UNIVERSITÉ DE NEUCHÂTEL

INSTITUT DE ZOOLOGIE

UNIVERSITÉ MONTPELLIER II

SCIENCES ET TECHNIQUES DU LANGUEDOC

THÈSE DE DOCTORAT

Réalisée en cotutelle entre l'Université de Neuchâtel et l'Université Montpellier II.

SPÉCIALITÉ : Biologie des Organismes et des Populations
FORMATION DOCTORALE : DEA Biologie de l'Évolution et Écologie (Montpellier)
ÉCOLE DOCTORALE : Biologie Intégrative (Montpellier)

PLANTES-HÔTES ET ORGANISATION DE LA DIVERSITÉ DES INSECTES PHYTOPHAGES, DES RADIATIONS ÉVOLUTIVES AUX PROCESSUS POPULATIONNELS : le cas des bruches du genre *Acanthoscelides* SCHILSKY (Coleoptera : Bruchidae)

présentée par

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Soutenue publiquement le 01.12.2004 à l'Université Montpellier II devant le jury composé de :

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IMPRIMATUR POUR LA THESE

Plantes-hôtes et organisation de la diversité des insectes phytophages, des radiations évolutives aux processus populationnels : le cas des bruches du genre *Acanthoscelides* Schilsky (Coleptera: Bruchidae)

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REALISEE EN COTUTELLE AVEC

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Neuchâtel, le 16 décembre 2004

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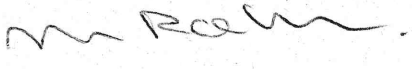

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I. INTRODUCTION

I.1. Contexte général

Les insectes représentent un des groupes d'organismes les plus diversifiés parmi tous les êtres vivants. Les estimations de leur diversité actuelle sont de l'ordre de 5 à 30 millions d'espèces (Erwin 1982, Stork 1997). Leur origine, qui date de la fin du Silurien il y a environ 420 millions d'années (Gaunt et Miles 2002), se situe lorsque leurs ancêtres arthropodes aquatiques colonisent les terres émergées. Cette origine coïncide avec l'arrivée des premières plantes vasculaires terrestres sur la terre ferme (Taylor et Taylor 1993, Edwards 2000). Les insectes sont donc parmi les premiers animaux à avoir quitté le milieu marin, puisque leur origine précède légèrement l'apparition des premiers tetrapodes (Labandeira et Sepkoski 1993). Ecologiquement, les premiers insectes se situent alors vraisemblablement dans la chaîne des décomposeurs, et jouent le rôle de détritivores (Southwood 1973). Pendant le Carbonifère, il y a environ 300 millions d'années, le nombre d'espèces d'insectes a connu une radiation massive – en parallèle avec l'importante diversification des plantes vasculaires – et les principaux super-ordres d'insectes actuels ont alors fait leur apparition (Gaunt et Miles 2002). C'est également de cette époque que datent les premiers fossiles de plantes présentant des dégâts liés à l'herbivorie (Labandeira 1998). Les insectes phytophages ont ainsi su développer la capacité de se nourrir des premières plantes vasculaires, dont, dès le Permien (il y a environ 250 millions d'années), les Gymnospermes qui dominaient alors la flore. Ces « paleo-phytophages » étaient essentiellement des piqueurs-suceurs, des défolieurs, des pollinivores, ainsi que des galligènes. Plus récemment, lors de la radiation des principaux ordres d'angiospermes au Crétacé (il y a environ 100 millions d'années), la diversification des insectes phytophages s'est poursuivie (Danforth *et al.* 1999), en entraînant l'émergence de nouvelles formes de phytophagie, comme par exemple, la

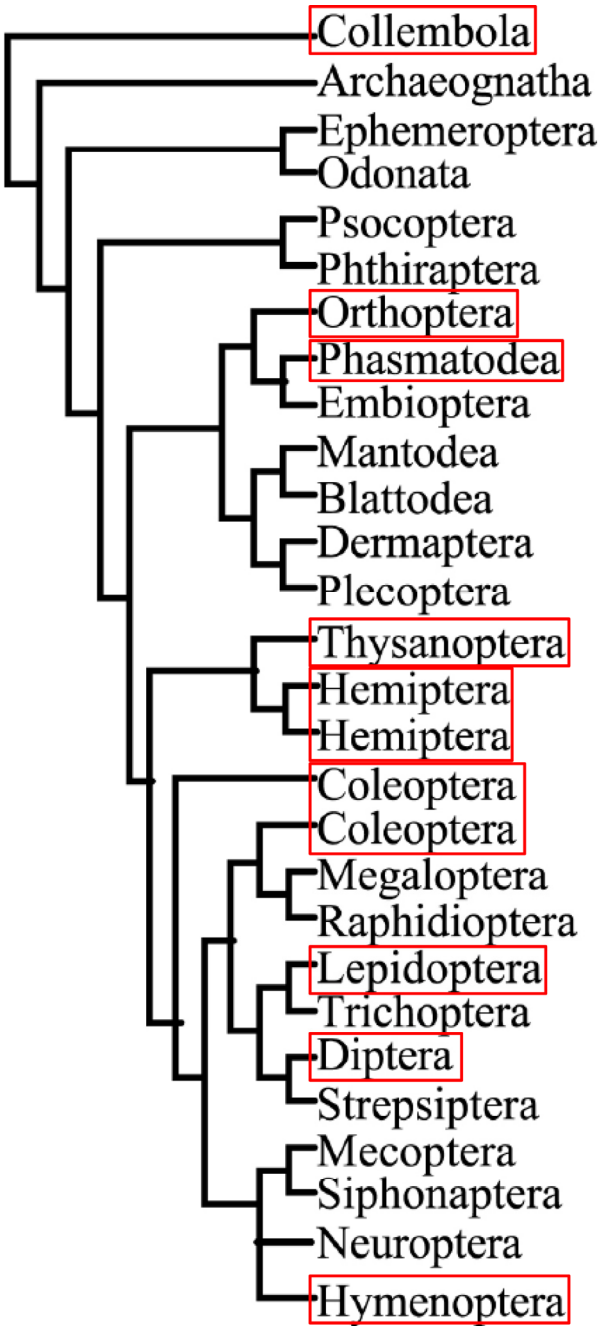

Figure 1

Phylogénie des principaux ordres d'insectes d'après Kirstensen (1999). Les ordres comprenant des espèces phytophages sont encadrés.

séminivorie[1], ainsi que d'autres types d'interactions entre les plantes et les insectes, tels que les mutualismes de pollinisation (Wyatt 1983).

Ainsi, tout au long de leur histoire, les insectes et les plantes semblent s'être diversifiés en parallèle, aussi bien au niveau phylogénétique qu'au niveau écologique. De nombreux auteurs ont proposé l'hypothèse selon laquelle la phytophagie serait à l'origine de l'incroyable diversification des insectes (Harborne 1988, Mitter *et al.* 1988), aux côtés d'autres facteurs tels que l'apparition de nouveaux substrats d'oviposition (Zeh *et al*. 1989), des associations mutualistes avec les angiospermes (Farrell 1998), et de la sélection sexuelle (Arnqvist *et al*. 2000). Mitter *et al.* (1988) ont en effet montré que dans différents groupes taxonomiques jumeaux, la diversité spécifique est significativement plus importante dans les groupes d'insectes phytophages que dans les groupes d'insectes non-phytophages. Il est également important de relever que la grande majorité des insectes phytophages sont oligophages (restreints à un faible nombre de plantes-hôtes), voire monophages (restreints à une seule espèce de plante-hôte), en raison de leur adaptation spécifique aux composés secondaires de leurs plantes-hôtes (Jaenike 1990).

Les insectes phytophages représentent aujourd'hui plus de la moitié de toutes les espèces d'insectes décrites (Strong *et al.* 1984). Néanmoins, seuls neuf ordres d'insectes parmi la trentaine décrite ont évolué vers la phytophagie (voir figure 1 ; Kristensen 1999). Ainsi, Strong *et al.* (1984) ont fait l'hypothèse suivante : "se développer sur des plantes supérieures représente une fantastique barrière évolutive que la plupart des groupes d'insectes n'ont pas réussi à franchir. Néanmoins, une fois cette barrière franchie, elle donne lieu à une incroyable radiation." Southwood (1973) a proposé deux types majeurs de barrières évolutives à l'émergence de la phytophagie. D'une part, le risque de dessiccation est plus grand chez les insectes phytophages que pour leurs ancêtres détritivores, qui eux peuvent profiter de l'humidité du sol et de la litière. D'autre part, les plantes vivantes constituent généralement un plus faible apport azoté que la matière organique en

[1] Chez les insectes, la séminivorie est caractérisées par la phytophagie obligatoire des semences, la plupart du temps lors des stades larvaires.

décomposition, et démontrent une composition déséquilibrée en acides aminés essentiels (Denno et McClure 1983).

Cependant, une fois que les insectes phytophages ont franchi de telles barrières, leur diversification est magnifiée par la diversité et l'importante biomasse que représentent les différents organes des plantes-hôtes, ainsi que par la faible compétition inter-spécifique des phytophages, essentiellement due à leur importante spécificité à un hôte donné (Strong et al. 1984). De nombreux auteurs développent par ailleurs l'idée selon laquelle la diversification des insectes phytophages et des plantes serait mutuellement renforcée (Ehrlich et Raven 1964) au travers de processus coévolutifs. Cette théorie a été soutenue par de nombreux travaux. Par exemple, aussi bien les espèces d'angiospermes riches en composés coumariques que les différentes familles de lépidoptères phytophages qui leur sont associées, semblent présenter une diversité spécifique plus importante que leurs groupes frères respectifs (Berenbaum 1983, Rausher 2001). Chez ces lépidoptères phytophages, l'innovation-clé qui leur a permis de contourner les défenses coumariques des plantes-hôtes – et donc d'exploiter un nouveau panel de ressources – réside en une mutation de leur cytochrome P450S, qui rend insensible leur chaîne respiratoire à l'effet inhibiteur des coumarines (Berenbaum *et al.* 1996). Il en résulte que les espèces phytophages phylogénétiquement proches sont le plus souvent associées à des plantes-hôtes proches, chimiquement et donc souvent taxonomiquement (Janz et Nylin 1998, Wahlberg 2001).

D'après le modèle de Ehrlich et Raven (1964), l'acquisition d'innovations permet donc aux insectes phytophages de contrer les défenses des plantes, en particulier les composés secondaires toxiques (Wittstock *et al.* 2004), d'exploiter de nouvelles ressources, et donc de se diversifier. Depuis l'émergence des techniques moléculaires appliquées aux reconstructions phylogénétiques, de nombreuses études ont mis en évidence un patron de conservatisme taxonomique lors de la diversification des insectes phytophages, ce qui signifie que des espèces d'insectes phylogénétiquement proches se retrouvent sur des espèces de plantes-hôtes également proches (par exemple, Lopez-Vaamonde *et al.* 2003). Un tel patron peut

être expliqué par des processus coévolutifs stricts, où les deux partenaires de l'interaction (l'insecte phytophage et sa plante-hôte) démontrent des processus d'adaptation et de diversification parallèles dans le temps. Dans ce cas, le développement de nouvelles défenses chez la plante sélectionne l'acquisition de résistances chez les insectes, résistances qui à leur tour vont entraîner l'émergence de nouveaux composés de défense chez la plante, et ainsi de suite. De nombreuses études ont mis en évidence des processus de co-évolution stricte aussi bien chez les insectes phytophages ennemis des plantes (par exemple, Gatehouse 2002) que chez les mutualistes (par exemple, Jousselin *et al.* 2003), qui aboutissent le plus souvent à une congruence phylogénétique élevée entre les deux groupes partenaires. Néanmoins, un tel patron peut aussi être expliqué par des processus phylogénétiques temporellement décalés entre les insectes phytophages et leur plante-hôte, au cours desquels la diversification des insectes est postérieure à celle des plantes (par ex. Janz et Nylin 1998). Dans ce cas, les insectes phytophages d'une même lignée peuvent se spécialiser sur des plantes hôtes chimiquement proches, entraînant la plupart du temps une diversification sur des plantes-hôtes également phylogénétiquement proches. Néanmoins, les processus de diversification dérogent parfois à la règle du conservatisme taxonomique, lorsqu'une lignée opère un « saut » sur d'autres plantes-hôtes phylogénétiquement éloignées, mais chimiquement proches (Ehrlich et Raven 1964, Becerra 1997, Termonia *et al.* 2001).

L'interaction que les insectes phytophages entretiennent avec leurs plantes--hôtes ne joue pas uniquement un rôle sur les patrons évolutifs à long terme, mais également sur les processus évolutifs à plus petite échelle de temps, comme la génétique des populations. En effet, les processus de dérive et de migration dans les populations d'insectes sont intimement liés à l'abondance et à la dispersion des populations de plantes-hôtes. Ainsi, des patrons de co-structuration ont parfois été mis en évidence chez les différents partenaires d'une interaction (Demasters et Haffner 1993, Dybdhal et Lively 1996, Jerome et Ford 2002, Anderson *et al.* 2004). A cette échelle de temps, un troisième partenaire, l'homme, peut parfois également entrer en jeu, notamment dans le cas des insectes phytophages d'importance

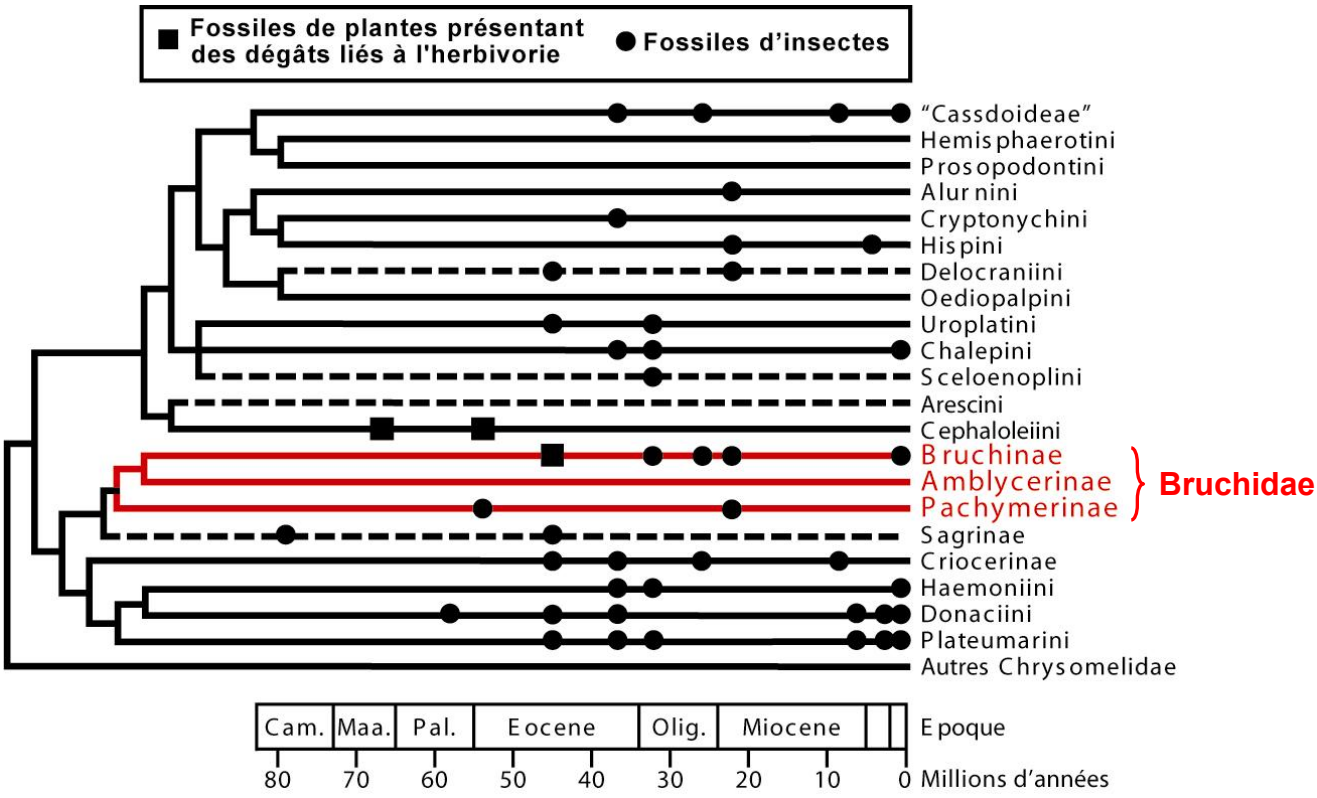

Figure 2 (d'après Wilf *et al.* [2000])

Phylogénie de certaines tribus et sous-familles de Chrysomelidae. Les fossiles d'insectes phytophages et de plantes présentant des dégâts d'herbivorie sont représentés sur le dendrogramme. Les époques géologiques sont abrégés comme suit : « Cam » : Campanien; « Maa » : Maastrichtien; « Pal » : Paleocène; « Olig » : Oligocène. Les deux dernières périodes (à droite de l'échelle) correspondent successivement au Pliocène et au Pleistocène.

économique. En effet, depuis l'apparition de l'agriculture, certaines espèces d'insectes phytophages spécialisées sur les ancêtres des plantes domestiquées sont devenues des ravageurs nuisibles. Les mêmes facteurs anthropiques agissent en premier lieu sur les populations de plantes domestiquées, puis indirectement sur les populations d'insectes phytophages ravageurs associés, en modifiant les paramètres d'abondance et de dispersion des plantes-hôtes. Par ailleurs, l'homme peut également jouer un rôle direct sur les populations d'insectes phytophages, en particulier au travers de toutes les pratiques agricoles visant à réduire la pression des ravageurs sur les cultures.

L'étude de ces interactions permet donc d'appréhender le rôle joué par les plantes-hôtes sur la diversité des insectes phytophages, à différentes échelles de temps. On peut se demander, par exemple, comment les plantes-hôtes peuvent-elles influencer les patrons de diversification de leurs insectes associés ? Ou encore, quelles sont leurs actions à une plus faible échelle de temps, lorsqu'elles interviennent au niveau des paramètres de génétique des populations des insectes ?

