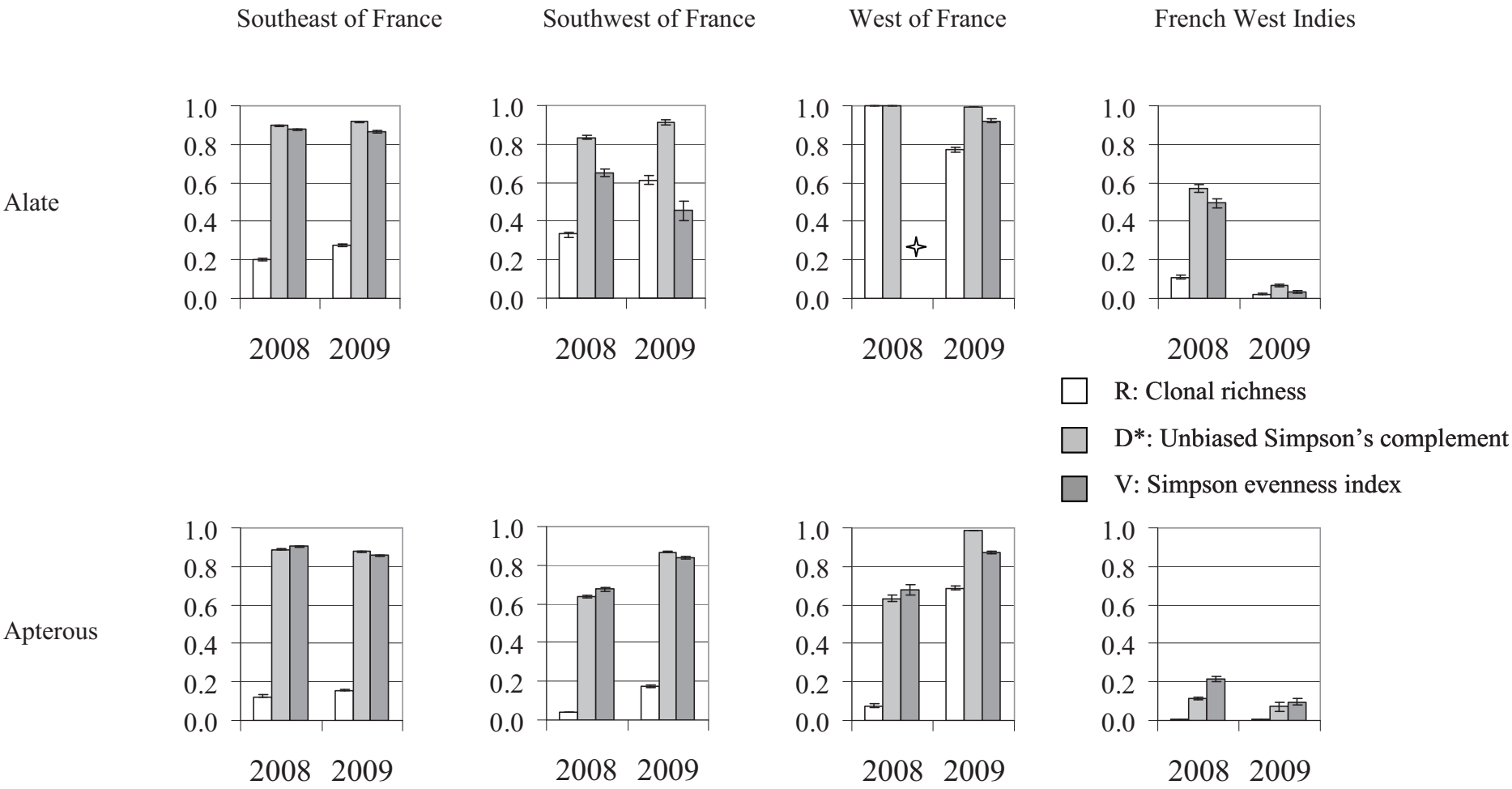


Figure 3: Clonal diversity of alate and apterous *A. gossypii* samples collected in 2008 and 2009 in four melon growing areas. The intervals of confidence derive from Jackknife procedures (p=0.05). *V* was not estimated because D* was maximal.

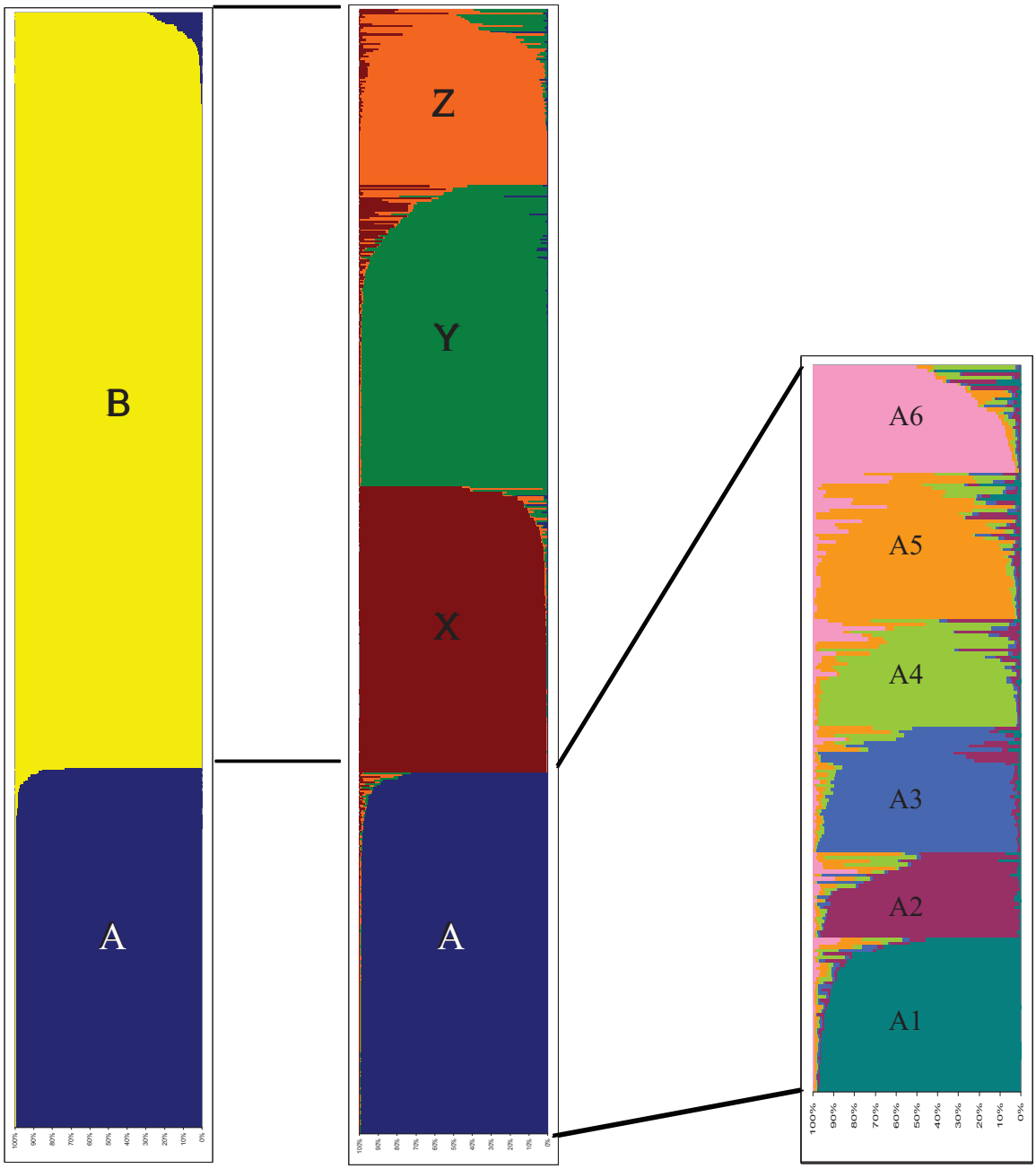
data of the four localities within the SE area for further analyses. The comparison of the four areas (Figure 3) revealed that the lowest diversity of alate populations was observed within populations from the FWI and the highest diversity within populations from W. Intermediate diversity was observed in populations from SE and SW. The clonal richness (*R*) was significantly higher in SW populations than in the SE populations while the Simpson evenness index (*V*) was significantly higher within populations sampled from SE than populations sampled from SW (no overlapping intervals of confidence at 5%, Figure 3). In all cases, a significant decrease of the clonal richness was observed from alate to apterous populations (Figure 3).

Genetic structure of *A. gossypii* populations

We investigated the aphid genetic structure by an AMOVA analysis considering the morph effect (alate *vs* apterous), the year of sampling effect, the growing area effect and the locality effect (Table 2). More than 75% of the variance was distributed within populations whatever the factor considered. The only factor exhibiting a significant effect among groups was the growing area factor that explained 20% of the variance. The AMOVA analysis confirmed the absence of significant difference of genetic structure between the SE data. We decided therefore to pool samples from all these localities.

To search for clustering of the MLGs found in this study, we analyzed these MLGs with the 44 MLGs previously described and assigned to host races (Carletto et al. 2009). Using a Bayesian structuring program, the likeliest numbers of genetic clusters were K = 2 and K = 4 (supplementary data, Figure Ba).

When K = 2, 203 MLGs of the 596 detected in our samples were assigned to the cluster A and represented only 5% of the aphids and 393 MLGs were assigned to the cluster B representing 95% of the aphids (Figure 4a). The F_{ST} value between both clusters was 0.254. All the MLGs belonging to the cluster A had never been described before. When K =4, the cluster A still consisted in the 203 MLGs but cluster B was divided in three (Figure 4a): cluster X consisted in 133 MLGs, representing only 4% of the individuals; cluster X also encompassed MLGs previously collected on *Solanum* spp., pepper, cotton, citrus, strawberry and *Hibiscus* spp.. The cluster Y gathered 163 MLGs representing 73.5% of the individuals sampled and 11 MLGs characteristic of aphids from the Cucurbitaceae race. Therefore the cluster Y will be called the cucurbit cluster. The cluster Z containing 97 MLGs representing 18% of the



a. b.

Figure 4: Results from Structure showing the estimated proportion of assignment of a. the 632 multilocus genotypes to K=2 and 4 and b. the 203 multilocus genotypes assigned to the cluster A to K=6.

individuals sampled, gathered NM1 and NM1-like MLGs also characterizing aphids from Cucurbitaceae along with MLGs previously identified on cotton (Ivo) and *Hibiscus* spp. (Hib4). Therefore, the cluster Z will be called the NM1 cluster.

We analysed the MLGs assigned to cluster B and those assigned to cluster A separately using the same parameters of Structure. The number of genetic sub-clusters that best fitted the data was K=3 for the cluster B (supplementary data, Figure Bb) and the structure was identical to the one described above for all the MLGs when K = 4 (Figure 4b). The number of genetic sub-clusters within cluster A was K = 6 (supplementary data, Figure Bc) and the 203 MLGs are quite uniformly distributed in the 6 sub-clusters (Figure 4b).

We considered the distribution of the alate and apterous individuals according to the existence of the four genetic clusters A, X, Y and Z, within each of the four geographic areas (Figure 5). In SE 70% of the alate and 80% of the apterous individuals were assigned to the cluster Y (cucurbit cluster). In SW, 60% of the alate and 80% of the apterous individuals are also assigned to the cluster Y. In both areas, 20 to 25% of the individuals alate or apterous, belong to the cluster Z (NM1 cluster). MLGs characteristic of the cluster A were significantly observed among alate individuals in SW (10%). In W, 5% of alate and 28% of apterous individuals had a MLG assigned to the cucurbit cluster, and 24 and 30% had a MLG assigned to the NM1 cluster and more or less 50% of individuals, whatever the morph, were assigned to the cluster A . In the FWI, most of alate and apterous individuals had a MLG assigned to the cucurbit cluster (91 to 100%) and these MLGs were not observed in the other growing areas. Whatever the area, 10 to 20% of the alate individuals exhibit a MLG from the cluster X (gathering MLGs characterizing host races other than cucurbit) but almost none among apterous. Moreover, whatever the area, the number of individuals that have a MLG assigned to the cucurbit cluster or to the NM1 cluster (known for its ability to colonize Cucurbitaceae) was much higher in the apterous populations than in the alate populations. It was worth noting that although the percentage of individuals belonging to the cucurbit host race was equivalent in SE and SW areas, the diversity among the cucurbit MLGs was considerably higher in SE (n=93) than in SW (n=28) (Figure 5).

Tracking genetic fingerprints of sexual reproduction

We examined the genetic variability of the alate populations to track genetic fingerprints of sexual reproduction. The data set was completed by the MLGs only observed within the

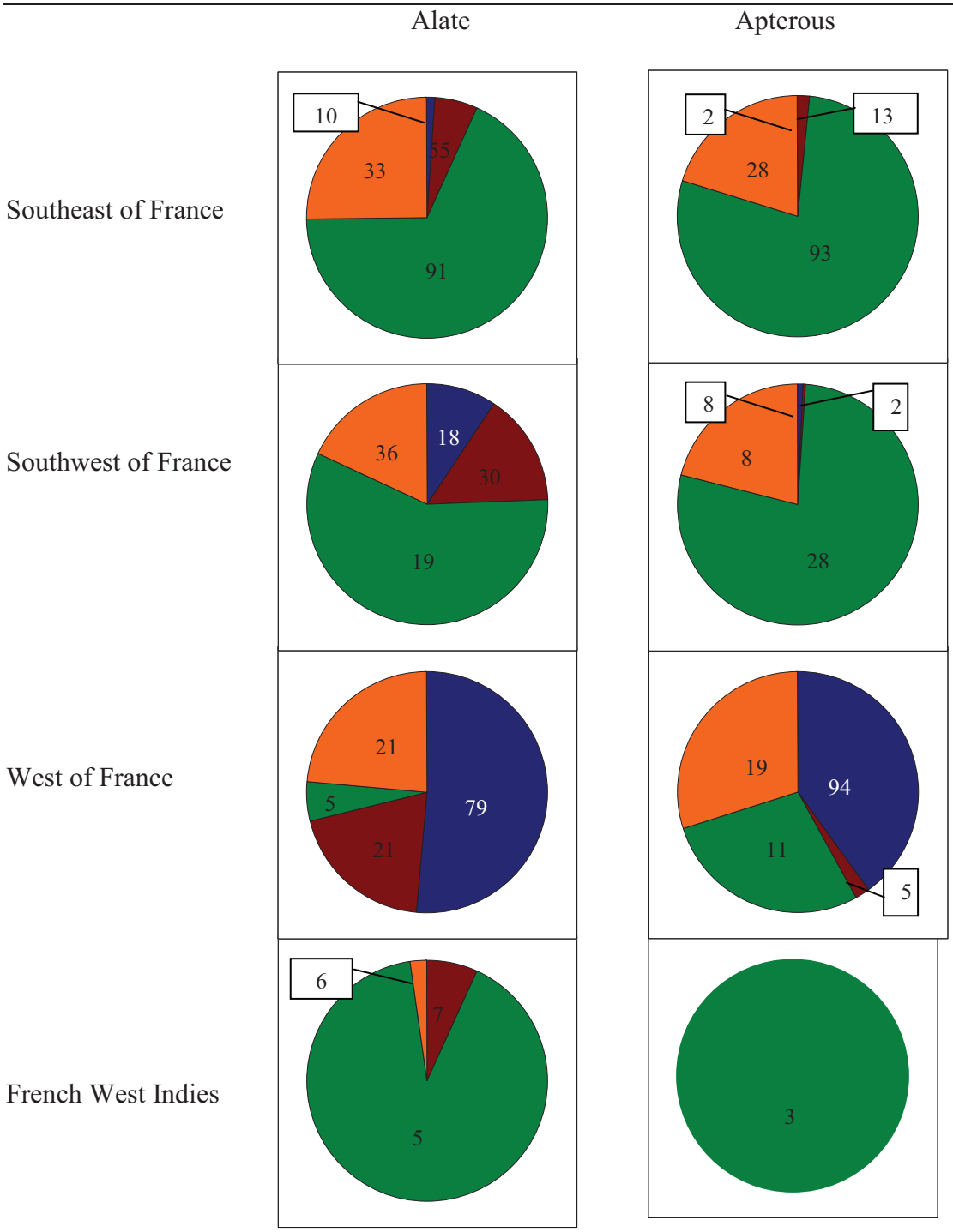


Figure 5: Distribution of alate and apterous *A. gossypii* individuals and numbers of MLGs collected in four melon growing areas according to the four genetic identified by Structure:

- The cluster A
- The cluster X
- Y, the cucurbit cluster
- Z, the NM1 cluster

apterous samples. We calculated three parameters: the Hardy Weinberg equilibrium (HWE), the linkage disequilibrium and the F_{IS} index. To reduce the Wahlund effect, we split the populations a) according to their cluster membership (A, X, Y and Z) and their area (SE, SW, W and FWI) (Table 3a); and b) according to the sub-clusters (A1 to A6) for the individuals assigned to the cluster A (Table 3b).

Departure from HW equilibrium, frequent LD and negative F_{IS} value were considered as fingerprints of obligate parthenogenesis. These characteristics were observed in the FWI subpopulations in the cluster X and the SE subpopulation in the cluster Z (NM1) and in all the sub-populations from the cluster Y (cucurbit) except for the W subpopulation.

Fingerprints of panmictic sexual reproduction were frequent HWE, infrequent LD and positive F_{IS} value close to 0. The only subpopulation showing these characteristics was the W subpopulation in the cluster Y.

Fingerprints of sexual reproduction by inbreeding were departure from HWE, frequent LD and positive F_{IS} value close to 1. They were observed in the SE subpopulation in the cluster X, the SW and W subpopulations in the cluster Z and the W subpopulation in the cluster A.

We observed a fourth situation with frequent HWE, rare LD and F_{IS} values ranging from 0.23 to 0.61 for the SW and W sub-populations assigned to the cluster X and the SE and SW subpopulations assigned to the cluster A.

When the cluster A was split into six sub-clusters, HWE was observed for several loci ($p>0.05$) and the number of significant LD was low (Table 3b). For the other loci, we observed heterozygote deficiency that is significant at 5% but not at 1%. Moreover, F_{IS} values across loci varied from 0.13 to 0.43 suggesting the occurrence of a sexual reproduction in these sub-populations.

Discussion

A. gossypii is considered as one of the most polyphagous species of aphid; it has been able to adapt successfully worldwide, in various agrosystems, to several crops belonging to many botanical families. Given this adaptability, the low genetic diversity of the species that has been reported in different studies is puzzling.

How wide is the diversity in *A. gossypii*?

This study was conducted on large samples within few agrosystems. With a set of eight microsatellites, we identified 226 alleles and 596 MLGs among 4792 individuals sampled in

Table 3: Number of individuals, N, number of multilocus genotypes, G, number of loci in Hardy Weinberg equilibrium, HW, number of pairs of loci in linkage disequilibrium, DL, and F_{IS} across all loci in alate *A. gossypii* populations and reproductive mode according to a. the sampling areas in France and to four genetic clusters and b. the six genetic clusters structured within the cluster A.

a.

Clusters	Areas	N	G	HW	DL	F_{IS}	Reproductive mode
A	SE	12	10	5/7	13/28	0.610	?
	SW	22	21	3/8	6/28	0.346	?
	W	182	172	0/8	21/28	0.451	Inbreeding
X	SE	84	61	1/8	27/28	0.310	Inbreeding
	SW	32	32	4/8	7/28	0.229	?
	W	37	37	5/8	6/28	0.321	?
	FWI	24	7	0/7	20/28	-0.027	Obligate parthenogenesis
Y, the cucurbit cluster	SE	819	130	0/8	27/28	-0.232	Obligate parthenogenesis
	SW	135	38	1/8	27/28	-0.180	Obligate parthenogenesis
	W	17	13	4/8	18/28	0.079	Panmictic sexual reproduction
	FWI	316	6	0/8	28/28	-0.694	Obligate parthenogenesis
Z, the NM1 cluster	SE	301	40	0/8	28/28	-0.451	Obligate parthenogenesis
	SW	43	23	0/8	28/28	0.189	Inbreeding
	W	55	35	0/8	28/28	0.565	Inbreeding
	FWI	8	6	3/8	25/28	0.051	?

b.

Clusters	n	g	HW	DL	F_{IS}
A1	47	44	2/8	9/28	0.434
A2	26	24	3/6	3/28	0.274
A3	39	34	2/8	12/28	0.335
A4	44	34	5/7	8/28	0.078
A5	43	41	3/7	7/28	0.244
A6	31	30	3/8	5/28	0.259

France. Among the 226 alleles observed in this study, 60 have been already observed and nine alleles described previously were not observed; among the 596 MLGs only 8 out the 44 described up to now have been already observed. The diversity present in the FWI appeared unique. Using the same set of microsatellites, only 42 alleles and 11 MLGs were identified among 1176 individuals collected in Cameroun and 559 individuals collected in Tunisia (Brevault et al., 2008; Charaabi et al., 2008). Using a set of four microsatellites, 16 alleles and 118 MLGs were identified among 245 individuals collected in Iran (Razmjou et al., 2010). Indeed, the investigation of *A. gossypii* diversity conducted up to now has been restricted to a small number of plants among the *A. gossypii* host races and the samples were only apterous individuals sampling among colonies. The genetic analyses, conducted in this study, highlighted a wider diversity than reported.

We aimed to resolve this wide diversity by investigating sexual reproduction in *A. gossypii* populations. Population genetics and the Hardy-Weinberg model has allowed to highlight traces of sexual reproduction in many species of aphids such as *S. avenae* (Simon et al., 1999), *Rhopalosiphum padi* (Delmotte et al., 2002) or *A. pisum* (Via, 1999). Within the *A. gossypii* species, the analyses of genetic diversity carried out on natural populations colonizing crops, *i.e.* in apterous colonies, have never demonstrated the existence of a sexual reproduction in this species. In accordance with previous studies, our study has not revealed genetic fingerprints of sexual reproduction when analysing apterous populations sampled on melon fields in different areas. We observed a high diversity among populations sampled in SE, SW and W of France and we tracked sexual reproduction fingerprints in spring migrant populations collected in these areas. If sexual reproduction occurred the previous autumn, these alate populations consisting in progenies deriving from few clonal cycles after the sexual reproduction cycle would be more favourable to exhibiting sexual reproduction fingerprints. We split each population in sub-populations according to the clusters derived from the assignment by a Bayesian structuring program. Among the MLGs assigned to the cucurbit cluster, 27% were persistent throughout the time and the individuals with these MLGs reproduced asexually. Among the MLGs assigned to the clusters A and X, almost 77% were observed in single or few copies, only one year and the individuals with these MLGs reproduced sexually. Our strategy revealed genetic recombination in French natural populations which is new information, but no genetic recombination in populations from the French West Indies. These results strongly suggest that sexual reproduction events occur over 40°N, which challenges the commonly shared view that *A. gossypii* has only clonal

reproduction in temperate regions (Blackman and Eastop, 2000). *H. syriacus* has been described as the host on which the sexual reproduction of *A. gossypii* can take place in the northeast of U.S and in Italy (Kring, 1959; Ferrari and Nicoli, 1994). *H. syriacus* is found in abundance in the three growing areas we investigated in France (Ebert and Cartwright, 1997) as well as in FWI and is the primary candidate to host sexual reproduction in France. Sexual reproduction occurrence that is commonly described as day-length and temperature dependent is still hypothetic in areas between 15° and 40°N. Investigations on spring migrant populations around the Mediterranean basin might allow detection of the geographical limit of sexual reproduction in the *A. gossypii* species.

Is specialisation in relation with the reproductive mode in *A. gossypii*?

Sexual reproduction fingerprints were not observed within populations belonging to the cucurbit cluster except in sub-populations collected in W. On one hand, the absence of breeding prints in these specialized populations is in accordance with previous genetic studies showing that parthenogenesis is the exclusive mode of reproduction occurring in the host-specialized *A. gossypii* clones (Brevault et al., 2008; Charaabi et al., 2008; Carletto et al., 2009). On the other hand, oviparous female and male morphs were induced under short days conditions in the lab from 24 out of 58 clones sampled on various plant species in Japan (Takada, 1988). In this study, all clones (11) sampled on *Hibiscus* sp. were able to produce oviparous female and/or male morphs, none of the clones (11) sampled on Rosaceae were able to produce oviparous female and male morphs and three clones out of 11 sampled on Cucurbitaceae were able to produce oviparous female and/or male morphs. Clones sampled on Cucurbitaceae in France were also reported to produce oviparous female and male morphs in the lab (Fuller et al., 1999). Among 56 *A. gossypii* clones sampled on different hosts (Malvaceae, Asteraceae, Cucurbitaceae) in Greece, only one from cotton proved capable of producing oviparous female and male morphs (Margaritopoulos et al., 2009). Thus, many clones were reported to be able to develop sexual morphs, and among them only a few clones specialized on Cucurbitaceae. In this study, the only cluster structured by host race is the cucurbit cluster. In this cluster, we showed that *A. gossypii* populations in France have different reproductive mode: i) obligatory parthenogenesis in SE and SW. ii) sexual reproduction in W. In case of sexual reproduction, host race formation requires, apart from divergent selection among secondary hosts, some mechanism of reproductive isolation through assortative mating on the primary host. In our case, the F_{IS} value of the cucurbit

cluster sampled in W did not suggest inbreeding. However, the effective of this sub-population was low. More investigations should be conducted on this sub-population, in particular the Cucurbitaceae specialization of the clones of this sub-populations needs to be checked.

The genetic clusters not assigned to host races consisted of asexual and sexual lineages. Aphids with MLGs belonging to these clusters probably originate from uncultivated host plants as these MLGs have never been observed in previous studies carried out on crops. Despite the "twofold cost of sex" which is one of the most prevalent debates in evolutionary biology, maintenance of sexuality exists in many organisms (with sexual and asexual reproduction) and results from advantages, such as the production of genetically different offspring in a heterogeneous environment (unpredictable environment in the lottery model and stable environment in the tangled bank hypothesis (Williams, 1975; Case and Taper, 1986)). Maintenance of sexuality in unspecialized genetic clusters of *A. gossypii* is in accordance with these models as uncultivated hosts could be considered as a heterogeneous environment. Pure asexually reproducing organisms are relatively rare and virtually all short-lived.

Among aphid species, a high proportion of the populations is composed of asexual lineages (Haack et al., 2000). Different hypotheses explain transitions to asexuality (Delmotte et al., 2001). Wolbachia-induced parthenogenesis is very unlikely since this bacterium has never been detected in aphids. There is no evidence for sperm-dependant asexuality in aphids. Two mechanisms could constitute routes to asexuality in *A. gossypii* species: mutations in the genes controlling the production of sexual forms, and recurrent gene flow via males produced by asexual lineages, which transmit asexuality genes. We hypothesize that this last route for asexuality is likely to exist within the *A. gossypii* species, because Takada (1988) observed some intermediate lineages producing both parthenogenetic females and males (no oviparous females). Strictly asexual lineages were observed in specialized aphid clones and this specialisation often results in better performance of lineages on their plant of origin (Carletto et al., 2009; Peccoud et al., 2009). Adaptations to different habitats and resources may induce reproductive isolation without the geographical separation of populations, an extrinsic barrier to hybridation that was long thought to be a requisite for speciation in animals (Via, 2001). Sympatric populations, in partial reproductive isolation, that are specialized to different host plants are considered as host races (Dres and Mallet, 2002). In phytophagous insects, "host races" with increasing reproductive isolation constitute intermediate stages of speciation. The

continuum of population differentiation toward complete speciation in sympatry was well documented within pea aphid populations (Peccoud et al., 2009); however we did not observe any continuum in the *A. gossypii* populations.

Which diversity is available for adaptation to agrosystems?

We showed that genetic diversity is geographically structured at the growing area level, which has not been observed up to now for *A. gossypii* species. Geographical differentiation is especially strong between FWI populations and those from France. In FWI, the relative isolation of small islands in the Caribbean basin, as well as the absence of sexual reproduction account for the very low clonal diversity found in the population sample from this area. It is a stable and confined environment where pure asexual reproduction is maintained year-round on Cucurbitaceae. Normark (2003) suggests that a temperate aphid lineage transported to the tropics may become obligatory parthenogenetic in the absence of a photoperiodic cue to switch to sexuality, but descendants reinvading the temperate zone may readily recover a sexual phase. Several hypotheses, other than the absence of sexual reproduction, the geographical isolation or the tropical climate, might explain the paucity of aphids in the FWI. One of them suggests that because plant diversity in the tropics is higher than in the northern hemisphere and because aphids locate their hosts randomly, aphids didn't found easily their specific host plants (Peccoud et al., 2010). Another hypothesis suggests that the few clones observed in the FWI could come from biological introductions where a small number of initial propagules were subject to founder events from their native range to a new area accompanied by a genetic bottleneck in the invaded area. Moreover, a drastic loss of genetic diversity was possible in introduced populations because a single individual can rapidly multiply asexually and then disperse (Zepeda-Paulo et al., 2010). The *A. gossypii* introduction in the FWI in unknown. Hypothese would be a recent introduction correlated with cultivated plant importation from Europe in the 17th during the colonization or with the triangular trade between Europe, Africa and America.

We showed that melon crops filtered the alate population and then, only clones specialized on Cucurbitaceae colonized the melon crops: the genetic diversity observed in alate populations is higher than the genetic diversity observed in apterous populations. This suggested that host-plant location and recognition in aphids act only after probing with their stylets before reaching the phloem, under the influence of chemical stimulants or repellents as suggested by Powell et al. (2006). This filter effect was amplified by the clonal multiplication. All the

colonies consisted in individuals with a MLG assigned to the cucurbit cluster or with the NM1 MLG. The apterous individuals with MLGs assigned to other clusters were sampled as isolated individuals. The filter effect did not appear as strong in SE and SW as in other growing areas; this suggests that selection by the host plant was not as strong in the SE and SW as in other growing areas. Indeed, clonal richness in alate populations was much lower in the SE area than in the other areas. Cucurbitaceae have been produced on large surfaces for a longer time in the SE than in the SW and W, this agrosystem may have had a selective effect on *A. gossypii* populations, leading to a dominance of specialized clones. So, only a part of the diversity available in the ecosystems is efficient in the agrosystems. The sources of the available diversity and the adaptive potential of this diversity for agrosystems has not been well identified. In France, recombination may occur on *H. syriacus* as observed in Italy. Eggplant and cotton-specialized aphids survived and reproduced on *Hibiscus* spp. (Liu et al., 2008; Carletto et al., 2009) and *A. gossypii* sampled on hibiscus reproduced on eggplant and cotton (Kim et al., 2008; Liu et al., 2008). The Cucurbitaceae specialized aphids survived and reproduced on *Hibiscus* spp. (Liu et al., 2008; Carletto et al., 2009) but *A. gossypii* sampled on hibiscus did not survive on cucumber (Kim et al., 2008; Liu et al., 2008). Lab experiments showed an alteration of the adaptation to Cucurbitaceae when *A. gossypii* clones sampled on Cucurbitaceae had over-wintered on hibiscus (Liu et al., 2008). So, even if the Cucurbitaceae specialized clones can grow on *Hibiscus* spp, hibiscus plants do not constitute a refuge plant for these clones and even less a source of individuals adapted to Cucurbitaceae. Altogether, these results suggest that only Cucurbitaceae specialized aphids over-wintering on Cucurbitaceae plants will be able to colonize Cucurbitaceae crops the following year. Therefore exchanges of genes between populations inhabiting the crops and the uncultivated environment are unlikely. Genetic differentiation of populations between growing areas and clonal reproduction of *A. gossypii* colonizing Cucurbitaceae should lead to a low evolutionary potential to face new agricultural practices. Nevertheless *A. gossypii* colonizing Cucurbitaceae have been able to adapt to pesticides: most of MLGs belonging to the cucurbit cluster are resistant to one or two insecticides ((Carletto et al., 2010) and unpublished data). Gene flow via males produced by asexual lineages may increase the evolutionary potential of the Cucurbitaceae host race. This hypothesis could be investigated by elucidating the genetic structure of *A. gossypii* populations sampled on hibiscus in Cucurbitaceae growing areas using mitochondrial and nuclear markers. Finally, if gene flow exists between cultivated and uncultivated parts of the ecosystem, then the uncultivated system may play the role of gene

reservoir, while the cultivated system may be responsible for genetic pollution of the adjacent environment (Martel et al., 2003).