I.2. Modèle biologique

Parmi les neuf ordres d'insectes comprenant des espèces phytophages, les coléoptères présentent de loin la plus importante diversité. Les Phytophaga, un sous-ordre des Coléoptères, comprennent en effet plus de 100'000 espèces phytophages décrites (Farrell 1998). Parmi celles-ci, 1700 appartiennent à la famille des bruches (Chrysomeloidea : Bruchidae), famille caractérisée par une synapomorphie remarquable, la séminivorie obligatoire au stade larvaire (Borowiec 1987), et pour laquelle les premiers fossiles – ainsi que les fossiles de plantes présentant des dégâts liés à la séminivorie – datent de l'Eocène (il y a environ 50 millions d'années ; voir Figure 2). Leur relation très étroite aux semences passe le plus souvent par un comportement monophage ou oligophage, en raison de la grande spécificité des caractères physiques (structure et épaisseur de la testa) et chimiques (nature et concentration des composés secondaires) des espèces de plantes attaquées (Johnson 1981). En raison de cette étroite interaction, les bruches représentent un modèle idéal

pour étudier les relations entre les insectes phytophages et leurs plantes-hôtes. La présente étude s'attache donc à traiter des relations évolutives existant entre espèces de bruches et plantes-hôtes associées, à plusieurs échelles de temps, en nous focalisant sur un genre du Nouveau Monde, *Acanthoscelides* Schilsky, qui est un des plus importants – en termes de nombre d'espèces – parmi les Bruchidae. Il contient, en outre, certaines espèces spécialisées sur des Fabaceae d'importance économique, comme les espèces du groupe *A. obtectus*, qui sont spécialisées sur les haricots du genre *Phaseolus*.

Traditionnellement divisées en six sous-familles (Amblycerinae, Bruchinae, Eubaptinae, Kytorhininae, Pachymerinae, Rhaebinae), les bruches ont vu leur statut de famille remis en question par certaines phylogénies moléculaires récentes, qui suggèrent un statut de sous-famille au sein des Chrysomelidae (C. D. Johnson, communication personnelle). Néanmoins, au vu de l'importante homogénéité de leur écologie, le rang de famille reste le plus communément accepté par les taxonomistes. Les bruches se développent essentiellement sur des semences de Fabaceae (84% des espèces décrites), même si une minorité d'espèces exploitent également une trentaine d'autres familles botaniques – parmi lesquelles des Malvaceae, des Convolvulaceae ou encore des Arecaceae. Des six sous-familles de bruches, la plus riche spécifiquement est de loin celle des Bruchinae, qui comprend plus de 80% des espèces de bruches décrites (Johnson 1989). La richesse spécifique de cette sous-famille est majoritairement (à hauteur d'environ 700 espèces sur 1300) due aux deux genres *Acanthoscelides* et *Bruchidius*, les seuls à avoir été décrits par Schilsky (1905). Au vu de l'absence de caractères morphologiques discriminants valides entre ces deux genres, et du fait de leur probable non monophylie (Johnson 1981), certains auteurs ont suggéré la nécessité de circonscrire les unités taxonomiques valides au sein de ces deux genres et d'autres genres morphologiquement proches (par ex. *Merobruchus*, proche de *Acanthoscelides* et *Tuberculobruchus*, proche de *Bruchidius* ; Borowiec 1987). En effet, Schilsky a différencié les deux genres sur la base d'un unique critère biogéographique, plaçant les espèces de l'Ancien Monde dans le genre *Bruchidius*, et celles du Nouveau Monde dans le genre *Acanthoscelides*.

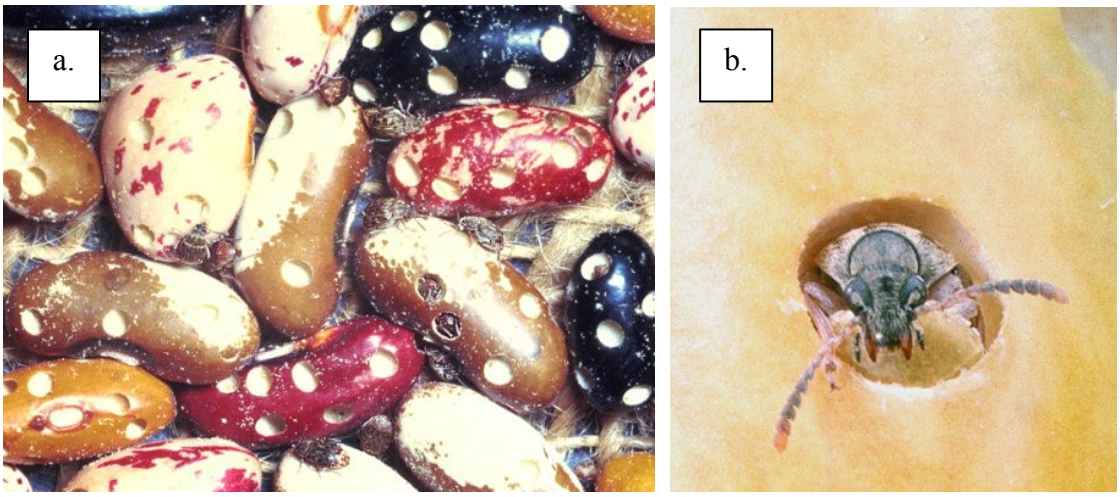

Figure 3

a. Grenier à grains de haricots infesté par *A. obtectus*.
b. Emergence d'un adulte de *A. obtectus*, peu après la mue imaginale de l'insecte.

Tableau 1.

Tableau des correspondances entre les espèces de bruches du groupe *A. obtectus* Say et leurs légumineuses associées. Les espèces de légumineuses suivies d'un « ? » doivent encore faire l'objet d'une vérification.

	Phaseolus	**Autres légumineuses**
A. obtectus Say	*P. vulgaris*, *P. coccineus*, *P. polyanthus*, *P. acutifolius*	*Pisum sativa*, *Lens esculenta*, *Vigna unguiculata*
A. obvelatus Bridwell	*P. vulgaris*, *P. coccineus*, *P. polyanthus*, *P. acutifolius*	-
A. argillaceus Sharp	*P. lunatus*	*Lablab purpurescens* ?
A. eriosemicola Johnson	-	*Eriosema* sp. ?
A. amplilobus Johnson	-	-

Les genres *Bruchidius* et *Acanthoscelides* ne présentent pas un nombre important de ravageurs économiques, à l'exception de quelques espèces dont *B. incarnatus* Boheman sur *Vicia faba* L. (Udayagiri et Wadhi 1989), et surtout des espèces du groupe *A. obtectus* Say sur des espèces de *Phaseolus* L.[2] (voir Johnson 1983, 1990) (voir Figure 3). D'origine néotropicale, les espèces du groupe *A. obtectus* Say sont spécialisées sur les plantes-hôtes de la sous-tribu des Phaseolinae (de la sous-famille des Faboideae). Parmi les cinq espèces décrites dans ce groupe, trois (*A. obtectus* Say, *A. obvelatus* Bridwell et *A. argillaceus* Sharp) sont inféodées aux haricots du genre *Phaseolus* et se développent aujourd'hui aussi bien sur des haricots sauvages que sur des haricots domestiqués. Ces trois espèces sont donc devenues des ravageurs du haricot : *A. obtectus* et *A. obvelatus* sont spécialisées sur les haricots du groupe *P. vulgaris* L. (qui comprennent notamment quatre des cinq espèces domestiquées de haricot, *P. vulgaris* L., *P. coccineus* L., *P. polyanthus* Greenman, *P. acutifolius* A. Gray.; voir Delgado-Salinas *et al.* 1999), tandis que *A. argillaceus* se développe exclusivement sur *P. lunatus* L., la cinquième espèce domestiquée du genre. Les deux autres espèces du groupe (*A. eriosemicola* Johnson et *A. amplilobus* Johnson) sont encore mal connues, et un important travail d'identification de leurs plantes-hôtes reste à réaliser (voir Tableau 1). Néanmoins, ces deux espèces sont très certainement inféodées uniquement à des espèces de plantes-hôtes qui n'ont pas été domestiquées (Johnson 1983, 1990).

I.3. Objectifs et moyens de l'étude

La grande diversité écologique des nombreuses espèces de Bruchinae et leur étroite relation à leurs plantes-hôtes rendent possible le test d'hypothèses évolutives à toutes les échelles de temps. Par ailleurs, l'existence d'un certain nombre d'espèces

[2] Les haricots du genre *Phaseolus* font partie des dix plantes les plus consommées sur la planète, et représentent la principale source d'azote dans de nombreuses régions du Tiers-Monde (Labeyrie 1981). Cinq espèces de haricot (*P. vulgaris* L., *P. coccineus* L., *P. lunatus P. polyanthus* Greenman et *P. acutifolius* A. Gray) ont été domestiquées dans le Nouveau Monde depuis le Néolithique. Pour chacune de ces espèces, il existe des formes sauvages et domestiquées qui peuvent coexister dans des environnements similaires. Alors que *P. vulgaris*, *P. coccineus* et *P. lunatus* sont fréquemment cultivées dans le monde entier, *P. polyanthus* et *P. acutifolius* semblent restreintes à des usages locaux au sein du Nouveau Monde.

d'importance économique – en particulier les espèces inféodées au haricot – permettent de mettre en évidence le rôle de l'homme sur l'évolution récente de ces espèces. Ainsi, cette étude traitera des points suivants.

1. Dans un premier temps, elle s'attachera à expliquer les patrons phylogénétiques des espèces du genre *Acanthoscelides* (obtenus à l'aide de marqueurs mitochondriaux), en fonction de facteurs liés aux plantes-hôtes et à la biogéographie. Au préalable, nous tenterons de circonscrire les genres *Acanthoscelides* et *Bruchidius* Schilsky, le genre frère de *Acanthoscelides*, et de déterminer les relations entre ces deux genres et d'autres genres apparentés de l'Ancien Monde et du Nouveau Monde (chapitre II).
2. Ensuite, elle se focalisera sur les espèces du groupe *A. obtectus*, afin de déterminer d'un point de vue morphologique et moléculaire si les différentes espèces associées à *Phaseolus* (*A. obtectus* Say, *A. obvelatus* Bridwell et *A. argillaceus*) représentent réellement des taxons différenciés (chapitre III).
3. Elle abordera par la suite la question de niche écologique des espèces jumelles du groupe *A. obtectus* – *A. obtectus* et *A. obvelatus* – afin de mettre en évidence des différences d'habitat entre ces espèces. Pour ce faire, nous utiliserons des approches écologiques (à l'aide de dénombrements sur le terrain) et de génétique des populations (chapitre IV).
4. Puis, l'origine de *A. obtectus* et son histoire biogéographique – en relation avec les flux migratoires induits par l'homme – feront l'objet d'une étude à l'aide de marqueurs mitochondriaux et microsatellites. Nous discuterons également de l'origine de chacune des deux espèces jumelles (chapitre V).
5. Enfin, l'effet des pratiques agricoles sur la génétique des populations de *A. obvelatus* sera plus précisément étudié à l'aide de marqueurs microsatellites à préciser. Nous comparerons en particulier les paramètres de génétique

des populations de bruches échantillonnées sur haricot sauvage, et ceux des populations échantillonnées sur haricot domestiqué (chapitre VI).

Pour répondre à ces questions, nous nous sommes livrés à une collecte d'individus qui a eu lieu essentiellement au Mexique, où *A. obtectus* et *A. obvelatus* sont présentes sur l'Altiplano, et où *A. argillaceus* est présente dans les régions de plus basse altitude, en particulier sur les côtes pacifique et atlantique. Ces espèces sont présentes aussi bien sur les types sauvages que sur les types domestiqués de haricot. L'échantillonnage des individus a été majoritairement réalisé à partir de la collecte de graines de haricot, desquels ont émergé les adultes de bruches, une fois leur développement larvaire à l'intérieur des semences terminé. Les individus de *A. obtectus* provenant d'autres régions biogéographiques (Pérou, Cameroun, France, Suisse, Espagne) ont été obtenus grâce à des collaborations extérieures. Enfin, les autres espèces de bruches étudiées proviennent pour la plupart de collections personnelles (C. D. Johnson, Texas A&M University, USA ; J. Romero Napoles, Colegio de Postgraduados de Texcoco, Mexico ; K.-W. Anton, Emmendingen, Allemagne) et du Muséum National d'Histoire Naturelle (Paris, France).

II. PROCESSUS DE DIVERSIFICATION ET PATRONS PHYLOGENETIQUES AU SEIN DU GENRE *ACANTHOSCELIDES*

La morphologie des espèces du genre néotropical *Acanthoscelides* a été étudiée par de nombreux taxonomistes, tels que J. M. Kingsolver (1968) et C. D. Johnson (1983, 1990). Bien que les espèces de ce genre présentent certains caractères en commun (notamment un nombre relativement constant d'épines fémorales, et des genitalia mâles particuliers [Borowiec 1987]), de nombreux auteurs ont suggéré la possible nature paraphylétique du genre (Johnson 1981, Borowiec 1987). En effet, il semble que les taxonomistes aient considéré comme un nouvel *Acanthoscelides* toute nouvelle espèce de Bruchinae néotropicale ne pouvant être classée dans aucun des 20 autres genres neotropicaux (Borowiec 1987). Par ailleurs, certains *Acanthoscelides* ne peuvent parfois pas être différenciés d'autres espèces du genre frère *Bruchidius* (distribué dans l'Ancien Monde uniquement), sur la base de caractères morphologiques. Pour les différencier, Borowiec (1987) a proposé que la différence-clé entre *Acanthoscelides* et *Bruchidius* soit de nature biogéographique, *Acanthoscelides* étant inféodé au Nouveau Monde, et *Bruchidius* à l'Ancien Monde. Pourtant, certains auteurs ont décrit des espèces d'*Acanthoscelides* eurasiatiques (Lukjanovitsch et Ter-Minassian 1957). Même si la plupart de ces espèces ont aujourd'hui été reclassées dans d'autres genres parmi lesquels *Bruchidius* (voir Egorov et Ter-Minassian 1981 ; Egorov 1990), il reste encore aujourd'hui trois espèces paléarctiques d'*Acanthoscelides*, dont le statut doit être vérifié. Si on admet la réalité biologique de ces genres et qu'on s'en tient au critère biogéographique de la clé de Borowiec (1987), tout laisse à penser que les genres *Acanthoscelides* (à l'exception des trois espèces décrites dans le Paléarctique) et *Bruchidius* ont divergé depuis que le Nouveau Monde et l'Ancien Monde se sont séparés sous l'effet des mouvements tectoniques. Deux événements géologiques pourraient expliquer leur séparation actuelle : soit la rupture du Gondwana par l'ouverture de l'Atlantique, il y a 90 millions d'années, soit la déconnection des ponts béringiens entre le Paléarctique oriental et le Néarctique occidental, il y a 35 millions d'années (Sanmartin 2001).

Le genre *Acanthoscelides*, avec 300 espèces décrites (voir Annexe du **Manuscrit I**), est l'un des genres de bruches qui présente le plus grand nombre d'espèces. La plupart des espèces décrites sont monophages ou oligophages (Johnson 1983, 1990). Parmi les espèces pour lesquelles une plante-hôte associée a été déterminée (soit environ 200 espèces), plus des trois quarts sont inféodées à 18 tribus de Fabaceae, essentiellement des Faboideae. Le quart restant se partage les plantes-hôtes de six autres familles, les Cistaceae, les Lythraceae, les Onagraceae, les Rhamnaceae, les Verbenaceae, et surtout les Malvaceae, sur lesquelles se développent une quarantaine d'espèces. De son côté, le genre *Bruchidius* présente un spectre d'hôtes proches, car il se développe sur 18 tribus de Fabaceae, ainsi que sur des Apiaceae et des Cistaceae. L'adaptation aux plantes-hôtes au travers d'innovations-clés a certainement été une force évolutive majeure au sein des Bruchinae, comme l'atteste leur capacité à se développer sur une importante diversité d'espèces végétales. La diversité des plantes-hôtes sur lesquelles se développent ces espèces, ainsi que la spécificité de leur relation, pourraient attester de processus de radiations liés à des groupes particuliers de plantes-hôtes.

Aussi bien les paramètres biogéographiques que les facteurs liés à la plante-hôte semblent donc pouvoir expliquer le patron phylogénétique des espèces des genres *Acanthoscelides* et *Bruchidius*. Pour vérifier cette hypothèse, nous avons reconstruit la phylogénie d'un échantillon d'espèces de chacun des deux genres (voir **Manuscrits I & II**). Nous avons séquencé différentes portions du génome mitochondrial (*12s rRNA*, *COI*, *cytb*) de 32 espèces de Bruchinae (**Manuscrit I**), avant d'élargir notre étude à 86 espèces (**Manuscrit II**). En tout, nous avons considéré 27 *Acanthoscelides* (dont 26 espèces néotropicales, et une espèce paléarctique, *A. plagiatus*) et 42 *Bruchidius*, mais aussi des représentants d'autres genres de Bruchinae, en nombres variables : *Algarobius* (1 espèce), *Gibbobruchus* (1), *Merobruchus* (1) et *Sennius* (1) qui proviennent du Nouveau Monde et *Callosobruchus* (4), *Conicobruchus* (1), *Decellebruchus* (1), *Palaeoacanthoscelides* (1), et *Tuberculobruchus* (2) qui sont originaires de l'Ancien Monde. Les reconstructions par des méthodes de maximum de vraisemblance et de parcimonie (**Manuscrit I**), puis par des méthodes bayésiennes

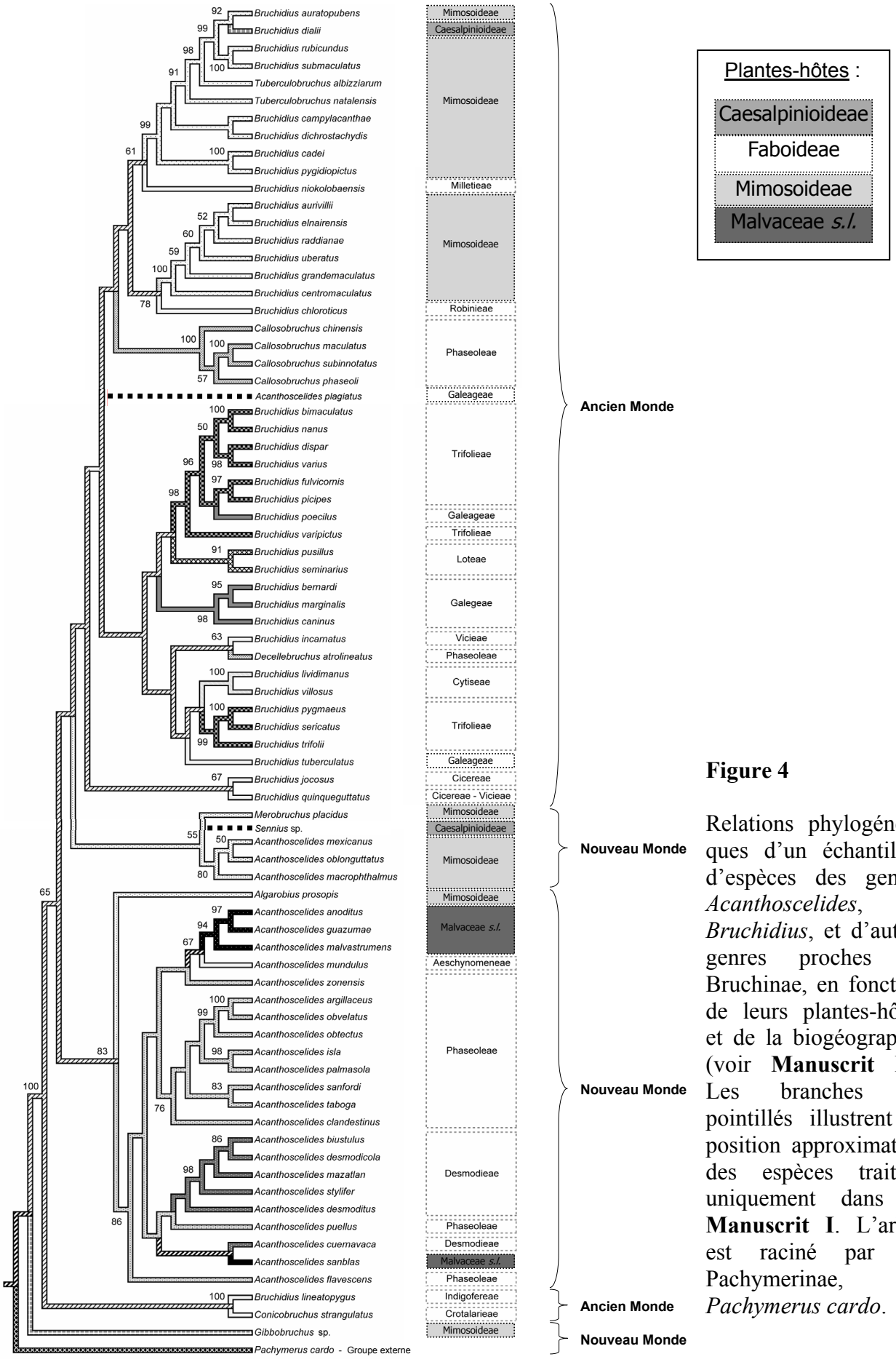

Figure 4

Relations phylogénétiques d'un échantillon d'espèces des genres *Acanthoscelides*, *Bruchidius*, et d'autres genres proches de Bruchinae, en fonction de leurs plantes-hôtes et de la biogéographie (voir **Manuscrit II**). Les branches en pointillés illustrent la position approximative des espèces traitées uniquement dans le **Manuscrit I**. L'arbre est raciné par un Pachymerinae, *Pachymerus cardo*.

(**Manuscrit II**), ont généralement donné des résultats congruents. A l'exception d'une minorité d'espèces dont nous discuterons le statut par la suite, les espèces du genre *Acanthoscelides* forment un groupe monophylétique (voir figure 4). Les espèces du genre *Bruchidius* sont quant à elles plutôt paraphylétiques, mais semblent former un groupe monophylétique avec les autres genres de l'Ancien Monde. Nous allons maintenant discuter des patrons phylogénétiques obtenus à l'échelle biogéographique et à l'échelle des radiations des branches terminales, en nous focalisant sur les espèces du genre *Acanthoscelides*.

II.1. Relations entre patrons phylogénétiques et biogéographie

La reconstruction phylogénétique consensus (voir Figure 4) montre une divergence globale ancienne, tous genres confondus, entre les espèces du Nouveau Monde et de l'Ancien Monde. Cette divergence est vraisemblablement le résultat de l'ouverture de l'Atlantique ou de la déconnection des ponts béringiens. D'un côté, on observe la quasi-totalité des espèces de *Bruchidius* (à l'exception de *B. lineatopygus*), les espèces des genres *Callosobruchus*, *Tuberculobruchus* et *Decellobruchus*, ainsi que *Acanthoscelides plagiatus*[3], et de l'autre côté, on retrouve 23 des 26 *Acanthoscelides* néotropicaux, ainsi que *Algarobius prosopis* (voir figure 4). Ces deux clades sont ancrés par un groupe hétérogène de trois espèces comprenant *B. lineatopygus*, *Conicobruchus strangulatus* et *Gibbobruchus* sp. Néanmoins, un petit clade comprenant cinq espèces néotropicales (trois *Acanthoscelides* – *A. macrophthalamus*, *A. mexicanus*, *A. oblongoguttatus* – ainsi que *Merobruchus plagiatus* et *Sennius* sp.) se positionne de façon inattendue. En effet, les espèces qui le composent font partie (analyse par maximum de vraisemblance) ou sont immédiatement à proximité (analyse bayésienne) du clade regroupant les espèces de l'Ancien Monde. La position inattendue de ce groupe néotropical, constitué de 5 espèces de trois genres différents se développant toutes sur Mimosoideae ou Caesalpinioideae, peut être expliquée soit par une hypothèse de vicariance, soit par une hypothèse de colonisation.

[3] Les *Acanthoscelides* de l'Ancien Monde semblent donc avoir été mal classifiés.

Selon l'hypothèse de vicariance, la divergence entre ce clade et les espèces de l'Ancien Monde serait antérieure à la dislocation du Gondwana (ou du moins antérieure à la rupture des ponts béringiens). Selon l'hypothèse de colonisation, ce clade serait issu de migrants de l'Ancien Monde, qui auraient atteint le Nouveau Monde après la dislocation du Gondwana (ou après la rupture des ponts béringiens). Cette colonisation d'un continent à l'autre pourrait s'être faite par migration aérienne (au travers de tempêtes ou de courants ascendants comme cela a été montré chez les papillons Monarques [Zalucki et Clarke 2004]) ou par une migration au travers des océans, surtout à des stades suivant de peu la rupture des connexions continentales. Une telle migration pourrait avoir par exemple emprunté des radeaux formés de troncs et d'autres matières organiques, comme décrit pour les rongeurs caviomorphes (Huchon et Douzery 2001) ou le genre de Clusiaceae *Symphonia* (Dick *et al.* 2003). Elle aurait aussi pu avoir lieu lors de leur stade larvaire séminivore, dans des graines en dérive, emportées par les courants marins.

II.2. Evolution parallèle entre Bruchinae du Nouveau et de l'Ancien Monde

D'une manière générale, notre étude phylogénétique montre une évolution parallèle entre espèces de l'Ancien Monde et du Nouveau Monde. Les mêmes tribus de plantes-hôtes (lorsque celles-ci ont une distribution transcontinentale) y sont exploitées par différentes espèces de Bruchinae, même s'il existe quelques exceptions (comme l'exploitation des Malvaceae par *Acanthoscelides* et des Apiaceae par *Bruchidius*). Cette colonisation indépendante des mêmes plantes-hôtes par ces différentes espèces de Bruchinae – de l'Ancien et du Nouveau Monde – est probablement due à des adaptations similaires (notamment aux défenses de leurs plantes-hôtes) dans leurs différentes aires de répartition. La colonisation d'environnements similaires par des groupes frères est observée chez les insectes phytophages, aussi bien au niveau infra-spécifique (par ex. chez différentes populations de phasmes de l'espèce *Timema cristinae* [Nosil *et al.* 2002]), qu'au niveau supra-spécifique (cf. notre étude). Les causes de telles radiations parallèles peuvent être expliquées par des prédispositions écologiques et génétiques. Dans notre étude,

les bruches démontrent le potentiel à se développer sur la grande majorité des semences qui présentent une testa dure. Elles peuvent donc théoriquement coloniser toutes les espèces qui présentent une telle caractéristique. Néanmoins, il existe certaines limites à cette colonisation. Par exemple, aucune des 130 espèces du genre pantropical *Erythrina* (Phaseoleae) – qui contiennent différentes érythroïdines toxiques [Neill 1993] – n'est attaquée par des bruches, alors que les espèces d'autres genres pantropicaux de Phaseoleae phylogénétiquement proches (comme *Teramnus* ou *Vigna*) sont consommées aussi bien par des espèces d'*Acanthoscelides* que de *Bruchidius* (Udayagiri et Wadhi 1989). Ainsi, on peut dire que les Bruchinae de l'Ancien et du Nouveau Monde ont colonisé l' « ensemble des possibles », en termes d'environnements physico-chimiques des plantes-hôtes. Cet « ensemble des possibles » semble donc lié à leur potentiel limité de détoxification, qui les empêche de coloniser certains groupes de plantes-hôtes contenant des composés toxiques particuliers, et qui les restreint à un nombre limité d'hôtes. Ceci explique probablement l'évolution parallèle observée chez *Acanthoscelides* et *Bruchidius*, dont les espèces ont exploré l' « ensemble des possibles » et colonisé les mêmes types de plantes-hôtes (ou presque) dans le Nouveau Monde et l'Ancien Monde respectivement.

On peut penser que des processus identiques de contraintes phylogénétiques jouent un rôle au niveau de la diversification écologique des Bruchidae. Les espèces de Bruchidae peuvent en effet coloniser toutes les espèces de plantes-hôtes dont elles peuvent potentiellement détoxifier les composés secondaires, mais pas celles produisant des composés secondaires qu'elles ne savent pas détoxifier, du moins, pas tant qu'une adaptation-clé spécifique n'aura pas évolué.

II.3. Radiations terminales et conservatisme taxonomique de l'association à la plante-hôte

En ce qui concerne les relations évolutives récentes entre plantes-hôtes et Bruchinae, le conservatisme taxonomique de la plante-hôte est le schéma le plus

souvent observé : les espèces de bruches phylogénétiquement proches se développent globalement sur des plantes-hôtes également phylogénétiquement proches. Les petites radiations caractéristiques des branches terminales (voir Figure 4) attestent probablement du fait qu'une adaptation-clé à une défense particulière d'une plante-hôte a permis à une lignée de bruches d'exploiter de nouvelles ressources (jusque-là non utilisées), et d'évoluer vers des processus de diversification grâce à une spécialisation plus fine encore. Un aspect particulièrement intéressant de notre étude est qu'au moins deux radiations terminales – d'une part dans un groupe d'espèces du Nouveau Monde et d'autre part dans un groupe d'espèces de l'Ancien Monde – concernent des espèces de bruches de genres différents, mais se développant sur un même groupe de plantes-hôtes. Ces deux groupes de Bruchinae sont :

- *Acanthoscelides mexicanus*, *A. macrophtalamus*, *A. oblongoguttatus*, *Merobruchus placidus* et *Sennius* sp., qui se développent sur Mimosoideae (ou Caesalpinioideae dans le cas de *Sennius* sp.).
- les deux espèces de *Tuberculobruchus* ainsi que huit espèces de *Bruchidius*, qui se développent également sur Mimosoideae (ou Caesalpinioideae dans le cas de *B. dialli*).

Il est intéressant de relever que dans ces deux groupes, l'adaptation aux espèces de Mimosoideae (ou de Caesalpinioideae) semble avoir entraîné une variation morphologique extrêmement importante. Ainsi, les espèces qui composent ces groupes sont si différentes morphologiquement que les taxonomistes les ont définies dans différents genres.

D'autres groupes d'espèces d'*Acanthoscelides* présentent également un conservatisme taxonomique en termes de plantes-hôtes. C'est notamment le cas des espèces se développant sur la tribu des Phaseoleae, qui forment des groupes monophylétiques au niveau du genre de plante-hôte. Selon les différentes méthodes de reconstruction phylogénétique utilisées, ce conservatisme est néanmoins plus ou moins strict. Les groupes monophylétiques les plus remarquables se développant sur

un même genre de Phaseoleae *sensu lato* (en incluant les *Desmodieae* ; voir Wink *et al.* 2003) sont les suivants :

- *A. argillaceus*, *A. obtectus* et *A. obvelatus* sur *Phaseolus* spp. (groupe monophylétique quelle que soit la méthode de reconstruction phylogénétique utilisée) ;
- *A. biustulus*, *A. desmodicola*, *A. mazatlan* et *A. stylifer* sur *Desmodium* spp. (groupe monophylétique quelle que soit la méthode de reconstruction phylogénétique utilisée) ;
- *A. flavescens*, *A. isla*, *A. palmasola*, *A. sanfordi* sur *Rhynchosia* spp. (groupe monophylétique selon la méthode de reconstruction phylogénétique par maximum de vraisemblance).

La monophylie de groupes d'espèces à l'échelle des tribus de Fabaceae est également le plus souvent respectée, que ce soit chez les espèces de l'Ancien Monde ou du Nouveau Monde. Outre les tribus de Fabaceae, un conservatisme taxonomique strict s'observe aussi chez les *Acanthoscelides* se développant sur des Malvaceae (autres que les Tilioïdeae).

Pourtant, dans certains cas, le conservatisme taxonomique n'est pas respecté, et les patrons phylogénétiques reflètent des événements de « sauts » d'un type de plante-hôte à un autre. C'est notamment le cas des espèces qui se développent sur Caesalpinioideae, qui aussi bien chez *Acanthoscelides* que chez *Bruchidius*, semblent s'être adaptées à un nouvel hôte à partir d'un ancêtre se développant sur Mimosoideae. De tels « sauts » peuvent avoir lieu entre des plantes-hôtes taxonomiquement éloignées mais chimiquement proches (Ehrlich et Raven 1964, Futuyma et McCafferty 1990, Becerra 1997, Termonia *et al.* 2001), ou au contraire résulter d'innovations-clés, qui permettent le passage vers un autre hôte taxonomiquement, mais aussi chimiquement, éloigné (Mardulyn *et al.* 1997). C'est probablement le cas pour les lignées ayant opéré un tel saut des Mimosoideae vers les Caesalpinioideae, car ces deux sous-familles de Fabaceae sont connues pour leurs

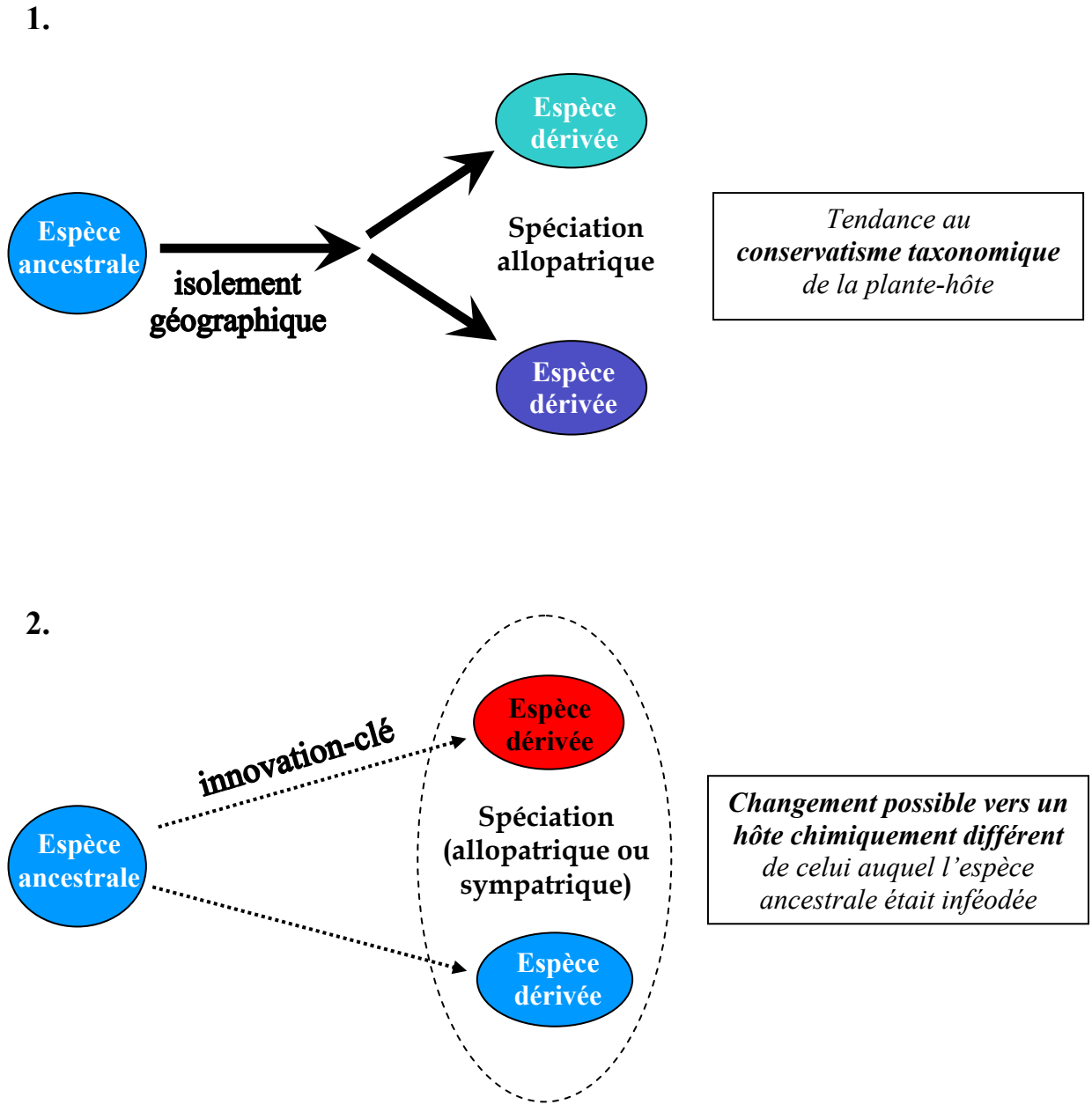

Figure 5

Représentation des deux schémas généraux de spéciation, rencontrés chez les différentes espèces de Bruchinae étudiées. Le schéma #1 est plus fréquent que le schéma #2.

différences de composés secondaires (Hegnauer 1994, Hegnauer et Hegnauer 1996, 2001).