Analysing relationships between populations living in cultivated and uncultivated areas is very important for pest management at the landscape scale, both for improving integrated and ecological control of pest populations and for forecasting the sustainability of any control methods such as transgenic crops, pesticides or natural genetic resistances (Gould, 1998). Uncultivated plants may constitute a source of natural enemies against *A. gossypii* pests as well as a refuge zone for the *A. gossypii* species but is not a source of colonizing Cucurbitaceae pests.

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Supplementary data:

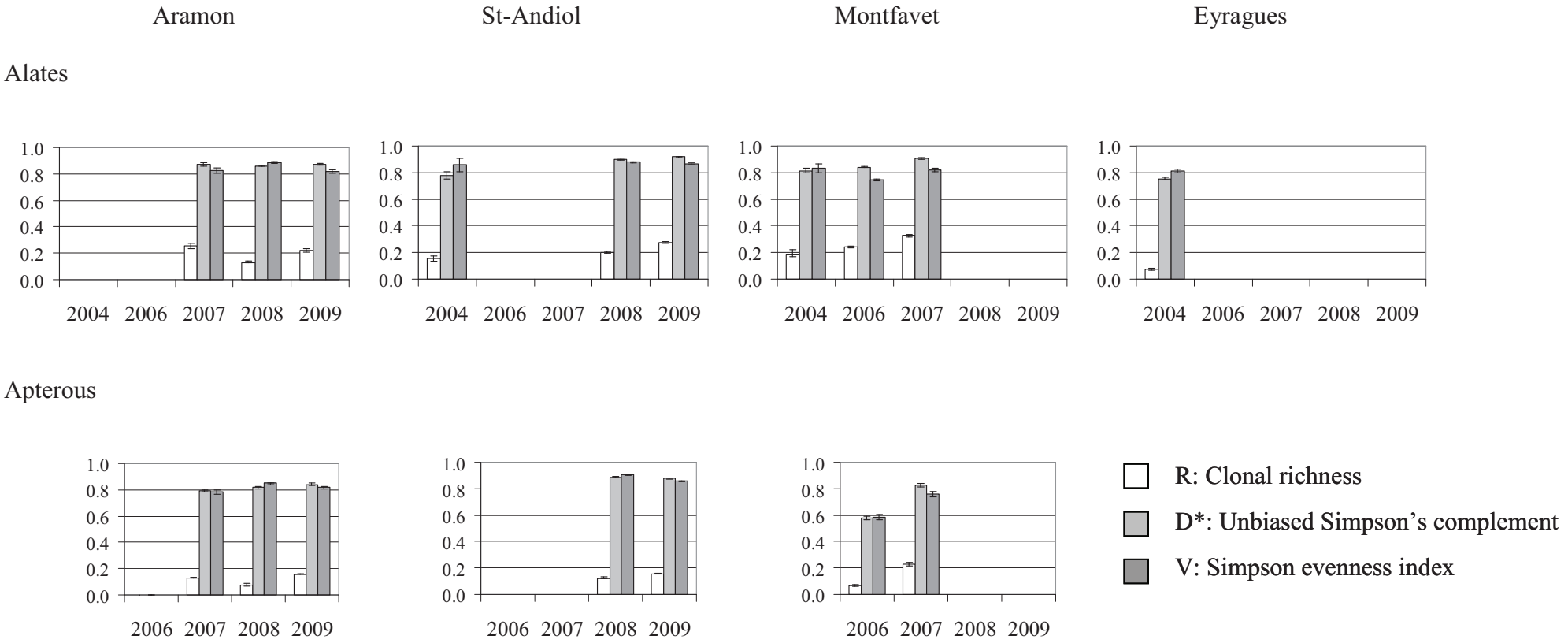
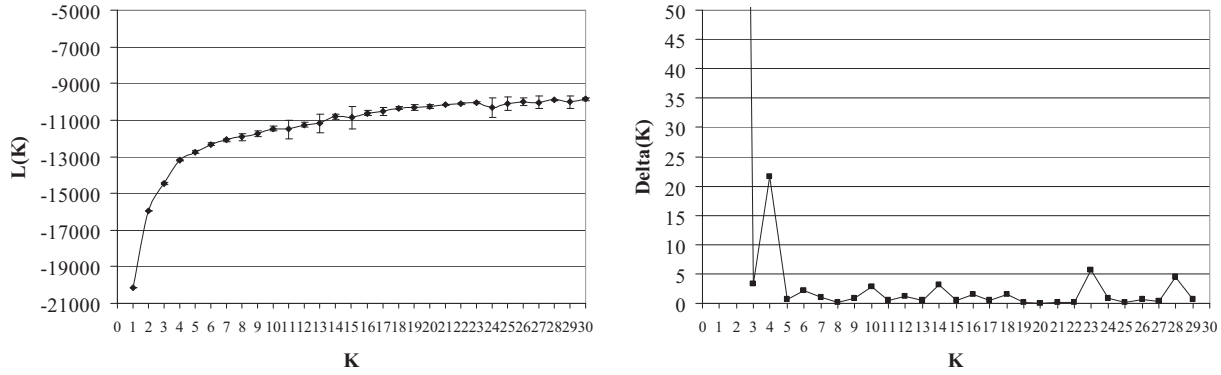
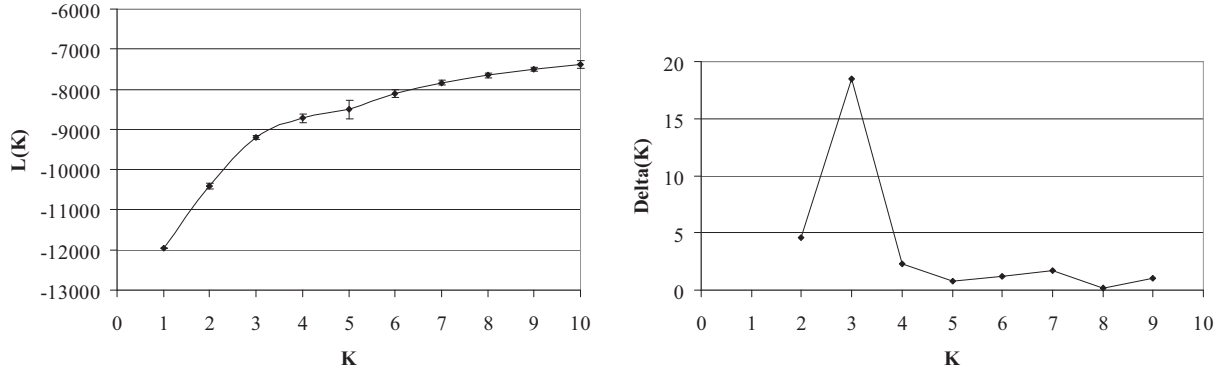


Figure A: Clonal diversity of alate and apterous *A. gossypii* samples collected from 2004 to 2009 in four localities in south-eastern France. The intervals of confidence derive from Jackknife procedures (p=0.05).



a.



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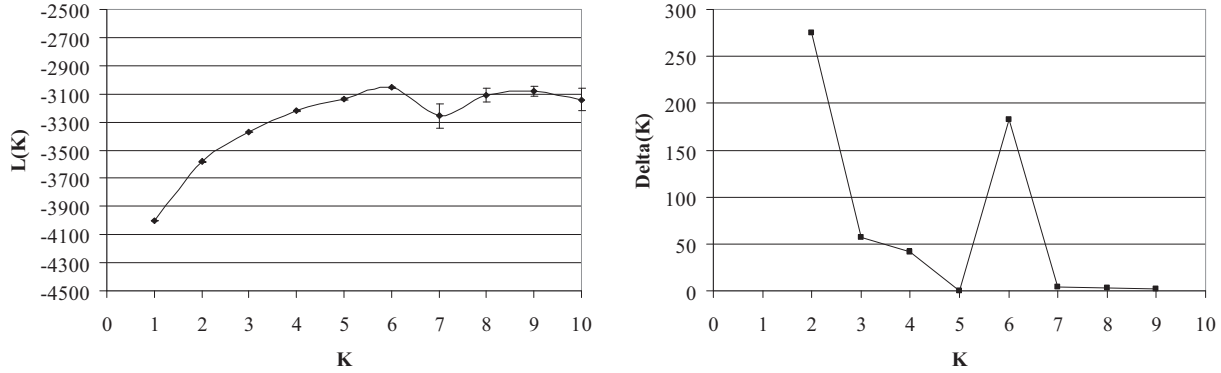

c.

Figure B: Mean value of ln likelihood and delta K obtained for 10 simulations of each K when we analysed with Structure a. all the MLGs; b. the MLGs assigned to the cluster B and c. the MLGs assigned to the cluster A.

CHAPITRE 3 : CONSTRUCTION DE COMBINAISONS DE RESISTANCE A *A. GOSSYPII* ET EFFET DES COMBINAISONS DE RESISTANCE SUR LES POPULATIONS D'*A. GOSSYPII*

Chapitre 3 : Construction de combinaisons de résistance à *A. gossypii* chez le melon et effet des combinaisons de résistance sur les populations d'*A. gossypii*

Dans ce chapitre, nous testons les hypothèses que la combinaison de gènes de résistance augmente leur durabilité comme l'a proposé Nelson (1978) et que les QTL diminuent la fitness des génotypes adaptés à la résistance contrôlée par un gène majeur dans la combinaison gène majeur-QTL (Pietravalle et al., 2006).

Le génome du melon a été largement cartographié à l'aide de marqueurs moléculaires ce qui a permis la mise en œuvre d'une cartographie de loci de résistance par marqueurs et le choix de RIL. Dans l'article 2, la détection de QTL de résistance à *A. gossypii* est décrite dans une première partie, puis une méthode de construction de combinaisons de QTL de résistance dans un fond génétique homogène est détaillée, ainsi que dans la validation de ces combinaisons de résistance, dans une seconde partie. Mon travail de thèse s'est situé dans cette seconde partie. Cet article a été publié dans Theoretical and Applied Genetics.

L'effet du gène *Vat* en plein champ a été étudié pour la régulation potentielle qu'il pouvait induire sur les maladies virales transmises par puceron (Gray et al., 1986; Lecoq and Pitrat, 1989). Cependant son effet sur la densité des populations de pucerons n'a jamais été caractérisé, ni, *a fortiori*, son effet sur la structure des populations. L'article 3 a pour objectif d'évaluer les pressions de sélection exercées par différentes combinaisons de résistance (gène *Vat* et QTL) sur les populations d'*A. gossypii* et le risque de contournement de ces résistances. Nous avons confirmé par des tests biologiques en conditions contrôlées les bénéfices attendus de l'association de gènes (majeur et QTL). La gestion de ces gènes de résistances *in situ* est envisagée. Ce travail sera complété par un jeu de données récoltées en plein champ en 2011 et présenté sous forme d'un article qui sera soumis à New Phytologist.

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Article 2 : Mapping and validation of QTLs for resistance to aphids and whiteflies in melon

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Mapping and validation of QTLs for resistance to aphids and whiteflies in melon

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Abstract

Aphis gossypii and *Bemisia tabaci* are severe hemipteran pests of melon crops and breeding for resistance to both insects is required to reduce pesticide use. Resistance was evaluated for its effect on behaviour and biotic potential of both hemipterans in a population of RILs derived from the cross Védrantais X PI 161375. Insect variability was considered using two *A. gossypii* clones and two *B. tabaci* populations. Two additive QTLs affected the whiteflies. Four additive QTLs and two couples of epistatic QTLs affected the aphids. Among them, a major QTL affects both behaviour and biotic potential of *A. gossypii* and therefore a same R gene induces both antixenosis and antibiosis. This major QTL colocalizes with the *Vat* gene belonging to the NBS-LRR gene family. No loci affected both aphids and whiteflies contrary to what was observed for the *Mi1.2* gene, a NBS-LRR gene in tomato. Original populations with different allelic compositions at QTLs affecting *A. gossypii* were built by one inter-crossing of RILs used for the mapping process. The genetic background was shown homogeneous between these populations what allowed validating QTLs and investigating the effect of allelic combinations at QTLs. Effects of QTLs were stronger than expected and some QTLs had a wider spectrum than expected. This strategy of validation appeared rapid and low cost.

Introduction

Hemiptera contains major pests of cultivated plants especially in three superfamilies: whiteflies (mostly pantropical), aphids (mostly in the northern temperate regions) and leafhoppers (worldwide). These pests have piercing-sucking mouthparts to probe plant tissues intra and intercellularly. They are phloem feeders and drain plant nutrients what causes direct damages. Moreover, because of their diet rich in sugars, they produce sticky and sugary excreta covering the foliage and serving as substrate to sooty mold fungi. They also deliver viruses and bacteria. Plant responses to hemipteran insects have substantial overlap with responses mounted against microbial pathogens (Kaloshian and Walling, 2005); even if genetic control of plant resistance to insects has been poorly studied compared to resistance to pathogens (Yencho et al., 2000), the heredity of resistance to hemipterans has been described in various plant species, mainly in cereals. Major genes have been identified in most of cases for hemipterans control. Only two genes of resistance to insects have been cloned so far and both belong to the NBS-LRR family resistance genes. The *Mi-1.2* gene, which confers resistance to the nematode *Meloidogyne incognita* and other species of nematodes in tomato, was also shown to confer resistance to the aphid *Macrosiphum euphorbiae* (Rossi et al., 1998) and to the whitefly *Bemisia tabaci* (Nombela et al., 2003). The *Vat* gene isolated in melon confers resistance to *Aphis gossypii* (Pauquet et al., 2004).

Aphids and whiteflies cause direct-feeding damages on melon. *A. gossypii* is the only aphid species able to colonize melon plants. *B. tabaci* is the most damaging whiteflies species in melon crops because of its huge and extending distribution. To control both species, insecticides have been frequently applied and both insects developed insecticide resistances. Because their geographical distribution overlaps in the main production areas, breeding for resistance to both insects is required to reduce the pesticide use. Several sources of resistance to *A. gossypii* and to *B. tabaci* have been identified in melon. *A. gossypii* resistant accessions have been largely described since the 1970s, particularly the Indian and Korean accessions PI 414723 and PI 161375 (Kishaba et al., 1971; Bohn et al., 1972; Lecoq et al., 1979; Pitrat and Lecoq, 1980); in both accessions, the resistance is controlled by the *Vat* gene (Pitrat and Lecoq, 1982). Nevertheless, resistance might be variable according to aphid clones. As early as 1971, Kishaba et al. (1971) pointed out that the melon resistance to the US south-eastern aphids was inefficient against the south-western aphids. In the same way, Soria et al. (2000) observed low resistance levels to *A. gossypii* clones from Spain in accessions that exhibited a high level of resistance to French *A. gossypii* clones. The *Vat* gene effect on different clones of *A. gossypii* is unknown so far. Resistance to *B. tabaci* was investigated more recently during the

1990's. Even if resistance and tolerance were described in several melon accessions few studies dealt with resistance to a characterized biotype. Sauvion et al. (2005) identified several accessions resistant to *B. tabaci* biotype B, among them PI 161375 and PI 414723, also resistant to *A. gossypii*. To our knowledge, inheritance of resistance to *B. tabaci* in melon has not been investigated.

In the present study, we used molecular markers to decipher *A. gossypii* and *B. tabaci* biotype B resistance in melon in quantitative trait loci. Variability of *A. gossypii* was considered using two genetically distant clones and variability of *B. tabaci* biotype B was considered using two natural populations. This study was conducted in a population of recombinant inbred lines (RILs) derived from the cross Védrantais X PI 161375 (resistant to both pests) to identify possible QTLs effective against a large spectrum of hemipterans. Populations with different allelic combinations at QTLs were built in a homogeneous genetic background in order to validate the QTL effects.

Materials and Methods

Plant material and genetic map

Védrantais is a commercial French line of Charentais type (Vilmorin, France). PI 161375 is a Korean accession, resistant to *B. tabaci* and *A. gossypii*. A recombinant inbred line progeny (RILs, F7, F8) was issued from the cross Védrantais X PI 161375.

A genetic map was built using the map produced by Périn et al. (2002) enriched by SSR markers developed by Ritschel et al. (2004) and Gonzalo et al. (2005). Among more than 800 markers available for this progeny in the lab, 216 markers were selected (88 SSR, 98 AFLP, 17 ISSR, 5 phenotypic, 5 RFLP and 3 RAPD) to genotype190 RILs. The linkage groups were determined with the 'group' command of Mapmaker (Lander et al., 1987), using a minimum logarithm of the odds ratio (LOD) of 8 as threshold for linkage detection. The order of the markers on each linkage group was determined using the 'order' command (minimum LOD 6) and marker's position was confirmed using the 'ripple' command. The map was drawn using the MapChart software. The assignment of the linkage groups were as in Périn et al. (2002).

Insects

The seedlings for the mass rearing of aphids and whiteflies were grown in insect proof greenhouses. Two clones of *A. gossypii*, NM1-Lab and 4-104, collected on cucurbits in south-eastern France, were used for the resistance tests. They were genotyped using 8 microsatellite markers and were shown to have a NM1 genotype and a C9 genotype respectively, as described by Carletto et al. (2009). The mass rearing of aphids was conducted on melon (cultivar Védrantais) in a room maintained at 24:18°C, 18:6h photoperiod. Two days after inoculation of plants by apterous adults, adults were removed to obtain 7 days later 5-7 day-old aphids for inoculation. Natural populations of *B. tabaci* were used because clonal lines can not be obtained as *B. tabaci* reproduces by arrhenotokous parthenogenesis. Two populations were caught in Guadeloupe during the cropping seasons 2000-2001 (Bt 2001) and 2001-2002 (Bt 2002) and identified as B biotype accordingly to De Barro and Driver (1997). The mass rearing of whiteflies was conducted on cabbage (*Brassica oleracea*, cultivar Copenhague) in a room maintained at 25-27°C and a 12:12h photoperiod. The day before inoculation, adults were removed from the cages to obtain newly hatched females (unmated) for inoculation.

Resistance to *A. gossypii* assays

All experiments were conducted in a room maintained at 24:18°C, 18:6h photoperiod. We evaluated behaviour and biotic potential components as different factors of resistance.

The behaviour component was the acceptance by aphids 48h after infestation of a plant. Ten 5-7 day-old apterous aphids were deposited on the first or second leaf of 2 week-old seedlings. Two days later, the number of *A. gossypii* remaining on each plant was recorded. Each experiment comprised one plant of 90 to 100 RILs and of Védrantais, PI 161375 and the F1 as controls. One hundred thirty-four RILs were observed. After obtaining 8-10 data per RIL, a new set of experiments were conducted with the RILs exhibiting a coefficient of variation over 30% (observed more frequently with the clone 4-104).Twenty-one experiments were conducted to obtain 8-16 data per RIL with the NM1-lab clone and 40 experiments were conducted to obtain 8-34 data per RIL with the 4-104 clone. The six

populations (A, B, C, D, E and F) built to validate QTLs (see below) were evaluated for acceptance using 35-50 plants per population in four independent tests (A, B, C with each clone on one hand and D, E, F with each clone on the other hand).

Biotic potential was explored through two life history parameters of aphids: the pre-reproductive period and the fecundity of an adult during a period equivalent to the pre-reproductive period. Two adult aphids were caged for egg-laying onto the leaves of a 2-3 week-old plantlet. The day after (d_0), 2-3 nymphs were kept and the adults and other nymphs were removed. The nymphs were daily observed until they died or reached the adult stage. When they produced their first progenies, the day d_n was scored. The pre-reproductive period was estimated as $d_{Ag} = d_n - d_0$. The first adult obtained in each cage was transferred onto a new leaf. The progenies laid out by this adult during a period equivalent to d_{Ag}, P_{Ag}, were counted and removed every two days. Each experiment comprised one plant of 90 to 100 RILs and of Védrantais, PI 161375 and the F1 as controls. One hundred thirty-eight RILs were observed. After obtaining 5-8 data per RIL, a new set of experiments were conducted with the RILs exhibiting a coefficient of variation of the parameters over 30%. Five to thirteen data per line were obtained for both parameters. A simplified test was used to evaluate aphid fecundity on populations combining QTLs (see below) : 2 adult aphids were deposited for egg-laying onto the leaves of a 2-3 week-old plantlet; a glue ring was placed around the peduncle of each leaf to prevent aphid escaping. The day after, 1 nymph was kept and the adults and other nymphs were removed. The progenies produced (when nymphs reached the adult stage) were scored as long as the aphid is alive.

Resistance to *B. tabaci* assays

Experiments were conducted at INRA in Guadeloupe island, Petit-Bourg (French West Indies), from May to August in 2001 and 2002. The seedlings were bred in an insect proof greenhouse. The acceptance, which is a behaviour parameter, was not observed for *B. tabaci* as mobile apterous forms does not exist on this species and acceptance tests with alates are not adapted for genetic studies. One month-old plants were transferred in a screenhouse. One newly hatched female of *B. tabaci* was caged onto a leaf for oviposition. The number of progenies per female, P_{Bt}, was estimated by counting empty puparium 15 to 30 days after infestation. Altogether, progenies of 7 to 17 females were observed for 111 RILs with the *B. tabaci* population Bt 2001 and for 68 among the 111 RILs with the population Bt 2002. Védrantais, PI 161375 and the F1 were included as controls in each experiment.

Data and QTL analyses

The phenotypic values in parental lines were compared taking into account the interval of confidence of the mean estimated as $IC = t_{(0,05,\ n-1)}s / \sqrt{n}$ with t the student value, n the number of data and s the standard error.

Narrow sense heritability of each trait (h^2) was calculated as follows: $h^2 = \sigma^2_g / (\sigma^2_g + (\sigma^2_e/n)$ where σ^2_g is the genetic variance, σ^2_e is the environmental variance and n is the mean number of replicate per genotype. We looked for transgressive RILs among the extreme resistant RILs exhibiting a [mean ± IC] not overlapping with [mean ± IC] of PI 161375 and among the extreme susceptible RILs exhibiting a [mean ± IC] not overlapping with [mean ± IC] of Védrantais. We selected the five most extreme RILs and we compared the data with the parent's data obtained in the same tests (unilateral Mann and Whitney test with exact p). The correlation between all traits observed in the RIL population was investigated using the r coefficient of Pearson.

The additive QTLs were detected using QTL Cartographer software (Basten et al., 1997) with the composite interval mapping procedure. Five markers, selected by stepwise regression analysis, were used as co-factors, with a window of 10 cM and a walking step of 2 cM. The thresholds of significant LOD scores were fixed after 1000 permutations. When several QTLs were detected within less 20 cM interval, only the marker with the highest LOD value was retained. When several markers were significantly associated with the resistance, we considered the overall region as a single QTL and indicated the linked marker exhibiting the highest R^2 value. The epistatic QTLs (digenic interactions) were detected using the two-way analysis of variance (ANOVA, procedure of S-Plus software) between the 216 markers. The p values were corrected for Bonferroni effect as $p_{cor.} = p\ (216 \times 215)/2$ and then, the threshold $p_{cor.} = 0.05$ was reached when $p = 2.15\ 10^{-6}$. For the detected QTLs, the homogeneity of the variances of the trait between the 4 genotype's classes was verified using a Levene

Table 1 Resistance parameter (mean ± CI 95%) to *A. gossypii* and *B. tabaci* observed on PI 161375, Védrantais and the F_1: acceptance (aphids remaining 48h after infestation by 10 aphids) and progenies produced by one female (during a time as long as the pre-reproductive period for *A. gossypii* and during all the life for *B. tabaci*)

	Acceptance by *Aphis gossypii*		Progenies produced by		
			Aphis gossypii	*Bemisia tabaci* biotype B	
	NM1-lab [a]	4-104 [a]	NM1-lab [a]	Bt 2001 [b]	Bt 2002 [b]
Védrantais	8.3 ± 1.0	7.1 ± 0.4	40.3 ± 12.7	112 ± 33	40 ± 12
PI 161375	2.6 ± 1.0	4.9 ± 0.8	19.7 ± 11.9	54 ± 27	17 ± 12
F_1	5.4 ± 1.7	5.8 ± 0.7	8.4 ± 7.6	53 ± 27	39 ± 18

[a] clone, [b] population

Table 2 Pearson correlation matrix between parameters of resistance to *A. gossypii* and *B. tabaci* calculated on a set of RILs derived from the cross Védrantais X PI 161375 (in bold the significant r at $p<0.05$)

	Aphis gossypii			*Bemisia tabaci*	
	Acceptance 4-104	d_{Ag} [x] NM1-lab	P_{Ag} [y] NM1-lab	P_{Bt} [z] Bt 2001	P_{Bt} Bt 2002
A. gossypii					
Acceptance NM1 lab	**0.81**	**-0.83**	**0.78**	-0.01	0.01
Acceptance 4-104		**-0.77**	**0.68**	-0.03	0.04
d_{Ag} NM1-lab			**-0.80**	0.02	0.08
P_{Ag} NM1-lab				-0.05	0.00
B. tabaci					
P_{Bt} Bt 2001					0.08

[x] pre-reproductive period of *A. gossypii*
[y] progenies produced by one female *A. gossypii* during a period as long as d
[z] progenies produce by one female *B. tabaci* during all its life

test. The QTLs were named as followed: the two first letters Ag for *A. gossypii* or Bt for *B. tabaci*, followed by A when the QTL controlled the acceptance or by B when the QTL controlled a biotic parameter, followed by two numbers, X.x, for the x^{th} QTL described on the X^{th} linkage group (in roman numeral). As an example, *AgA-V.1* is the first QTL described located on the linkage group V that controls an *A. gossypii* traits, the acceptance. We calculated the adjusted global R^2 from an ANOVA, taking into account the markers with the highest LOD from an ANOVA for each QTL.

Breeding melon populations combining resistance QTLs to *A. gossypii* in a homogeneous genetic background

We built new families of plants carrying various allelic combinations at QTLs in a homogeneous genetic background. We chose to create a homogeneous background at a population level by obtaining a heterozygous background between Védrantais and PI 161375 at most loci. The first step was to select RILs derived from the cross Védrantais X PI 161375 on the basis of the allelic composition of the markers at the resistance loci to *A. gossypii*. Seventy RILs were selected and divided into 2 families. The family 1 comprises the RILs selected on the basis of 4 QTLs affecting the acceptance by the NM1-lab clone (2 additive QTLs and one couple of epistatic QTLs), the family 2 comprises the RILs selected on the basis of 2 additive QTLs affecting the acceptance by the 4-104 clone. Each family was composed of three groups: i) the RILs with all the resistant alleles (15 RILs in the family 1 and 11 RILs in the family 2); ii) the RILs with the resistant allele at the major QTL and the susceptible alleles at the others QTLs (11 RILs in the family 1 and 13 RILs in the family 2); iii) the RILs with the susceptible allele at the major QTL and the resistant alleles at the others QTLs (9 RILs in the family 1 and 11 RILs in the family 2). The RILs belonging to a same group were inter-crossed using the pollen mixture technique. Six populations named A, B, C, D, E and F were constructed by mixing an equal quantity of seeds collected on every RIL belonging to a same group. The populations A, B and C derived from the RILs of the family 1 and the RILs D, E and F derived from the RILs of the family 2 (see table 4).

To evaluate the homogeneity of the genetic background within the families, we calculated the expected heterozygosity at each marker in each population derived from the inter-crossing process. Within the group of RILs constitutive of a population, at the marker i, the allele PI 161375 has a frequency p_i, and the allele Védrantais has a frequency q_i such as $p_i+q_i = 1$. The expected heterozygosity in the derived population at the marker i is $H_i=2p_iq_i$. When $p_i=q_i=0.5$ within the group of RILs constitutive of a population, the expected heterozygosity in the derived population is maximum ($H_i =0.5$), i.e. 50% of the plants are heterozygous at the marker i, and 25% of the plants are homozygous for each allele. Then, the genetic background is homogeneous between the populations of a family when $H_i = 0.5$. To calculate the expected heterozygosity, we selected sets of markers without missing data in the studied population. The number of markers took into account was 101, 195 and 104 for the populations A, B and C and 285, 228 and 267 for the populations D, E and F.

The acceptance of each population was predicted according to the allelic combination of the homozygous QTLs in the population. For each QTL selected, the markers with the highest LOD from an ANOVA were included in a linear model to predict the phenotypic value of this population.

Results

Acceptance by *A. gossypii* and biotic potential of *A. gossypii* and *B. tabaci* in Védrantais and PI 161375

Resistance parameters were observed on PI 161375, Védrantais and the F1 with two clones of *A. gossypii* and two natural populations of *B. tabaci* biotype B.

The acceptance, which is a behaviour parameter, was only observed for *A. gossypii* for technical reasons. We observed the mean number of adults remaining on plants 48h after infestation by 10 aphids (Table 1). Acceptance was significantly reduced on PI 161375 compared to Védrantais, 70% with the NM1-lab clone and 30% with the 4-104 clone. The acceptance of the F_1 was intermediate between the parents for both clones of *A. gossypii*.

The number of progenies produced by one female, which is a biotic parameter, was observed for both pests (Table 1). The *A. gossypii* NM1-lab clone produced two fold less progenies on PI 161375 than on Védrantais (t test, p= 0.02). The number of progenies produced on the F1 was close to the

Table 3 QTLs with an additive effect (Composite interval mapping) and an epistatic effect (ANOVA) on the acceptance by *A. gossypii* and the biotic potential of *A. gossypii* NM1-lab clone (NM1 genotype) and 4-104 clone (C9 genotype) and on biotic potential of *B. tabaci* biotype B.

Trait	QTL	LG[a]	Marker[b]	Nb[c] Ind	Position[d] cM	LOD[e] Value	P[f]	Resistant[g] allele
Aphis gossypii acceptance by NM1-lab clone								
	AgA-V.1	V	Vat	118	78.1-82.6	39.5***		PI 161375
	AgA-IX.1	IX	H36M42_12	120	31.1-37.2	5.9***		PI 161375
	AgA-VII.1-XI.1	VII	H36M41_9	106	0		0.03	Epistasis trans[h]
		XI	E46M48_4		26.1			
A. gossypii acceptance by 4-104 clone								
	AgA-V.1	V	Vat	120	78.2-81.6	35.7***		PI 161375
	AgA-IX.2	IX	E35M35_10	126	40.5-58.5	3.7**		PI 161375
A. gossypii biotic potential NM1-lab clone								
d_{Ag}	*AgB-V.1*	V	E33M40_13	112	78.1-82.0	27.2***		PI 161375
	AgB-IV.1	IV	CM122	79	31.2-42.6	5.8**		Védrantais
	AgB-VII.1-XII.1	VII	E_850	75	126.3		0.03	Epistasis cis[h]
		XII	CMTCN14		20.0			
P_{Ag}	*AgB-V.1*	V	E39M42_23	108	86.3-89.5	19.5***		PI 161375
Bemisia tabaci biotic potential population 2001								
P_{Bt}	*BtB-VII.1*	VII	E43M44_15	37	85.8-94.2	3.6*		PI 161375
B. tabaci biotic potential population 2002								
P_{Bt}	*BtB-IX.1*	IX	H36M37_14	59	72.0-82.0	4.0**		PI 161375

[a] Linkage group
[b] The nearest flanking marker to QTL
[c] The number of RILs genotyped for the identified QTL-linked marker
[d] Estimated position of the QTL within ± 1 LOD unit
[e] LOD value for the additive QTLs with significance at 5% (*),1% (**), 0.1% (***) after 1000 permutations
[f] P value corrected for Bonferroni effect for the epistatic QTLs (See materials and methods)
[g] Parental allele which contributed to the resistance
[h] Trans: Resistant alleles are Védrantais X PI 161375 or PI 161375 X Védrantais, Cis: Resistant alleles are Védrantais X Védrantais or PI 161375 X PI 161375

number of progenies produced on PI 161375. The population Bt 2001 of *B. tabaci* produced more progenies than the population Bt 2002 on Védrantais as well as on PI 161375. Both populations produced two fold less progenies on PI 161375 than on Védrantais (t test $p<0.01$ for both populations), The number of progenies on the F1 was close to the number of progenies produced on PI 161375 with Bt 2001 and close to the number of progenies produced on Védrantais with Bt 2002.

Resistance to *A. gossypii* and *B. tabaci* in a RIL population (Védrantais X PI 161375)

One hundred thirty-four RILs were assessed for the acceptance by two aphid clones, NM1-lab and 4-104. The heritability of the acceptance was 0.92 for the NM1-lab clone and 0.96 for the 4-104 clone. No significant transgressive line was observed for the acceptance by the NM1-lab clone. Acceptance by the 4-104 clone was reduced on the transgressive line, RIL181, compared to PI 161375 ($p=0.02$) with only 2.3 adults in average remaining on the RIL181 *vs* 4.2 adults on PI 161375. The acceptance by the NM1-lab clone was correlated to the acceptance by the 4-104 clone (Table 2), suggesting common genetic factors for the resistance control toward both clones.

One hundred and twenty seven RILs were assessed for two biotic parameters of the *A. gossypii* NM1-lab clone, the duration of the pre-reproductive period, d_{Ag}, and the number of progenies produced by one female, P_{Ag}. The heritabilities were 0.78 for d_{Ag} and 0.85 for P_{Ag}. We observed a transgressive RIL for P_{Ag}: aphids produced 64.2 progenies on the RIL208 and 46.0 on Védrantais ($p=0.03$). The two components assessed for the biotic potential of *A. gossypii,* d_{Ag}, and P_{Ag}, were negatively correlated (Table 2), the shortest d_{Ag} and the highest P_{Ag} inducing the highest biotic potential. This correlation suggested the involvement of common genetic factors for the control of these two traits. Moreover, d_{Ag} and P_{Ag} were correlated to the acceptance parameter (Table 2), suggesting that common genetic factors control the acceptance by *A. gossypii* and the biotic potential of *A. gossypii*.

One hundred and eleven RILs were assessed for a biotic parameter, the progenies produced by a whitefly, $P_{Bt,}$ with two natural populations of *B. tabaci*. The heritabilities of P_{Bt} were 0.62 with Bt 2001 and 0.74 with Bt 2002. We observed a transgressive RIL ($p=0.04$): *B. tabaci* Bt 2002 produced 103 progenies on the RIL140 and only 40 progenies on Védrantais. The number of progenies produced by the whiteflies Bt 2001 was not correlated to the number of progenies produced by the whiteflies Bt 2002 (Table 2), suggesting an independent genetic control of these traits. The component assessed for resistance to *B. tabaci*, P_{Bt}, was not correlated to any components assessed for *A. gossypii*, suggesting an independent genetic control for resistance to *A. gossypii* and to *B. tabaci* (Table 2).

Mapping QTLs of resistance to *A. gossypii* and *B. tabaci*

Genetic map

The 216 markers designed a framework map consisting in 12 linkage groups (corresponding to the basic number of chromosomes in melon) and covering 1312 cM (Kosambi) (Figure 1). The median distance between two markers was 5.1 cM (3.3 cM for the first quartile and 7.8 cM for the third quartile). Therefore the melon genome was well covered by the marker set.

Resistance to A. gossypii

Several QTLs controlled the acceptance by *A. gossypii* (Table 3 and Figure 1). Three additive QTLs, *AgA-V.1*, *AgA-IX.1* and *AgA-IX.2*, affected the acceptance by the NM1-lab and the 4-104 clones. The resistant allele originated from PI 161375, the resistant line, for these three QTLs. The major QTL *AgA-V.1* colocalized with the *Vat* locus. *AgA-V.1* equally affected the NM1-lab and 4-104 clones (R^2=71% and R^2=66%). *AgA-IX.1* reduced the acceptance by the clone NM1-lab (R^2=6.0%) whereas *AgA-IX.2* reduced the acceptance by the clone 4-104 (R^2=4.2%). A couple of epistatic QTLs *AgA-VII.1-XI.1* reduced the acceptance by the NM1-lab clone with a R^2 value over 20%, nevertheless this R^2 value (issued from an ANOVA) can not be compared to the R^2 calculated for additive QTLs (issued from composite interval mapping analysis). The global R^2 was estimated at 82% for acceptance by *A. gossypii* NM1-lab clone, 74% for acceptance by *A. gossypii* 4-104 clone.

Several QTLs affected the biotic potential of *A. gossypii*. NM1-lab clone (Table 3 and Figure 1). One major and additive QTL, *AgB-V.1*, controlled d_{Ag}, the duration of the pre-reproductive period, and P_{Ag}, the number of progenies produced by one female. Its effect (R^2) was 55% on d_{Ag}, and 67% on P_{Ag}. *AgB-V.1* peaked at 1.4 cM of the *Vat* locus for d_{Ag} and 8.0 cM of the *Vat* locus for P_{Ag}, its resistant

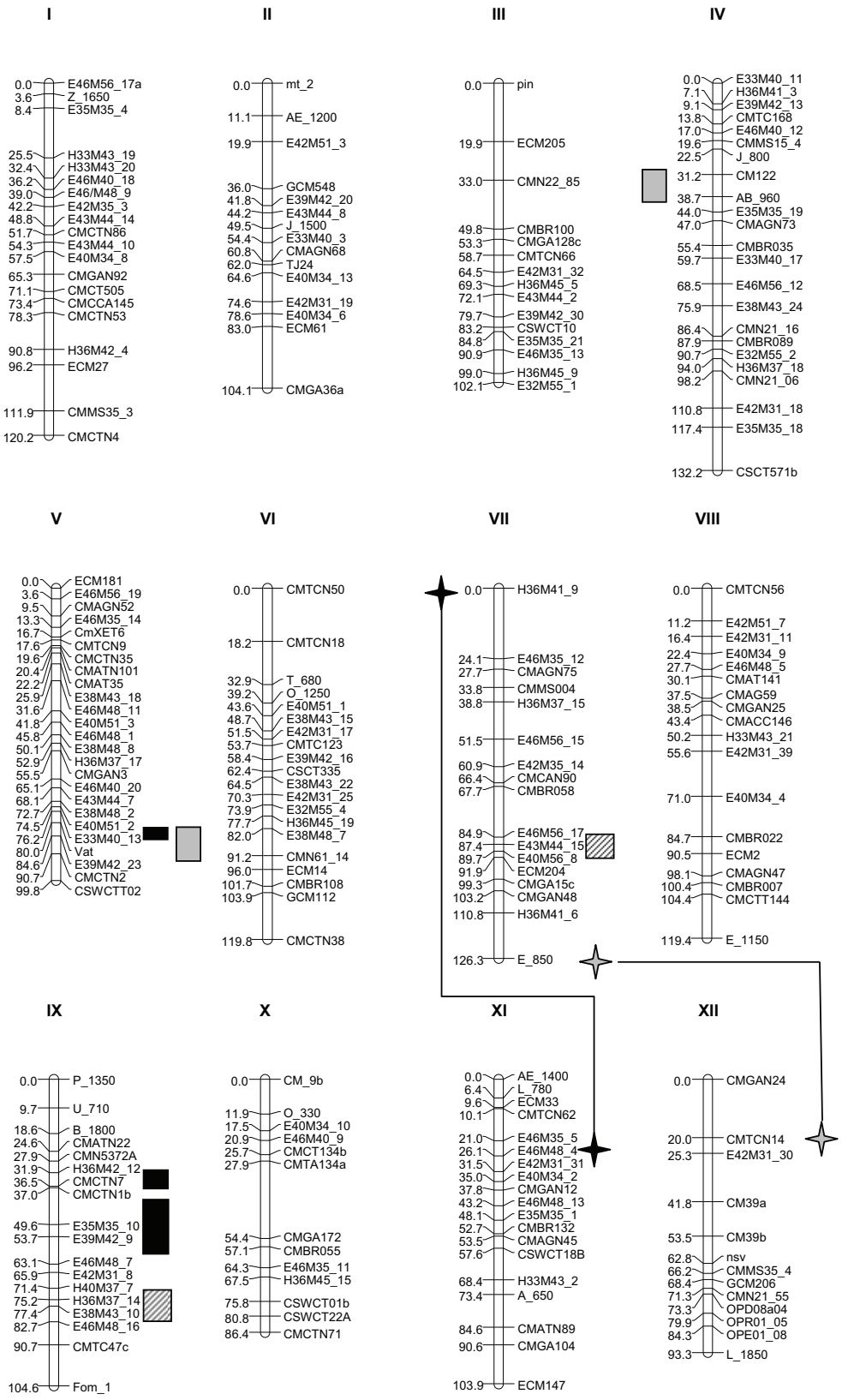

Fig. 1 Genetic map of melon (Védrantais X PI 161375) and QTLs affecting hemipterans: plain: *A. gossypii* and striped: *B. tabaci*, black: acceptance, grey: biotic potential, rectangle: additive QTL (right side: PI 161375 allele for resistance, left side: Védrantais allele for resistance) and star: epistatic QTL (same side of the two linkage groups: cis effect, opposite of the two linkage groups: trans effect).

allele originated from PI 161375. One minor QTL *AgB-IV.1* had an additive effect (R^2=9.3%) on d_{Ag}, its resistant allele originated from the susceptible line Védrantais. A putative QTL (not shown in the table 3) affecting the acceptance by the NM1-lab clone (R^2=2.9%, p=0.066) colocalized with AgB-IV.1. A couple of epistatic QTLs, *AgB-VII.1-XII.1* affected over 20% d_{Ag} (value not comparable to the R^2 calculated for additive QTLs). The global $R^2$of the QTLs was estimated at 68% for d_{Ag}, and at 62% for P_{Ag}.

Resistance to B. tabaci biotype B

Two additive QTLs affected the number of progenies produced by one female of *B. tabaci*, P_{Bt} (Table 3 and Figure 1). *BtB-VII.1* affected the Bt 2001 population (R^2=17.9%) and *BtB-IX.1* the Bt 2002 population (R^2=13.8%). The resistant alleles at both QTLs originated from PI 161375. No epistatic QTL affecting *B. tabaci* was detected. The poor QTL detection for resistance to *B. tabaci* is linked to a weak heritability of P_{Bt}. The inflated phenotypic variance in some RILs may be due to the fact that we used unexpected mated females that usually produce more progenies, instead of unmated females. Some RILs that were phenotyped for their *B. tabaci* resistance, were not fully genotyped and therefore, for some markers, the analysis was affected by a reduced effective size of the sample of RILs.

QTL validation in 6 populations combining QTLs of resistance to A. gossypii

Populations were built displaying different allelic combinations at the QTLs affecting either the aphid NM1-lab clone (populations A, B and C, family 1) or the aphid 4-104 clone (populations D, E and F, family 2). The genetic background is homogeneous within a family when the expected heterozygosity is H=0.5 at all the markers. In order to check the homogeneity of the genetic background within a family, the expected heterozygosity at each marker was estimated in each population (Figure 2). The heterozygosity was over 0.4 for about 70% of the markers in the populations A, B and C and for about 80% of the markers in the populations D, E and F. Less than 7% of the markers have a nil heterozygosity whatever the population, these markers are in the vicinity of the QTLs selected to build the populations and therefore are homozygous as expected. For the last 10-15% of the markers, the allelic composition was unbalanced (0.1<H<0.3). Thus, we considered that the populations A, B and C on one hand and the populations D, E and F on the other hand have a homogeneous genetic background.

The phenotypic value was predicted for each population according to its allelic composition at each QTL (Table 4). As expected, the population A which contains the resistant allele at all the QTLs affecting the NM1-lab clone (*AgA-V.1*, *AgA-IX.1, AgA-VII.1-XI.1*) was predicted to exhibit a reduced acceptance by the NM1-lab clone when compared with the population B which contains only the resistant allele at the major QTL (*AgA-V.1*). The population B was predicted to exhibit a reduced acceptance by the NM1-lab clone when compared with the population C which contains only the resistant allele at the minor QTLs (*AgA-IX.1*, *AgA-VII.-XI.1*). In the same way, the population D that contains the resistant allele at the two QTLs affecting the 4-104 clone (*AgA-V.1*, *AgA-IX.2*) was predicted to exhibit a reduced acceptance by the 4-104 clone when compared to the population E which contains only the resistant allele at the major QTL (*AgA-V.1*). The population E was predicted to exhibit a reduced acceptance by the 4-104 clone when compared to the population F which contains only the resistant allele at the minor QTL (*AgA-IX.2*).

The six populations were evaluated for acceptance by the NM1-lab and 4-104 *A. gossypii* clones (Table 4). In order to observe the effect of the major QTL *AgA-V.1,* we compared two couples of populations with the same allelic composition except at the *AgA-V.1* locus, i.e. A *vs* C and D *vs* F. The resistant allele at *AgA-V.1* reduced the acceptance from 54% (A *vs* C) to 61% (D *vs* F) of the NM1-lab clone. In the same way, the resistant allele at *AgA-V.1* reduced the acceptance by the 4-104 clone from 31% (A *vs* C) to 55% (D *vs* F). These results confirmed the strong effect of *AgA-V.1* on the acceptance by the NM1-lab clone as well as by the 4-104 clone. Its effect appeared slightly stronger on the NM1-lab clone than on the 4-104 clone.

In order to observe the effect of the minor QTLs *AgA-IX.1* and *AgA-VII.1-XI.1,* we compared a couple of populations with the same allelic composition except at *AgA-IX.1* and *AgA-VII.1-XI.1* loci, i.e. A *vs* B (Table 4). The resistant alleles at *AgA-IX.1* and *AgA-VII.1-XI.1* reduced the acceptance by the NM1-lab clone of 24% as expected and, surprisingly, reduced the acceptance by the 4-104 clone of

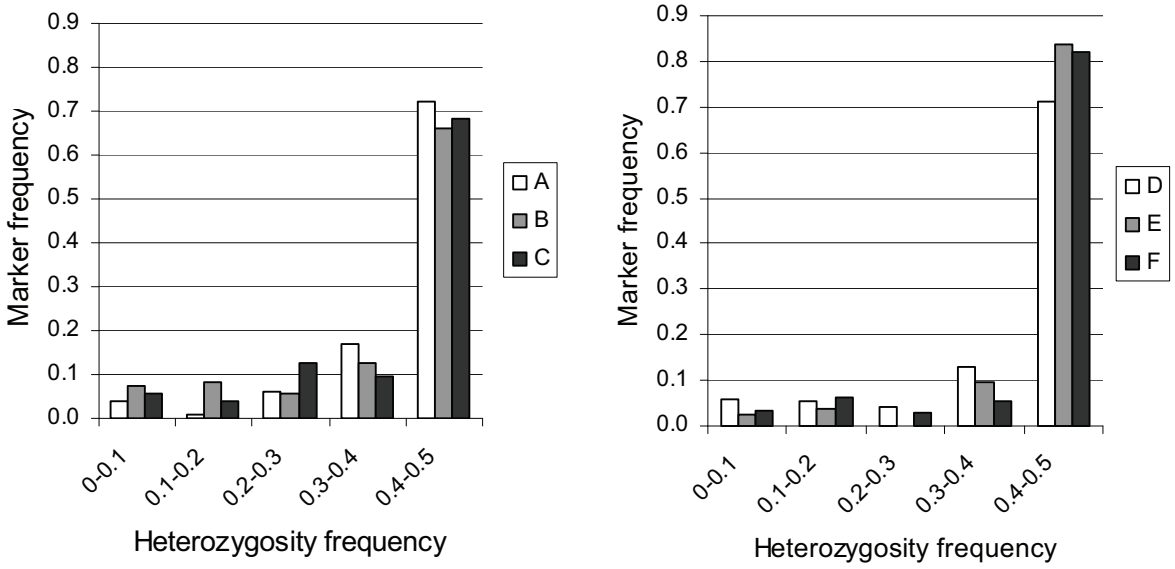

Fig. 2 Heterozygosity frequency in the populations of the family 1 combining the 4 QTLs, (populations A, B, C), and in the populations of the family 2 combining 2 QTLs, (populations D, E and F) for a set of markers (from 101 to 195 markers in the first family and 225 to 285 markers in the second family).

31%. This effect of the minor QTLs *AgA-IX.1* and *AgA-VII.1-XI.1* was not predicted on the 4-104 clone. In order to observe the effect of the minor QTL *AgA-IX.2*, we compared a couple of populations with the same allelic composition except at the *AgA-IX.2*, i.e. D *vs* E (Table 4). The resistant allele at *AgA-IX.2* reduced the acceptance by the 4-104 clone of 20% as predicted, but this difference was not significant (p=0.11). Surprisingly, the resistant allele at *AgA-IX.2* reduced the acceptance by the NM1-lab clone of 29%. Its effect was not predicted on the NM1-lab clone.

AgB-V.1, the only QTL predicted to affect the number of progenies, mapped very close of *AgA-V.1*. We checked the allelic composition at the nearest marker of *AgB-V.1* in the 6 populations built to validate the acceptance. The populations D, E and F appeared appropriate for validating *AgB-V.1*, i.e their allelic composition is homogonous at AgB-V.1 (the same than at AgB-V.1). As expected the number of progenies produced was strongly reduced when the resistant allele at *AgB-V.1*is present*:* 2.4 progenies were produced on D and 35.6 on F ($p<0.0001$). As expected the fecundity was not affected by the allelic composition at *AgA-IX.2* (D vs E)*:* 2.4 progenies produced on D and 3.1 on E (p=0.84). We used a simplified test to evaluate the populations instead of the test used for the RILs evaluation. Therefore, the magnitude of the predicted effect (from the QTL analysis) of the populations cannot be compared to the magnitude of the observed effects of the populations for the fecundity parameter.

Discussion

Plant resistance to insects is mostly quantitatively inherited and most of the QTLs identified control traits that are a response of the plant to the pest attack (usually scored as damages) (Yencho et al., 2000). Few QTLs that affect the insect biology have been described so far (Maliepaard et al., 1995; Yencho et al., 1996; Alam and Cohen, 1998; Yamasaki et al., 1999; Duan et al., 2007), very few affecting aphids (Castro et al., 2005). In our study, we focused on resistance parameters that reveal an effect on the behaviour and the biotic potential of the insect, because we consider that these effects are a more direct and reliable measure of the resistance than damages. Moreover these parameters may allow further modelling of the impact of plant genotypes on insect dynamics. We identified 10 genome locations on 5 linkage groups of the melon genome involved in resistance to hemipterans.

<u>The same locus affects both the behaviour and the biotic potential of aphids</u>

Since Painter (1951), entomologists have distinguished two mechanisms of resistance to insects: antixenosis that affects the behaviour of insects and, antibiosis that affects their biotic potential. Insect behaviour is a complex trait that involves physical and chemical interactions with hosts, parasites and environment. The biotic potential of insects depends on different life traits as fecundity, mortality, duration of larval development etc… In our study, two major QTLs affecting either the behaviour or the biotic potential of *A. gossypii* colocalized with the *Vat* gene and should correspond to this gene. In the same way, the tomato *Mi-1.2* gene alters the feeding behaviour of the aphid *M. euphorbiae* and drastically decreases its fecundity and longevity (Kaloshian et al., 1997). The AKR gene in *Medicago truncatula* induces deterrence and low biotic potential of the aphid *Acyrthosiphon kondoi* (Klingler et al., 2005). The behaviour and the biotic potential of several aphid species appear affected by a same major R gene. Thus, antixenosis and antibiosis should be considered as two responses of aphids to R genes. The melon *Vat* gene and the tomato *Mi-1.2* gene belong to the NBS-LRR family of R genes (Milligan et al., 1998; Pauquet et al., 2004), and NBS-LRR genes are also candidates for the *M. truncatula* AKR locus (Klingler et al., 2005). NBS-LRR proteins have been shown to be involved in the recognition of pathogens (McHale et al., 2006) and are therefore probably involved in the recognition of aphids. This recognition induces a complex plant response which, interestingly, leads aphids to modify their behaviour (antixenosis effect). On a *Vat*-resistant melon plant, *A. gossypii* seldom reaches the phloem, stops feeding in phloem when reached (Chen et al., 1996; Klingler et al., 1998), and then the starvation affects its biotic potential.

QTLs affecting the biology of several hemipterans have been described in tomato (Maliepaard et al., 1995), wheat (Castro et al., 2005) and rice (Alam and Cohen, 1998; Wang et al., 2004; Duan et al., 2007). These QTLs affect the behaviour, the biotic potential, or both of their targets. Here we showed that minor additive or epistatic QTLs affected either the behaviour or the biotic potential of *A. gossypii*. One of these minor QTLs, *AgB-IV.1,* affected the biotic potential of *A. gossypii* and was a putative QTL affecting the acceptance by the NM1-lab clone. Therefore, as for major genes,

Table 4 Acceptance by NM1-lab and 4-104 *A. gossypii* clones (adults 48h after infestation by 10 adults) on 6 populations combining QTLs of resistance: predicted from the QTL analysis (ANOVA) and observed on the populations.

					Acceptance by two *A. gossypii* clones			
	Allele at the resistance locus[y]				Predicted values from QTL analysis		Observed values on the population[z]	
Population	*AgA-V.1*[l]	*AgA-IX.1*[m]	*AgA-VII.1-XI.1*[m]	*AgA-IX.2*[n]	NM1-Lab	4-104	NM1-lab	4-104
A	R	R	R	H	4.3	5.1	3.8[a]	4.6[a]
B	R	S	S	H	4.6	5.1	5.0[b]	5.8[b]
C	S	R	R	H	8	7.3	8.4[c]	6.7[c]
D	R	H	H	R	4.6	4.6	2.6[a]	3.1[a]
E	R	H	H	S	4.6	5.1	3.7[b]	3.9[a]
F	S	H	H	R	8.5	6.8	6.7[c]	6.9[b]

[l] QTL detected with both aphid clones
[m] QTL only detected with the NM1-lab aphid clone
[n] QTL only detected with the 4-104 aphid clone
[y] R: homozygous for the resistant allele, H: either homozygous (R or S alleles) either heterozygous, S homozygous for the susceptible allele
[z] means significantly different at 5% within column for each family (A, B and C family 1 or D, E and F, family 2).

antixenosis and antibiosis should be considered as two responses of hemipterans to QTLs of resistance.

Specificity of the resistance loci to hemipterans

Several major genes for resistance to aphids have been described. More often the resistance conferred by these genes is biotype-specific, such as the resistance to *Amphorophora idaei* in raspberry (Sargent et al., 2007), the resistance to *Schizaphis graminum* and *Diuraphis noxia* in wheat (Berzonsky et al., 2003) and, the resistance to *Dysaphis devecta* in apple trees (Alston and Briggs, 1977). In our study, we used two distantly related clones of the *A. gossypii* species, the 4-104 clone with a C9 genotype, and the NM1-lab clone with a NM1 genotype (Carletto et al., 2009). We identified a major QTL that reduces acceptance by both *A. gossypii* clones. This major QTL colocalizes with the *Vat* gene, which has been characterized so far using the NM1-lab clone (NM1 genotype). We showed here that the *Vat* gene also reduces acceptance by a C9 clone. Moreover, we used two hemipteran species, *A. gossypii* and *B. tabaci* to track QTLs with a broad effect on piercing sucking insects. No QTL affecting both *A. gossypii* and *B. tabaci* was detected in the RIL population we used. The *Vat* gene did not confer any resistance to *B. tabaci* biotype B as already suggested by Sauvion et al. (2005). These results contrast with the spectrum of the tomato *Mi-1.2* gene, which confers resistance to different pests such as nematodes, aphids, whiteflies and psyllids (Milligan et al., 1998; Nombela et al., 2003; Casteel et al., 2006) but confers resistance to a single clone of the aphid *M. euphorbiae* (Goggin, 2007). Specificity of resistance to hemipterans remains poorly studied, but knowledge from the *Mi-1.2* and *Vat* genes suggested that the NBS-LRR genes offer an unpredictable spectrum of resistance against hemipteran species.

Effect of allelic combinations at QTLs

To validate the QTLs, we opted to compare populations with a homogeneous genetic background (at the population level) and different allelic combinations at QTLs. The populations were derived from RILs used for the QTL mapping by inter-crossing set of RILs with the same allelic combinations at QTLs. This original strategy offers several advantages: i) the new populations are obtained in one generation, the expected homogeneity between populations corresponds to the expected homogeneity after 5 to 6 back-crosses between a line and a recurrent parent, ii) it is not necessary to carry on any new genotyping, iii) the effects of the QTLs (and of the combinations of QTLs) can be evaluated within a confidence interval and, iv) the effect of the detected QTLs on different clones can be investigated. This strategy requires the inter-crossing of enough RILs with the same allelic combinations to obtain a high heterozygosity level in the population. The expected heterozygosity can be checked before inter-crossing, especially if the number of RILs available is low. If needed, the heterozygosity can be inflated by inter-crossing each RIL used as a female by a pollen mixture excluding its own pollen. This strategy also requires the phenotyping of at least 30 plants per population because the genetic background is homogeneous at the population level (the genetic background of each plant is distinct to each other).

In this study, the validation procedure allowed confirming the strong effect of the major QTL *AgA-V.1* on acceptance by *A. gossypii*; its effect appeared even slightly stronger than expected. We showed that the combination of minor QTLs (additive and epistatic) have a significant effect on acceptance by *A. gossypii*; this combination effect appeared stronger than expected (acceptance reduced of 6% according to the predicted values and of 24% according to the observed values). Moreover, according to the clone, we observed a significant but unexpected effect of some combinations (acceptance reduced of 30% with the NM1-lab clone while no reduction was expected). New clones representative of all the *A. gossypii* variability will be used to investigate the spectrum of efficiency of these combinations of QTLs. The effect of associating a major QTL with minor QTLs on durability of the major QTL will be investigated.

Melon breeding perspectives

Aphids, as whiteflies, invade crops in low numbers early in the season and their population increase gradually over generations before reaching damaging levels. Kennedy et al. (1987) suggested that, for such pests, even low or moderate levels of all types of resistance could increase the time necessary to the population to reach a damaging level.

Although *B. tabaci* is considered as a devastating pest on several crops, loci affecting the biology of *B. tabaci* has been only characterized in tomato (Nombela et al., 2003). In our study, two minor QTLs, each of them specific to a population of *B. tabaci* biotype B, were detected. Most likely, the lack of control over the variability of *B. tabaci* biotype B impaired the detection of QTL. *B. tabaci* has been structured in 12 major clades according to the COI sequences (Boykin et al., 2007). Intra-clade or intra-biotype variation has been only investigated in whiteflies from Asia-Pacific region (de Barro, 2005). Thus, more efforts are needed to improve genetic studies i) to characterize the intra and inter biotype variability of *B. tabaci* populations infecting melon crops and ii) to control the breeding of *B. tabaci* in the mass rearings in the aim of inflating heritability of resistance in biological tests.

The *A. gossypii* genotypes that colonize cucurbits crops belongs either to the NM1 genotype, up to now only identified in France, or to a cluster of a dozen of related genotypes (Carletto et al., 2009). In this cluster, the C9 genotype is the most frequent and is worldwide distributed. In our study, we showed that the *Vat* gene affects *A. gossypii* NM1-lab clone, with a NM1 genotype, and 4-104 clone with a C9 genotype. For some clones having a C9 genotype (including the clone 4-104), Lombaert et al. (2009) did not observe any significant difference in residence time (a parameter comparable to acceptance) on *Vat*- and non *Vat*-melon. This lack of consistency with our results could be due to a lack of power to reveal difference (β risk) in the biological test used by Lombaert et al. (2009). Moreover, we showed that the accession PI 161375, the accession carrying the resistant allele at the *Vat* locus, was resistant to different clones belonging to the NM1 or C9 genotypes (Boissot et al., 2008). Altogether, these results suggest that the *Vat* gene affects different clones of NM1-lab and C9 genotypes of *A. gossypii* and it appears as a solid basis for breeding resistance for all production areas. Moreover, the resistance could be reinforced by minor additive and epistatic QTLs whose efficiency when combined to the *Vat* gene was proved in our study. We will investigate the effect of these minor QTLs on the durability of the *Vat* gene using the populations combining the *Vat* gene with different QTLs.

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Article 3 : Effects of resistance combinations on density and genetic diversity of *Aphis gossypii* populations in melon crops

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INTRODUCTION

Aphids are one of the major hemipteran pests of cultivated plants. These pests are sap-sucking: during probing, their stylet penetrates into the phloem and they feed on plant nutrients rich in sugars. They cause direct damages on infested leaves which become crumpled and distorted. Aphids excrete honeydew causing indirect damages since sooty mould develops on leaves and fruits coated with the sticky and sugary excreta. Their extremely high growth and developmental rates, allow aphid populations to rapidly reach levels that are damaging to crop plants and heavy infestations can results in yield losses. The chemical control during the last 50 years resulted into the mismanagement of pest populations through overdosing with pesticides resulting in i) appearance of resistant pest population, ii) loss of biological control through the use of broad-spectrum pesticides and iii) loss of habitat diversity in agroecosystems. This lead to the concept of 'Integrated Pest Management' (IPM) including chemical control, biological control, host-plant resistance and cultural control of aphids (Dedryver et al., 2010).

Host-plant resistance seems to be an efficient and environmentally friendly means when controlling pests, including aphids. For example, rootstocks of vineyard *Vitis* species resistant to phylloxera, *Daktulosphaira vitifoliae,* have been used extensively all over the world since the 1870s and no virulent genotype of grape phylloxera has been described up to now on resistant rootstocks (Granett et al., 2007; Korosi et al., 2007). Nevertheless, resistance-

breaking genotypes have occurred in several plant-aphid systems. Because of their short life cycles by clonal reproduction and extremely high reproductive rates, a female produces genetically identical offspring generating colony with hundreds of individuals in few generations. This reproductive mode facilitates the propagation of mutant variants able to break resistance. For example, breakdown of resistance conferred by *Dn4* gene in wheat to the Russian wheat aphid, *Diuraphis noxia*, occurred in Europe (Shufran and Payton, 2009) as well as breakdown of resistance conferred by *Ag1* gene in raspberry to the raspberry aphid, *Amphorophora agathonica* (Keep, 1989). Different strategies for deployment of resistance genes have been suggested in order to reduce the probability of emergence and growth of new resistance-breaking clones such as continual description and introduction of new resistance genes or combinations of many resistance genes (Lindhout, 2002). Alternative is to identify and introduce resistance controlled by multiple, quantitative loci or by recessive loci demonstrated to be more durable in other pathosystems such as plant/potyviruses (Ayme et al., 2007; Palloix et al., 2009) or plant/fungi (Brun et al., 2010). Among aphids, *Aphis gossypii* (melon aphids) is by far the most important of the direct pests of Cucurbitaceae. Cucurbitaceae crops cover about 20,000 ha in France and 4,000 ha in the French West Indies (FWI). According to the surfaces used for production, Cucurbitaceae are the main vegetables cultivated in France. About 70% of theses surfaces are dedicated to melon crops principally in four growing areas (south-eastern, south-western and western) and in the French West Indies. The *Vat* gene that confers resistance to *A. gossypii* was isolated in the Korean accession PI 161375 in the 1980s (Pitrat and Lecoq, 1980, 1982). This gene belongs to the NBS-LRR gene family (Pauquet et al., 2004). Several sources of resistance to *A. gossypii* have been identified in melon. The majority of the 50 melon accessions that display resistance to *A. gossypii,* carries the same resistance *Vat* allele, whatever their geographical origins (Dogimont et al., 2010). This low variability of major resistance alleles in melon reinforces the importance to develop strategies to preserve the *Vat* resistance gene.

The *Vat* gene effect has been particularly studied in laboratory using two *A. gossypii* clones named NM1-lab and 4-104. Additionally, three additive QTLs and one couple of epistatic QTLs, affecting the acceptance of NM1-lab and 4-104 clones, were located on the melon genome and validated in breeding progenies. Among them, a major QTL affects both the behavior and the biotic potential of these two *A. gossypii* clones; it colocalizes with and likely corresponds to the *Vat* gene (Boissot et al., 2010). The NM1-lab and 4-104 clones are specialized on Curcurbitaceae and belong to two genetically distinct clusters on the basis of

eight microsatellite markers (Carletto et al., 2009). NM1-lab is characterized by the multilocus genotype (MLG) called NM1 and 4-104 by the MLG C9. Up to now, the resistance conferred by *Vat* or by QTLs has only been tested with these two clones, but the genetic diversity of *A. gossypii* populations specialized on cucurbits is large and distributed in the two genetic clusters made each of more than 100 MLGs (Thomas et al., submitted). Therefore, the response of *A. gossypii* field populations to the melon resistance factors might be variable according to the aphid clones and their MLGs. Since the 1990s, the *Vat* resistance allele has been used extensively, incorporated into more than 30 cultivars in France and representing over 40% of the area of melon cultivation in France. Melons are produced in three areas in France, in southeast (SE), in Southwest (SW) and in the French West Indies (FWI). The use of resistant varieties is marginal in SW and FWI whereas their use is majoritary (80% of cultivated varieties) in SE for early melon crops. Therefore, the selection pressure is strong and breakdown of resistance may occur in SE. Several colonies were episodically observed in SE on resistant melons, without propagating to the whole crop (Lombaert et al., 2009). They corresponded to seven distinct MLGs that could not be assimilated to resistance-breaking MLGs as no extension has been observed.