Ainsi, pour les espèces d'insectes phytophages étudiées ici, les processus de diversification peuvent être :

- liés à un conservatisme taxonomique (et probablement chimique) des plantes-hôtes ;
- associés à de rares changements d'hôtes (vers des hôtes chimiquement, et souvent taxonomiquement, différents).

Ces deux schémas correspondent à deux facettes complémentaires du rôle de l'écologie dans les processus de spéciation. Dans le premier schéma – le plus fréquemment observé – on observe un conservatisme de niche au niveau phylogénétique : les lignées divergent en allopatrie (par vicariance ou par dispersion) et ne démontrent pas d'adaptation locale, la niche ancestrale étant conservée chez chacune des lignées (Webb *et al.* 2002). Dans un tel cas, différents facteurs tels que le manque de variabilité génétique, les flux de gènes ou la pléïotropie empêchent chacune des lignées de s'adapter différentiellement à leur nouvel environnement (Wiens 2004). Ainsi, les lignées divergentes de phytophages restent associées à des espèces de plantes-hôtes chimiquement (et donc le plus souvent taxonomiquement) proches. Dans le second schéma, c'est au contraire l'adaptation locale à un nouvel environnement (en allopatrie ou en sympatrie) qui se révèle le moteur de la spéciation : la sélection divergente (sur des traits phénotypiques) entre populations, dans des habitats contrastés, conduit de façon directe ou indirecte à l'évolution d'un isolement reproducteur (Schluter 2000, Via 2002) (voir Figure 5).

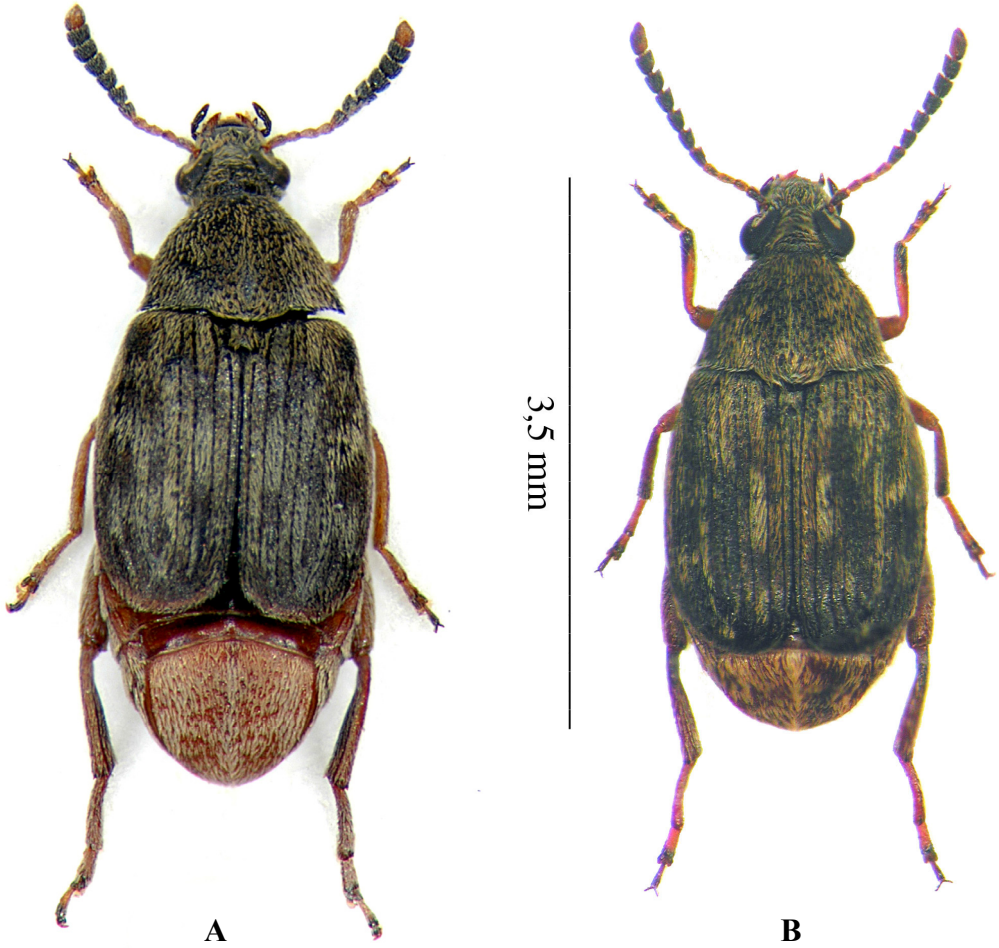

Figure 6

Habitus de *A. obtectus* (A) et de *A. obvelatus* (B).
Note: l'espace situé entre les élytres et le pygidium de A. obtectus *est artéfactuel et ne doit pas être pris en consideration.*

III. STATUT DES ESPECES SPECIALISEES SUR LES HARICOTS DU GENRE *PHASEOLUS*

Afin d'explorer à un niveau de détail plus important le rôle de la plante-hôte sur l'évolution des *Acanthoscelides* associés, nous avons focalisé notre étude sur les espèces associées au genre *Phaseolus* : *A. argillaceus*, *A. obtectus* et *A. obvelatus*. Ces trois espèces d'*Acanthoscelides* présentent un nombre très important de ressemblances morphologiques et écologiques – d'où leur appartenance au même groupe *A. obtectus* (Johnson 1983, 1991) (voir Tableau 1) – qui laissent supposer leur proximité phylogénétique. Alors que *A. argillaceus* présente une coloration générale rouge vif, *A. obtectus* et *A. obvelatus* démontrent une pigmentation beaucoup plus foncée, tendant vers le brun-gris. *Acanthoscelides obtectus* et *A. obvelatus* sont très difficilement différenciables d'un point de vue morphologique (voir Figure 6), puisque seules des variations de leur coloration (au niveau du pygidium, du fémur et des antennes), de la forme des antennes, ainsi que de la forme des genitalia pour les mâles, permettent de les distinguer. Elles ont par ailleurs souvent été confondues dans la littérature portant sur l'écologie ou la génétique de la bruche du haricot, d'autant qu'elles partagent parfois le même habitat. Cette confusion perdure jusqu'à la fin des années 1970 (Huignard et Biémont 1978), avant que leur statut d'espèces jumelles ne soit finalement reconnu (Leroi *et al.* 1990). Ces deux espèces semblent néanmoins se différencier par un trait écologique majeur, le voltinisme (ou le nombre de générations par année). Alors que *A. obvelatus*, comme *A. argillaceus*, est univoltine, *A. obtectus* démontre un multivoltinisme obligatoire, qui lui a permis de devenir un ravageur cosmopolite, comme nous le verrons. En effet, de nos jours, alors que *A. obvelatus* et *A. argillaceus* sont toujours restreintes à une distribution mesoaméricaine, *A. obtectus* a colonisé d'autres continents, et est capable de se développer sur d'autres plantes-hôtes domestiquées, telles que *Vigna unguiculata* (L.) Walp., *Pisum sativum* L. ou *Lens esculenta* Moench (Jarry et Bonet 1982). Jusqu'à présent, le statut des deux espèces jumelles *A. obtectus* et *A. obvelatus* est toutefois resté obscur : alors qu'une étude assez récente menée à l'aide de marqueurs allozymiques a mis en évidence une

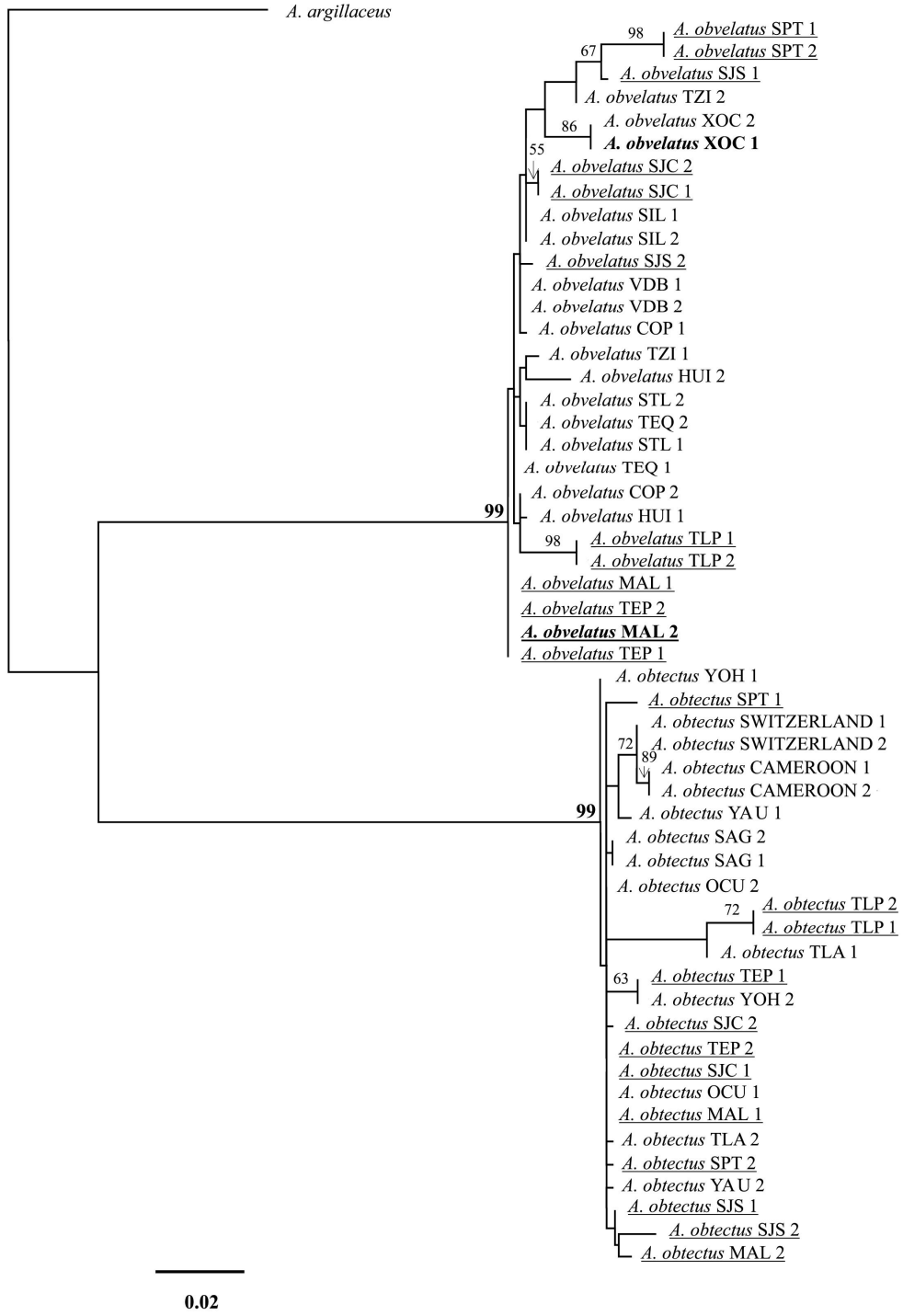

Figure 7

Relations phylogénétiques entre individus des espèces *A. obtectus* et *A. obvelatus* (basées sur *COI*). Les individus soulignés proviennent de sites où les deux espèces sont sympatriques. Les individus en gras démontrent des malformations antennaires, mais présentent des genitalia de type *A. obvelatus*. L'arbre est raciné par *A. argillaceus*.

différenciation génétique entre les deux espèces (Gonzalez-Rodriguez *et al.* 2000), une autre étude plus ancienne (Biémont *et al.* 1986), a démontré la capacité de ces deux espèces à s'hybrider en laboratoire, mais seulement après traitement hormonal restaurant entre autres un synchronisme des fertilités. De plus, ces croisements ont démontré un faible taux de fécondité et une forte proportion de descendants présentant des malformations (notamment au niveau des antennes). Cette dernière étude a également postulé que la présence d'individus présentant des malformations antennaires sur le terrain, pouvait être un argument en faveur de l'hybridation *in natura* de ces deux espèces. Cette question se pose d'autant plus qu'on a observé des individus fertiles des deux espèces à la même période de l'année.

III.1. Relations phylogénétiques entre *A. obtectus* et *A. obvelatus*

Avant d'étudier le rôle de la plante-hôte sur l'évolution de ces espèces, le statut de *A. obtectus* et *A. obvelatus* doit donc être mieux connu. Ces deux espèces représentent-elles des lignées différenciées ? Existe-t-il des événements d'hybridation qui tendent à homogénéiser les pools génétiques des deux espèces ? Les deux espèces sont-elles en réalité une seule et même espèce, présentant un polymorphisme aussi bien écologique (voltinisme) que morphologique (couleur et forme des antennes) ? Pour répondre à ces questions, nous avons analysé différents gènes mitochondriaux (*COI*, *12s rRNA*) et nucléaire (*28s rRNA*) d'un échantillon d'individus des deux espèces (54 individus répartis dans 20 populations). L'étude des séquences obtenues montre sans ambiguïté la répartition en deux clades bien différenciés des individus de chacune des espèces, pour chacun des gènes analysés **(Manuscrit III)**. Quant aux individus présentant des antennes malformées, mais ayant des genitalia caractéristiques de *A. obvelatus*, ils se retrouvent tous dans le clade de cette espèce (voir figure 7). A l'aide d'une horloge moléculaire mise au point sur *Timarcha*, un autre genre de coléoptère de la famille des Chrysomelideae, très proche des Bruchidae (Gomez-Zurita *et al.* 2000), nous avons inféré la divergence nucléotidique (~15%) entre ces deux clades à 22 millions d'années. Evidemment, cette estimation ne saurait prétendre être précise, car les événements biogéographiques utilisés pour

calibrer l'horloge de *Timarcha* ne sont pas communs à *Acanthoscelides*. Néanmoins, cette estimation nous permet d'affirmer que les deux espèces sont différenciées depuis plusieurs millions d'années. Par ailleurs, une étude morphologique et génétique sur un plus grand échantillon (540 individus) – cette fois-ci à l'aide de marqueurs microsatellites spécifiques à chacune des deux espèces (voir **Manuscrits IX et X**) – a confirmé l'absence d'individus issus d'hybridation, et a mis en évidence la validité des caractères antennaires pour diagnostiquer les individus de l'une ou l'autre espèce. *Acanthoscelides obtectus* et *A. obvelatus* sont donc deux espèces distinctes au sens biologique. Par contre, les colorations d'autres parties de l'organisme se sont révélées être de mauvais prédicteurs de l'identité spécifique.

La validité du diagnostic obtenu par les traits antennaires confirme l'importance de tels caractères dans la reconnaissance intra-spécifique des individus. Les antennes des *Acanthoscelides* possèdent en effet des soies qui jouent un rôle dans l'interception des signaux phéromonaux (Halstead 1973). Ainsi, la reconnaissance de phéromones – sexuelles ou d'agrégation – spécifiques à l'une ou l'autre espèce est très probablement liée à des caractéristiques éco-physiologiques particulières, qui pourraient se refléter par des modifications d'ordre morphologique au niveau des antennes. On pourrait objecter que les coléoptères phytophages tendent à sécréter des phéromones chimiquement proches des composés volatiles émis par leur plante-hôte (Byers *et al.* 1979), ce qui dans le cas de *A. obtectus* et *A. obvelatus* ne devrait pas conduire à l'évolution vers des phéromones très différentes, puisque les deux espèces se développent sur les mêmes plantes. Néanmoins, certaines espèces jumelles (comme par ex. *Drosophila* spp. [Coyne et Charlesworth 1997]) parviennent à se reconnaître intra-spécifiquement (et à se différencier inter-spécifiquement) uniquement sur la base de différences infimes dans la structure de phéromones de la même famille chimique.

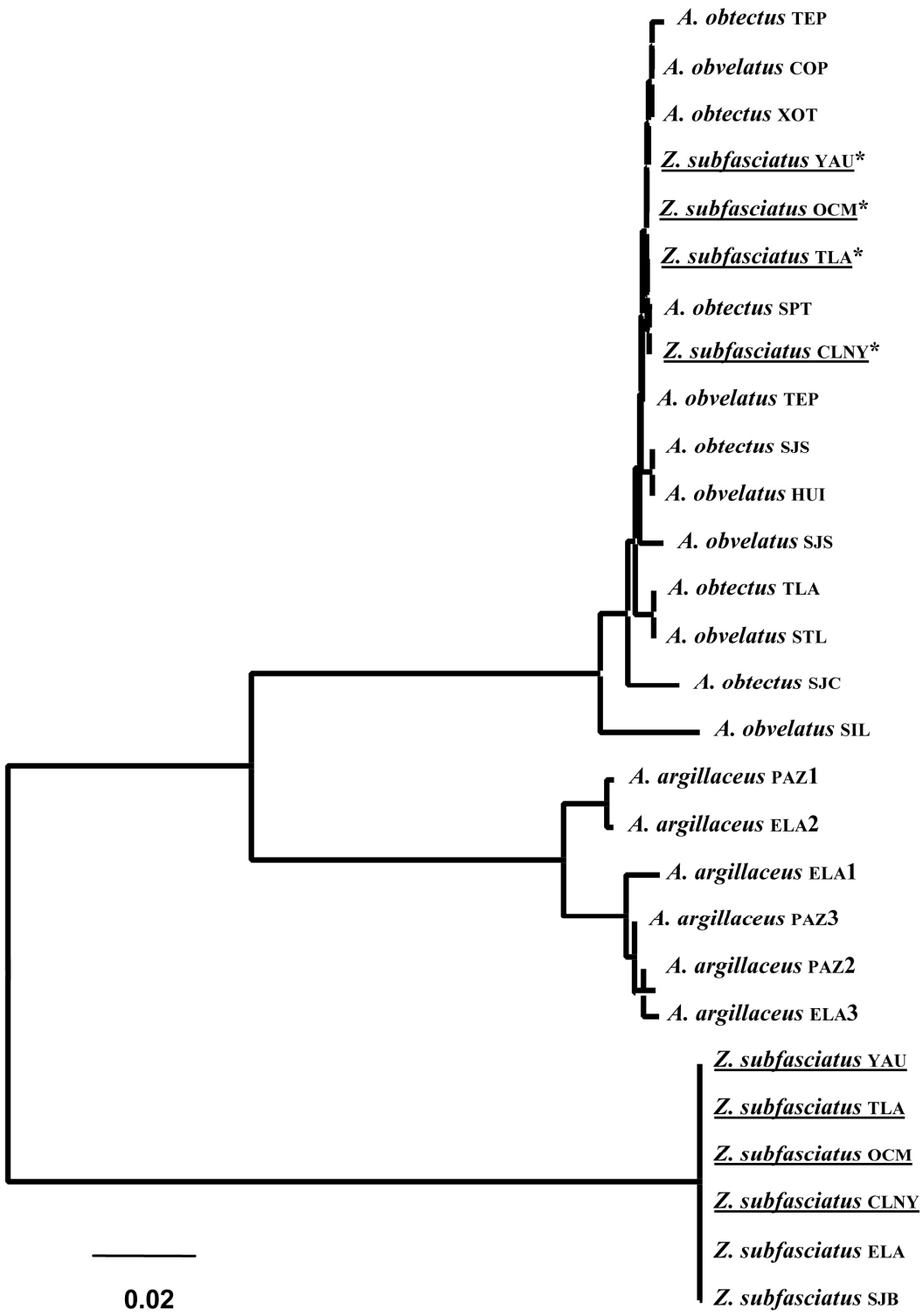

Figure 8

Relations phylogénétiques entre différents individus de *A. obtectus*, *A. obvelatus*, *A. argillaceus* et *Z. subfasciatus*, obtenues à l'aide du *cytb*. Les individus de *Z. subfasciatus* soulignés proviennent de l'Altiplano mexicain (où ils sont en sympatrie avec *A. obtectus* et *A. obvelatus*), et ceux suivis d'un astérisque correspondent à la séquence du *cytb* « étranger », similaire aux séquences du clade *A. obtectus* - *A. obvelatus*.

III.2. La patron atypique révélé par le gène mitochondrial *cytb*

L'examen d'un autre gène mitochondrial (*cytb*) a montré des résultats totalement contradictoires avec les patrons phylogénétiques obtenus pour tous les autres gènes. En effet, pour ce gène particulier, tous les individus de *A. obtectus* et *A. obvelatus* analysés se comportent comme s'ils appartenaient à la même espèce, et présentent des séquences très proches – voire identiques – entre individus d'espèces différentes (voir figure 8 ; **Manuscrit IV**). Au contraire, les individus de *A. argillaceus* se groupent tous à une distance attendue du clade regroupant les individus de *A. obtectus* et *A. obvelatus*, de la même façon que lors de l'analyse d'autres gènes. Un fait remarquable est que ce clade atypique comprend également certains individus d'une espèce d'Amblycerinae, *Zabrotes subfasciatus* Boheman, que nous avions initialement utilisée comme un groupe externe. Cette espèce est également présente au Mexique, où elle peut se développer aussi bien sur les haricots du groupe *P. vulgaris*, que sur *P. lunatus*. Ainsi, elle partage tantôt le même habitat que *A. obtectus* et *A. obvelatus*, tantôt le même habitat que *A. argillaceus*. Or, les seuls individus de *Z. subfasciatus* qui se positionnent dans le clade atypique, sont ceux prélevés sur l'Altiplano – sur des populations de haricot de *P. vulgaris* – où ils sont sympatriques avec *A. obtectus* et *A. obvelatus*. Au contraire, les individus de *Z. subfasciatus* provenant de la côte Pacifique – où ils se développent sur *P. lunatus* en sympatrie avec *A. argillaceus* – se sont positionnés à une distance considérable des autres individus, de la même façon qu'un groupe externe classique. Par la suite il s'est révélé que les *Z. subfasciatus* contenus dans le clade atypique présentaient en réalité également un autre *cytb* (mis en évidence avec d'autres paires d'amorces), qui les plaçait aux côtés des *Z. subfasciatus* de la côte Pacifique. Ainsi, les *Z. subfasciatus* analysés provenant de l'Altiplano possèdent deux *cytb*, qui diffèrent entre eux par plus de 20% de divergence nucléotidique. Or, ces deux gènes semblent être sous sélection et transcrits par une mitochondrie, comme l'attestent le très important nombre de mutations silencieuses (89.4 %) et l'absence de codons stops lorsque le gène est traduit avec le code génétique mitochondrial des invertébrés. Ce résultat est d'autant plus surprenant qu'il traite de différences considérables en ce qui concerne les

patrons phylogénétiques obtenus avec deux gènes mitochondriaux différents (*COI* et *cytb*), deux gènes liés sur un même génome non-recombinant[4].

Le moyen emprunté par ce gène atypique – provenant vraisemblablement de *A. obtectus* ou *A. obvelatus* – pour arriver dans une espèce congénérique, ou aussi éloignée qu'un Amblycerinae, n'est certainement pas l'hybridation, comme l'attestent les résultats précédents sur le statut de *A. obtectus* et *A. obvelatus*, ainsi que l'incompatibilité évidente des genitalia de *Z. subfasciatus* avec celles des *Acanthoscelides*. En réalité, il semble que les patrons phylogénétiques obtenus ne puissent être expliqués que par l'occurrence de transferts horizontaux *via* un vecteur externe. Celui-ci pourrait être de nature virale, bactérienne ou eucaryote, et être parasite ou symbionte. Les candidats à de tels transferts sont aujourd'hui encore peu connus. Néanmoins, des transferts horizontaux similaires ont été mis en évidence chez les angiospermes (Bergthorsson *et al.* 2003), chez les algues (Archibald 2003) ou encore chez les protistes (Andersson *et al.* 2003). Dans notre cas, il n'est pas exclu que le vecteur soit encore associé aux bruches, et que le *cytb* atypique provienne en fait de cet organisme exogène, qui aurait capturé le *cytb* d'un *Acanthoscelides*. Ce vecteur pourrait être de nature procaryote (comme les bactéries endosymbiotiques qui abondent chez les insectes [Buchner 1965]), ou alors de nature eucaryote (comme les différents protozoaires du tube digestif). Il est intéressant de relever que récemment, Kondo *et al.* (2002) ont mis en évidence un événement de recombinaison entre une *Wolbachia* symbiotique et son hôte, un Bruchinae du genre *Callosobruchus*. Le point essentiel de notre analyse reste le fait que ce transfert horizontal se produit entre des espèces démontrant une proximité écologique, et non phylogénétique.

[4] La possible recombinaison de la mitochondrie animale est un sujet actuellement débattu et très controversé (Awadella *et al.* 1999, Ladoukakis et Zouros 2001, Maynard-Smith et Smith 2002, Rokas *et al.* 2003). En revanche, la recombinaison est fréquente chez les eumycètes, le groupe frère des animaux (Saville *et al.* 1998).

IV. DIFFERENCES DE DISTRIBUTION ECOLOGIQUE ENTRE LES ESPECES JUMELLES *A. OBTECTUS* ET *A. OBVELATUS*

Acanthoscelides obtectus et *A. obvelatus* représentent deux entités génétiques distinctes qui ne s'hybrident pas. Néanmoins, leur coexistence en sympatrie – des individus des deux espèces peuvent émerger de la même gousse, voire de la même graine de haricot – pose le problème de leur différenciation écologique. Ces deux espèces, qui démontrent une ressemblance morphologique frappante, semblent en effet partager la même niche écologique, ce qui est en contradiction avec le principe de différenciation nécessaire des niches écologiques, suggéré par Hutchinson (1957). Selon ce principe, deux espèces peuvent coexister si, et seulement si, certaines dimensions de leur niche diffèrent. En d'autres termes, si deux espèces présentent une niche écologique identique en tous points, la plus compétitive des deux exclura l'autre. Cependant, Hubbell (2001) a proposé une alternative au principe de Hutchinson (1957), au travers de la théorie neutre de la structure des communautés. Selon ce nouveau paradigme, deux espèces écologiquement identiques pourraient coexister transitoirement, sans que la différenciation de leur niche soit nécessaire, car les dynamiques d'exclusion ou de coexistence sont lentes pour des compétiteurs identiques (Zhang *et al.* 2004).

La coexistence de *A. obtectus* et *A. obvelatus* pourrait bien être expliquée par un tel paradigme. Cependant, il est possible que certains facteurs différencient les niches de ces deux espèces, malgré leur apparente similitude. Leroi *et al.* (1990) ont proposé que les deux espèces se différencient par l'espèce de plante-hôte à laquelle elles seraient associées, *A. obtectus* étant d'après eux mieux adapté à *P. vulgaris*, et *A. obvelatus* à *P. coccineus*. Ces deux espèces de haricot sont présentes en sympatrie sur l'Altiplano Mexicain. Néanmoins, elles se répartissent essentiellement selon un gradient altitudinal, *P. vulgaris* se développant jusqu'à 2200m, et *P. coccineus* ne poussant pas en dessous de 1750m. Ainsi, l'altitude est un facteur confondant de la plante-hôte, bien qu'à des altitudes intermédiaires (de 1750m à 2200m), les deux espèces de haricot peuvent coexister. Un autre argument en faveur de la ségrégation

des deux espèces sur des plantes-hôtes différentes – cette fois-ci en termes de type de plante-hôte (domestiqué *versus* sauvage) – résulte de la différence de voltinisme entre *A. obtectus* et *A. obvelatus*. En effet, le multivoltinisme de *A. obtectus* lui permet de se développer jusqu'à épuisement des ressources, ce qui la prédispose aux stocks de semences de haricot, et donc *a fortiori*, à être plutôt adaptée au haricot domestiqué. Au contraire, *A. obvelatus* présente une diapause reproductive obligatoire qui l'empêche de réaliser plus d'une génération par an. Ainsi, il semble que les individus de *A. obvelatus* soient parfaitement synchronisés à la phénologie des haricots sauvages, et on peut s'attendre à ce qu'ils s'y développent préférentiellement.

Quelle que soit l'hypothèse formulée, la différenciation de niche écologique entre *A. obtectus* et *A. obvelatus* en fonction de la plante-hôte pourrait faire appel à des processus d'adaptation locale, ou d'évolution de races d'hôtes, processus qui ont été mis en évidence dans de nombreux autres modèles biologiques (Caillaud et Via 2000, Nason *et al.* 2002, Pelozuelo *et al.* 2004). Ils sont également connus chez les parasitoïdes d'*Acanthoscelides*, dont au moins une espèce semble démontrer une préférence pour l'une ou l'autre espèce de haricot[5]. En réalité, une telle adaptation semble également attendue chez les bruches, qui, de par leur biologie en interaction étroite avec les semences des plantes-hôtes, les rendent très sensibles à toute modification des substances secondaires des graines de haricot. En effet, aussi bien au niveau de l'espèce (Calderon *et al.* 1992) que du type – domestiqué *versus* sauvage – (Smartt 1988), les haricots présentent d'importantes différences en termes de composés secondaires[6] qui pourraient aboutir à des processus d'adaptation et de différenciation chez les espèces qui leur sont inféodées (Benrey *et al.* 1998).

[5] Les parasitoïdes semblent dans le cas présent plus étroitement liés à l'espèce de plante-hôte qu'à l'espèce de bruche, probablement en raison du fait qu'ils repèrent la présence de bruches en étant attirés par les composés volatils de la plante-hôte (Aebi *et al.* in prep).

[6] Quelle que soit l'espèce considérée, les haricots sauvages se différencient notamment des haricots domestiqués par des concentrations bien plus élevées en arceline, en inhibiteurs de l'alpha-amylase, et en composés cyanogéniques (Smartt 1988). Quant à *P. coccineus* et *P. vulgaris*, ils se distinguent en particulier par des compositions différentes en acides aminés, *P. coccineus* étant plus riche en lysine et en méthionine (Calderon *et al.* 1992).

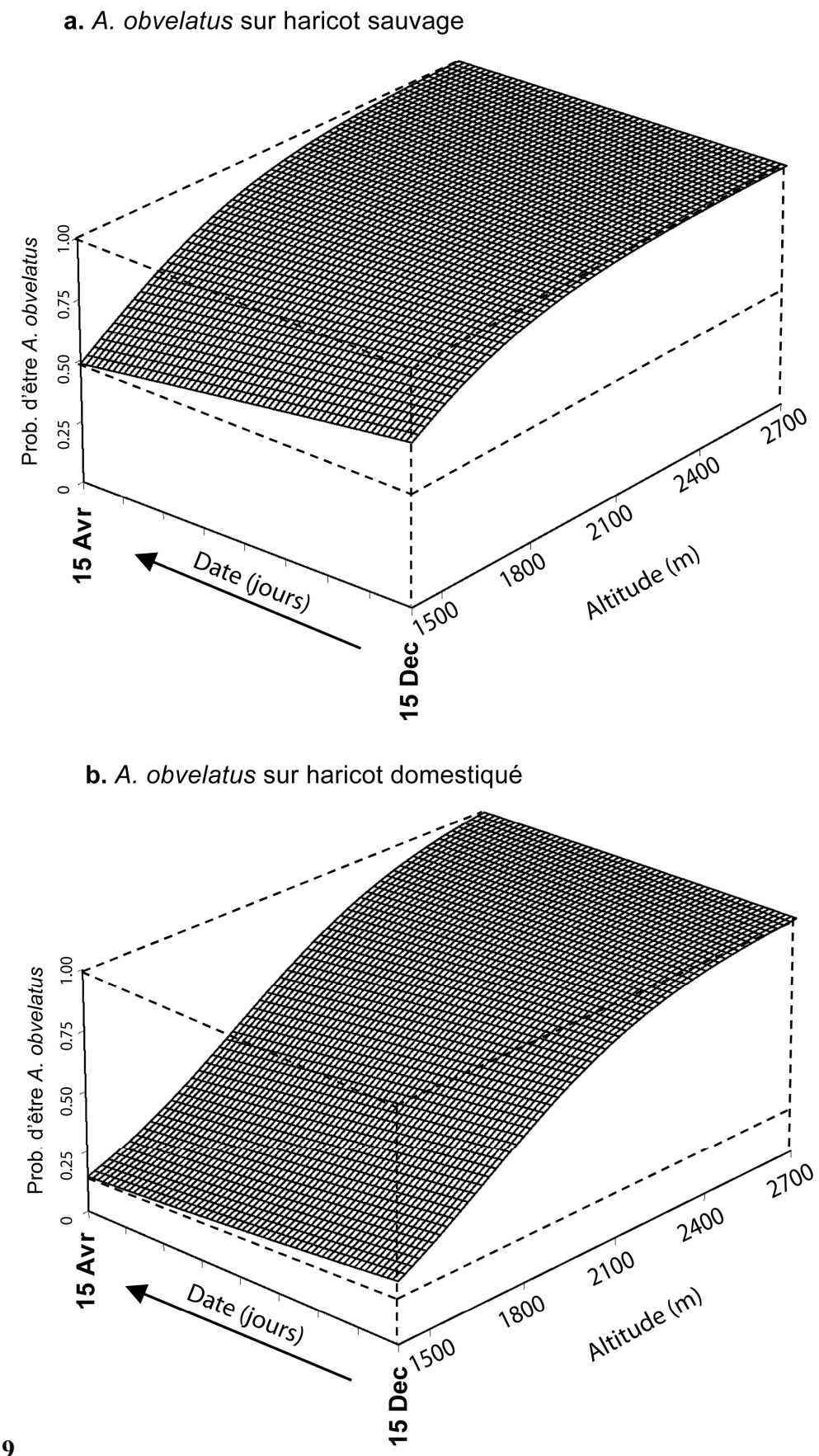

Figure 9

Distribution écologique de *A. obvelatus* (ou probabilité qu'une bruche échantillonnée dans les populations de haricot de l'Altiplano mexicain soit *A. obvelatus* [$P_{obvelatus}$]) en fonction du type de plante-hôte (sauvage [a] versus domestiqué [b]), de la date, et de l'altitude. La probabilité contraire (1-$P_{obvelatus}$) correspond à la distribution écologique de *A. obtectus*.