In the present paper, we tested the combinations of aphid-resistance *Vat* gene and QTLs in melons constructed by Boissot *et al.* (2010) in various environments among French melon growing areas. The objectives were i) to challenge the efficiency of the source of resistance and reveal the occurrence of resistance-breaking clones and ii) to investigate the impact of distinct resistance combinations on the density and genetic changes of *A. gossypii* populations.

MATERIALS AND METHODS

Plant material: resistance combinations

We investigated *in situ* the effect of the *Vat* gene and minor QTLs using melon populations combining the *Vat* gene with different QTLs in a homogeneous genetic background.

Two families of resistance combinations combine the resistant or susceptible allele at the *Vat* locus with resistant or susceptible allele at different QTLs. The family I comprises the resistance combinations A, B and C, combining the *Vat* gene and/or minor QTLs affecting the acceptance by the NM1-lab and 4-104 clones. The family II comprises the resistance combinations D, E and F combining the *Vat* gene and/or minor QTLs affecting only the acceptance by the NM1-lab clone (Table 1). These melon populations derived from inter-

Table 1: Allelic composition at the resistance loci (*Vat* gene and QTLs) of six melon combinations and acceptance by NM1-lab and 4-104 *A. gossypii* clones (remaining adults 48 h after infestation by 10 adults).

Family	Group	*Vat* gene	AgA-VII.1-XI.1	AgA-IX.1	AgA-IX.2	Acceptance by	
						NM1-lab	4-104
I	A	R^x	R	R	H	3.8^a	4.6^a
	B	R	S	S	H	5.0^b	5.8^b
	C	S	R	R	H	8.4^c	6.7^c
II	D	R	H	H	R	2.6^a	3.1^a
	E	R	H	H	S	3.7^b	3.9^a
	F	S	H	H	R	6.7^c	6.9^b

From (Boissot et al., 2010)

R homozygous resistance allele, S homozygous susceptibility allele, H heterogenous

^{abc} Means significantly different at 5% within column for each family

Table 2: Characteristics of the localities and resistant combinations tested in each locality.

Areas	Localities	Coordinates	Simpson evenness index *V* within alate populations[a]	Climate	Resistant combinations	
					2008	2009
Southeast	Aramon	43°53N	[0.78-0.86]	Mediterranean	ABC	ABC
	Saint-Andiol	43°50N	[0.85-0.91]		DEF	ABC DEF
Southwest	Moissac	44°05N	[0.67-0.84]	Oceanic	DEF	DEF
French west Indies	Petit-Canal	16°22N	[0.09-0.22]	Tropical	DEF	ABC DEF

[a] From Thomas *et al*, submitted, *V* represents an equitability index which describes the repartition and relative amount of alate clones, it varies from 0 (no diversity) to 1.

crossing RILs carrying a same allelic combination at QTLs. The genetic backgrounds between melon combinations were shown homogenous, equivalent to genome homogeneity obtained after 5 to 6 back crosses between a line and a recurrent parent (Boissot et al., 2010).

Sampling locations, melon growing areas description and field experiments

The melon combinations combining resistance loci were grown in 2008 and 2009 in two locations in southeast of France (SE), Saint-Andiol and Aramon, in one location in other areas, Moissac in Southwest of France (SW) and Petit Canal in the French West Indies (FWI). In each location, one or both families of melon combinations were grown (Table 2). SE, SW and FWI are Cucurbits producing areas exhibiting variable climates and agrosystems (Table 2). Seeds of melon were sown in 60 ml pots filled with disinfected soil in a greenhouse. Seedlings were transplanted in the field on black plastic mulch, 14 to 22 days later depending on the trials. The distance between rows was 2 m with 50 cm between plants. All fields were drip irrigated. Each field was divided into three plots corresponding to the three resistance combinations (ABC or DEF). Each plot contained 150 plants and the plots were separated by 30 Védrantais plants (susceptible to aphids).

Estimation of *A. gossypii* population density

We estimated the aphid density twice a month using the qualitative visual counting method of *A. gossypii* developed by Boll et al. (2002). We tagged 16 or 17 counting areas among the plants in each plot where we scored the aphid density on one m^2. Approximately 10% of each plot surface was scored. Four density data obtained during the culture were used to build a demographic curve and the area under the curve (AUC) was computed for each plot. The AUC corresponded to the amount of insects present during all the cultivation period.

Aphid sampling, DNA analysis and MLG assignment

One aphid population is defined as a sample collected on a single plot in a given field at a given year. Three weeks after plantation we sampled aphids every week. Each colony was sampled and tagged to avoid any re-sampling. We collected 34 to 123 apterous morphs per plot which corresponded either to singletons or to one individual from a colony produced by a single asexual founding mother (foundress). We sampled 2596 aphids among fields (Table 3). DNA from aphids was extracted using a 5% (w/v) Chelex resin solution as described by Fuller et al. (1999). DNA amplifications at 8 microsatellite loci specific of the *A. gossypii*

genome (Vanlerberghe-Masutti et al., 1999) were performed in two PCR reactions as described by Carletto et al. (2009). The allele size at each locus was identified by comparison with molecular size standard using the software GeneMapper v3.7 and a multilocus genotype (MLG) was subsequently assigned to each aphid.

Diversity analyses

Clonal diversity

The clonal diversity of *A. gossypii* population of N individuals was calculated using the Shannon–Wiener index: $H = -\Sigma_i\ p_i\ \ln\ p_i$ where pi represents the relative frequency of the i^{th} multilocus genotype. It was expressed by e^H as proposed by Vanoverbeke and De Meester (1997) to take into account the number of individuals in the sample and the evenness in relative abundance of the different MLGs. The values of e^H range from 1 (all individuals have the same MLG) to N (all individuals have a different MLG). To eliminate the sampling bias due to unbalanced number of data from the different plots, we create a balance set of 40 bootstrapped data by each data set: 40 values were randomly selected with replacement (*i.e.*, each sampled insect was returned to the data pool before another insect was sampled). Random sampling and calculation of H were repeated 40 times. Then we computed from the mean *H* the e^H which can vary from 1 to 40 for every plots.

Genetic structure analyses

The MLGs identified in the different populations were analyzed together using the Bayesian program STRUCTURE (Pritchard et al., 2000). We used the admixture model with a burn-in of 500000 and a subsequent Markov Chain of 250000 iterations. Ten replicate runs for each putative number of clusters *K* (*K* varying from 1 to 10) were compared to check for the consistency of estimates and to determine the likeliest number of genetic clusters.

Statistical analyses

Our aim was to assess the effect of the resistance loci on demography (AUC), diversity (e^H) and genetic structure of *A. gossypii* populations.

Because our plant populations had a homogenous genetic background, to assess the *Vat* effect we compared the group *Vat*/QTL to the group -/QTL within a single family, *i.e.* A *vs* C in the family I and D *vs* F in the family II. To assess the QTLs effect we compared the group

Vat/QTL to the group *Vat*/- within a single family, *i.e* A *vs* B in the family I and D *vs* E in the family II.

Statistically, fields were considered as raters of the resistance loci effect on three parameters: AUC for the effect on aphid density, e^H for the effect on aphid genetic diversity and the cluster assignation for the effect on aphid genetic structure. The agreeable (concordant) and non agreeable (discordant) rank of the AUC according to the melon combinations combining resistance loci was assessed using W, the Kendall coefficient of concordance (Kendall, 1955). W can be interpreted as the difference between the probability that the variables vary in the same direction and the probability that they vary in the opposite direction. On the same way, W were computed for e^H and the cluster assignation

Biological characterization of clones developing colonies

Beside the lab-maintained clones NM1-lab and C9/4-104 collected in Southeast France respectively in 1978 and 2004, we collected one clone (MLG CUC6) in SE in 2009 on the melon combination B, one clone (MLG GWD) in FWI in 2009 on the melon combination D and one clone (MLG CUCU3) in SW in 2009 on the melon combination F. Synchronous mass rearing of these clones was conducted on susceptible melon Védrantais at 24: 18°C under a 16h: 8h photoperiod. Five to seven-day-old aphids were used to infest plantlets at two-leaf stage for resistance biotests conducted at 24:18°C under a 16h: 8h photoperiod.

We investigated *in vivo* the effect of the *Vat* gene and minor QTLs using melons combining the *Vat* gene with different QTLs. To assess 'Acceptance of plant' of the different *A. gossypii* clones, 10 adults were deposited on a two-leaf plantlet. Two days later, the number of aphids remaining on the plantlet was recorded as Acceptance parameter. We tested 40 to 120 plants per resistance combination per clone; the normality of the Acceptance parameter was checked using a Shapiro-Wilk test and homogeneity of the variances with a Levene test. ANOVA and the test of Fisher multiple comparisons were performed to test the effect of the *Vat* gene and minor QTLs on a clone. Because plant populations had a homogenous genetic background, to assess the *Vat* effect we compared the group *Vat*/QTL to the group -/QTL within a single family, *i.e.* A *vs* C in the family I and D *vs* F in the family II. To assess the QTLs effect we compared the group *Vat*/QTL to the group *Vat*/- within a single family, *i.e.* A *vs* B in the family I and D *vs* E in the family II.

Table 3: Number of individuals, N, number of alleles, n, number of multilocus genotypes, G, number of multilocus genotypes with frequencies higher than 5%, g_1, with frequencies were lower than 5%, g_2, and number of single MLG, g_3, in total *A. gossypii* sample and according to the fields.

	fields	N individuals	n alleles	G	g_1 (>5%)	g_2 (<5%)	g_3 (single)
Total	All	2596	103	109	5	44	60
Family I ABC	Aramon 2008	244	36	20	5	7	8
	Aramon 2009	249	51	32	4	13	15
	Saint-Andiol 2009	161	67	30	5	10	15
	Petit-Canal 2009	345	28	2	1	1	0
Family II DEF	Saint-Andiol 2008	225	39	21	6	8	7
	Saint-Andiol 2009	211	59	28	4	11	13
	Moissac 2008	209	31	8	4	2	2
	Moissac 2009	267	51	33	5	9	19
	Petit-Canal 2008	346	31	3	2	1	0
	Petit-Canal 2009	339	29	4	2	0	2

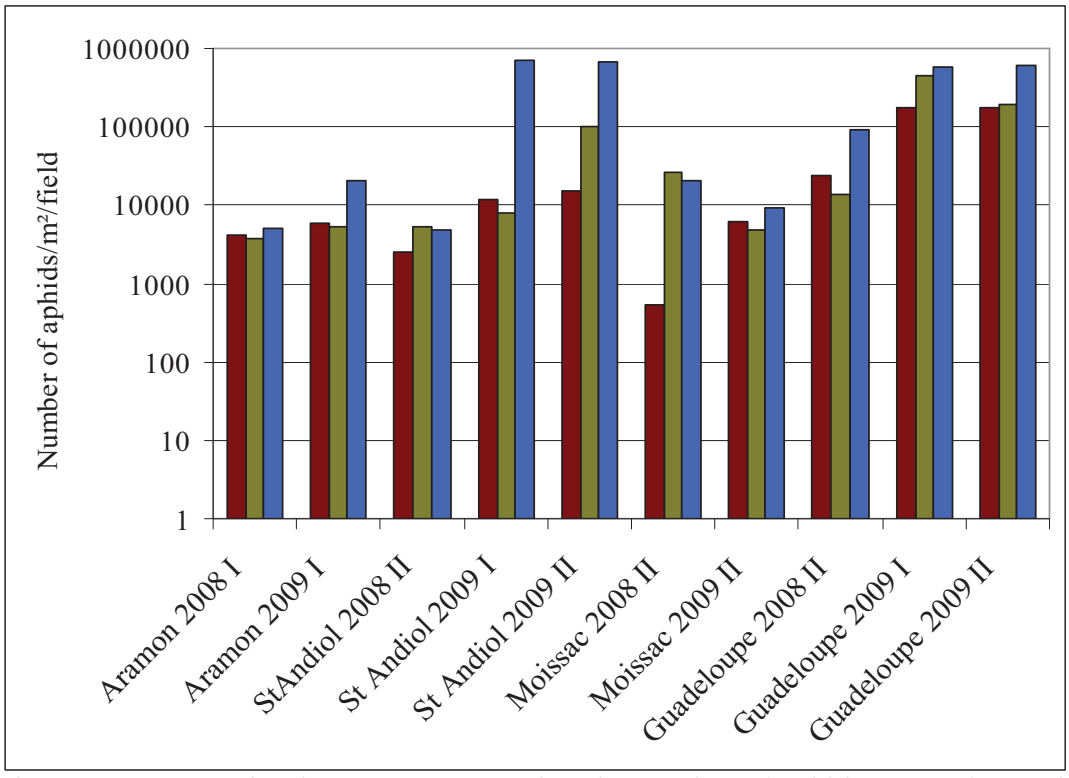


Figure 1: Areas under the curve representing the number of aphids per surface unit per fields according to the resistance combinations: *Vat*/QTL in red, *Vat*/- in green and -/QTL in blue.

RESULTS

Effects of melon resistances on *A. gossypii* density

We compared the aphid density on the resistance melons combining resistance loci grown in fields from three melon producing areas over two consecutive years. Ten fields were used to investigate the effect of the resistance loci on aphid density, five in SE, two in SW and three in the FWI (Figure 1). The *A. gossypii* densities were variable among fields, from 547 aphids/m² in Moissac in 2008 to 694000 aphids/m² in Saint-Andiol in 2009. To test the *Vat* gene effect we compared the aphid density on plants with resistance alleles at *Vat* and QTL *i.e.* belonging to the *Vat*/QTL melon combination with the aphid density on plants combining resistance alleles at QTL *i.e.* belonging to the -/QTL combination. Whatever the field, the highest aphid density was observed within the melon combination -/QTL without the resistant allele at the *Vat* gene. Statistically, the fields were considered as raters of the resistance loci effect. We observed a significant positive concordance ($\alpha = 0.02$) between the ranks of the aphid density on the *Vat*/QTL melon combination and the -/QTL melon ($W = 1$). In this case all aphid densities varied in the same direction *i.e.* density of aphids was inferior on the *Vat*/QTL melon combination than on the -/QTL melon combination. This reduction is particularly strong in Saint-Andiol in 2009 and in Moissac in 2008. On the other hand, to test the QTL effect we compared the aphid density on plants with resistance alleles at *Vat* and QTL *i.e.* belonging to *Vat*/QTL melon combination with the aphid density on plants with resistance allele only at the *Vat* locus *i.e.* belonging to *Vat*/- melon combination. We observed a null concordance ($W=0$, $\alpha=1$) between the ranks of the aphid density on the *Vat*/QTL combination and the aphid density on the *Vat*/- melon combination, signifying that the probability that the aphid densities varied in the same direction in all fields was significantly equal to the probability that they varied in the opposite direction.

In conclusion, this analysis revealed an effect of the *Vat* gene on *A. gossypii* density but no effect of the QTLs.

Effects of melon resistances on *A. gossypii* clonal diversity

We collected 2596 individual apterous aphids in 10 melon fields. We identified 102 MLGs and 103 alleles across all loci (Table 3).

Only five MLGs were observed in the FWI, of which two were detected both year, C6 (freq~0.5) and GWD (freq~90%). These 5 MLGs have never been observed in mainland France. Out of the other 97 MLGs, 19 were found both in SE and SW of which 9 were present

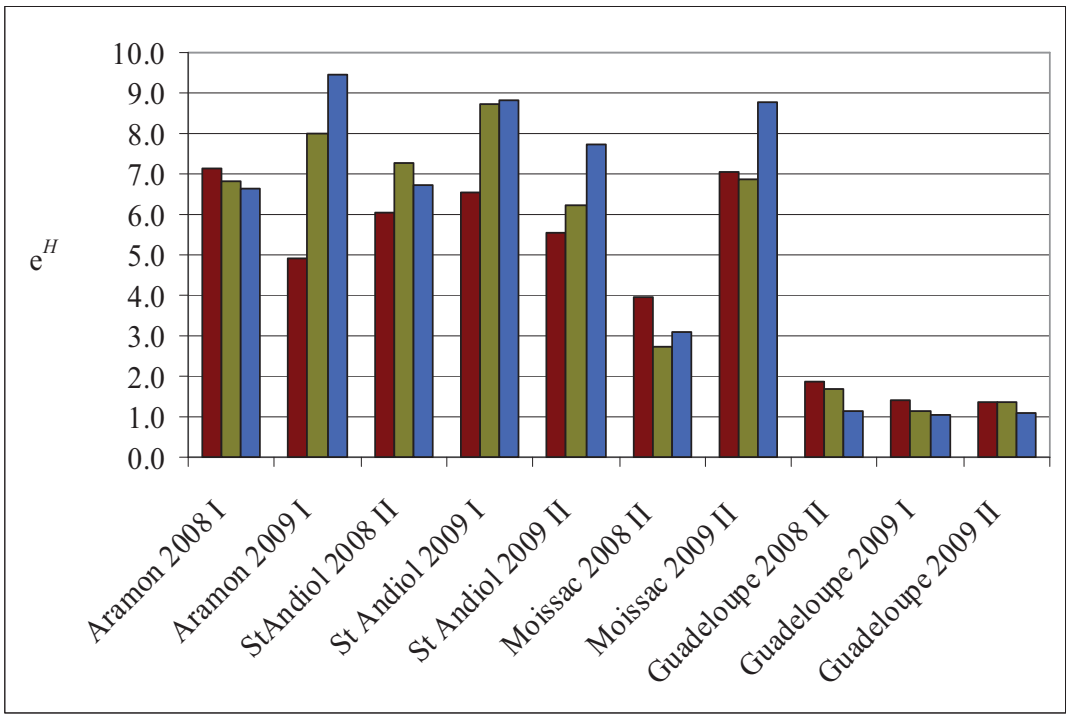


Figure 2: Clonal diversity (Shannon index which can vary from 1 to 40) of *A. gossypii* collected on 10 melon fields according to the resistance combinations: *Vat*/QTL in red, *Vat*/- in green and -/QTL in blue.

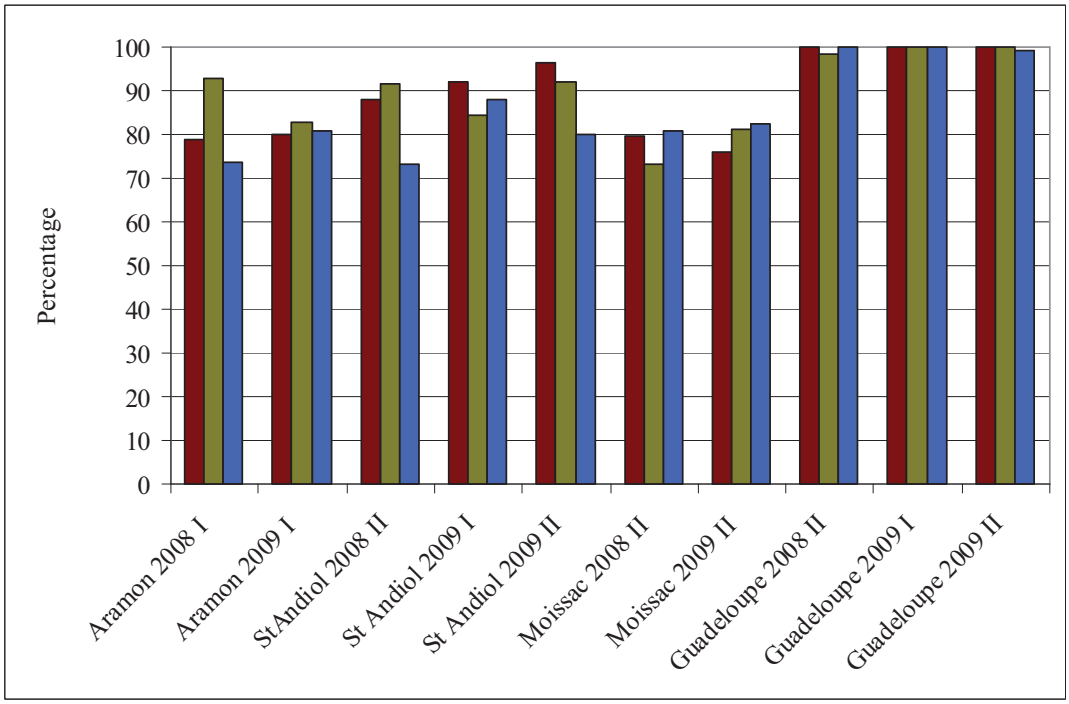


Figure 4: Percentage of *A. gossypii* individuals with a MLG assigned to the cucurbit cluster collected in 10 melon fields according to the resistance combinations "*Vat* +QTL" in red, *Vat*/- in green and -/QTL in blue.

each year, four of them at a frequency > 5% (NM1, CUC6, CUC3, CUC1) and five at a lower frequency (C9, C11, CUC5, GEL2 and GEL6). We observed 54 singletons MLGs *i.e.* one copy of MLG sampled only once.

To estimate the genetic diversity of *A. gossypii* samples on melon resistance combinations, we considered a clonal diversity index: the Shannon index e^H (Figure 2). We observed the lowest clonal diversity in the FWI (e^H <1.9). In SE and SW fields, the Shannon index varied from 2.7 to 9.4. To test the *Vat* gene effect on clonal diversity, we compared e^H on plants belonging to the *Vat*/QTL melon combination with e^H on plants belonging to the -/QTL melon combination. To test the QTL effect we compared the e^H on plants belonging to the *Vat*/QTL melon combination with the e^H on plants belonging to the *Vat*/- melon combination. In both cases, when we considered all the fields, we observed a null concordance (W=0, α =1) between the pairs *Vat*/QTL *vs* -/QTL and *Vat*/QTL *vs Vat*/-. However when we considered only the five SE fields we observed a positive concordance between both pairs *Vat*/QTL *vs* -/QTL and *Vat*/QTL *vs Vat*/- (W=0.36). This correlation was not significant (α =0.18) probably because of the low number of fields and a lack of power of the statistical test. Indeed, for 4 out of 5 fields, the e^H on the resistance combinations varied in the same direction. Only one repetition in SE would be necessary to make this correlation significant at the 10% significance level. We assume that both the resistant allele at the *Vat* locus and at the QTLs loci reduced the diversity. The resistance loci did probably not affect the genetic diversity of *A. gossypii* populations in areas SW and FWI.

Effects of melon resistances on *A. gossypii* genetic structure

We described the *A. gossypii* genetic structure of the 109 MLG**s** using a Bayesian structuring program (Figure 3). The likeliest numbers of genetic clusters K was two. Among the 109 MLGs discriminated in this study, 77 MLGs with 87.4% of the individuals were assigned to the cluster Y and 32 MLGs with 12.6% of individuals were assigned to the cluster Z. The MLGs assigned to the cluster Y were clustered with 7 MLGs (C4, C5, C6, C9, C10, C11, and C15) characteristic of aphids from the Cucurbitaceae race; therefore it will be called the cucurbit cluster. The cluster Z gathered NM1-like MLGs (NM1 MLG has been previously found only on Cucurbitaceae). Therefore, the cluster Z will be called the NM1 cluster.

We investigated the distribution of *A. gossypii* individuals on resistance combinations in relation with their MLG assignment to the two genetic clusters: cucurbit cluster or NM1 cluster (Figure 4). To test the *Vat* gene effect, we compared the percentage of individuals

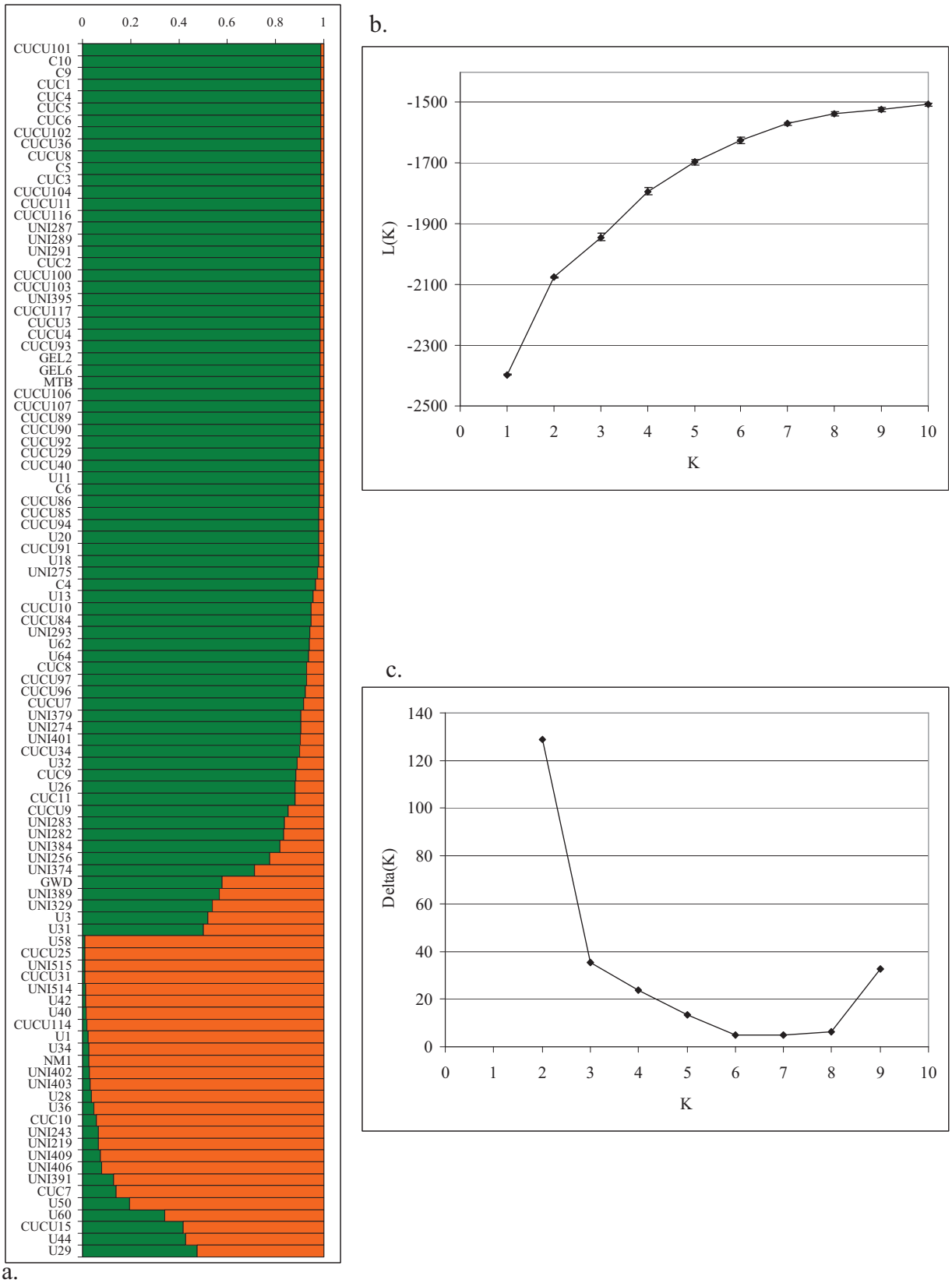


Figure 3: a. Result from Structure showing the estimated proportion of assignment of the 109 multilocus genotypes to K=2 clusters: the NM1cluster in orange and the cucurbit cluster in green. Mean value of: b. ln likelihood and c. delta K obtained for 10 simulations of each K when we analysed with Structure.

sampled on *Vat*/QTL melons to the percentage of individuals sampled on -/QTL melons, within the cucurbit cluster. To test the QTL effect, the same comparison was done between individuals sampled on *Vat*/QTL melons and individuals on *Vat*/- melons, within the cucurbit cluster. In both cases, we did not observe any concordance between the pairs *Vat*/QTL *vs* -/QTL (W=0.05, α =0.48) and *Vat*/QTL *vs Vat*/- (W=0, α =1). However when we considered only the five SE fields, we observed a positive correlation between pairs *Vat*/QTL *vs* -/QTL with a Kendall's concordance coefficient W of 0.36 (α =0.18). As previously, only one repetition in SE would be necessary to make this correlation significant at the 10% significance level. We assumed in this area a stronger effect of the *Vat* gene on individuals assigned to the NM1 cluster than on individuals assigned to the cucurbit cluster.

Selective effect of melon resistances on MLGs of *A. gossypii*

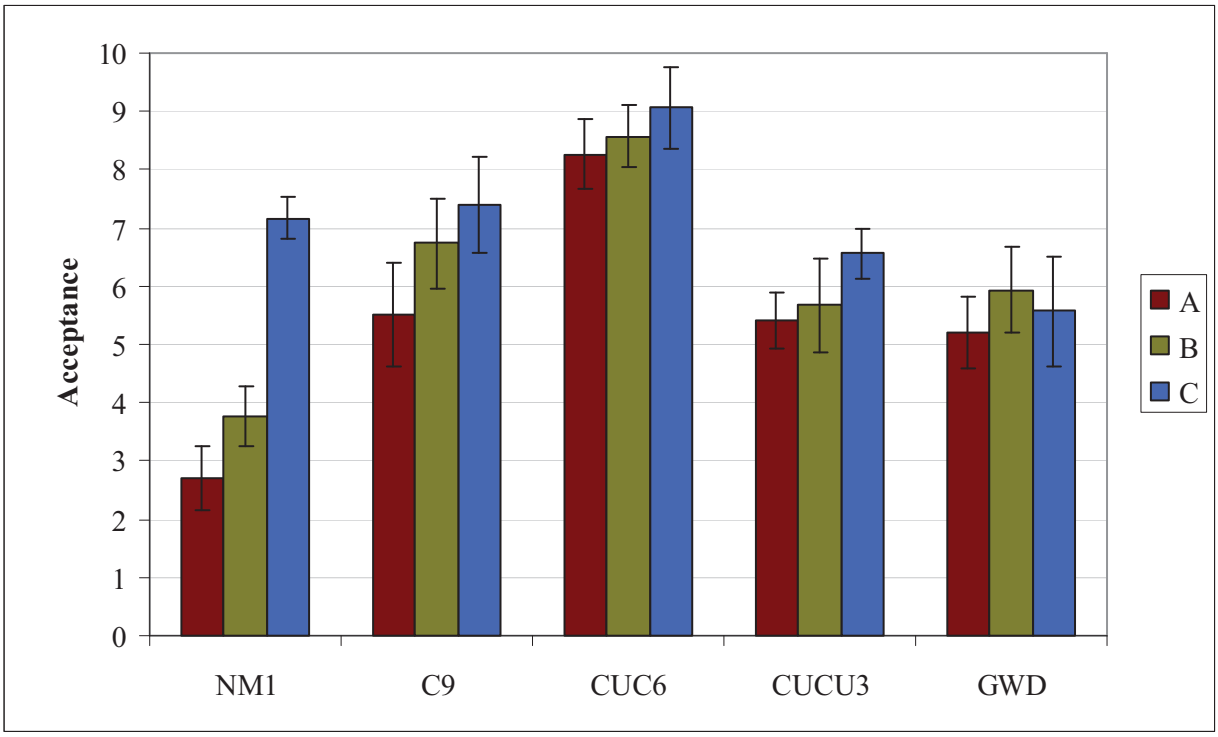
Among the ten fields we observed twice more colonies on plants without the *Vat* gene than on plants with the *Vat* gene (Table 4). This difference is particularly marked in SE with three colonies on *Vat*/QTL melons within five fields against 20 colonies on -/QTL melons. On the contrary the number of colonies was high on all melon combinations in the FWI.

Table 4: Multilocus genotypes of 125 aphid colonies and resistance profile of the plants on which colonies were sampled.