IV.1. Ségrégation des niches écologiques des deux espèces jumelles

Afin de déterminer si *A. obtectus* et *A. obvelatus* diffèrent effectivement par leur adaptation à l'espèce ou au type – domestiqué *versus* sauvage – de plante-hôte (ou par d'autres facteurs tels que l'altitude), nous avons échantillonné près de 30'000 individus des deux espèces entre décembre 2001 et mars 2002 sur l'Altiplano mexicain (**Manuscrit V**). Les résultats de l'analyse sur l'ensemble des individus montrent que les deux espèces ne semblent pas se différencier en fonction de l'espèce de haricot. Nous avons en effet échantillonné un certain nombre de bruches se développant sur des populations de haricot dans lesquelles coexistaient *P. vulgaris* et *P. coccineus*, et les fréquences relatives de l'une ou l'autre espèce jumelle de bruche ne diffèrent pas en fonction de l'espèce de haricot. Par contre, elles varient en fonction du type de haricot, de l'altitude, et de la date de prélèvement (voir figure 9) :

- l'altitude semble être le facteur qui ségrège le mieux les deux espèces, puisque dès l'altitude de 2200m, on ne retrouve plus que *A. obvelatus* – quel que soit le type de haricot – alors que *A. obtectus* devient de plus en plus fréquent à des altitudes inférieures ;
- à des altitudes inférieures, la fréquence de *A. obtectus* augmente en effet progressivement, et ce, d'autant plus si on considère les individus associés au haricot domestiqué, sur lequel *A. obtectus* devient majoritaire dès qu'on se trouve au-dessous de 1700m ; en revanche, sur haricot sauvage, la proportion de *A. obtectus* ne dépasse jamais 50%, même aux altitudes les plus basses de notre échantillonnage.
- la date de prélèvement influe également sur la proportion des deux espèces, *A. obtectus* devenant plus fréquente au fur et à mesure que la saison avance[7].

[7] Sur haricot sauvage, les premières bruches émergent dès le mois de décembre, lorsque les premières gousses viennent de mûrir. L'émergence prend fin en avril, lorsque s'achève la période de fructification du haricot. Sur haricot domestiqué, l'émergence continue tout au long de l'année, tant que des ressources sont disponibles.

Il n'est donc pas nécessaire d'invoquer la théorie de Hubbell pour expliquer la coexistence de ces deux espèces jumelles : leurs niches sont significativement différenciées. D'une manière générale, il est important de mettre l'accent sur le fait que *A. obvelatus* est l'espèce majoritaire dans presque tous nos échantillons, à l'exception de populations de haricot domestiqué de basse altitude. Il est également nécessaire de préciser ici que la différenciation de niche observée (en fonction de l'altitude, du type de haricot et de la date) n'est pas systématique quel que soit le site considéré. Ainsi, certains stocks de haricot domestiqué à des altitudes intermédiaires présentaient moins d'1% de *A. obtectus*, alors que certaines populations de haricot sauvage hébergeaient presque exclusivement des *A. obvelatus*. Cependant, on peut considérer que les niches écologiques des deux espèces sont significativement différentes. Le multivoltinisme de *A. obtectus* explique aisément pourquoi la fréquence de l'espèce est plus importante sur haricot domestiqué, et augmente au fil de la saison. En effet, alors que *A. obvelatus* ne se reproduit qu'une fois par an, *A. obtectus* se développe exponentiellement tout au long de l'année, ce qui en fait aujourd'hui un ravageur bien plus important que *A. obvelatus*. La ségrégation altitudinale des deux espèces peut également être en partie expliquée par leurs différences de voltinisme. L'univoltinisme de *A. obvelatus* est en effet associé à une diapause reproductive (et *a fortiori* un ralentissement métabolique) de plusieurs mois. A haute altitude plus encore qu'à basse altitude, l'univoltinisme permet donc aux individus de *A. obvelatus* de mieux passer la mauvaise saison. Au contraire, à plus basse altitude, l'univoltinisme n'est pas autant sélectionné car les conditions climatiques sont moins extrêmes au cours de la mauvaise saison. De surcroît, la proportion de haricot domestiqué – sur lequel il est avantageux de se reproduire continuellement – est bien plus importante en basse altitude[8]. Ainsi, le multivoltinisme de *A. obtectus* est plus adaptatif en basse altitude.

Néanmoins, les différences de voltinisme entre les deux espèces (et par là même également leur ségrégation altitudinale) ne semblent pas être issues de la compétition entre les deux espèces, mais plutôt de caractéristiques physiologiques

[8] L'altitude est donc un facteur confondant du type de haricot (sauvage versus domestiqué).

propres, acquises dans des environnements différents. Comme nous le verrons par la suite (V.1, V.2), *A. obtectus* et *A. obvelatus* semblent avoir divergé en allopatrie (**Manuscrit VII**).

La différence de voltinisme des deux espèces jumelles ne reste pas moins une distinction fondamentale qui pourrait entraîner la spécialisation de *A. obtectus* sur les stocks de haricot domestiqué (où les ressources sont constantes) et de *A. obvelatus* sur haricot sauvage :

- d'une part, les populations de haricot sauvage représentent des puits pour les individus de *A. obtectus*, qui sont incapables de rentrer en diapause reproductive et doivent impérativement se reproduire et pondre rapidement sur de nouveaux haricots ; à moins que certains d'entre eux ne parviennent à migrer transitoirement vers un stock de haricot domestiqué, les populations de *A. obtectus* s'éteignent donc systématiquement sur haricot sauvage, puisque les 8 mois par an, au cours desquels le haricot sauvage ne fructifie pas, représentent une durée bien supérieure à leur espérance de vie maximale (de l'ordre de 4 mois [Jarry 1984]) ;
- d'autre part, les individus de *A. obvelatus*, même s'ils peuvent se développer sur haricot domestiqué, ne peuvent pas rester dans les stocks de haricot ; ils rentrent en effet obligatoirement en diapause pour une durée de huit à dix mois (c'est-à-dire qu'ils sont incapables de se reproduire pendant ce laps de temps) et doivent rejoindre un environnement naturel, risquant au passage de se perdre ou d'être sujets à la prédation par d'autres animaux, afin de se nourrir du nectar de différentes plantes, ce qui est indispensable à leur survie jusqu'à la prochaine saison de fructification des haricots (Pichard *et al.* 1991) – en d'autres termes, *A. obvelatus* est maladapté aux populations de haricot domestiqué, qui représentent des puits pour ses individus.

Ainsi, alors que les populations de haricot sauvage représentent des « puits » pour *A. obtectus*, les populations de haricot domestiqué représentent des « puits » pour *A. obvelatus*. Chacune des deux espèces de bruches semble donc plus adaptée que l'autre à un type de haricot. Néanmoins, l'existence de flux bidirectionnels, chez chacune des deux espèces, entre populations « sources » et populations « puits » – bien que plus importants dans le sens « sources » → « puits » que dans le sens « puits» → « sources » – peut prévenir l'évolution d'adaptations spécifiques, comme le suggère Wiens (2004). Il est également possible que la capacité de chacune des deux espèces de bruches à se développer sur les deux types de haricot ne soit contre-sélectionnée à aucun moment, tant les densités de populations et les flux de migrants des sources vers les puits sont importantes.

Néanmoins, il serait intéressant d'étudier plus précisément les potentielles adaptations au haricot domestiqué acquises par les individus de *A. obtectus* évoluant sur les autres continents. Hors du Nouveau Monde, *A. obtectus* est uniquement connu pour être un ravageur des cultures, et n'a pas été décrit sur des plantes sauvages[9]. Dans un tel environnement, et au vu de l'absence de flux de gènes avec des conspécifiques ayant émergé de populations de plantes-hôtes sauvages, des adaptations particulières au haricot domestiqué pourraient se fixer dans les populations.

IV.2. Adaptation locale *versus* contre-sélection par les plantes-hôtes

Les différences de niches écologiques entre *A. obtectus* et *A. obvelatus* sont liées à différents facteurs biotiques et abiotiques. Néanmoins, les mêmes facteurs qui agissent sur la ségrégation écologique entre espèces peuvent également agir sur la différentiation des populations à l'intérieur d'une espèce, notamment *via* des processus d'adaptation locale (Kuussaari *et al.* 2000, Ballabeni *et al.* 2003, Ruiz-Montoya *et al.* 2003). Afin de déterminer si les populations de bruches de chaque espèce ne se différenciaient pas en races d'hôtes sur les deux espèces de haricot *P.*

[9] À l'exception d'un relevé anecdotique de *A. obtectus* sur plantes sauvages de *Vigna* spp. au Sénégal (M. Jarry, communication personnelle).

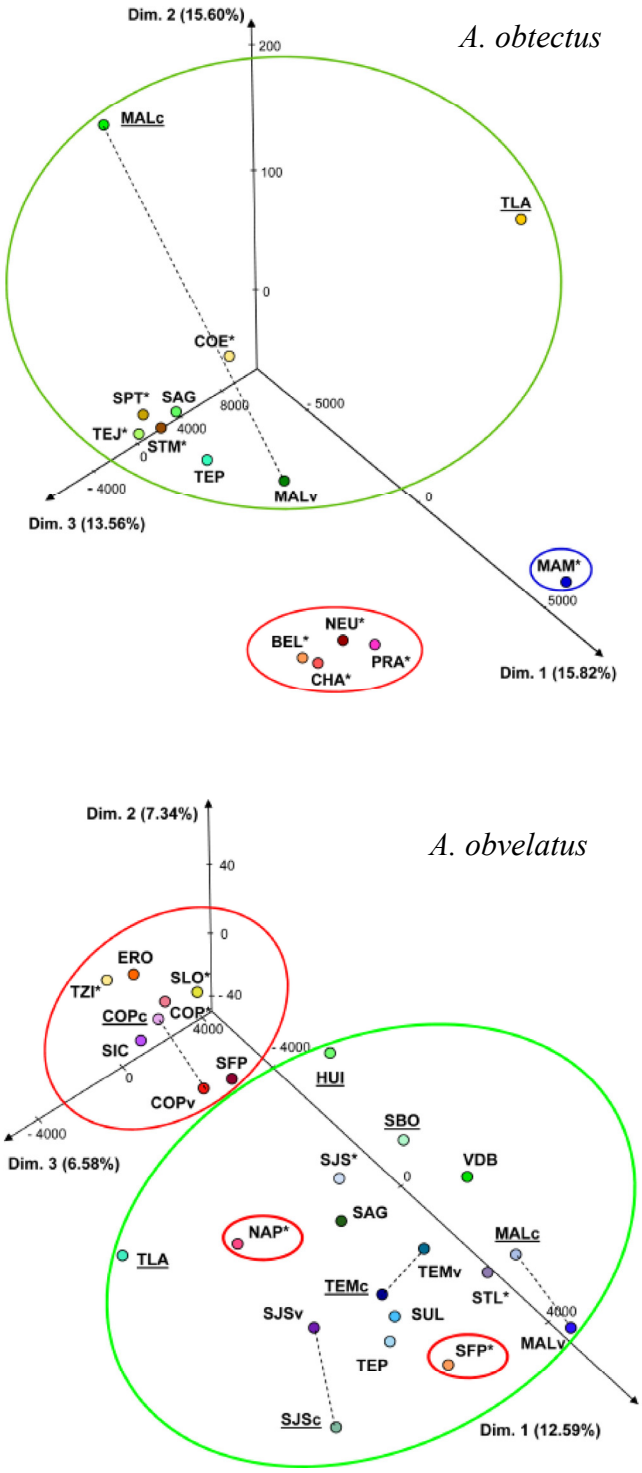

Figure 10

Analyses factorielles des correspondances basées sur les fréquences alléliques au sein des populations pour différents locus microsatellites chez *A. obtectus* (en haut) et *A. obvelatus* (en bas). Les populations soulignées proviennent de plantes-hôtes de haricot de l'espèce *Phaseolus coccineus*, les autres provenant de *P. vulgaris*. Dans les populations mixtes de haricot (où sont présentes ensemble *P. coccineus* et *P. vulgaris*), les individus prélevés sur *P. coccineus* ou sur *P. vulgaris* ont été considérés comme faisant partie de deux populations différentes. Dans de tels cas, ces populations d'un même site sont reliées par des pointillés. Pour *A. obtectus*, les populations mexicaines sont entourées en vert, les populations européennes en rouge, et la population péruvienne en bleu. Pour *A. obvelatus*, les populations provenant de l'ouest de la zone d'échantillonnage sont entourées en rouge et celles qui proviennent de la zone centre et sud sont entourées en vert.

vulgaris et *P. coccineus*, nous avons analysé environ 200 individus de *A. obtectus* et 600 individus de *A. obvelatus* – provenant respectivement de 14 et 21 populations – à l'aide de marqueurs microsatellites spécifiques à chaque espèce (voir **Manuscrits IX et X**). L'analyse de la différenciation génétique entre populations montre un rôle très important de la répartition géographique dans la structuration génétique, mais par contre, elle ne met pas en évidence de différenciation génétique, ni en fonction de l'espèce de plante-hôte, ni en fonction du type de plante-hôte (voir **Manuscrit VI** et figure 10).

Néanmoins, le cas de *A. obtectus* sur les différentes espèces de haricot (*P. coccineus* et *P. vulgaris*) mérite d'être un peu plus explicité : pour cette espèce de bruche, les deux populations provenant de *P. coccineus* présentent un profil très différent des autres populations (d'après une analyse des correspondances basée sur les fréquences alléliques ; voir populations MALc et TLA sur la figure 10). Cependant, ces deux populations (dont l'une provient de *P. coccineus* prélevés dans une population mixte de haricots) sont encore plus éloignées entre elles qu'elles ne le sont des autres populations se développant sur *P. vulgaris*, qui quant à elles se regroupent de façon homogène (voir figure 10). Ainsi, il ne semble pas qu'il y ait formation de races d'hôtes sur l'une ou l'autre espèce de haricot, à moins que cette adaptation existe à un niveau très local. Une autre hypothèse pourrait être que les individus de *A. obtectus* soient peu adaptés à se développer sur *P. coccineus*, et présentent une forte mortalité sélective. De ce fait, l'action de la dérive est susceptible d'être très forte dans de telles populations, ce qui pourrait entraîner un patron allélique différent de celui observé dans d'autres populations. Cette hypothèse est encore renforcée par le fait que dans chacune des deux populations sur *P. coccineus* que nous avons analysées, nous avons génotypé moins de dix individus (respectivement six et huit). Bien que l'analyse des prélèvements sur les populations mixtes de haricot ne révèle pas de différences de fréquences entre *A. obtectus* et *A. obvelatus* (voir IV.1), il est donc probable que *A. obtectus* soit fortement contre-sélectionnée sur *P. coccineus*. Une hypothèse alternative à la contre-sélection de *A. obtectus* sur *P. coccineus* pourrait être une adaptation locale à très petite échelle

spatiale. Une telle adaptation, au vu des très petites tailles de populations associées à *P. coccineus* – entre zero et vingt individus par site (ce qui est très faible relativement aux centaines d'individus de *A. obvelatus* qui émergent de certaines populations sur *P. coccineus*) – aurait néanmoins peu de chances de se maintenir.

Il est intéressant de relever qu'à cette probable contre-sélection de *A. obtectus* sur *P. coccineus*, s'ajoute la maladaptation de cette espèce à des altitudes supérieures à 2000m comme nous l'avons vu antérieurement. En effet, alors que la diapause reproductive de *A. obvelatus* lui permet de mieux résister lors de la mauvaise saison (de surcroît en altitude), *A. obtectus* a peu de moyens adaptatifs pour passer cette mauvaise saison. En outre, même si *P. coccineus* et *P. vulgaris* se retrouvent parfois ensemble sur un même site, *P. coccineus* a tendance à être inféodée aux environnements ombragés et légèrement humides, tandis que *P. vulgaris* se retrouve plutôt dans les milieux ouverts et plus secs. Ainsi, la contre-sélection de *A. obtectus* sur *P. coccineus* provient peut-être des mêmes limitations de sa niche écologique, que celles qui l'empêchent de coloniser de plus hautes altitudes, à savoir une mauvaise adaptation physiologique pour passer la mauvaise saison dans des zones relativement peu ensoleillées et/ou relativement froides.

Ainsi, aucune des deux espèces jumelles ne semble se décliner en deux races d'hôtes liées à une espèce particulière de *Phaseolus*. *Acanthoscelides obvelatus* paraît suffisamment généraliste pour se développer aussi bien sur *P. vulgaris* que sur *P. coccineus*, tandis que *A. obtectus* semble contre-sélectionnée sur *P. coccineus* (sans parvenir à s'adapter à cette espèce). La répartition en mosaïque des populations sauvages – voire des individus – des deux haricots *P. vulgaris* et de *P. coccineus* peut être invoquée pour expliquer l'absence d'évolution vers une spécialisation. En effet, les populations de bruches (d'une même espèce) associées à *P. vulgaris* et *P. coccineus* s'échangent constamment des migrants, et une adaptation spécifique à l'un ou l'autre haricot ne pourrait pas apparaître en raison de flux de gènes incessants, de la même façon que nous l'avons vu pour l'absence d'adaptation à un type (domestiqué *versus* sauvage) de haricot.