	Vat/QTL	*Vat*/-	-/QTL
NM1	0	0	5
C9	0	0	1
C11	0	0	1
CUC1	1	0	0
CUC6	2	3	12
MTB	1	3	2
CUCU3	0	0	2
CUCU91	0	0	2
C6	6	1	1
GWD	32	19	31
SE	3	3	20
SW	1	3	4
FWI	38	20	32
Total	42	26	57

Ten MLGs were identified among 125 colonies observed across the 10 fields. Five MLGs (C11, C9, CUCU3, CUCU91 and NM1) were identified only among colonies developed on -/QTL melons. These results suggested that those clones were not able to develop colony on

a.



b.

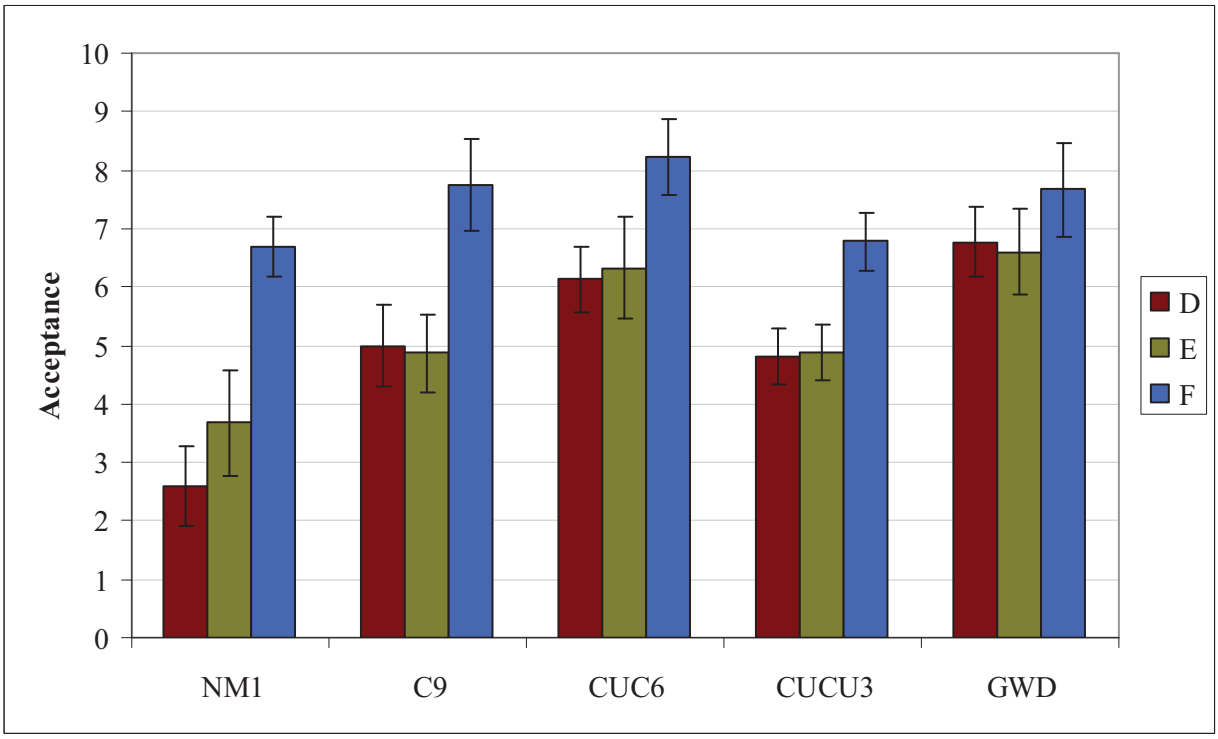


Figure 5: Acceptance estimated by the number of aphid on plantlets of melon combinations 48h after infestation by 10 aphids: A resistance combination "*Vat* +QTL" in red, B resistance combination *Vat*/- in green and C resistance combinations -/QTL in blue. a. Family I and b. family II.

melon combinations carrying the resistant allele at the *Vat* locus. Only 5 MLGs were identified among colonies developed on melons carrying the resistant allele at the *Vat* locus (*Vat*/QTL or *Vat*/-). Out of these 5 MLGs, one (CUC1) was identified only among colonies developed on *Vat*/QTL melons, while the four others were identified all melon combinations. Among them MTB and GWD had the same frequency across the melon combinations, CUC6 was more frequent on -/QTL and C6 was more frequent on *Vat*/QTL. In these cases, we did not demonstrate significant difference in the distribution of colonies between the resistance combinations. This may be due to a low power of the test because of the low number of field trial repetitions.

Effect of melon resistance on clonal performance of three clones of *A. gossypii*

The six melon combinations were evaluated for acceptance by five *A. gossypii* clones, two reference clones, NM1-lab and C9/4-104 and three clones collected in fields (CUC6, CUCU3, GWD) (Figure 5). In order to observe the effect of the *Vat* locus, we compared two couples of combinations with the same allelic composition except at the *Vat* locus, *i.e*. A *vs* C and D *vs* F. The resistant allele at the *Vat* locus reduced the acceptance by the clones observed in SE and SW: - 45 and -41% ($p<0.0001$) for the NM1 clone, - 19 and - 28% ($p< 0.0001$) for the C9 clone, - 12 and 20% ($p\sim0.0001$-0.06) for the CUCU3 clone and – 8 and - 21% ($p\sim0.0001$-0.02) for the CUC6 clone. The GWD clones, only observed in the FWI, was not affected by the *Vat* locus as the number of aphids still observed on plantlets 48h after infestation by 10 aphids was similar when comparing A *vs* C and D *vs* F ($p=0.39$ and $p=0.07$).

The CUC6 clone performed better than the other clones observed in SE and SW. On melon combinations -/QTL more than 8 aphids were still observed on plantlets 48h after infestation by 10 aphids (less than 8 aphids were still observed on plantlets 48h after infestation by 10 aphids with the 3 other clones). On melon combinations *Vat*/QTL more than 6 aphids were still observed on plantlets 48h after infestation by 10 aphids, (2-7) aphids were still observed on plantlets 48h after infestation by 10 aphids with the 3 other clones.

In order to observe the effect of the QTLs, we compared a couple of combinations with the same allelic composition except at QTLs, *i.e*. A *vs* B and D *vs* E. The QTLs present in the family I affected only the NM1 and C9 clones: - 10% ($p=0.04$) and - 12% ($p=0.005$) respectively. The QTLs present in the family II only affected the NM1 clone: - 11% and $p=0.03$.

DISCUSSION

The general objective of this paper was to approach the impact of selection by hosts differing from their resistance loci on population genetic structure and demography of *A. gossypii* at a local level. Very few studies have been conducted *in situ* on the effect of resistant cultivars on pathogen populations, and none effect of resistant cultivars on pest populations. The originality of our strategy was to test resistant plants with a homogenous genetic background in field trials.

Does the host resistance affect *A. gossypii* populations?

We showed that the *Vat* gene affects *A. gossypii* populations in field by reducing the development of the aphid populations. We observed this effect in three melon growing areas, SE, SW of France and the FWI. Up to now, the effect of the *Vat* gene has been only shown in laboratory tests. It reduces acceptance and biotic potential of the NM1–lab and 4-104 clones having a NM1 and C9 MLG respectively (Boissot et al., 2010) and some accessions carrying the *Vat* gene were shown resistant to clones collected in Spain or in EU (Klingler et al., 1998; Soria et al., 2003). Our laboratory study confirmed the strong effect of the *Vat* locus on the acceptance by the NM1-lab clone as well as by the 4-104 clone. Its effect appeared slightly stronger on the NM1-lab clone than on the others clones. Finally, the effect of the *Vat* gene appeared high in laboratory test on the NM1 clones and partial to the clones assigned to the cucurbit cluster. In our study none of the colonies with a MLG assigned to the NM1 cluster was observed on plants with the *Vat* gene. Similar results were obtained by Lombaert et al. (2009) in a melon crop survey in SE. Because the SE growing area is the only one among the three areas studied that has infesting populations (spring migrants) with more than 25% of the individuals with a MLG assigned to the NM1 cluster (Thomas et al., submitted), we assumed that the *Vat* gene has an effect on the clonal diversity and the structure of populations in SE: the *Vat* gene reduced the clonal diversity of the populations and the percentage of individuals with a MLG assigned to the NM1 cluster. Contrarily, very few spring migrants had a NM1 MLG in SW and the FWI and, we hypothesize that the *Vat* gene has no effect on the clonal diversity and the structure of aphid populations in these growing areas.

We didn't show an effect of the QTLs on demography, clonal diversity and structure of aphid populations. These QTLs were shown to reduce the acceptance of NM1 and C9 clones tested in the laboratory (Boissot et al., 2010) and confirmed in this study. On the other hand, no effect of QTLs was observed on acceptance in the lab of CUC6, CUCU3 and GWD.

Therefore because NM1 and C9 clones are not dominant in the field, the QTL did not affect demography, diversity and structure of aphid population *in situ*.

Consequences for the durability of *A. gossypii* resistance in melon

Our data are not sufficient to conclude about host adaptation in the long term in melon growing area in France. In SE France, the resistance has been deployed without breaking down for 20 years. Few biotypes adapted to the *Vat* gene were observed in SE (Lombaert et al., 2009), but no extension of resistance-breaking biotypes has been observed up to now in farmer crops. Meta-population dynamics, most notably gene flow and local extinction can swamp local selective effect (Burdon and Thrall, 1999; Kaltz and Shykoff, 2002). Gene flow is one of the important factors influencing the durability of resistance, *i.e.* the speed of population response to selection by resistant host (McDonald and Linde, 2002). Gene flow is dependant of dispersion ability and reproduction mode; it is a process in which particular alleles (genes) or individuals (genotypes) are exchanged among geographically separated populations. Aphids are known for their efficient dispersal, nevertheless a recent study in France showed that aphid populations are geographically structured, suggesting no exchange occurrence between populations (Thomas et al., submitted). This study also showed that sexual reproduction may occur within *A. gossypii* populations from France; but its fingerprints have been essentially detected within individuals assigned to genetic clusters not specialized on Cucurbitaceae (Thomas et al., submitted). The rare or absence of sexual reproduction in populations assigned to the cucurbit cluster may explain the durability of the *Vat* gene. Absence of sexual reproduction suggests that individuals assigned to the cucurbit cluster are anholocyclic (only asexual reproduction occurs) and overwinter parthenogenetically in glasshouses for example. Such overwintering may lead to heavy local extinction as hosts are very rare. In our study, the most aggressive clones, CUC6, was found the most frequently on the melon combinations. However, despite aphid colony growth among resistant crops, the most frequent biotypes are not necessarily the fittest ones over the whole season or over several seasons, but possibly during the winter conservation phase. Indeed, it is possible that highly aggressive biotypes, which will be favoured during the epidemic season, will be under a negative selection pressure during the winter. All the more, since CUC6 has been observed both years of the study it has proven his ability to overwinter. Biological tests suggested no cost to the resistance-breaking of CUC6 clones and the risk of extension of these clones appeared high.

Sexual reproduction does not occur within *A. gossypii* populations from the FWI (Thomas et al., submitted) and therefore gene flow is not an evolutionary driver of *A. gossypii* populations in this area. In the FWI, there is a tropical climate with very little difference between the hottest and the coldest months, Cucurbitaceae are grown over year and therefore no local extinction are expected during the cold season. In the FWI, the resistance has never been deployed up to now. However, the most frequent clones on the melon combinations, GWD, was shown very aggressive on all melon combinations (*Vat*/QTL, *Vat*/QTL and -/QTL) in the laboratory study. These results suggest local adaptation of this clone to the resistance without cost to the resistance-breaking or more probably, because the *Vat* gene has never been deployed in the FWI, pre-adaptation of local clones to the *Vat* gene.

Quantitative resistance is generally assumed to be more durable than gene-for-gene resistance (Johnson, 1984). Few studies demonstrated the efficiency of quantitative resistance to increases the durability of a major gene: experimental evidence for a field crop attacked by a fungal disease (Brun et al., 2010) and a greenhouse work with a virus disease (Palloix et al., 2009). Our study does not provide experimental evidence in crops that the combinations of qualitative effective major gene and quantitative polygenic resistance to *A. gossypii* improve control of the aphid populations. Our preliminary results did not suggest the reduction of resistant-breaking clone emergence when a major gene was combined to quantitative resistances. Breeders may be reluctant to put in extra effort required to combine polygenic quantitative resistance with an effective major gene resistance in new cultivars. The QTLs we tested affect the acceptance by *A. gossypii*, others QTLs affecting the biotic potential of *A. gossypii* were described in melon. These last QTLs could be combined with the *Vat* gene in order to obtain a cumulative effect on *A. gossypii* development. These combinations could be studied in similar experimental design developed in this work.

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CHAPITRE 4 : PERFORMANCE CLONALE ET CARACTERISATION DE CLONES CONTOURNANT LA RESISTANCE CONTROLEE PAR LE GENE *VAT*

Chapitre 4 : Performance clonale et caractérisation de clones contournant la résistance contrôlée par le gène *Vat*

Dans ce dernier chapitre, nous avons caractérisé des clones parmi les différents génotypes d'*A. gossypii* ayant développés des colonies dans des champs de melon, à l'aide de deux types de test : un test évaluant la virulence et un test évaluant l'acceptation de la plante par *A. gossypii* et son agressivité.

Pour justifier cette approche, l'article 4 présente la relation existant entre un génotype d'*A. gossypii,* et sa réponse biologique face à la diversité du melon. Nous avons vérifié que des clones prélevés sur une large échelle de temps et de lieu, et qui présentent un même génotype défini par les huit marqueurs microsatellites utilisés tout au long de cette thèse, ont un même phénotype sur l'espèce melon. Le melon présente une très large diversité morphologique et génétique (Kirkbride, 1993; Pitrat et al., 2000; Mliki et al., 2001; Decker-Walters et al., 2002; Dhillon et al., 2007). Nous avons donc choisi des accessions de différentes origines géographiques dans la plupart des types botaniques décrits. Cet article a été publié dans la revue Arthropod-Plant Interactions (DOI : 10.1007/s11829-011-9155-2).

Dans l'article 5, nous avons caractérisé différents génotypes d'*A. gossypii* contournants, c'est-à-dire capables de développer des colonies sur des plantes de melon résistants en plein champ. Nous avons utilisé les critères de virulence face au gène *Vat*, définis dans l'article 4, et nous avons caractérisé la fitness des clones. En utilisant le double phénotype contrôlé par le gène *Vat*, résistance au puceron et résistance au virus qu'il transmet, nous avons acquis des précisions sur les mécanismes de contournement de la résistance conférée par le gène majeur *Vat*. Cet article sera soumis à BMC Plant Biology.

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Article 4 : Association between *Aphis gossypii* genotype and phenotype on melon accessions

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ABSTRACT

Development of molecular markers has allowed the characterization of several host-aphid interactions. We investigated the usefulness of microsatellite markers to characterize the plant- resistance interaction in the model *Aphis gossypii*/*Cucumis melo*. Six aphid clones, collected in different localities and years and belonging to two multilocus genotypes (MLGs) based on eight microsatellite markers were phenotyped on a set of 33 melon accessions, some of them known to carry the *Vat* gene. Three parameters were used: acceptance of plant, ability to colonize the plant and resistance to virus when inoculated by aphids. Concordance and correlation analyses showed that aphid clones sharing a same MLG exhibited a very agreeable phenotype on the set of accessions for acceptance of plant and resistance to virus when inoculated by aphids. From host point of view, melon accessions were grouped in four clear categories, resistant to aphids of both MLGs, only resistant to the NM1 MLG, only resistant to the C9 MLG, susceptible to both MLGs and another group of unclear characteristics. The four categories revealed different patterns of virulence for NM1 and C9 MLGs, that are likely controlled by a single avirulence gene in accordance with a gene for gene interaction. In contrast, the ability to colonize the plant appeared slightly variable among clones sharing a same MLG. We hypothesize it is due to the putative polygenic control of this aphid trait. Because the phenotypic variability of *Aphis gossypii* matched the genetic variability revealed by eight microsatellite markers, these markers could be used to infer the frequency of biotypes in field experiments and help to elucidate the allele diversity of melon resistance genes.

KEYWORDS: Plant resistance, Biotype, *Vat* gene, virus resistance, aphid resistance

INTRODUCTION

In the past 15 years, DNA molecular markers have been developed to characterize the diversity of aphids. They have been widely used to resolve aphid species identity as well as to characterize the genotypes of individuals within populations, revealing patterns of genetic variation in relation with life cycle, ecology, demography, and climate (see Loxdale et al. (2007) for a review). Molecular markers allowed the characterization of host-plant associations in several aphid species (Frantz et al. 2006; Carletto et al. 2009) but very few studies have been reported that used molecular markers to investigate the interaction between plant resistance and aphids. The phenotypic variability of *Acyrtosiphon pisum* on resistant alfalfa cultivar was shown to match the genetic variability of *A. pisum* revealed by allozymes and RAPD markers (Bournoville et al. 2000). Although clones of *Diuraphis noxia* have overcome the *Dn4*-resistance in wheat, no genetic variability was revealed by seven SSR markers between avirulent and virulent populations (Shufran et al. 2009). Fifty putative SNPs between two biotypes of *Aphis glycines* ,virulent and avirulent on the Rap2 soybean resistance gene, were recently identified (Bai et al. 2010).

The cotton or melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a cosmopolitan species colonizing more than 600 host plants. It is a major pest of cucurbits and cotton (Blackman et al. 2007). Since the 1990s, molecular markers have been developed to characterize *A. gossypii* clones (Vanlerberghe-Masutti et al. 1999). They have allowed the description of a host race organization of the species (Carletto et al. 2009). Two hundred and eighty MLGs have been identified among individuals sampled on cucurbit crops; very few of them have been observed frequently and developed colonies (Thomas 2011). Among the clones adapted to cucurbit crops, 11 MLGs are closely related whereas one of them (named NM1) is distantly related to the other ones. Colonization of cucurbits by *A. gossypii* causes stunting and severe leaf curling that can result in plant death. Aphids also excrete honeydew on leaves and fruits, which serves as a growth medium for sooty mould. Moreover, they are efficient virus vectors and thus contribute to spread viral diseases. *A. gossypii* resistant melon accessions have been described since the 1970s, and a major gene, the *Vat* gene, and several QTLs controlling aphid resistance have been localized on the melon genome (Périn et al. 2002; Dogimont et al. 2004; Boissot et al. 2010). The *Vat* gene has the unique feature of conferring resistance to non-persistent viruses when vectored by *A. gossypii* (Lecoq et al. 1979; Lecoq et al. 1980). As early as 1971, Kishaba et al. reported the existence of biotypes of *A. gossypii* in US: they pointed out that melon resistance to the south-eastern biotype of

aphids was inefficient against aphids of the south-western biotype. In the same manner, MacCarter et al. (1974) observed that melon resistance to the US south-western biotype of aphids was inefficient against aphids of the south-eastern biotype. In Europe, Soria et al. (2000) observed a low resistance level to *A. gossypii* clones from Spain in melon accessions that exhibited a high level of resistance to French *A. gossypii* clones. In this context, the use of diverse aphid genotypes could be a new tool to better characterize the spectrum of efficiency of plant resistance genes. A major prerequisite is to know whether aphids sharing a same genotype (MLG) exhibit a same phenotype towards plant resistance.

In this study, we investigated the relationship between genotype and phenotype in *A. gossypii* species using six clones characterized by microsatellite markers on one hand and by biological tests on 33 melon accessions on the other hand. The six clones were selected into two MLGs observed on cucurbit crops: the MLG C9 is found worldwide and groups with 11 other MLGs, whereas the MLG NM1 is restricted to France, it is genetically very distant of the C9 MLG. The set of melon accessions was built to represent i/ the diversity of resistance sources known so far, ii/ a wide diversity of geographical origins (Asia, Africa, America and Europe) and iii/ the botanical groups (Pitrat et al. 2000). The phenotypic variability of the six clones was described using three parameters: acceptance of the plant, ability to colonize the plant and resistance to virus when inoculated by aphids.

MATERIAL AND METHODS

Aphid clones and rearing

Synchronous mass rearings of six *A. gossypii* clones collected on cucurbits were conducted on melon Védrantais at 24: 18°C under a 16h: 8h photoperiod. NM1-lab and 4-106 were collected in south-eastern France in 1978 and 2004, respectively; A8 was collected in western France in 2008. NM1-lab and 4-106 were genotyped using 15 microsatellite markers (Lombaert et al. 2009) and A8 was genotyped using 8 microsatellite markers. These three clones share a same MLG, NM1. C9-lab, 3-99 and 4-104 were collected in south-eastern France in 1988, 2003 and 2004, respectively. They were genotyped using 15 microsatellite markers and they share a same MLG, C9 (Lombaert et al. 2009). Five-seven day-old aphids, apterous adults, were used for biological tests.

Melon accessions

Thirty-three accessions of *Cucumis melo* Linné were used to reveal *A. gossypii* variability. They belong to 12 out of the 17 botanical groups described in the melon species

Table 1. Geographical origins and botanical groups of 33 melon accessions used to characterize *A. gossypii* clones. In bold, the lines with aphid resistance known to be conferred by the *Vat* locus.

Botanical groups	Asia	Africa	America	Europe
Chinensis	Chenggam			
	Miel Blanc			
	PI 161375			
	PI 255478			
	PI 266935			
Conomon	Shiro Uri Okayama			
Makuwa	Ginsen Makuwa			
	K 5442			
	Kanro Makuwa 1			
	Kanro Makuwa 2			
	Shiro Nashi Makuwa			
Momordica	MR-1			
	PI 414723			
Acidulus	**90625**	**PI 482420**		
Ameri	PI 164323			Persiski BR5
Agrestis	PI 164320			
	PI 164723			
Cantalupensis				**Charentais Vat R**
				Charentais T
				Margot
				Védrantais
Chito			Meloncillo	
Flexuosus		Fegouss 1		
Reticulatus		PI 224770	Smiths' Perfect	
		PI 234607		
Inodorus				**Anso AL77**
				Invernizo 8427
unknown	Durgapura Madhu	PI 282448		Escrito 8429

(Pitrat et al. 2000) and they originate from a wide diversity of geographical regions (Table 1). Some of them were chosen for their resistance to *A. gossypii*. The accession PI 161375, from which the *Vat* gene was cloned, was shown resistant to clones having either a NM1 or a C9 MLG (Boissot et al. 2010). It is also resistant to non-persistent viruses when vectored by *A. gossypii* clone NM1-lab (Lecoq et al. 1980). Margot and Charentais Vat R are Charentais lines with aphid resistance originating from PI 161375. The aphid resistance in PI 414723 were shown to be controlled by the *Vat* locus (Klingler et al. 2001) and the aphid resistance and the virus resistance when inoculated by *A. gossypii* in PI 482420 were suggested to be controlled by the *Vat* locus (Sarria et al. 2008). Anso AL77 and 90625 were suggested to carry other alleles of resistance at the same *Vat* locus (Pitrat et al. 1988; Boissot et al. 2008; Dogimont et al. 2008).

Biological characterization of *A. gossypii* clones

Plantlets were grown in insect-proof greenhouse until they develop one or two leaves and then used in two types of biological tests. The first one allows characterizing the acceptance of the plant by *A. gossypii* and its ability to colonize the plant; the second one allows characterizing resistance to virus when inoculated by the *A. gossypii* aphid clones.

To assess acceptance of the plant and ability to colonize the plant of *A. gossypii* clones, 10 apterous adults were deposited on a plantlet. The plantlets were then grown in a chamber at 24:18°C under a 16h: 8h photoperiod. To prevent aphids leaving a plantlets from reaching another one, plantlets were isolated by putting each of them on a petri dish placed in a water-filled tray. Three days later, the number of aphids remaining on the plantlet was recorded as the 'Acceptance' parameter. Seven days after infestation, the adults were counted and the density of nymphs was estimated using a 0-6 scale (0 no nymph, 1 1~20 nymphs, 2 20~50 nymphs and 3 more than ~50 nymphs observed on the infested leaf and on the rest of the plantlet; the two indices were added) . The 'Ability to Colonize' parameter at seven days was calculated as [density of nymphs + ln(number of adults + 0.001)]; ‘Ability to colonize’ gives a balance weight to the number of adults (used for infestation and produced after seven days) and the number of nymphs (produced after seven days) observed on a plantlet seven days after infestation. ‘Acceptance’ and ‘Ability to Colonize’ parameters were collected on 33 accessions for the clones NM1-lab and 4-104 and on 10 to 25 accessions for the others clones (see Online Resource for details). The parameters were collected on 8-30 plantlets per accession.

To assess resistance to virus when inoculated by aphids, we first checked the CMV susceptibility of the accessions, we mechanically inoculated ten plants of each accession with the CMV isolate I17F. All accessions exhibited clear symptoms of CMV 15 days after mechanical inoculation. Then, to assess resistance to virus when inoculated by aphids, aphids from mass rearings were transferred to CMV (isolate I17F)-infected leaves of melon 'Védrantais' for 10 min virus acquisition. Batches of 10 aphids were deposited on plantlets for inoculation. After 15 min, the aphids were removed, and plants were sprayed with aphicide, pyrimicarb (NM1 MLGs) or endosulfan (C9 MLGs), and placed into an insect proof glasshouse. The occurrence of infected plants was determined 20 days after inoculation by visual assessment of symptoms. 'Resistance to virus when inoculated by aphids' parameter was collected on 33 accessions for the clones NM1-lab and 4-104 and on 10 to 25 accessions for the others clones (see the Online Resource for details). Nine to 40 plantlets were tested per accession.

Data analysis

Numerous tests were conducted from 2004 to 2010 what induced specific variations (season effect on plant growth, aphid fitness ...). To be able to compare all the tests, two lines, Védrantais (a Charentais line susceptible to aphids) and Margot (a Charentais line with aphid resistance introgressed from PI 161375), were inoculated in all tests (8 to 15 plantlets). Data obtained on both lines were used to define references.

The first step was to determine a score for ‘Acceptance’, (A), for each clone on Védrantais and Margot. For that, we pooled the data of 'Acceptance' obtained in all the tests on Védrantais and Margot and we analyzed the clone and melon line effects using a non parametric multiple comparisons proposed by Dunn and described by Siegel et al. (1998). According to the group identified by the statistical analysis, scores were given to each pair 'aphid clone x Védrantais', $(A)_V$, and 'aphid clone x Margot', $(A)_M$. The second step was to determine a (A) score for each pair 'aphid clone x accession'. For that, for each clone, 'Acceptance' of each melon accession was compared to 'Acceptance' observed on Védrantais and Margot in the same test by non parametric multiple comparisons proposed by Dunn. From these analyses, for a given clone, each pair 'aphid clone x accession' was scored either as $(A)_V$ or as $(A)_M$ or intermediate between $(A)_V$ and $(A)_M$. The third step was to identify the significantly associated clones for (A) using the Kendall’s coefficient of concordance (W) (Kendall 1955). (W) is usually used for assessing agreement among raters; in this study, accessions are considered as raters of aphid clones. Kendall's W ranges from 0 (no agreement)

to 1 (complete agreement). The strength of the relationship between pairs of clones was assessed by the coefficient of correlation of Kendall ($-1<\tau<1$), that better takes into account ex-aequos than the coefficient of correlation of Spearman. The same procedure was applied to the parameter 'Ability to Colonize' and a score, (C), was given to each pair 'aphid clone x accession'.

We analyzed the clone and melon line (Margot and Védrantais) effects on resistance to CMV when aphid-transmitted from data obtained in all the tests using Monte Carlo exact test on χ^2 statistics (comparison of the proportion of infected and symptomless plantlets). According to this statistical analysis, (V) scores were given to each pair 'aphid clone x Védrantais', $(V)_V$, and 'aphid clone x Margot', $(V)_M$. For each clone, 'Resistance to Virus when inoculated by aphids' of each melon accession was compared to 'Resistance to Virus when inoculated by aphids' of Védrantais and Margot observed in the same test by Monte Carlo exact test on χ^2 statistics. Because there were two comparisons per accession, p was fixed at 0.025 for significant differences. From these analyses, each pair 'aphid clone x accession' was scored either as $(V)_V$ or as $(V)_M$ or intermediate between $(V)_V$ and $(V)_M$. The association between clones was assessed as described above for (A) and (C).

RESULTS

To compare six clones of *A. gossypii* we investigated three traits, the acceptance of the plant by *A. gossypii* and its ability to colonize the plant as well as resistance to virus when inoculated by *A. gossypii* aphids. These traits were measured on a set of melon accessions that were considered as raters of the aphid clones.

Characterisation of six *A. gossypii* clones on the melon lines Védrantais and Margot

Acceptance and ability to colonize plant: Twenty-one to 74 melon plants of Védrantais and Margot, which carries the *Vat* gene, were infested by aphids belonging to the clones 3-99, C9-lab, 4-106 and A8 and more than 150 plants of both lines were infested by aphids belonging to the clones NM1-lab and 4-104. All clones did significantly less accept Margot than Védrantais (Figure 1). Seven to nine aphids stayed on Védrantais plantlets 72 hours after infestation; two to seven aphids stayed on Margot plantlets. The ‘Ability to Colonize’ was calculated from the number of nymphs and adults on a plantlet seven days after infestation; it ranged from -0.3 to 7.8 (Figure 2). All clones did significantly less colonize Margot than Védrantais.

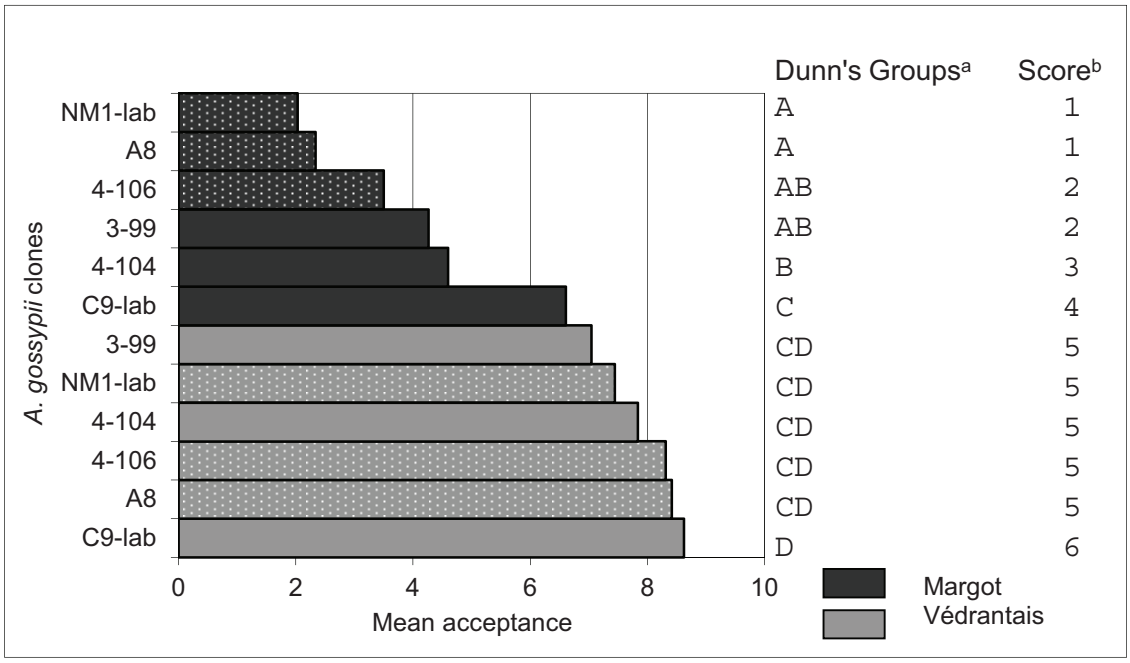

Figure 1: Acceptance by six clones of *A. gossypii* of two melon lines, Védrantais (in grey) and Margot (in black). Acceptance = number of aphids on the plant 72 hours after infestation by 10 aphids, spotted NM1 MLG, plain C9 MLG. [a] significant difference (p = 0.05, non parametric test (Dunn procedure) corrected for the Bonferroni effect), [b] scores assigned to each clone on Védrantais and Margot.