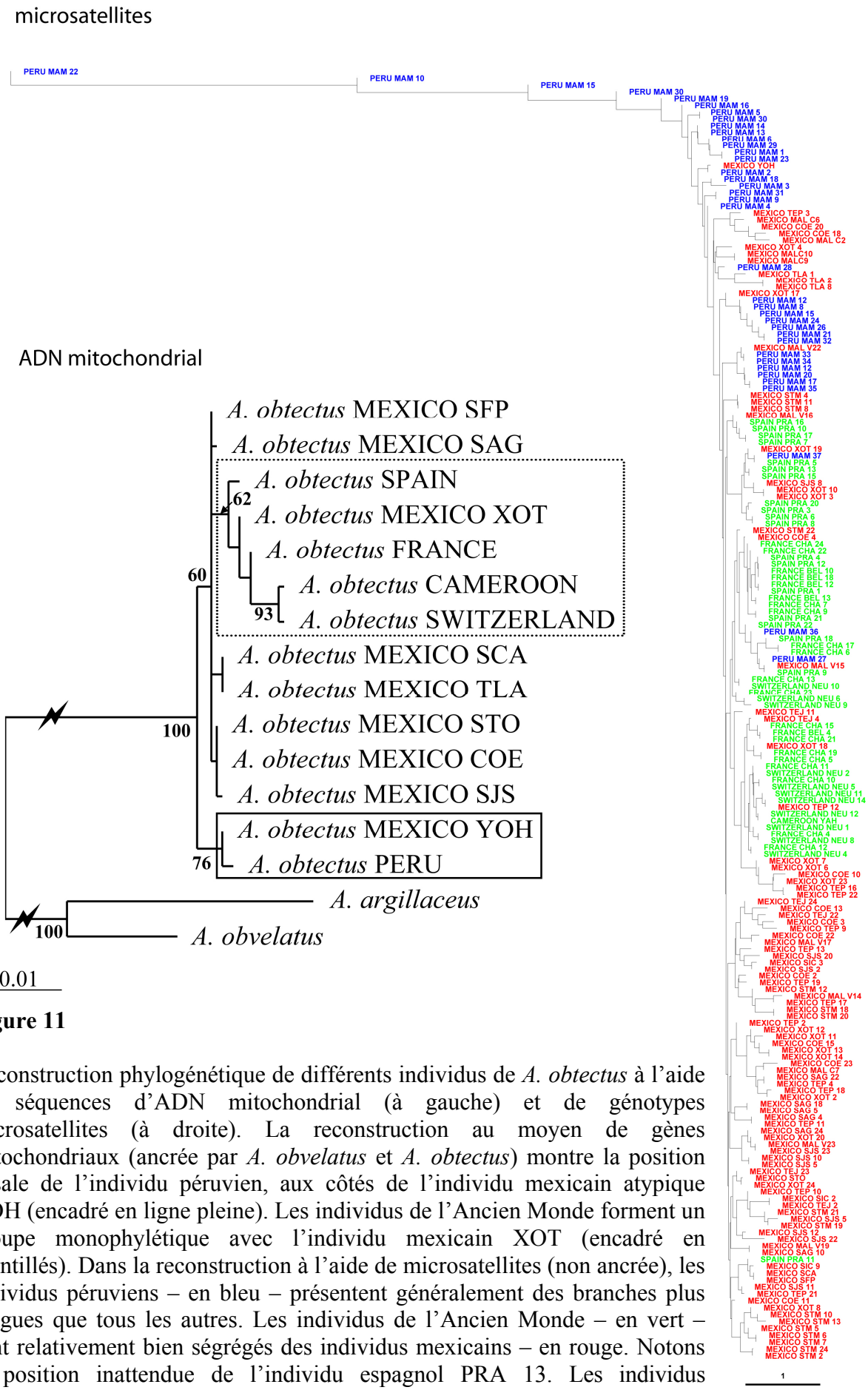

Figure 11

Reconstruction phylogénétique de différents individus de *A. obtectus* à l'aide de séquences d'ADN mitochondrial (à gauche) et de génotypes microsatellites (à droite). La reconstruction au moyen de gènes mitochondriaux (ancrée par *A. obvelatus* et *A. obtectus*) montre la position basale de l'individu péruvien, aux côtés de l'individu mexicain atypique YOH (encadré en ligne pleine). Les individus de l'Ancien Monde forment un groupe monophylétique avec l'individu mexicain XOT (encadré en pointillés). Dans la reconstruction à l'aide de microsatellites (non ancrée), les individus péruviens – en bleu – présentent généralement des branches plus longues que tous les autres. Les individus de l'Ancien Monde – en vert – sont relativement bien ségrégés des individus mexicains – en rouge. Notons la position inattendue de l'individu espagnol PRA 13. Les individus mexicains et péruviens se positionnent à des endroits divers au sein de l'arbre.

V. ORIGINE DES DEUX ESPECES JUMELLES ET ANALYSE PHYLOGEOGRAPHIQUE DE *A. OBTECTUS*, L'ESPECE COSMOPOLITE

La répartition cosmopolite de *A. obtectus* démontre son adaptation actuelle aux stocks de haricot domestiqué. Néanmoins, l'ancienne divergence entre *A. obtectus* et *A. obvelatus* ne permet pas d'envisager le multivoltinisme comme une adaptation aux stocks de haricot bien que certains auteurs (par ex., Labeyrie 1990) aient proposé que le multivoltinisme ait été à l'origine de la spéciation des deux espèces, lors de la domestication du haricot il y a environ 7000 ans (Kaplan et Lynch 1999). Ainsi, l'origine du multivoltinisme doit probablement être antérieure à la domestication du haricot, et avoir été favorisée par un environnement particulier.

V.1. Origine péri-andine de *A. obtectus*

Au travers d'une étude phylogéographique d'individus de *A. obtectus* provenant du Mexique, du Pérou, d'Espagne, de France, de Suisse, et du Cameroun, nous avons cherché à déterminer l'origine de *A. obtectus* (**Manuscrit VII**). Pour ce faire, nous avons utilisé différents marqueurs mitochondriaux (*COI*, *16s rRNA* et *12s rRNA*) ainsi que des marqueurs microsatellites propres à *A. obtectus* (**Manuscrit X**). Les deux méthodes de reconstruction utilisées (maximum de vraisemblance pour les marqueurs mitochondriaux ; *â* de Rousset [10] (2000) pour les marqueurs microsatellites) ont toutes deux placé les individus péruviens en position basale (voir figure 11) :

- dans l'analyse sur marqueurs mitochondriaux (pour laquelle un seul individu par population a été séquencé), l'individu péruvien ancre tous les autres individus, à l'exception d'un individu mexicain atypique (dont la position sera discutée plus tard)[11].

[10] *â* est un paramètre de différenciation génétique entre individus, analogue à l'estimation de la différenciation entre populations « Fst/(1-Fst) ».
[11] Cet arbre a pu être raciné à l'aide de séquences d'individus de *A. obvelatus*.

- dans l'analyse à l'aide de marqueurs microsatellites (pour laquelle plusieurs individus par population ont été génotypés), les individus péruviens présentent les branches les plus longues ; ainsi, bien que l'arbre phylogénétique basé sur les génotypes microsatellites ne puisse être ancré à l'aide d'un groupe externe, les individus péruviens démontrent vraisemblablement une histoire évolutive plus longue que tous les autres individus, qui présentent quant à eux des branches relativement courtes.

Le statut ancestral des individus péruviens nous permet de suggérer une hypothèse pour expliquer l'évolution du multivoltinisme. En effet, dans les vallées centrales des Andes, la saisonnalité est très faible et certaines espèces de haricot peuvent fructifier toute l'année. Aujourd'hui, c'est notamment le cas de la forme sub-spontanée de *P. polyanthus* Greenman, qui est appelée « frijol de toda la vida » par les péruviens – ce qui signifie « haricot de toute la vie » – en raison de sa fructification continue tout au long de l'année. Dans un tel environnement, une bruche qui évoluerait vers le multivoltinisme serait fortement sélectionnée. Cette hypothèse corrobore la probable origine centro-andéenne de *A. obtectus*. A l'inverse, de telles conditions ne sont pas du tout présentes au Mexique, où la forte saisonnalité du haricot ne favorise pas l'évolution vers le multivoltinisme. Dans un environnement à saisonalité marquée, la diapause reproductive obligatoire de *A. obvelatus* est fortement adaptative, puisqu'elle lui permet de traverser la mauvaise saison, et d'être parfaitement synchrone avec la phénologie du haricot.

V.2. Origine de *A. obvelatus* et arrivée de *A. obtectus* au Mexique

En raison du caractère peu adaptatif du multivoltinisme dans une région où la saisonnalité est marquée, il est improbable que *A. obtectus* ait pu se trouver au Mexique avant la domestication du haricot. Il est donc probable que l'espèce n'ait pas été adaptée à l'environnement mesoaméricain avant la domestication du haricot. En revanche, dès que l'homme a commencé à stocker ses récoltes, le multivoltinisme de

A. obtectus a pu se révéler une préadaptation capitale, qui a permis à cette espèce de se développer dans des régions où la forte saisonnalité l'empêchait de se maintenir. Ainsi, on peut penser que l'arrivée de *A. obtectus* au Mexique est postérieure au Néolithique, et a fait suite aux échanges de semences entre civilisations pré-colombiennes. En datant l'arrivée des *A. obtectus* dans l'Ancien Monde au XVè siècle (Sturtevant 1887), nous pouvons inférer une horloge moléculaire qui situerait son arrivée au Mexique il y a moins de mille ans. Néanmoins, il est très délicat d'inférer des horloges moléculaires sur une période si courte, d'autant plus que le polymorphisme étudié est peut-être ancestral, et pourrait très fortement post-dater les événements étudiés. En outre, le fort effet fondateur consécutif à l'arrivée de *A. obtectus* dans l'Ancien Monde pourrait avoir eu des effets importants sur la dérive, et avoir par conséquent entraîné une accélération de l'horloge moléculaire. Cependant, la comparaison des diversités de séquences entre *A. obtectus* et *A. obvelatus* au Mexique a clairement fait apparaître une diversité allélique plus importante chez *A. obvelatus*. Même si la diversité génétique est soumise à des processus stochastiques, ce résultat va dans le sens d'une plus longue histoire évolutive de *A. obvelatus* que de *A. obtectus* au Mexique. Ainsi, il semble plausible que *A. obtectus* soit passée par un goulot d'étranglement à son arrivée au Mexique, alors que *A. obvelatus* est probablement originaire de cette région mesoaméricaine. L'origine mésoaméricaine de *A. obvelatus* est appuyée par son aire de répartition actuelle, qui se limite au Mexique, au Guatemala et à la Colombie (Johnson 1983, 1990). En effet, alors que grâce à son multivoltinisme, *A. obtectus* a pu coloniser les stocks de haricot du monde entier, *A. obvelatus* est restée confinée à la région mésoaméricaine caractérisée par une forte saisonnalité, où elle se développe principalement sur les haricots sauvages.

V.3. Colonisation de l'Ancien Monde par *A. obtectus* et migrations induites par les échanges de semences

Lors de l'analyse à l'aide de marqueurs mitochondriaux, les individus de l'Ancien Monde se sont révélés former un groupe quasi-monophylétique (à l'exception du seul individu mexicain de Xochitlan qui s'est retrouvé dans le même

clade). Ceci laisse présager la colonisation de l'Ancien Monde par une migration unique à partir du Nouveau Monde (voir **Manuscrit VII**). La reconstruction phylogénétique à l'aide des marqueurs microsatellites montre également le regroupement des individus de l'Ancien Monde (voir **Manuscrit VII** ; voir figure 11), de même que l'analyse factorielle des correspondances basée sur les fréquences alléliques (voir **Manuscrit VI** ; voir figure 10). Cette colonisation trouverait vraisemblablement son origine au Mexique, comme le montre la proximité des individus mexicains – notamment de la population de Xochitlan – et des individus européens. L'arrivée de *A. obtectus* en Europe pourrait s'être faite *via* l'Espagne, dont la population échantillonnée semble présenter une diversité allélique supérieure aux autres populations de l'Ancien Monde. En effet, le seul individu européen qui se dégage des autres en venant s'ancrer au milieu d'individus mexicains provient de la population de Prado, en Espagne, ce qui est logique compte-tenu des importants échanges ayant eu lieu entre ce pays et la région mesoaméricaine.

Il est intéressant de relever qu'un événement récent de migration du Pérou vers le Mexique a également été mis en évidence dans le cas de l'individu mexicain de Yohualichan – qui s'ancre aux côtés d'individus péruviens – alors que cet individu provient d'une monoculture intensive de haricot. Ceci révèle la continuité des migrations à longue distance d'individus de *A. obtectus*, *via* les échanges de semences. Ainsi, l'histoire biogéographique de *A. obtectus* est fonction de la faculté de sa plante-hôte à être disséminée sur tous les continents, suite à sa domestication par l'homme. La biologie de l'insecte qui colonise les semences le prédispose tout particulièrement à profiter de la dissémination de son hôte.

VI. CONSEQUENCES DES PRATIQUES AGRICOLES SUR LES PARAMETRES POPULATIONNELS ET SUR LA GENETIQUE DES POPULATIONS DE *A. OBVELATUS*

Alors que la dispersion du haricot *via* les échanges humains de semences a façonné le patron phylogéographique de *A. obtectus*, il ne semble *a priori* pas en être de même pour *A. obvelatus*, dont la distribution est aujourd'hui encore mesoaméricaine. Néanmoins, les pratiques agricoles peuvent avoir modifié certains processus populationnels, et par là même, les paramètres de génétique des populations de *A. obvelatus*. Comme nous l'avons vu, la domestication du haricot n'a pas entraîné la formation de races d'hôtes chez *A. obvelatus*, chez qui la structuration génétique est essentiellement expliquée par la répartition géographique. Pourtant, il semble évident que les pratiques agricoles doivent également modifier les paramètres de migration et de dérive des phytophages, comme elles modifient la génétique des populations des plantes domestiquées. Les échanges de graines, les pratiques liées à la sélection des semences (qui déterminent l'effectif efficace des reproducteurs), ou les systèmes de reproduction des plantes (par exemple, la propagation par boutures/tubercules ou la dissémination par semences), sont des exemples où les pratiques agricoles influencent de façon directe la structure des populations de plantes domestiquées (voir **Manuscrit XI**).

VI.1. Rôle des pratiques agricoles sur l'isolement par la distance entre populations

Le rôle des pratiques agricoles sur la dynamique des populations des bruches associées à des plantes cultivées intervient essentiellement au niveau des processus de dispersion des individus. En effet, les bruches peuvent parcourir d'importantes distances pendant leur stade larvaire, si elles se trouvent dans des semences qui font l'objet d'un commerce ou d'un échange entre paysans. Au Mexique, nous avons vu à de nombreuses reprises, des paysans de l'état de Guerrero venir vendre leur récolte, visiblement infestée par des *Acanthoscelides*, environ 100km plus loin, au marché de

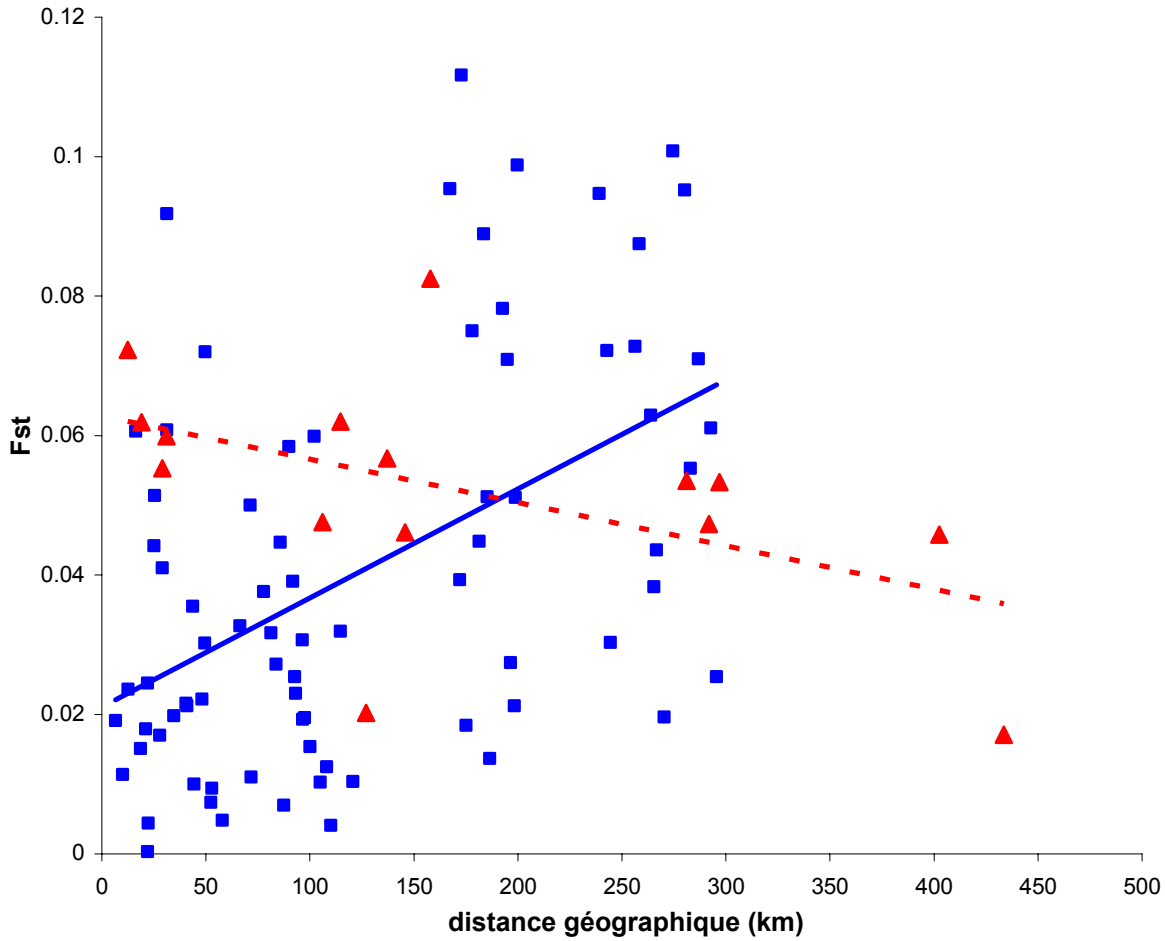

Figure 12

Isolement par la distance (km → Fst) pour les 190 paires de populations de *A. obvelatus* examinées. Les paires de populations sur haricot sauvage sont représentées en bleu ; les paires sur haricot domestiqué sont représentées en rouge. La droite bleue correspond à la régression linéaire ($P = 0.0005$) obtenue pour les paires de populations sur haricot sauvage. La droite rouge en pointillés correspond à la régression linéaire (non significative) pour les paires de populations sur haricot domestiqué.

Tenango dans l'Etat de Mexico. Les acheteurs de leur récolte repartaient à leur tour dans les villages avoisinants, où le haricot était soit consommé, soit gardé pour la semence d'une nouvelle parcelle qui serait plantée l'année suivante. Dans un cas comme dans un autre, les *Acanthoscelides* ont migré des dizaines de fois plus loin que ne l'aurait permis leur dispersion intrinsèque par le vol. Ainsi, on s'attend à ce que les pratiques agricoles modifient substantiellement l'isolement par la distance des populations de bruches associées au haricot.