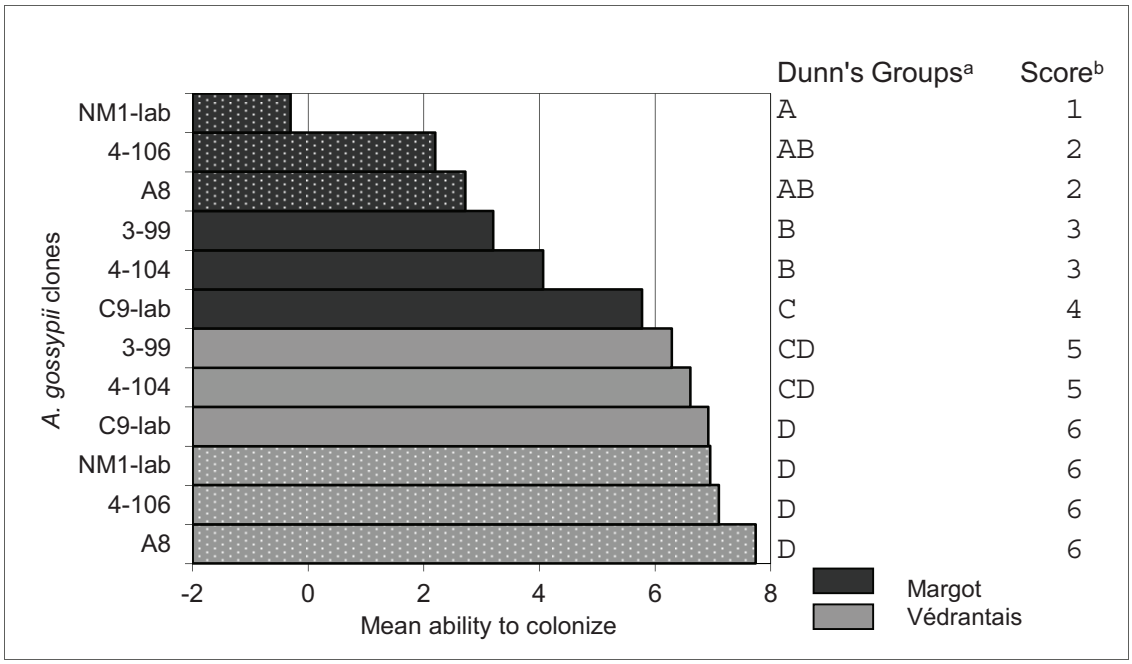

Figure 2: Ability of six clones of *A. gossypii* to colonize two melon lines, Védrantais (in grey) and Margot (in black) observed seven days after infestation by 10 aphids, spotted NM1 MLG, plain C9 MLG [a] significant difference (p = 0.05, non parametric test (Dunn procedure) corrected for the Bonferroni effect), [b] scores assigned to each clone on Védrantais and Margot.

Resistance to virus when inoculated by aphids: CMV was inoculated to 30 to 50 melon plants of Védrantais and Margot using the clones 3-99, C9-lab, 4-106 and A8 as vectors and to more than 100 plants of both lines using the clones NM1-lab and 4-104 as vectors. The percentage of plants exhibiting CMV symptoms varied from 73 to 94% on Védrantais; the percentage of plants exhibiting CMV symptoms was less than 10% on Margot whatever the aphid clone (Figure 3). The results indicate that this trait may be considered as qualitative.

Scores assignation to each combination 'clone x reference melon line': To be able to compare the parameters for several clones on a large set of melon accessions, the parameters observed for each clone on both lines Védrantais and Margot, inoculated in each test, were used as references. A (A) score for 'Acceptance' and a (C) score for 'Ability to Colonize' was assigned to each combination 'clone x reference melon line' on the basis of non-parametric analyses (Figures 1 and 2). For (A) as well as for (C), the scores on Védrantais were from 1 to 4 and the scores on Margot from 5 to 6. The χ^2 tests conducted to compare the proportion of plants exhibiting CMV symptoms for each pairs of 'vectoring clone x reference melon line' revealed a highly significant effect of the melon line (Védrantais *vs* Margot) and no significant effect of the clone (Figure 3). Thus, the (V) score assigned to the combinations was 1with Védrantais and was 5 with Margot, whatever the clone. For the three parameters (A), (C) and (V) the scores established for each combination 'clone x reference melon line' were used as references to establish the scores of the three parameters (A), (C) and (V) for each combination 'clone x accession'.

A. gossypii clones characterization on a set of 33 melon accessions

Ten to 33 melon accessions were infested with the six clones of *A. gossypii* and were given (A), (C) and (V) scores (see Online Resource for details). The Kendall's coefficients of concordance (W) were used to identify groups of clones exhibiting the same pattern of phenotypes on a set of melon accessions. For (A), (C) and (V) scores, (W) was calculated including the six clones or including only the clones sharing a same MLG (Table 2). (W) of (A), (C) and (V) scores are weak (<0.75) when considering the six clones. Therefore, when considering the six clones, the ranking of the accessions was not agreeable for (A), (C) and (V), i.e. the clones did not have a concordant pattern of phenotypes on the set of accessions for 'Acceptance', 'Ability to Colonize' and, 'Resistance to Virus when inoculated by aphids'. In contrast, (W) of (A) and (V) scores are remarkably high (over 0.95) when considering the clones sharing either a C9 MLG or a NM1 MLG and the mean r of (A) and (V) are over 0.9.

Table 2. Kendall's coefficients of concordance, W, of phenotypic traits of *A. gossypii* clones measured on n melon accessions (=raters). Three clones share a C9 MLG and three clones share a NM1 MLG. r = mean coefficient of correlation.

Traits		All Clones	Clones having a C9 MLG	Clones having a NM1 MLG
Acceptance	W	0,74	0,95	0,95
	r	0,68	0,93	0,92
	n	8	14	15
Ability to colonize	W	0,74	0,72	0,92
	r	0,69	0,58	0,88
	n	9	13	14
Resistance to virus when inoculated by aphids	W	0,68	0,96	0,95
	r	0,61	0,95	0,93
	n	8	12	14

Therefore, when considering the clones having a same MLG, either C9 or NM1, the ranking of the accessions was agreeable for (A) and (V), i.e. the accessions the most accepted by a clone of a given MLG were also the accessions the most accepted by other clones sharing the same MLG; on the same way, accessions exhibiting virus symptoms after inoculation by a clone of a given MLG exhibited also virus symptoms after inoculation by the other clones sharing the same MLG. (W) of (C) is weak (< 0.75) when considering the clones sharing a C9 MLG. (W) of (C) is high (0.92) when considering the clones sharing a NM1 MLG but the mean r of (C) is under 0.9 for these clones. The clones did not have a highly concordant pattern of phenotypes for (C) on the set of accessions whatever the group of clones we considered.

Concordance analyses showed that aphid clones sharing a same MLG exhibited a very agreeable (A) and (V) on a set of melon accessions. We measured the strength of the relationship of pairs of clones for both traits using the Kendall's coefficient of correlation τ. For 'Acceptance', τ are from 0.8 to 0.9 between (A) obtained with the clones sharing a same MLG (Table 3). For 'Resistance to Virus when inoculated by aphids', τ are from 0.9 to 1 between (V) obtained with the clones sharing a same MLG (Table 4). In contrast, for (A) and (V) τ were weak when considering clones not sharing a same MLG. Therefore, correlation analyses confirmed the conclusion of the concordance analyses. For both traits, 'Acceptance' and 'Resistance to Virus when inoculated by aphids', the pattern of clones having on one hand a C9 MLG and on the other hand a NM1 MLG can be illustrated by the phenotypes of the 4-104 and NM1-lab clones that were used to infest 33 melon lines. When considering 'Resistance to Virus when inoculated by aphids', a trait that may be considered as qualitative, the clone 4-104 exhibited (V) = 5, on 12 out of the 33 melon lines , the clone NM1-lab

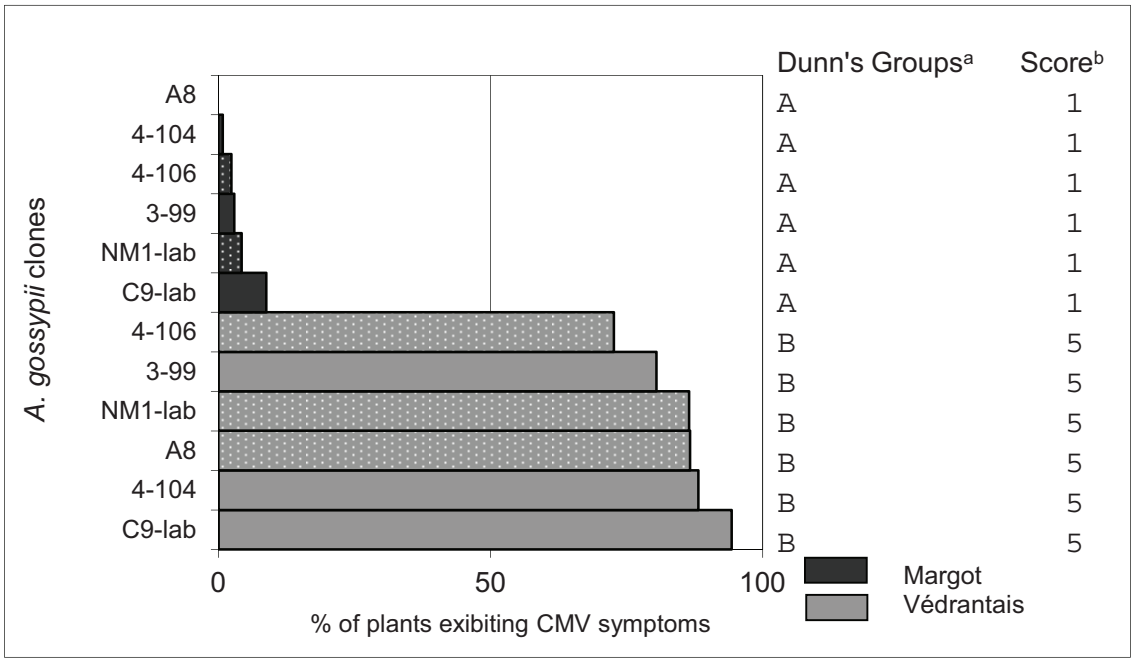

Figure 3: Percentage of melon plants, Védrantais (in grey) and Margot (in black), with CMV symptoms after inoculation by six clones of *A. gossypii* aphids, spotted NM1 MLG, plain C9 MLG. [a] significant difference (p = 0.05) based pairwise comparison by χ^2 statistics corrected for the Bonferroni effect (p_{cor}=0.003), [b] scores assigned to each clone on Védrantais and Margot.

Table 3. Coefficients of correlation between acceptance scores (A) after infestation of melon accessions by six clones of *A. gossypii*: 3-99, 4-104 and C9-lab (C9 MLG), 4-106, A8 and NM1-lab (NM1 MLG). Right part: Kendall coefficient (in bold when p<0.01); left part: number of accessions observed for each pair of clones.

	3-99	4-104	C9-lab	4-106	A8	Nm1-lab
3-99		**0.9**	**0.9**	0.7	0.6	0.7
4-104	10		**0.8**	0.5	0.2	0.3
C9-lab	10	19		0.4	0.3	0.5
4-106	10	19	13		**0.9**	**0.8**
A8	9	21	15	15		**0.9**
Nm1-lab	10	33	19	19	21	

Table 4. Coefficients of correlation between scores after CMV inoculation of melon accessions (V) by six clones of *A. gossypii*: C9-lab, 3-99 and 4-104 (C9 MLG), NM1-lab, 4-106 and A8 (NM1 MLG). Right part: Kendall coefficient (in bold when p<0.01); left part: number of accessions observed for each pair of clones.

	3-99	4-104	C9-lab	4-106	A8	NM1-lab
3-99		**0.9**	**0.9**	**0.7**	**0.5**	**0.7**
4-104	28		**0.9**	**0.5**	**0.6**	**0.5**
C9-lab	12	15		0.6	**0.6**	0.3
4-106	23	25	**9**		**1.0**	**0.9**
A8	16	19	**13**	14		**0.9**
NM1-lab	28	33	**15**	25	**19**	

exhibited (V) = 5 on seven out of the 33 melon lines (see Online Resource for details). Finally, according to (V), accessions could be ordered into five classes. The first one comprised 17 accessions resistant to both clones, such as Margot; the second one comprised a single accession (Smiths' Perfect) which is only resistant to the 4-104 clone; the third one comprised six accessions only resistant to the NM1-lab clone, such as 90625; the fourth one comprised seven accessions susceptible to both clones such as Védrantais. Two accessions were not clearly assigned to one of these four classes. (A) scores observed in the first class (resistant to both clones) varied from 1 to 4 except for Miel blanc and PI 164323 ((A) from 4 to 6). (A) scores observed for the clones sharing a C9 MLG appeared higher than (A) scores observed for the clones sharing a NM1 MLG. (A) scores observed in the third class (resistant to the clones having a NM1 MLG, susceptible to the clones having a C9 MLG) were 1 or 2 with the clones having a NM1 MLG and 3 to 6 with the clones having a C9 MLG.

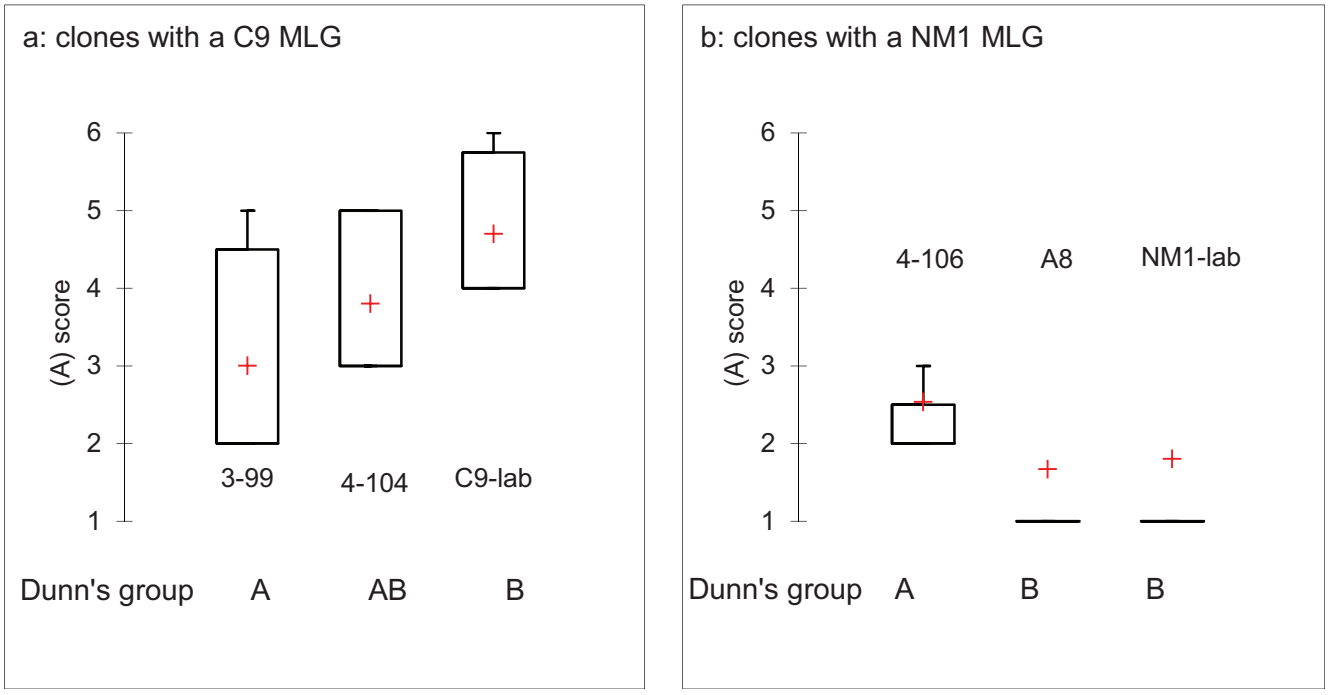

Figure 4: Box plots (75% of the data in the box) of acceptance (A) scores obtained for pairs 'melon accessions x *A. gossypii* clones' a: 10 melon accessions x 3 clones having a C9 MLG (3-99, 4-109 and C9-lab) and b: 15 melon accessions x 3 clones having a NM1 MLG (4-106, A8 and NM1-lab). Cross: Mean score; Dunn's group: significant difference (p = 0.05, non parametric test (Dunn procedure) corrected for the Bonferroni effect).

In order to investigate the aggressiveness of clones within a MLG, we took into account (A) obtained on a sub-set of accessions infested by the three clones sharing a same MLG (Figure 4). Among the three clones sharing a C9 MLG, the C9-lab clone exhibited a

significantly higher aggressiveness than the 3-99 clone, while the 4-104 clone showed an intermediate aggressiveness between the C9-lab and 3-99 clones. Among the three clones sharing a NM1 MLG, the 4-106 clone exhibited a significantly higher aggressiveness than the A8 and NM1-lab clones.

DISCUSSION

To investigate the aphid phenotypic diversity between MLGs and within a same MLG, our strategy was to offer a large variability of hosts (33 melon accessions, some known for their resistance to *A. gossypii*) to several clones having two genetically distant MLGs, the MLG C9 found worldwide and, the MLG NM1 restricted to France. These two MLGs have been regularly sampled on cucurbit crops in France. For each MLG, we used clones collected on a large scale of time (from 1988 to 2008) and in different growing areas in Southern France. Melon accessions were considered as raters of the aphid clones.

Concordance and correlation analyses showed that clones collected in different localities and different years, but sharing a same MLG, had a same pattern of responses on a large set of melon accessions for their 'Acceptance' and 'Resistance to Virus when inoculated by aphids'. On the same way, aphid resistance pattern observed on several melon accessions infested with clones from Sudan and France, sharing a C9 MLG, were consistent (Boissot et al. 2008) and as well as virus resistance pattern (unpublished data). Clones with distinct MLGs did not exhibit a concordant pattern of response for (A) and (V). Therefore, the eight microsatellite markers used to designed MLGs, appeared enough to differentiate two groups of *A. gossypii* facing melon accessions. These two groups can be considered as biotypes. This result could be puzzling, but has to be considered in accordance with the genetic structure of *A. gossypii* species. Lineages observed on cucurbits in southern France have a parthenogenetic reproduction (Thomas 2011) and therefore no gene flow may occur between lineages.

Several accessions from the set (Margot, Charentais Vat R, PI 161375, Anso AL77, 90625, PI 414723 and PI 482420) studied here are known to carry the *Vat* allele or another allele at the same locus. Based on the widely assumed knowledge on the NBS-LRR proteins, Dogimont et al. (2010) speculated that the *Vat*-mediated resistance results from the recognition (direct or indirect) between the product of the *Vat* gene and that of an avirulence gene of *A. gossypii*, which activates a cascade of plant responses, leading on one hand to the inhibition of plant virus infection and on the other hand to non acceptance of plants by the

aphids and to a low ability to colonize the plant. In our study, acceptance and resistance to viruses when inoculated by aphids appeared dependent on the aphid MLG, in accordance with the gene-for-gene relationship between aphid and melon genotypes predicted by the molecular feature of the *Vat* gene.

For 'Ability to Colonize', we only observed concordance of phenotypes for the clones sharing a NM1 MLG. This trait, that is a result of acceptance, daily fecundity, time for pre-adult development and, mortality of clones, exhibited a slight variability among the clones sharing a C9 MLG. Consistently, a variable phenotype for daily fecundity was reported for 24 clones sharing a C9 MLG and sampled in south-eastern France (Lombaert et al. 2009). To explain the variation among clones sharing a same phenotype, we favour the hypothesis that the ability to colonize plant, resulting from several life traits (including acceptance), is likely controlled by several aphid genes. If true, a few of these genes may have different allelic forms, due to mutations, in a same MLG defined by eight to 15 microsatellite markers and this genetic variability could lead to a slight variability in 'Ability to Colonize'. Aphids harbour several secondary symbiotic bacteria and there is growing evidence that they may modulate various important adaptive traits of their host such as host plant use (Frantz et al. 2009). However, secondary symbiotic bacteria are unlikely involved in phenotypic variability within a MLG in *A. gossypii* in southeastern France as individuals of *A. gossypii* sampled in this area were shown free of three secondary symbionts (Carletto et al. 2008). A third hypothesis concerns the epigenetic control of phenotypic plasticity. Phenotypic plasticity is defined as the development of different phenotypes from a single genotype depending of environment. Unfortunately, the mechanisms that underlie the development of alternative phenotypes are still largely unknown for many systems (see Aubin-Horth et al. (2009) for review).

Biotypes are strains of insects with inherited differences in their ability to use a host species. They are convenient and useful designations for applied problems in agricultural pest management especially involving plant resistance. Typically in agriculture, whenever a resistant crop variety is no longer resistant, a new pest biotype is considered. Among aphid pests, this terminology has been largely used to describe relationship between plant resistance and pest. Most aphid resistances were shown biotype-specific, such as resistance to the greenbug, *Shizaphis graminum*, conferred by the *Gb* genes, to the European raspberry aphid, *Amphorophora idaei*, conferred by the *Ag* genes, to the soybean aphid, *Aphis glycines*, conferred by the *Rag* genes, and to the woolly apple aphid, conferred by the *Er* genes

(Berzonsky et al. 2003; Sargent et al. 2007; Bus et al. 2008; Kim et al. 2008). The genotypic diversity of some of these aphid species has been recently studied but few relationships have been established between biotypes and genotypes. As previously observed for the interaction between *A. pisum* and alfalfa (Bournoville et al. 2000), we showed that biotype and genotype have a strong convergence in *A. gossypii* species in the frame of the interaction with the melon species. Therefore, the genotypic data, that can be obtained on a large number of individuals could be used to infer the frequency of biotypes in field experiments, and to study the effects of resistance gene deployment on the diversity of aphid populations.

In melon, the *Vat* gene confers resistance to the aphid *A. gossypii* and it has also the unique feature of conferring resistance to non-persistent viruses when inoculated by *A. gossypii* (Lecoq et al. 1979; Lecoq et al. 1980). We observed this double phenotype in accessions from all geographical origins and all botanical groups; nevertheless the double phenotype observed in the cantalupensis accessions from Europe is due to resistance introgression from the accession PI 161375. The accession PI 161375, from which the *Vat* gene was cloned, was shown resistant to four clones having either a NM1 or a C9 MLG (Boissot et al. 2008 ; Boissot et al. 2010). By enlarging the set of clones, the *Vat* allele from PI 161375 appeared to confer resistance to *A. gossypii* clones with a NM1 and C9 MLGs. By the same way, the allele at the *Vat* locus carried by the accession 90625 appeared to confer resistance only to *A. gossypii* clones with a NM1 MLG. In this study, we also showed that the accession Smiths' Perfect was only resistant to clones with a C9 MLG. In these three accessions, the acceptance and colonization by an aphid clone is associated with resistance to viruses when inoculated by this aphid clone. In this study, we identified several accessions, which did not exhibit this double phenotype. Thus, the accession PI 164323 exhibited resistance to virus when inoculated by aphids but was rather susceptible to aphids. This particular phenotype was observed on PI 164323 with both MLGs, it was also observed on Miel Blanc with aphids having a C9 MLG. On the contrary, Escrito exhibited susceptibility to virus when inoculated by aphids having a NM1 MLG but was rather susceptible to aphids having a NM1 MLG. Altogether, these results strongly suggest that other alleles at the *Vat* locus that those already known and/or new loci of resistance exist within the melon diversity.

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Article 5 : Molecular and biological characterization of *Aphis gossypii* clones reveals different types of adaptation to a resistance mediated by a NBS-LRR gene

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INTRODUCTION

Among the 4000 aphid species known in the world, about 100 species have successfully exploited the agricultural environment. It is their ability to rapidly exploit the ephemeral habitats constituting most agricultural landscapes that makes aphids serious pest (Dedryver et al., 2010). This ability results from their short generation time, their potential for rapid growth, their dispersal capacities, all characteritics conferring to aphid a high potential of adaptation. Aphids have been mainly fought by aphicide sprays and the deployment of plant resistances. Screening germplasm for aphid resistance led to the discovery of resistant accessions in several crop species against various aphid species. However, sources of aphid resistance are limited and usually scarce (Dogimont et al., 2010). More often, aphid resistance was identified in unimproved landraces, in wild accessions or even in related species, thus requiring a long breeding process to introduce the resistance into cultivated varieties. The relative high number of resistant accessions discovered in certain species should not mask the fact that aphid resistance usually relies on a small number of genes with limited numbers of resistance alleles. In most cases, the genetic studies have still to be done to determine if the selected accessions are sources of novel resistance genes. Up to now, only two aphid resistance genes have been isolated and both genes, *Vat* and *Mi-1*, are members of the nucleotide-binding-site and leucine-rich repeat region (NBS-LRR) family of resistance genes (Dogimont et al., 2010), to which belong the majority of the genes, isolated to date, conferring resistance to bacteria, viruses, fungi and nematodes (Dangl and Jones, 2001). The

identification of signalling cascades activated by aphid resistance genes is still at its infancy, but the data available to date indicate they partially overlap with those activated by pathogens (Kaloshian and Walling, 2005). Evidence of biotypes in some aphid species suggests potential for breakdown of major gene resistance to aphids. To study aphid adaptation to plant resistance we choose the *Aphis gossypii*/*Cucumis melo* interaction, the only model with a aphid resistance gene characterized and a well-known deployment of resistance.

The cotton or melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a cosmopolitan species colonizing more than 600 host plants. It is a major pest of many crops, including Cucurbitaceae and cotton (Blackman and Eastop, 2007). Since the end of the 1990s, molecular markers have been developed to characterize *A. gossypii* clones (Vanlerberghe-Masutti et al., 1999). They have allowed the description of a host race organization of the species (Carletto et al., 2009); 18 major multilocus genotypes (MLGs) have been identified among clones colonizing melon crops (Carletto et al., 2009; Thomas, 2011). Colonization of Cucurbitaceae by aphids causes stunting and severe leaf curling and can result in plant death. Aphids may also excrete honeydew on the leaves and the fruits, which serves as a growth medium for sooty mould. Moreover, they are efficient virus vectors and thus contribute to spread diseases (Ng and Perry, 2004). Intensive use of insecticides to control aphids in Cucurbitaceae culture has led to emergence of resistant clones of *A. gossypii* (Delorme et al., 1997). *A. gossypii* resistant melon accessions have been largely described since the 1970s, and a major gene, *Vat*, and QTLs controlling the resistance have been localized on melon genome (Dogimont et al., 2004; Boissot et al., 2010). The *Vat* gene belongs to the NBS-LRR family (Pauquet et al., 2004). The resistance genes belonging to this family are widely assumed to be involved in a specific recognition of the bioaggressor and in the activation of plant defence responses (Dangl and Jones, 2001). The *Vat* gene has the unique feature of also conferring resistance to non-persistent viruses when vectored by *A. gossypii i.e.* the melon plants carrying the resistance *Vat* allele are susceptible to viruses when inoculated mechanically (Lecoq et al., 1979; Lecoq et al., 1980). Dogimont et al. (2010) speculate that the *Vat*-mediated resistance results from the recognition (direct or indirect) between the product of the *Vat* gene and that of the avirulence gene of *A. gossypii*, which activates a cascade of plant responses leading on one hand to the inhibition of infection by the virus and on the other hand to no acceptance of plants by the aphids and a low ability to colonize the plant. *A. gossypii* clones sharing a same MLG were shown exhibiting a similar response for acceptance of plant

and were shown triggering similar virus-resistance when inoculated by aphids on a set of melon accessions (Thomas et al., accepted).

In this study we investigated adaptation of *A. gossypii* clones to resistance conferred by different alleles of resistance at the *Vat* locus in melon, in relation with the position of the clones in the genetic structure of *A. gossypii* species. To this aim, on one hand, we chose five clones of *A. gossypii* collected in different melon producing areas where the resistance has been deployed or not and, on the other hand, we selected melon accessions carrying different alleles at the *Vat* locus. Our strategy was to molecularly characterize the aphid clones and to characterize the interaction aphid/melon by taking advantage of the double phenotype mediated by the *Vat* resistance gene: aphid resistance and resistance to viruses when inoculated by *A. gossypii*. The fitness of the clones was evaluated and in conjunction with their adaptation to the resistance conferred by the *Vat* gene.

MATERIAL AND METHODS

Aphid clones collection and rearing

Aphid clones were collected in France and in the French West Indies. In these areas, all varieties cultivated are of Charentais type, in which aphid resistance has been introgressed since several years (50 to 80% of new melon cultivars are resistant to aphids). The resistance has been deployed for about 15 years in southern France but as far as we know, no commercial variety of melon with resistance to aphids have been deployed in Guadeloupe. Even if different resistance alleles are available in the melon species, the one from the PI 161375 line has been the most used or the only used in improved Charentais varieties for different reasons i/ it was the first identified in 1979 (Lecoq et al., 1979) and ii/ this allele was quickly introgressed in a Charentais line so-called Margot (commercialized in 1987) that has been largely used to create new Charentais type varieties resistant to aphids. All clones were collected on Cucurbitaceae. The NM1-lab, 4-104 and DRB clones were collected in South eastern France in 1978, 2004 and 2009 respectively; the CEFEL clone was collected in South western France in 2009 and the Gwada clone was collected in the French West Indies (Guadeloupe) in 2009. The 4-104 clone was collected on a commercial melon variety resistant to aphids, the DRB and Gwada clones were collected in field trials on melon plants carrying the resistance allele from the line PI 161375 at the *Vat* locus.

Synchronous mass rearings of the five *A. gossypii* clones were conducted on melon 'Védrantais' at 24: 18°C under a 16h: 8h light-night photoperiod. In these conditions

A. gossypii has a viviparous reproduction via parthenogenesis. Five to seven day-old aphids were used to infest plantlets at two-leaf stage for resistance biotests.

Molecular characterization of *A. gossypii* clones

DNA from aphids was extracted using a 5% (w/v) Chelex resin solution as described in (Fuller et al., 1999). DNA amplifications at eight microsatellite loci specific of the *A. gossypii* genome (Vanlerberghe-Masutti et al., 1999) were performed in two PCR reactions as described in Carletto et al. (2009). The allele size at each locus was identified by comparison with molecular size standard using the software GeneMapper v3.7 and a multilocus genotype (MLG) was subsequently attributed to each aphid.

We assigned each MLG to a cluster using the Bayesian program STRUCTURE (Pritchard et al., 2000). We used the admixture model with a burn-in of 500,000 and a subsequent Markov Chain of 250,000 iterations. Ten replicate runs for each value of the putative number of clusters, K (varying from 1 to 10), were compared to check the consistency of estimates and to determine the likeliest number of genetic clusters. In our assignment procedure, we refer to the four host races that have been unambiguously identified (Cucurbitaceae, cotton, Solanum and pepper) analysing 44 MLGs (Carletto et al., 2009). To this reference set, we added 7 MLGs (Table S1) identified among colonies collected on melon crops (article 3).

Plant material

Four melon lines were used to reveal the phenotype of the five *A. gossypii* clones. The Védrantais and Margot French lines belong to the botanical group of 'cantalupensis', the Anso AL 77 Spanish line belongs to the 'inodorus' group. The 90625 line belongs to the 'acidulus' group, it originates from India. Védrantais is a line susceptible to *A. gossypii*. Margot, Anso AL 77 and 90625 are resistant to *A. gossypii* (Boissot et al., 2008). Margot is a Charentais line in which resistance to *A. gossypii* was introgressed from PI 161375 (a Korean line) and Margot is closely related to Védrantais. The Anso AL 77 line was assumed to carry a resistant allele at the *Vat* locus based on heredity studies (Pitrat and Lecoq, 1988). The 90625 line was shown to carry an allele at the *Vat* locus different from the one present in the PI 161375 line based on molecular studies (Dogimont et al., 2008).

In addition, four susceptible melon lines were used to evaluate the fitness of the aphid clones, they were chosen for their belonging to the same botanical groups of melon than the four lines used to reveal the phenotypic variability of *A. gossypii*. The Charentais T line belongs to the

'cantalupensis' group, the WMR 29 and Bola de Oro 2 lines belong to the 'inodorus' group, Charentais T and Bola de Oro 2 lines originate from Europe, the WMR 29 originate from the USA. The AM 39 line belongs to the 'acidulus' it originates from India.
Plantlets were grown in insect-proof greenhouse until they developed one or two leaves and used for biological tests.

Biological characterization of *A. gossypii* clones

To characterize the phenotypes of the aphid clones according to the resistance allele at the *Vat* locus, we conducted two types of test. The first one allowed characterizing the acceptance of the plant by *A. gossypii*, and the second one allowed characterizing the plant resistance to Cucumber Mosaic Virus (CMV, isolate I17F) when inoculated by *A. gossypii*. To assess the acceptance of plant, 10 adult aphids were deposited on plantlets which were transferred in a climatic room at 24: 18°C under a 16h: 8h (light-night) photoperiod. Three days later, the number of aphids remaining on the plantlet was recorded as the 'Acceptance' parameter. The five aphid clones were used in independent tests, each test comprised the susceptible Védrantais line, the resistant Margot line and one of the resistant lines, Anso AL 77 or 90625. The Védrantais, Margot, Anso AL 77 and 90625 lines were previously shown to be susceptible to the CMV when the virus is mechanically inoculated; they exhibit very clear mosaic symptoms or necrosis. To assess the plant resistance to CMV when inoculated by aphids, aphids from mass rearings were transferred to CMV-infected leaves of melon 'Védrantais' for 10 min virus acquisition. Batches of 10 aphids were deposited on plantlets for inoculation. After 15 min, the aphids were removed, and the plantlets sprayed with an aphicide, pyrimicarb (NM1-lab clone) or endosulfan (4-104, DRB, CEFEL and Gwada clones), and placed into an insect proof glasshouse. The occurrence of infected plants was determined 20 days after inoculation by visual assessment of symptoms. The five aphid clones were used in independent tests, each test comprised the susceptible Védrantais line, the resistant Margot line and one of the other resistant lines Anso AL 77 or 90625.
To characterize the fitness of aphid clones, we estimated their ability to colonize plants of four aphid-susceptible lines, Charentais T, WMR29, Bola de Oro 2 and AM 39. Ten adult aphids were deposited on plantlets which were transferred in a climatic room at 24:18°C under a 16h: 8h (light-night) photoperiod. Three days later, the number of aphids remaining on the plantlet was recorded. Only the plantlets on which 7-9 adults remained were kept, in order to avoid the acceptance effect and the resulting experimental bias on the following step

of the experiment. Seven days after infestation, the adults were counted and the density of nymphs was estimated using a 0-6 scale (0 no nymph, 1 1~20 nymphs, 2 20~50 nymphs and 3 more than ~50 nymphs observed on the infested leaf and on the rest of the plantlet; the two indices were added) . The 'Fitness' parameter was calculated as [nymph score + ln(number of adults + 0.001)] that gives a balance weight to the number of adults (used for infestation and produced) and the number of nymphs (produced) observed on a plantlet seven days after infestation. The five aphid clones were used in a same test for each susceptible line. The four melon lines were tested in independent tests.

Data analysis

To investigate the acceptance of plant and the resistance to CMV when inoculated by *A. gossypii* aphids on four melon lines, each resistant line (Margot, 90625 and Anso AL 77) was compared, for both traits, to the susceptible line Védrantais. 'Acceptance' trait was compared using a non parametric test (Mann & Whitney) and resistance to CMV trait was compared using a Monte Carlo exact test on χ^2 statistics.

To investigated the fitness of the clones, for each susceptible line, Charentais T, WMR 29, Bola de Oro 2 and AM 39, the fitness of the five *A. gossypii* clones was compared using Kruskal and Wallis non parametric test and Steel-Dwass-Critchlow-Fligner's multiple comparisons described by Hollander and Wolfe (1999).

RESULTS

Assignation of MLGs to *A. gossypii* host races

Genotyping of the five aphid clones with eight SSR markers allowed identifying five MLGs, of which two already described, NM1 for the NM1-lab clone and C9 for the 4-104 clone (see Table S1). The three other clones had a MLG already characterized in the lab, CUC6 for the DRB clone, CUCU3 for the CEFEL clone and GWD for the Gwada clone. The allelic composition at each locus is given in the Table S1. The name of MLGs will be used in the following parts of the manuscript.

To place the five MLGs within *A. gossypii* diversity, they were analysed with MLGs previously assigned to host races and known for their ability to develop colonies on crops. Using a Bayesian structuring program, the likeliest numbers of genetic clusters K were three (Fig. 1). Within the first cluster, the C9, CUC6, CUCU3 and GWD MLGs were assigned with 14 MLGs characteristic of the cucurbit cluster, *i.e.* a cluster grouping MLGs identified in

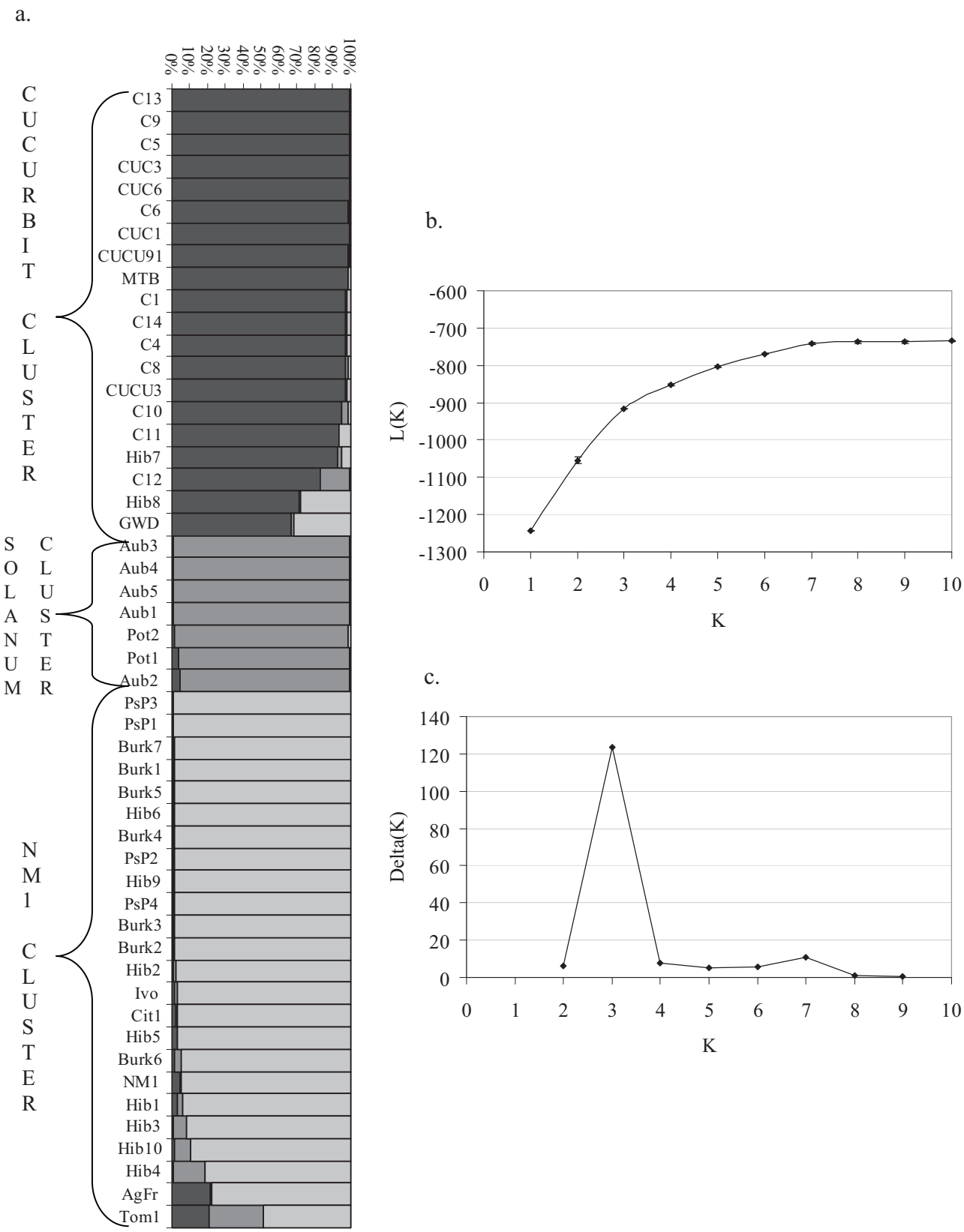


Figure 1: a. Results from Structure showing the estimated proportion of assignment of a. the 51 multilocus genotypes to K=3: the NM1cluster in grey, the *Solanum* cluster in medium grey and the cucurbit cluster in dark grey. Mean value of: b. ln likelihood and c. delta K obtained for 10 simulations of each K when we analysed with Structure.

individuals collected on Cucurbits. The C9, CUC6 and CUCU3 MLGs had a high assignation to this cluster (>97%) while the GWD MLG was assigned only at 67% to this cluster. The NM1 MLG shares several alleles with the C9, CUC6, CUCU3 and GWD MLGs, it has only half of its allelic combinations common with the allelic combinations observed for MLGs assigned to the Cucurbit cluster. The NM1 MLG belongs to the third genetic cluster. It groups MLGs previously described from aphids collected on pepper, cotton, citrus, strawberry and *Hibiscus spp.*. The NM1 MLG was assigned at 95% to this cluster. The second cluster assigned MLGs previously described from aphids collected on *Solanum spp.*

Phenotypic characterisation of five *A. gossypii* clones on resistant melon lines

To investigate the adaptation of *A. gossypii* clones to the *Vat*-mediated resistance, we characterized the resistance of melon plants to CMV when inoculated by aphids and the acceptance of melon plants by aphids, on three melon lines carrying a resistant allele at the *Vat* locus: Margot, 90625, and Anso AL 77.

First, we characterized resistance to CMV and acceptance on Védrantais and Margot lines. To assess resistance to CMV when inoculated by 10 aphids, CMV was inoculated to 35 to 40 melon plants of both lines, using the clones CUC6, CUCU3 and GWD as vector, and CMV was inoculated to more than 100 melon plants of both lines using the C9 and NM1 clones as vector. The percentage of plants exhibiting CMV symptoms was over 85% on the Védrantais line and less than 5% on the Margot line whatever the aphid clones used as vectors (Fig. 2a). Védrantais and Margot exhibited a binary response for resistance to CMV when inoculated by aphids. To assess acceptance, 34 to 45 melon plants of Védrantais and Margot were infested by 10 aphids belonging to the CUC6, CUCU3 and GWD clones and more than 70 plants of both lines were infested by 10 aphids belonging to the NM1 and C9 clones. All clones accepted significantly less the Margot line than the Védrantais line (Fig. 2b). Nevertheless, this reduction of acceptance was strong with the clones NM1 (-67%), C9 (-54%) and CUCU3 (-40%) and weak with the clones GWD (-21%) and CUC6 (-15%). Therefore, the Margot line, that carries the *Vat* allele from PI 161375, exhibited resistance to CMV when inoculated by all the clones and this resistance was qualitative (yes/no). This resistance to CMV was not triggered when Margot plants were inoculated mechanically. The Margot line exhibited high resistance to acceptance by the NM1, CUCU3 and C9 clones but appeared rather susceptible to acceptance by the GWD and CUC6 clones.

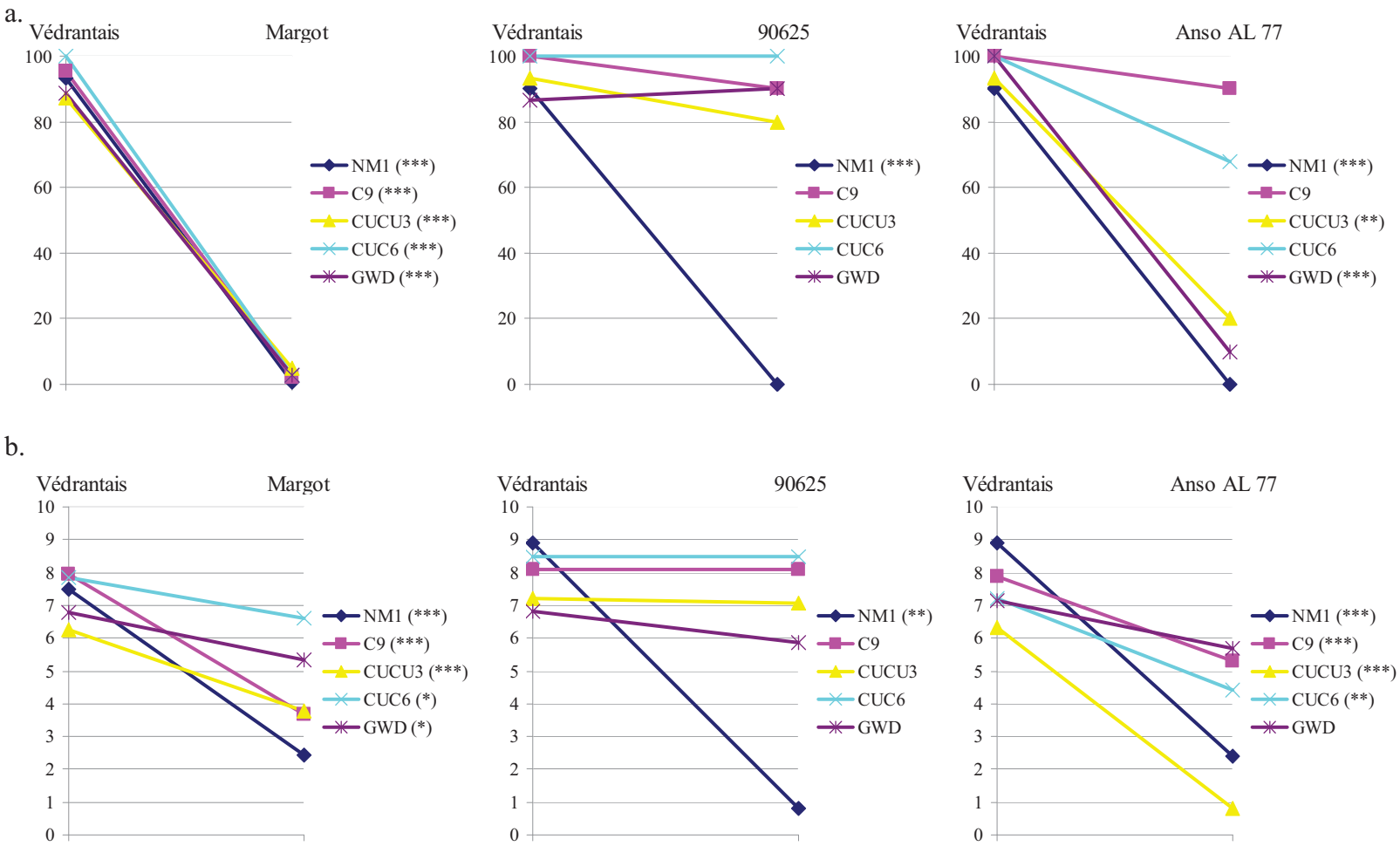
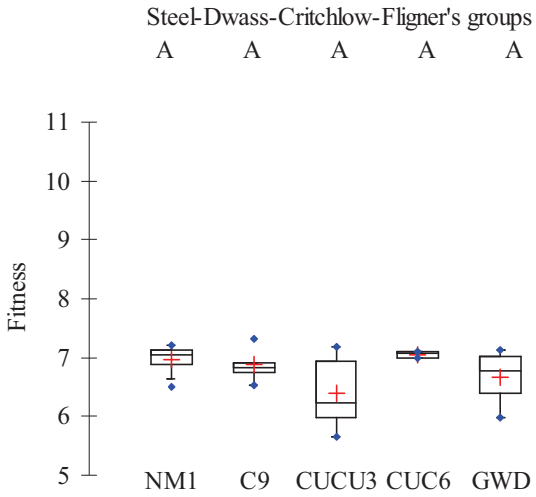


Figure 2: a. Characterization of five clones of *A. gossypii* on three melon lines (Védrantais used as reference) a. Percentage of CMV- infected plants after inoculation by aphids. b. Acceptance (number of aphids on the plant 72 hours after infestation by 10 aphids). *** $p<0.0001$, ** $p<0.001$, * $p<0.01$ based pairwise comparison by χ^2 statistics for a. and non parametric test (Mann & Whitney) for b.

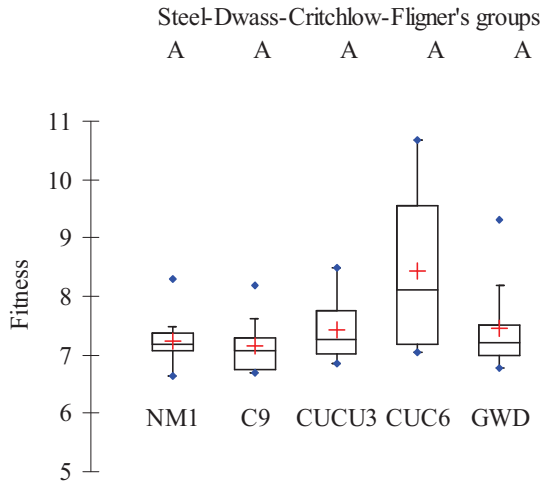
Second, we characterized resistance to CMV and acceptance on the Védrantais and 90625 lines. To assess resistance to CMV when inoculated by 10 aphids, CMV was inoculated to 10 to 20 melon plants of both lines, using each clone as vector. The percentage of plants exhibiting CMV symptoms was over 85% on the Védrantais line whatever the aphid clones used as vectors (Fig. 2b). None of the plants exhibited CMV symptoms on the 90625 line when the virus was inoculated by the NM1 clone and more than 80% of plants exhibited CMV symptoms when the virus was inoculated by the other clones. Védrantais and 90625 exhibited a binary response for resistance to CMV when inoculated by aphids. To assess acceptance, 10 to 30 melon plants of Védrantais and 90625 were infested by 10 aphids belonging to each clone. The NM1 clone did significantly less accept the 90625 line than the Védrantais line (-91%, Fig. 2b). Acceptance of the clones C9, CUCU3, CUC6 and GWD was similar on the 90625 and Védrantais lines. The 90625 line, already known for carrying another allele at the *Vat* locus than the allele present in the PI 161375 line, a binary response for both traits: resistant to the NM1 clone for acceptance and resistant to CMV when inoculated by the NM1 aphid clone (resistance to CMV was not triggered when 90625 plants were inoculated mechanically), but susceptible to the four other clones for both traits.

Third, we characterized resistance to CMV and acceptance on Védrantais and Anso AL 77. To assess resistance to CMV when inoculated by 10 aphids, CMV was inoculated to 10 to 25 melon plants of both lines with each clone. The percentage of plants exhibiting CMV symptoms was over 90% on the Védrantais line whatever the aphid clones used as vectors (Fig. 2c). The percentage of plants exhibiting CMV symptoms on the Anso AL 77 line was over 90% when the virus was inoculated by the C9 clone and 68% when the virus was inoculated by the CUC6 clone. The percentage of plants exhibiting CMV symptoms was less than 20% on the Anso AL 77 line when the virus was inoculated by the NM1, CUCU3 and GWD clones. To assess acceptance, 10 to 35 melon plants of both lines were infested by 10 aphids belonging to each clone. The clone GWD accepted the Anso AL 77 line as well as the Védrantais line. The other clones significantly less accepted the Anso AL 77 line than the Védrantais line (Fig. 2c). Nevertheless, this reduction was stronger with the clones CUCU3 (-87%,) and NM1 (-73%) than with the clones CUC6 (-39%) and C9 (-33%). The Anso AL 77 line was observed resistant to the NM1 and CUCU3 clones for acceptance and resistance to CMV when inoculated by aphid. This line exhibited resistance to CMV when inoculated by the GWD clone but susceptibility for ‘Acceptance’ by this clone. resistance to CMV was not triggered when Anso AL 77 plants were inoculated mechanically**.** This line exhibited

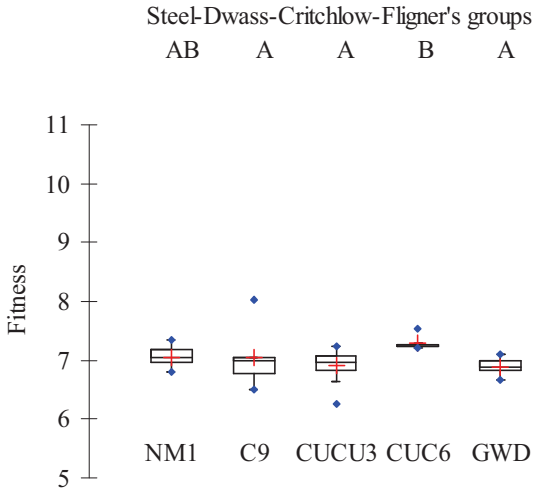
a. Charentais T (p=0.021)



c. Bola de Oro 2 (p=0.254)



b. WMR 29 (p=0.006)



d. AM 39 (p=0.769)

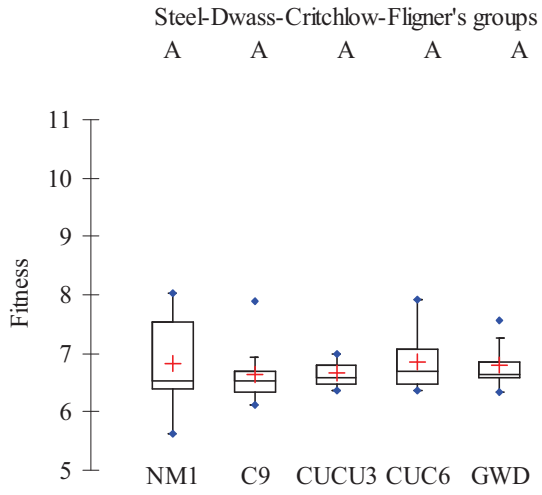


Figure 3: Fitness of the five clones of *A. gossypii* on four susceptible lines of melon, Fitness = nymph score + ln(number of adults +0.001) observed seven days after infestation by 7-9 aphids (p exact (Monte Carlo) from Kruskal and Wallis test). Box plots (75% of the data in the box) and groups according to Steel-Dwass-Critchlow-Fligner's procedure (non parametric test corrected for the Bonferroni effect). The test on the Bola de Oro 2 line had a weak power (the power to discriminate fitness from 6.5 to 7.5 was 46 %). The test of on the AM 39 line had a satisfying power (to discriminate fitness from 6.2 to 7.2 the power was 76 %).

susceptibility to virus when inoculated by the C9 and GWD clones and resistance to acceptance by these two clones.

Characterisation of five *A. gossypii* clones on susceptible melon lines

'Fitness' of the NM1, C9, CUC6, CUCU3 and GWD clones was measured on four susceptible lines Charentais T, WMR 29, Bola de Oro 2 and AM 39 (Fig. 3). Fitness was considered as a measure of the biotic potential independently of aphid behaviour, the test was built to avoid the individual differences in acceptance and experiment bias to reach a high reproducibility of the measure. If all adults are dead after seven days without have produced any progenies, the fitness parameter measure will be -7. If all adults are alive after seven days without have produced any progenies, the fitness parameter measure will be the Fitness parameter measure will be about 2, if they have produced some progenies the Fitness parameter measure will be about 3. We observed a mean fitness from 6.0 to 7.3 on Charentais T plants (cantalupensis group), 6.3 to 8.0 on WMR 29 plants and from 6.6 to 10.7 on Bola de Oro 2 plants (inodorus group) and, from 5.6 to 8.0 on AM39 plants (acidulus group). On the WMR 29 line, the fitnesses of the C9, CUCU3 and GWD clones was significantly lower than that of the CUC6 clone. The fitness of the NM1 clone was intermediate. On the Charentais T, Bola de Oro 2 and AM 39 lines, we did not observed any significant fitness differences between the five aphid clones although the CUC6 clone displayed a better fitness than the other clones.

DISCUSSION

We showed in the Margot line resistance to CMV when inoculated by all the clones, in the 90625 line resistance to CMV only when inoculated by the NM1 clone and in the Anso AL 77 line resistance to CMV when inoculated by the NM1, CUCU3 and GWD clones. We showed in the Margot line resistance to acceptance by the NM1, CUCU3 and C9 clones in Margot, in the 90625 line resistance to acceptance only by the NM1 clone and in the Anso AL 77 line resistance to acceptance by the NM1 and CUCU3 clones.

The *Vat* locus has alleles with different specificities

Considering both phenotypes of plant resistance (low acceptance by aphids and virus-resistance when inoculated by aphids) and five aphid clones, we revealed in this study three contrasted patterns of resistance in three melon lines carrying a resistance allele at the *Vat*

locus, Margot, 90625 and Anso AL 77, suggesting that the three lines carry different alleles at the *Vat* locus. Heredity studies, carried in the lab with a NM1 clone, showed that the resistance to the NM1 aphid clone was controlled by the *Vat* locus in the 90625 line (unpublished data) and molecular studies showed that the resistance allele at the *Vat* locus is different in the 90625 line and in the PI 161375 line, the resistance donor for the Margot line. In the 90625 line, a *Vat* analogue was identified which has a similar size as *Vat* in the PI 161375 line (5802 bp) (Dogimont et al., 2008). These two alleles encode proteins that share 92.3 % identity. Concerning the Anso AL 77 line, heredity studies carried with a NM1 clone showed that the aphid resistance to the NM1 clone was controlled by the *Vat* locus or a very near locus (Pitrat and Lecoq, 1988). The pattern of virus resistance on the Anso AL 77 line, different of the patterns observed on the Margot and 90625 lines, suggested that the Anso AL 77 line carries an allele different of those carried by the Margot and 90625 lines.

Therefore, the *Vat* allele present in the Margot line (*i.e.* the *Vat* allele present in the PI 161375 line) mediated resistance to CMV when inoculated by a wide spectrum of *A. gossypii* clones belonging to different genetic clusters, specialised or not on Cucurbits. The *Vat* allele present in the Anso AL 77 line mediated resistance to CMV when inoculated by clones belonging to different genetic clusters (specialised or not on Cucurbits); two closely related clones, C9 and CUC6, did not mediate the CMV resistance. The *Vat* allele present in the 90625 line mediated resistance to CMV when inoculated by only one clone, NM1, belonging to a genetic cluster comprising clones collected on different plant species.

Moreover, because the Anso AL 77 line exhibited some resistance to aphid (low acceptance) for the C9 and CUC6 clones but did not exhibit resistance to CMV when inoculated by those clones, this line could carry other loci, involved in the low acceptance, than the *Vat* locus. Heredity studies should allow verifying this hypothesis.

A. gossypii displayed two types of adaptation to the *Vat*-mediated resistance

Allelic series are common among the R genes in particular for those belonging to the NBS-LRR gene family such as in the flax and flax rust system that has been the basis for Flor's 'gene-for-gene' model. In this model, the product of the host resistance gene recognises the product of the pathogen avirulence gene what initiates defence responses leading to host resistance. On the contrary, when the product of the host resistance gene does not recognise the product of the pathogen avirulence gene, the plant is susceptible, *i.e.* the pathogen is adapted to the host resistance system. The flax/flax rust model has been an enduring model

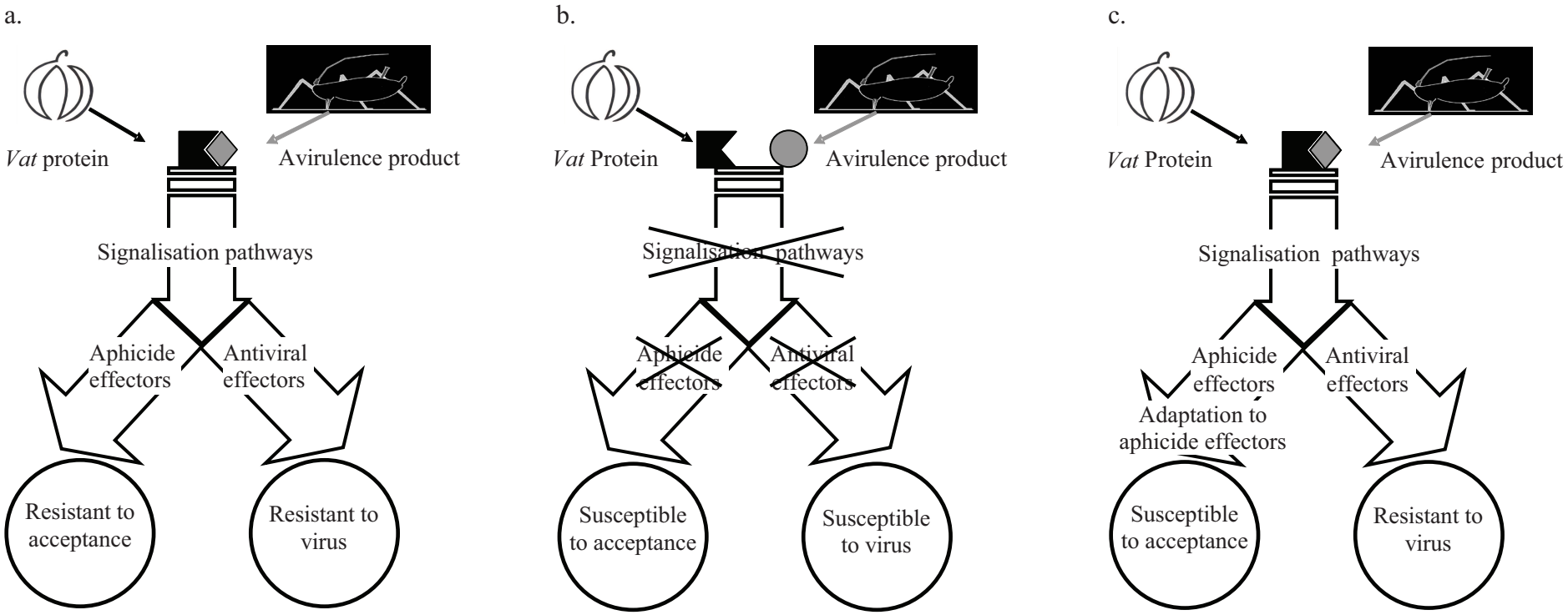


Figure 4: a. *Vat*-mediated resistance, leading to resistance to acceptance by *A. gossypii* and resistance to virus when inoculated by *A. gossypii,* results from the recognition between the product of the *Vat* gene and that of the avirulence gene of *A. gossypii*, which activates a cascade of plant responses. b. No recognition between the product of the *Vat* gene and that of the avirulence gene of *A. gossypii* leading on susceptibility to acceptance by *A. gossypii* and susceptibility to virus when inoculated by *A. gossypii*. c. *Vat*-mediated resistance with adaptation to aphid resistance effectors by *A. gossypii* leading only on suppression of the resistance to acceptance by *A. gossypii.*

with 11 alleles of resistance characterized at the *L* locus and at least four *Avr* locus exhibiting several haplotypes (Dodds et al., 2007). This resistance-breaking mode is known for NBS-LRR-like gene in interaction with several pathogens (Belkhadir et al., 2004). Aphid/plant interactions, that have been shown sharing defence pathways with pathogen/plant interactions (Kaloshian and Walling, 2005), have been much less studied and, up to now, no *Avr* genes have been characterized in aphid. Dogimont et al. (2010) speculate that the *Vat*-mediated resistance, controlled by a NBS-LRR gene, results from the recognition (direct or indirect) between the product of the *Vat* gene and that of the avirulence gene of *A. gossypii*, which activates a cascade of plant responses leading on one hand to the inhibition of infection by the virus and on the other hand to non acceptance of plants by the aphids and a low ability to colonize the plant (Fig 4.a). Although the *Vat* gene belongs to the NBS-LRR R gene family, our study, based on the double phenotype mediated by the *Vat* gene, *i.e* low acceptance by aphids and virus-resistance when inoculated by aphids, showed that the melon/*A. gossypii* system does not completely fit the gene-for-gene model. On one hand, the phenotypes we observed on the 90625 line with five aphid clones fit the gene-for-gene model. The Avr product of the NM1 clone is assumed to be recognised by the product of the host resistance gene in the 90625 line, leading to plant resistance for both phenotypes (Fig. 4.a); the Avr products of the C9, CUCU3, CUC6 and GWD clones are assumed to be not recognised by the product of the host resistance gene in the 90625 line, leading to plant susceptibility for both phenotypes (Fig. 4.b). On the same way, the phenotypes we observed with two aphid clones on the Anso AL 77 line fit the gene-for-gene model. The Avr products of the NM1 and CUCU3 clones are assumed to be recognised by the product of the host resistance gene in the Anso AL 77 line, leading to plant resistance for both phenotypes (Fig. 4.a); the Avr products of the C9 and CUC6 clones are assumed to be not recognized by the product of the host resistance gene in the Anso AL 77 line, leading to plant susceptibility only for CMV when inoculated by aphids (Fig. 4.b). We observed Anso AL 77 rather resistant for 'Acceptance' by these two clones. This unexpected phenotype could have two explanations: i) this partial resistance should be conferred by other loci than the *Vat* locus ii) the C9 and CUC6 clones partially overcome the recognition step (low affinity between the partners involved in the recognition) and then the resistance is only partially triggered. On the other hand, the phenotypes we observed with several aphid clones on the Margot and Anso AL 77 lines did not fit the gene-for-gene model. The GWD clone accepted quite well the Margot and Anso AL 77 lines, i.e. both lines are susceptible to these aphid clones, but the GWD clone triggered

the virus-resistance in both lines when inoculated by this clone. It strongly suggests that the Avr product of the GWD clone is recognised by the products of the host resistance gene in the Margot and Anso AL77 lines, leading to resistance to virus when inoculated by the GWD clone, but the GWD clone appeared not affected by the aphid resistance effectors that are produced as long as the Avr product of the GWD clone is recognised by product of the host resistance gene (Fig. 4.c). Therefore the GWD clone did not adapt to the Margot and Anso AL 77 allele at the *Vat* locus according to the 'gene-for-gene' model. On the same way, we assumed that the CUC6 clone is not affected by the resistance effectors triggered by the *Vat* allele in the Margot line. We already observed on other melon pedigrees that the *Vat* allele in the PI 161375 line (the same allele than in Margot) did not affect acceptance by the CUC6 and GWD clones (article 3). The adaptation to resistance effectors could result from different mechanisms, such as aphid resistance to insecticides (Foster et al., 2007). Identification of aphid *Avr* genes in aphid and identification of *Vat*-mediated effectors against aphid in plant would strongly help resolving aphid adaptation to R genes.

Does *A. gossypii* adaptation to the *Vat*-mediated resistance induce a loss of fitness ?

Comparison of fitness of clones differing only for gene(s) involved in transition from avirulent to virulent genotype has been an ideal tool to assess potential cost of pathogen adaptation to plant resistance. Those types of clones are not available for aphid/plant interaction and will not be available in a short time as long as the gene(s) involved in transition from avirulent to virulent genotype are unknown. Comparison of fitness of clones differing for more than gene(s) involved in transition from avirulent to virulent genotype would bias evaluation of resistance adaptation cost if some aphid genes, not involved in transition from avirulent to virulent phenotype, are involved in different fitness on the susceptible melon line used for the fitness evaluation. Therefore, to pass over this bias and to approach the potential cost of adaptation, we evaluated fitness of the clones on a set of melon lines genetically distant and representative of the botanical groups in which aphid resistance was observed.

Deleterious pleiotropic interactions with ancestral physiological functions should occur when adaptive differences are evolutionary responses to new environment. Fitness costs related to the plant resistance breaking have been detected in several plant pathogen such as *Xanthomonas oryzae* pv *oryzae* (Bai et al., 2000), Turnip Mosaic virus (Jenner et al., 2002), Potato virus Y (Janzac et al., 2010), Tobamovirus (Fraile et al., 2011) or *Phytophtora*

infestans (Montarry et al., 2010). Among aphid/plant interactions, that have been much less studied than plant pathogen interactions, no data have been available on fitness costs related to adaptation to the plant resistance breaking.

Margot is the only line carrying a resistance allele deployed in areas where the five clones used in this study were collected. These clones triggered resistance to virus when Margot plants were inoculated by aphids and two of them, the CUC6 and the GWD clones, both assigned to the Cucurbit cluster, appeared adapted to the aphid resistance effectors. The GWD clone was collected in 2009 in Guadeloupe, an island of the French West Indies, where no commercial variety of melon with resistance to aphids has been deployed. Therefore, we considered that the GWD clone is pre-adapted to aphid resistance effector(s) triggered by the resistance allele from the Margot line. No cost for adaptation to resistance was expected for this pre-adapted clone; in accordance with the theory, the GWD clone did not exhibit a lower fitness on susceptible melon lines than the not adapted clones, such as the CUCU3 or NM1 clones. The CUC6 clone was collected in 2009 in Southeastern France. In this producing area, aphid resistant varieties have been deployed since the end of the 1980^{ties}, and about 80% of the early melon crops consisted in aphid-resistant varieties the last ten years. The CUC6 clone was not observed in surveys conducted in south-eastern France in 2003 and 2004 in melon crops (Carletto et al., 2009; Lombaert et al., 2009) as well as in surveys we conducted from 2004 to 2007 in south-eastern France; it has been observed in field trials since 2008 in France mostly in South-eastern France (Thomas, unpublished data). The CUC6 clone could be an adaptive clone emerging from this new environment *i.e*. the CUC6 clone could be considered as breaking the *Vat* mediated resistance. The CUC6 clone had a comparable or a higher fitness on susceptible melon lines than the other clones, and particularly an higher fitness than the CUC3 clone, a genetically very closed clone. This suggest no fitness cost, at least for biotic potential, related to this adaptation to aphid resistance effectors.

The NM1 MLG was the only clone affected by the three alleles of resistance at the *Vat* locus we studied. It has never been observed developing colony on *Vat*-melon in crops (article 3). This clone exhibited a fitness comparable to the fitness of the four other clones on four susceptible melon. It has been observed susceptible to all insecticides as far as known (Carletto et al., 2010). The NM1 MLG is the single MLG no assigned to the Cucurbit cluster that has been identified in individuals colonizing Cucurbitaceae. It belongs to a genetic cluster with MLGs collected on various plant families: this cluster has not been assigned to a host race. Our study showed it is the only clone affected by the resistance conferred by three

alleles known at the *Vat* locus and, the reduction of ability to colonize on resistant accessions was very stronger for the NM1 clone than for the others clones (data not shown). Altogether, on one hand the NM1 clone appeared a specialized clone within an unspecialized cluster of clones, on the other hand the NM1 clone appeared susceptible to several resistant alleles present in the melon species and as well as to all aphicides. This could reflects a trade-off between an ancestral specialization and ability to adapt to new constraints such as plant resistances or pesticides.

The CUC6 and GWD clones, were found the most frequently on *Vat* melon (article 3). However, despite aphid colony growth among resistant crops, the most frequent biotypes are not necessarily the fittest ones over the whole season or over several seasons, but possibly during the winter conservation phase. Indeed, it is possible that highly aggressive biotypes, which will be favoured during the epidemic season, will be under a negative selection pressure during the winter. All the more, since the CUC6 clone has been observed several years, it has proven its ability to overwinter; the GWD clone, that have been only observed in the French West Indies, can develop over year under tropical climate. Biological tests suggested the CUC6 and GWD clones had a fitness comparable to other clones and then, the risk of extension of these clones appeared high. Polymorphism of host resistance and pathogen virulence are commonly explained by the fact that both resistance and virulence gens are costly. Nevertheless stochastic simulations show that the polymorphism in plant pathogen systems can be explained without costs (Salathe et al., 2005).

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Supplementary data

Table S1: Multilocus genotypes identified as different allelic combinations at the eight microsatellite loci in 18 clones collected on Cucurbitaceae and used in the assignation process: first part described by Carletto et al. (2009), second part described among melon crops (article 3). The size of each allele is indicated in base pairs. The MLGs in bold are those identified in the five clones characterized in this study.

MLG	**Ago24**	**Ago53**	**Ago59**	**Ago66**	**Ago69**	**Ago84**	**Ago89**	**Ago126**
C1	153-157	116-116	182-182	143-152	109-114	112-112	150-150	166-176
C4	153-153	116-116	180-182	152-152	109-114	112-116	150-150	176-176
C5	153-153	116-116	182-182	152-152	109-114	112-118	150-150	176-176
C6	153-153	116-116	180-182	152-152	109-114	112-118	150-150	176-176
C8	153-153	116-116	182-182	152-152	109-114	112-118	150-158	176-176
C9	**153-157**	**116-116**	**182-182**	**152-152**	**109-114**	**112-118**	**150-150**	**176-176**
C10	157-157	116-116	182-182	152-152	108-109	118-118	150-150	176-176
C11	153-157	116-116	182-200	152-152	109-109	108-112	150-150	176-176
C12	157-157	116-116	161-182	148-152	102-114	112-118	150-150	176-176
C13	153-153	116-116	182-182	152-152	109-114	112-112	150-150	176-176
C14	153-153	116-116	182-188	152-152	109-111	112-112	150-150	176-176
NM1	**153-153**	**113-116**	**184-217**	**152-156**	**109-115**	**116-116**	**150-158**	**166-177**
CUC1	153-157	116-116	182-207	147-152	109-114	112-124	150-150	176-176
CUC3	153-157	116-116	182-207	147-152	109-114	112-112	150-150	176-176
CUC6	**153-157**	**116-116**	**182-182**	**147-152**	**109-114**	**112-124**	**150-150**	**176-176**
CUCU3	**157-157**	**110-116**	**147-147**	**147-152**	**109-116**	**118-118**	**150-152**	**176-176**
CUCU91	153-153	116-116	000-000	147-152	109-114	112-112	150-150	176-176
MTB	157-157	110-116	182-182	147-152	109-116	118-118	150-152	176-176
GWD	**157-157**	**113-116**	**163-182**	**152-152**	**109-115**	**124-124**	**150-150**	**166-176**

CHAPITRE 5 : DISCUSSION GENERALE ET PERSPECTIVES

Chapitre 5 : Discussion générale et perspectives

Le temps évolutif et celui qui rythme les activités agricoles sont longtemps apparus à des échelles très éloignées l'une de l'autre et par conséquent la théorie de la sélection naturelle a mis du temps à être intégrée à l'agronomie. On réalise maintenant que les milieux agricoles et les milieux naturels sont soumis à d'intenses modifications environnementales et subissent des perturbations rapides. Les changements évolutifs qui s'y produisent sont alors étudiés « en temps réel ». Dans ce travail de thèse, différentes échelles de temps d'évolution de la relation puceron/melon ont été prises en compte : d'une part les pressions de sélection anciennes ont été abordées par une approche de génétique des populations de pucerons et d'autre part les pressions de sélection récentes, comme celles exercées par le déploiement de gènes de résistance par une approche expérimentale.

Les résultats majeurs et originaux obtenus au cours de ce travail de thèse sont :

- La mise en évidence d'une grande diversité génétique d'*A. gossypii* (Article 1, 596 MLG observés contre 44 MLG décrits jusqu'alors).
- La mise en évidence de reproduction sexuée chez certaines populations d'*A. gossypii* en France alors que jusqu'ici l'espèce était considérée comme clonale (Article 1), ce qui explique en partie la très large diversité observée.
- La mise en évidence d'une diversité originale aux Antilles (Article 1).
- La mise en évidence d'une relation forte entre la diversité génétique estimée par 8 SRR et le phénotype d'*A. gossypii* face aux résistances chez le melon (Article 4) qui permet d'inférer des biotypes en utilisant les marqueurs moléculaires.
- La démonstration *in situ* d'un effet du gène majeur *Vat* qui entraîne une diminution de la densité des populations naturelles et modifie leur structure génétique (Article 3).
- La démonstration de l'absence d'effet *in situ* de QTL sur les populations de pucerons (densité et structure, Article 3).
- La mise en évidence de différents processus de contournement d'un gène majeur de type NBS-LRR : contournement au niveau de la reconnaissance ou des effecteurs de la résistance (Article 5).

Dans cette discussion générale nous aborderons trois points :

- Quelle part de la diversité d'*A. gossypii* constitue un réservoir pour l'adaptation de cette espèce aux résistances du melon ?

- Comment combiner les locus de résistances chez le melon pour éviter les contournements ?
- Que révèle le double phénotype contrôlé par le gène *Vat* ?

Quelle part de la diversité d'*A. gossypii* constitue un réservoir pour l'adaptation de cette espèce aux résistances du melon ?

A. gossypii a une gamme de plantes hôtes extrêmement large appartenant à différentes familles de plante cultivées : Cucurbitacées, coton, aubergine, pomme de terre, piment, fraise et Citrus. L'analyse génétique à huit loci microsatellites sur des échantillons d'*A. gossypii* prélevés dans des colonies de pucerons aptères, a révélé que l'espèce est structurée par la plante hôte et constituée de MLG spécialisés sur certaines plantes cultivées (Carletto et al., 2009). Dans l'article 1, le même type d'analyse, menée sur des individus d'*A. gossypii* ailés prélevés uniquement sur melon, a révélé que l'espèce est structurée à la fois par la plante hôte et par le mode de reproduction. La richesse génotypique observée parmi les ailés, qui atterrissent sur des plants de melon en champ, est très importante et diversifiée en début de culture, alors que la richesse clonale des aptères est réduite en fin de culture et composée de MLG majoritairement spécialisés sur Cucurbitacées. Ceci démontre que le choix de la plante hôte ne se fait pas à distance mais après que l'individu ailé ait atterri, d'une part, et que la sélection exercée par la plante hôte est forte sur les populations infestantes ailées, d'autre part. La très large diversité d'*A. gossypii* révélée dans l'article 1 et l'étude génétique menée dans ce même article, nous ont permis de mettre en évidence l'existence d'évènements de reproduction sexuée chez cette espèce, moteur de la création de variation génétique. L'augmentation de la variation génétique par le sexe présente quatre avantages i) rassembler les mutations bénéfiques dans le même individu (aide du sexe dans la diffusion de traits avantageux) ; ii) rassembler des mutations délétères pour créer les individus non viables qui sont alors éliminés de la population (aide du sexe dans la purge des gènes délétères) ; iii) diminuer la compétition entre apparentés ; iv) éliminer des dommages par la réparation de l'ADN, lors de la recombinaison méïotique. Mais il existe un coût associé à la recombinaison : en effet dans la descendance il y aura ségrégation du génotype le plus favorable entrainant le désappariement des combinaisons multilocus favorables.

Chez *A. gossypii*, un polymorphisme de reproduction semble avoir lieu, avec une coexistence de populations sexuées et asexuées. L'hypothèse serait que les évènements de reproduction

sexuée aient lieu sur *Hibiscus syriacus*, mais nous ne disposons d'aucune connaissance sur les hôtes secondaires potentiels de ces populations sexuées. En théorie, l'avantage du sexe est considéré supérieur au coût de la recombinaison si : i) l'environnement est variable dans l'espace, il y aura une faible compétition entre descendants génétiquement différents (hypothèse du « tangled bank »). En effet, si l'environnement est hétérogène la diversité génétique permet l'occupation d'un plus grand nombre de niches. ii) L'environnement est en constante évolution, il y aura un avantage à produire continuellement de nouvelles combinaisons multilocus (Théorie de la Reine rouge). Chez *A. gossypii*, les analyses de génétique des populations donnent des valeurs de F_{is} très positives suggérant des taux élevés de consanguinité. Une hypothèse serait l'existence d'accouplements préférentiels entre individus génétiquement proches *i.e.* individus apparentés. Dans le cas des environnements homogènes et stables, comme peuvent l'être les agrosystèmes, cette stratégie réduirait la compétition entre les individus apparentés et maintiendrait les combinaisons favorables.

Dans l'article 1, l'étude des populations aptères, qui peuvent être des individus issus de colonies ou isolés, prélevées sur des cultures de melon montre qu'elles contiennent des MLG presque exclusivement assignés au cluster Cucurbitacées ou proches de NM1. Ces MLG semblent avoir perdu la capacité à se reproduire de façon sexuée sauf dans l'Ouest. Ces populations contiennent de très nombreux MLG, dont la majeure partie n'avait jamais été observée dans les études portant sur des individus prélevés dans des colonies. En fait, seulement 10% de ces MLG sont capables de développer des colonies sur melon (Article 3). Le maintien d'une large diversité dans le cluster Cucurbitacées plaide pour une reproduction sexuée, au moins chez une partie des individus suite à l'apparition ponctuelle de mâles sexués comme suggéré par les observations de Ferrari and Nicoli (1994), mais nous n'avons pas mis en évidence ces phénomènes par notre analyse de génétique des populations. La pression de sélection qui s'exerce au sein même du cluster cucurbitacées pourrait s'expliquer par des effets de dilution des gènes d'adaptation à la plante hôte lors de ces évènements ponctuels de reproduction sexuée. Les clones capables de développer des colonies seraient issus de parthénogenèse stricte, la perte de phase sexuée pourrait être la conséquence de la spécialisation sur la ressource. En effet, les combinaisons de gènes coadaptés des différents MLG ne sont pas détruites par la recombinaison et sont transmises intégralement à la descendance via la parthénogenèse. Ainsi la valeur sélective d'un clone spécialisé sur une plante hôte reste maximale de génération en génération ce qui permet l'infestation rapide d'une parcelle cultivée suite à l'apparition ponctuelles de mâles sexués. Même si la

reproduction asexuée pourrait être un désavantage face aux pressions de sélection exercées par la plante et les pratiques culturales, ce désavantage peut être compensé par une démographie explosive et l'apparition rapide de mutants.
Il apparaît finalement que la diversité efficace pour s'adapter aux pressions anthropiques telles que les résistances est relativement réduite.

Comment combiner les locus de résistances pour éviter les contournements ?

Nous avons testé l'hypothèse que la durabilité d'un gène majeur est augmentée quand il est combiné à des QTL de résistance. Pour le puceron, son adaptation dépendra de sa capacité à évoluer face aux différents facteurs de résistance, ainsi que de l'aptitude des mutants à se multiplier, à coloniser et à se disperser sur les plantes. Dans l'article 3, nous avons montré que très peu de MLG d'*A. gossypii* développaient des colonies sur des melons possédant l'allèle de résistance au locus *Vat*, ce qui démontre que la plante résistante exerce une forte pression de sélection sur les populations colonisantes aptères. Nous avons montré que les QTL expérimentés n'avaient un effet que sur deux clones ayant eux-mêmes servi à la sélection de ces QTL (article 2), et donc ces QTL présentent peu ou pas d'efficacité en plein champ. Ceci révèle la spécificité des QTL vis-à-vis des clones. De plus, ces QTL ne semblent affecter ni la diversité ni la structure des populations. Ces résultats semblent indiquer qu'ils présentent peu d'intérêt pour la sélection variétale.

La résistance à l'acceptation de la plante par *A. gossypii* est contournée par des clones collectés dans le sud-est de la France et en Guadeloupe (Article 5). Nous avons réalisé, *in vivo*, des mesures de valeur sélective sur des melons sensibles. Nous montrons des différences de colonisation par les pucerons appartenant aux différents clones, sans pour autant démontrer l'existence d'un coût engendré par le contournement de la résistance. De plus, nous avons démontré qu'un même MLG présentait une certaine plasticité phénotypique pour son agressivité face à une même accession de melon (Article 4). Pour relier un coût de la virulence et la durabilité des résistances chez le melon il serait nécessaire de tester de nombreux clones isolés *in situ* et présentant des distances génétiques plus ou moins grandes, afin de voir l'effet du fond génétique du puceron. La présence de clones contournants dans les différents champs étudiés ne suffit pas pour prédire que ces résistances ne sont pas durables.

En effet, la durabilité ne sera compromise que si les clones contournants deviennent prévalents en conditions naturelles. Les MLG contournants la résistance contrôlée par le gène *Vat* (Article 5) se maintiennent d'une année sur l'autre (Article 3). De plus, nous avons observé une structuration géographique de la diversité d'*A. gossypii* dans les bassins de production de melon et des clones originaux en Guadeloupe (Article 1). Il apparaît nécessaire de mener une sélection différentielle pour les variétés cultivées aux Antilles et en France. Pour les bassins de productions français, à cause du potentiel de dispersion à longue distance des pucerons par l'intermédiaire des morphes ailés, le risque d'extension de MLG contournants est omniprésent en particulier en ce qui concerne le MLG CUC6 (clone capturé dans le sud-est et MLG observé dans le sud-ouest).

Nous manquons de ressources génétiques efficaces contre les clones CUC6 et GWD. D'autres accessions disponibles au laboratoire présentent des spectres de résistance à *A. gossypii* différents de ceux observés dans les accessions étudiées dans l'article 5 (données non publiées). On ne sait pas si ces résistances sont contrôlées par le locus *Vat.* Une perspective serait de mener une sélection pour la résistance à *A. gossypii* sur une large gamme d'accessions vis-à-vis des nouveaux clones caractérisés, puis d'établir l'intérêt de ces nouvelles sources de résistance pour la création variétale. Dans l'article 3, les analyses sur cinq clones et six combinaisons de résistances *Vat* et QTL montrent qu'il existe une bonne concordance entre l'acceptation des clones observés en laboratoire sur les combinaisons de résistance et le développement de ces clones en plein champ sur ces mêmes combinaisons de résistance. Ceci laisse penser qu'il serait possible de prédire via un modèle démo-génétique le développement de différents clones en plein champ à partir de données acquises en laboratoire. Des données expérimentales (potentiel biotique et acceptation) ont déjà été acquises au cours de cette thèse (données non présentées dans ce mémoire). Trois principaux processus, impliqués dans l'infestation des cultures, seraient considérés dans le modèle démo-génétique. Leurs paramètres dépendraient des MLG d'*A. gossypii* et des combinaisons de résistance de la plante. Le premier processus, modélisé par la probabilité d'installation des ailées sur la plante, représenterait l'acceptation par un MLG donné sur une résistance donnée. Le second processus représente la dynamique des populations dans le champ. Elle serait modélisée en utilisant l'estimation de la descendance produite chaque jour par une femelle d'un MLG donné sur une résistance donnée. Il s'agit d'utiliser des courbes d'émergence obtenu pour chaque MLG basé sur des modèles de croissance des populations. Pour la dernière étape, l'émigration de pucerons au champ, nous considérerions que la capacité à se disperser dépend de la densité de population (par exemple un seuil de densité pour la différenciation des

morphes ailés). La structure génétique de la population de pucerons qui infestent (populations ailées) et les combinaisons de gènes de résistance déployées dans le champ de melons seraient considérées comme des variables explicatives. Ce modèle serait utilisé sur les combinaisons de résistance, l'indépendance entre les effets loci permettrait de prévoir l'effet de combinaisons possibles. Une analyse de sensibilité permettrait de dégager des règles pour la sélection en choisissant les meilleures combinaisons de facteurs de résistance en fonction des populations de pucerons infestantes. Ceci suggère qu'il serait possible de modéliser l'effet de combinaisons de résistance ou de multilignées portant plusieurs allèles de résistance déployés en plein champ sur les populations d'*A. gossypii* et en particulier d'évaluer les risques d'émergence de contournants.

Que révèle le double phénotype contrôlé par le gène *Vat* ?

Nous avons montré dans l'article 5 que la résistance au CMV lorsqu'il est inoculé par *A. gossypii*, résistance conférée par le gène *Vat,* a une efficacité sur un large spectre de clones d'*A. gossypii* spécialisés sur Cucurbitacées. Des expérimentations, non publiées, menées sur des clones d'*A. gossypii* « non-Cucurbitacées », montrent que lorsque ces clones inoculent le CMV à des melons porteurs du gène *Vat* les plantes sont sensibles au même titre que lorsque les CMV est inoculé par une autre espèce de puceron, comme le démontre les expériences de transmission de CMV par *Myzus persicae*, ou lorsque le virus est transmis mécaniquement. Ces résultats, associés à ceux exposant la très large diversité des ailés qui atterrissent sur les plantes de melon (Article 1), permettent d'expliquer les épidémies virales observées chaque années dans les cultures de melon porteur du gène *Vat*.

Dans le chapitre 4, la caractérisation de clones d'*A. gossypii* appartenant à cinq MLG sur des accessions de melon a confirmé l'existence de trois allèles de résistance différents au locus *Vat*. La relation gène pour gène (Résistance/Avirulence) n'a pas été formellement caractérisée à ce jour pour la résistance des plantes aux pucerons. La virulence dans le cadre des pucerons peut être définie comme la capacité à coloniser une plante. L'acquisition de la virulence peut se faire par deux mécanismes indépendants : i) soit la virulence est acquise par détoxification des effecteurs de la résistance comme c'est le cas dans l'adaptation aux insecticides (Walling, 2008). ii) Soit la virulence est acquise par la perte de la reconnaissance entre le gène de résistance et le gène d'avirulence. Chez *A. gossypii* l'utilisation du double phénotype de *Vat* a permis de mettre en évidence différents niveaux de contournement en fonction d'une

accession et d'un clone donné : i) au niveau de la reconnaissante R/Avr ; dans ce cas, on a à la fois perte de l'effet aphicide et perte de l'effet antiviral. ii) au niveau des effecteurs de la cascade de signalisation déclenchée par la reconnaissance R/Avr ; dans ce cas, on a seulement perte de l'effet aphicide.

Le contournement au niveau de la reconnaissance se situe dans le cadre de la coévolution gène pour gène (R/Avr) qui est sous le contrôle d'une force diversifiante au niveau moléculaire appelée aussi sélection darwinienne positive. Ce moteur d'évolution génère de la biodiversité. Par exemple, chez le lin plus de 30 allèles de résistance complémentaire de gènes d'avirulence ont été décrit au niveau du locus de résistance (Flor, 1971 ; Ellis et al., 2007). Chez les insectes, les premier gènes d'avirulence ont été clonés chez la mouche de Hesse (Stuart and Chen, 2011). Au moins quatre gènes, impliqués dans la virulence, codent pour une superfamille de protéines appelée SSGP-11 pour secreted-salivary-gland proteins 11. La présence d'un peptide signal de sécrétion exclusivement exprimé au stade larvaire, seul stade infectieux, indique que cette superfamille pourrait jouer un rôle dans la virulence/avirulence chez la mouche de Hesse (Chen et al., 2006). A ce jour aucun gène de virulence chez les pucerons n'a été caractérisé ni chez d'autres hémiptères. Le cadre de l'interaction gène pour gène chez les hémiptères a conduit à l'hypothèse que le produit du gène d'avirulence doit se trouver dans les composants de la salive (Kaloshian et al., 2000). Chez *A. gossypii*, il n'y a pas de contournement de la résistance au niveau de la reconaissance de l'allèle de résistance de PI 161375au locus *Vat* par tous les clones étudiés. Pour les allèles de *Vat* portés par les accessions Anso AL 77 et 90625, il y a perte de la reconnaissance avec les produits du gène d'avirulence de certaines MLG. Il existe, face à une accession donnée, des clones possédant l'allèle de virulence et des clones possédant l'allèle d'avirulence. Ces profils d'avirulence/virulence semblent être corrélés avec la structure génétique d'*A. gossypii*, constituant un matériel dans l'objectif du clonage de gène d'avirulence complémentaire de *Vat* à partir de gènes candidats exprimés dans la salive.

Le contournement des effecteurs de la résistance peut-être considéré dans le cadre des réponses de défenses induites par les insectes. Des voies de signalisation, mises en évidence lors d'interactions plantes-pathogènes (voie du salicylate) ou lors d'interactions plantes-herbivores (voie du jasmonate et de l'éthylène), semblent conjointement impliquées dans le cadre des réponses de défenses induites par les insectes (Thompson et Goggin, 2006). Dans ce contexte, divers travaux suggèrent l'existence d'une voie spécifique de la réponse de défense des plantes aux insectes phloémophages ainsi que l'inhibition d'une partie de ces réponses par les pucerons, probablement à travers leur mode alimentaire. Cette hypothèse est très

largement supportée par diverses études qui soulignent la variabilité tant quantitative que qualitative de l'accumulation de peptides suivant que l'agent inducteur est un lépidoptère, un coléoptère, un diptère mineur, un acarien ou encore un aphide. La dérégulation de divers mécanismes de défense par les salives permettrait aux aphides d'exploiter la sève (Moran and Thompson, 2001; Divol et al., 2005).

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CONCLUSION

Conclusion

Les sources naturelles de résistances aux ravageurs sont limitées et rares et devraient être utilisées de manière à maintenir leur efficacité sur le long terme. Bien que le gène *Vat* soit déployé depuis plusieurs années, il reste efficace sur le terrain. Mais nous avons montré au cours de cette thèse que cette efficacité pouvait être mise à mal par des clones contournant les effecteurs de la résistance. Les QTL ne permettent pas de défavoriser ce contournement. Il faudra se tourner vers d'autres stratégies pour préserver l'efficacité du gène *Vat.* Pendant longtemps le développement de variétés résistantes aux insectes s'est heurté à un certain cloisonnement entre disciplines et a fait que les améliorateurs et les entomologistes ont conduit leurs recherches de manière indépendante, largement indifférents aux avancées de l'autre discipline. Ce cloisonnement est en train de se lever permettant d'intégrer différents concepts pour une même problématique. La modélisation pourrait être une démarche plus prédictive pour le choix de gènes de résistance et leur déploiement.

Pressions de sélection exercées par les résistances génétiques du melon sur les populations d'*Aphis gossypii*

La réponse adaptative de populations de bioagresseurs aux pressions de sélection exercées par les activités agricoles détermine la durabilité des moyens de lutte. Chez le melon, le gène *Vat* qui confère la résistance à *Aphis gossypii* étant déployé depuis plus de 10 ans, on craint son contournement. L'enjeu est de proposer des éléments stratégiques aux semenciers sur le risque d'évolution des pucerons vers la virulence, pour développer de nouvelles variétés avec des résistances durables. Dans le cadre de cette thèse, nous avons :

i) Estimé la diversité génétique disponible dans des populations d'*A. gossypii* de différentes régions de production de melon. Elle est structurée géographiquement. La grande diversité observée en France aurait en partie pour origine des évènements de reproduction sexuée suggérant un potentiel évolutif élevé d'*A. gossypii*.

ii) Estimé la pression de sélection exercée par différentes combinaisons de résistance (gène *Vat* et QTL) sur ces populations. Les densités de population sont plus faibles sur les plantes *Vat* que sur les plantes non *Vat* et la structure génétique des populations est modifiée dans certaines régions de production quand le gène *Vat* est présent. Les clones se multipliant sur les plantes *Vat* ont une forte fitness et le risque de leurs extensions est grand. Aucun effet de QTL de résistance n'a été mis en évidence en plein champ.

iii) Caractérisé les clones contournant le gène *Vat*. Nos résultats suggèrent que l'adaptation des clones s'effectue soit par modification du gène d'avirulence du puceron soit par l'adaptation du puceron aux effecteurs de la résistance.

De nouvelles stratégies de gestion de la résistance *Vat* sont proposées.

Mots-clés : *Cucumis melo*, *Aphis gossypii, Vat*, QTL, durabilité des résistances, contournement, méthodes d'expérimentation au champ et/ ou en conditions contrôlées, génétique des populations, génétique quantitative.

Selection pressures exerted by the genetic resistances of melon on *Aphis gossypii* populations

The adaptive response of pest populations to selection pressures exerted by agricultural activities determines the sustainability of control methods. In melon, the *Vat* gene that confers resistance to *Aphis gossypii* has been deployed for over 10 years, so there are fears it will be overcome. The challenge is to provide strategic elements to plant breeders, concerning the risk of development of virulent aphids, in order to develop new varieties with durable resistances. In the context of this PhD, we have:

i) Estimated the available genetic diversity in populations of *A. gossypii* from different melon growing areas. The diversity is structured geographically. The great diversity observed in France would have its origine in part from the events of sexual reproduction, suggesting a high evolutionary potential of *A. gossypii*.

ii) Estimated the selection pressure exerted by different resistance combinations (*Vat* gene and QTLs) on these populations. Population densities are lower on Vat^R plants than Vat^S plants and population genetic structure is altered in certain growing areas when the Vat^R gene is present. The clones multiplying on Vat^R plants have good fitness and the risk of their spreading is great. No effect of QTLs has been identified in the field.

iii) Characterized the clones overcoming the Vat^R gene. Our results suggest that the adaptation of clones made either by alteration of the avirulence gene of aphids or by adaptation of aphids to resistance effectors.

New strategies for *Vat* resistance management are proposed.

Keywords: *Cucumis melo*, *Aphis gossypii*, *Vat*, QTL, resistance durability, overcoming, field experiments and / or controlled conditions, population genetics, quantitative genetics.