Pour étudier l'effet des pratiques agricoles sur la génétique des populations de *A. obvelatus*, nous avons génotypé à l'aide de marqueurs microsatellites environ 600 individus de 20 populations mexicaines, parmi lesquelles 13 se développent sur haricot sauvage, et 7 sur haricot domestiqué. Comme attendu, ces populations ne se sont pas révélées être structurées génétiquement en fonction du type (domestiqué *versus* sauvage) de haricot, alors qu'elles démontrent un isolement par la distance significatif. Cependant, en comparant les paires de populations sur haricot sauvage avec les paires de populations sur haricot domestiqué, il s'est avéré que seules les premières démontraient une corrélation entre distance géographique et différenciation génétique. Les populations sur haricot domestiqué ne présentent quant à elles pas de structuration en fonction de la distance géographique (voir figure 12 et **Manuscrit VIII**). Cet absence d'isolement par la distance sur haricot domestiqué provient très certainement des échanges de semences, qui peuvent se faire sur plusieurs centaines de kilomètres d'un marché régional à un autre. A l'échelle de notre étude (environ 500 km), les individus émergeant de haricot domestiqué se comportent donc comme s'ils étaient en panmixie, et on peut considérer l'ensemble des individus sur haricot domestiqué comme faisant partie d'un seule et même population. Au contraire, les individus émergeant de haricot sauvage (dont la distance maximale de dispersion en volant est de l'ordre de 2km [Jarry 1984]) ne peuvent migrer que de proche en proche. Ainsi, l'isolement par la distance générale, constaté sur l'ensemble des populations, est uniquement dû au patron observé pour les populations sur haricot sauvage.

VI.2. Conséquences des pratiques agricoles sur la diversité allélique

Le rôle joué par les pratiques agricoles sur la génétique des populations de *A. obvelatus* ne s'arrête pas aux patrons de migration. En effet, les goulots d'étranglement se produisant à chaque génération dans les populations de bruches sur haricot domestiqué entraînent une forte action de la dérive. Ces goulots d'étranglement sont notamment causés par la consommation humaine de semences. En effet, si les agriculteurs consomment rapidement leur récolte de haricot, ils consomment en même temps les larves de bruches. A cette forte mortalité par consommation humaine s'est ajouté, plus récemment, l'usage d'insecticides dans les stocks. Par ailleurs, la séparation spatiale entre les champs de haricot dans lesquels pondent les bruches, et les greniers desquels leurs descendants émergent est un autre facteur de stochasticité démographique : lorsqu'une bruche adulte quitte le grenier pour rejoindre un champ, sa probabilité de ne pas retrouver sa population d'origine est importante. L'adulte peut en effet être sujet à la prédation lors de sa migration, ou encore se retrouver dans un champ différent de celui dans lequel il a été pondu. Ainsi, il existe non seulement une forte mortalité d'une année sur l'autre, mais en plus, les populations recomposées dans les champs ne reflètent pas nécessairement le mélange des lignées de l'année précédente. Ainsi, on peut s'attendre à ce que de tels goulots d'étranglement d'une année à l'autre soient responsables d'une diminution de la richesse allélique à l'intérieur des populations. Or, nos analyses montrent que le nombre d'allèles dans chaque population (pondéré par la taille des populations) n'est pas significativement différent entre populations sur haricot domestiqué et populations sur haricot sauvage. Cependant, une analyse plus fine de cette diversité allélique montre que le « compartiment sauvage » contient significativement plus d'allèles que le « compartiment domestiqué ». Elle montre par ailleurs que les allèles présents dans 10% au plus des populations se retrouvent en nombre significativement plus grand dans les populations sauvages.

Les pratiques agricoles entraînent donc des processus populationnels différents entre bruches sur haricot sauvage et bruches sur haricot domestiqué, qui conduisent à un isolement par la distance différent, mais aussi à une diversité

allélique également différente. Cependant, ces différents processus populationnels aboutissent à la même richesse allélique dans les populations des deux types. Ainsi, la continuité démographique des populations sur haricot sauvage – les populations de plantes-hôtes restant stables temporellement et spatialement – et leur faible taux de migration (reflété par l'isolement par la distance observé) permettent le maintien des allèles rares et d'une certaine diversité allélique au sein des populations. A l'inverse, les importants goulots d'étranglement empêchent le maintien d'allèles rares dans les populations issues de haricot domestiqué, alors que le fort taux de migration associé aux échanges de semences permet d'homogénéiser la diversité génétique, et de maintenir ainsi un nombre d'allèles aussi important dans les populations sur haricot domestiqué que dans les populations sur haricot sauvage.

VI.3. Conséquences des patrons de migration sur l'acquisition d'adaptations favorables

Les bruches issues de haricot domestiqué se comportent comme si elles appartenaient à une seule et même population, en raison des échanges humains de semences de haricot. Ainsi, toute adaptation sélectionnée envahira rapidement le « compartiment domestiqué ». Dans le contexte actuel du débat sur les OGM, un tel résultat semble primordial en termes d'acquisition de résistances. En effet, si un individu évolue vers la résistance à un insecticide produit par sa plante-hôte, ce caractère se répandra très rapidement, d'une part car la sélection peut agir sur un effectif efficace plus important, et d'autre part, car les individus de tout le « compartiment domestiqué » sont capables de migrer à très grande échelle (dans la limite des 500km de notre étude). Plus généralement, toute adaptation favorisant la valeur sélective des individus pourra envahir très rapidement toute l'aire de distribution de l'espèce. Les flux de semences à longue distance représentent donc le moyen, pour les bruches, de fixer plus rapidement les adaptations.

VII. DISCUSSION GENERALE

Comme nous l'avons vu au cours de ce travail, les plantes-hôtes jouent un rôle à deux niveaux de l'évolution de leurs bruches associées : (1) au niveau des processus de spéciation et de diversification ; (2) au niveau des processus populationnels. Ces deux niveaux sont eux-mêmes liés, puisque les processus populationnels influencent parfois directement les processus de spéciation. Néanmoins, ils opèrent à des échelles de temps différentes, et les processus populationnels ne jouent pas systématiquement un rôle sur les processus de spéciation. C'est pourquoi nous les considérerons ici comme deux types de processus différents.

D'après notre étude au sein de la sous-famille des Bruchinae, nous avons vu que les processus de diversification sont essentiellement le fait – outre les événements biogéographiques – d'apparition d'innovations-clés contrant probablement les défenses naturelles des plantes-hôtes. Au contraire, comme nous l'avons vu chez *A. obtectus* et *A. obvelatus*, les processus populationnels semblent principalement influencés par les paramètres de dispersion des plantes-hôtes. Néanmoins, l'association entre défenses de la plante-hôte et processus de spéciation des phytophages d'une part, et entre dispersion de la plante-hôte et processus populationnels des phytophages d'autre part, n'est pas stricte. En effet, la défense des plantes-hôtes peut influencer les processus populationnels, tandis que les paramètres de dispersion des plantes-hôtes peuvent jouer un rôle sur les processus de spéciation, comme nous allons le discuter maintenant.

VII.1. Défenses de la plante-hôte

Le rôle des défenses de la plante-hôte sur les processus de diversification des insectes phytophages s'appuie sur le concept d'innovation-clé. Heard et Hauser (1995) définissent une innovation-clé comme la modification évolutive d'un trait d'un individu, qui est causalement liée à une augmentation du taux de

diversification du clade résultant (pour lequel cette adaptation-clé est une synapomorphie). L'innovation-clé qui permet à une lignée d'insectes de contourner les défenses d'une nouvelle plante-hôte, entraîne en effet la possibilité d'exploiter de nouvelles ressources, et de diminuer la compétition inter-spécifique, qui représentent des conditions favorisant la diversification spécifique. Néanmoins, pendant un certain temps, une lignée peut être incapable de contourner une défense particulière d'une plante-hôte potentielle donnée. Dans ce cas-là, la défense de la plante-hôte n'influence pas les processus de diversification (puisqu'aucune adaptation-clé n'est encore apparue), mais contre-sélectionne les populations d'insectes qui essaient de s'y développer. Ainsi, dans une telle situation, la défense de la plante-hôte modifie-t-elle les paramètres génétiques des populations des insectes. C'est notamment le cas, en ce qui concerne notre étude, des populations de *A. obtectus* sur *P. coccineus.* Si on admet que l'adaptation locale y est faible ou nulle (c'est-à-dire qu'il n'y a pour l'instant pas d'adaptations-clés chez *A. obtectus*, pour contourner les défenses de *P. coccineus*), ces populations sont des puits, où la dérive – suite à de forts goulots d'étranglement – est très forte.

En généralisant, on peut donc proposer l'hypothèse suivante : tant qu'une lignée n'a pas acquis d'adaptation lui permettant de contrer la défense d'une plante-hôte, cette défense influence essentiellement les paramètres de génétique des populations de cette lignée ; si un individu de cette lignée acquiert une adaptation-clé lui permettant de contourner la défense de la plante-hôte – et donc d'exploiter de nouvelles ressources – alors cette défense aura agi comme un moteur de diversification.

VII.2. Dispersion de la plante-hôte

Les différentes possibilités de migration des bruches les situent dans une position intermédiaire, entre les phytophages sessiles et les espèces bonnes voilières (qu'elles soient phytophages, carnivores ou décomposeurs). En effet, les bruches peuvent migrer par deux moyens : leur moyen intrinsèque, à savoir le vol au stade

adulte, et un moyen extrinsèque, à savoir la migration lors de leur stade larvaire séminivore, au travers de la dispersion des semences. Ni la migration par le vol, ni la migration au travers des semences n'assurent à une bruche de se retrouver dans une nouvelle population de plantes-hôtes. Néanmoins, ces deux modes de dispersion diffèrent de par la distance parcourue. La dispersion intrinsèque se fait généralement sur quelques kilomètres (à part certains cas exceptionnels de migrations à longue distance *via* des courants ascendants ou *via* des tempêtes), alors que la migration extrinsèque, à travers les semences, peut se faire sur de beaucoup plus grandes distances. En effet, deux vecteurs de migration peuvent générer des dispersions extrinsèques à une importante échelle spatiale. Tout d'abord, les bruches à l'intérieur des semences dispersées par zoochorie[12], tels que certains acacias par des ongulés (Miller 1994), peuvent migrer sur des distances de plusieurs dizaines de kilomètres ; de même, les bruches à l'intérieur de semences dispersées par l'homme peuvent parcourir des distances de l'ordre de plusieurs centaines de kilomètres. Les courants marins jouent le rôle du deuxième vecteur, permettant des migrations à une plus grande échelle encore. En effet, à condition que les semences de leur plante-hôte flottent, les bruches peuvent migrer d'une île à une autre ou d'un continent à un autre (voir **Manuscrit I**). Ainsi, alors que les dispersions à petite échelle spatiale modifient les patrons de migration, et donc la génétique des populations des espèces considérées, les migrations à plus grande échelle spatiale peuvent conduire, si elles mènent à l'isolement de certaines populations, à des processus de spéciation.

On peut donc globalement différencier deux niveaux (les mêmes que pour les défenses des plantes) au travers desquels la dispersion de la plante-hôte modifie les processus évolutifs des bruches associées. Tout d'abord, les dispersions à faible échelle spatiale modifient la génétique des populations des espèces de bruches associées, de même que le font les paramètres d'abondance et de variation démographique interannuelle des plantes-hôtes. Ensuite, les dispersions à plus grande échelle spatiale – par zoochorie à longue distance ou hydrochorie *via* les courants marins – peuvent modifier les histoires biogéographiques des espèces, et,

[12] La dispersion des graines se fait *via* un vecteur animal.

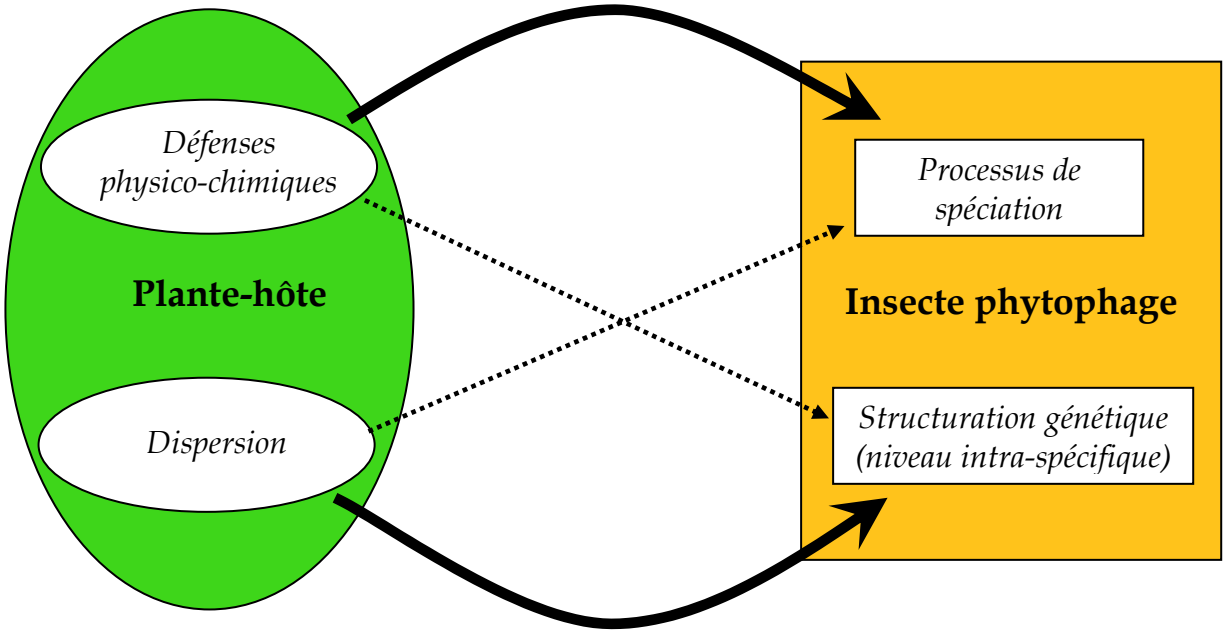

Figure 13

Rôle des plantes-hôtes sur l'organisation de la diversité des bruches associées. La taille des flèches est proportionnelle à la fréquence des événements représentés.

lorsque cette dispersion conduit à l'isolement des populations, mener à des processus de spéciation. C'est notamment le cas des différentes espèces d'*Acanthoscelides* présentes dans les îles du Nouveau Monde. Parfois, cette dispersion est récurrente (ou récente), et la différenciation des populations n'a pas (encore) entraîné de processus de spéciation. C'est par exemple le cas de *A. argillaceus* – qui se développe sur *P. lunatus*, une plante-hôte dont les semences de la forme sauvage flottent (A. Delgado-Salinas, communication personnelle) – et qui se retrouve sur le continent américain, mais aussi à Haïti et sur Trinidad. De même, *A. flavescens* se retrouve sur le continent, mais également en Jamaïque, sur Aruba, et à Grenade. Cependant, si la dispersion à longue distance est telle qu'elle permet aux processus de dérive de conduire à l'isolement des populations, alors elle peut conduire à des processus de spéciation. Les endémismes tels que *A. fuscomaculatus* (endémique des Galapagos) et *A. quadratus* (endémique de Cuba) illustrent probablement le rôle joué par la dispersion des plantes-hôtes sur la spéciation des phytophages.

VII.3. Conclusion

L'interaction entre les insectes phytophages et leurs plantes-hôtes a entraîné un important processus de diversification, depuis près de 400 millions d'années. Elle a notamment modifié profondément l'organisation de la diversité des insectes phytophages, des radiations évolutives aux processus populationnels. Dans le cas des bruches, nous avons vu que ces processus peuvent être liés à la défense physico-chimique, mais également à la capacité de dispersion des plantes-hôtes, en interaction avec les forces tectoniques (on entend par là, la conformation des continents, des îles et des courants marins) (voir Figure 13). Dans le cas des espèces inféodées aux plantes domestiquées, cette dispersion est également liée, depuis peu, à l'histoire humaine et aux flux des semences (*via* les échanges ou le commerce). Au cours de cette étude, nous avons illustré l'évolution des insectes phytophages dans ces différents cas de figure, en nous focalisant sur les Bruchinae. Du fait de leur étroite relation à leur plante-hôte, les bruches représentent un modèle remarquable pour estimer le rôle de l'interaction plante-insecte sur l'évolution des espèces

phytophages. Evidemment, cette étude s'est focalisée sur un nombre restreint d'espèces, et n'a pas répondu à toutes les questions envisageables sur le sujet. Néanmoins, elle a traité des processus évolutifs d'un insecte phytophage sur un continuum temporel, en partant d'une échelle de temps géologique, pour arriver aux processus populationnels. De ce fait, elle pose les bases d'une étude plus complète qui s'intéresserait par exemple à la compréhension globale des patrons évolutifs observés sur l'ensemble des bruches actuelles. Du fait du nombre limité d'espèces de cette famille (environ 1700), une telle étude pourrait être envisageable, même si elle requerrait beaucoup de temps et une panoplie d'analyses différentes. Présentes sur tous les continents (excepté l'Antarctique), et ayant colonisé de nombreuses familles botaniques, les bruches se révèlent donc être un modèle prometteur à l'étude intégrée des interactions plante-insecte, à toutes les échelles de temps.

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MANUSCRIT I

Phylogenetic relationships in the Neotropical bruchid genus *Acanthoscelides* Schilsky.

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Phylogenetic relationships in the Neotropical bruchid genus *Acanthoscelides* (Bruchinae, Bruchidae, Coleoptera)

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Short title: Phylogeny of the bruchid genus *Acanthoscelides*
Author's name: Alvarez *et al.*

Word count and manuscript information:
Abstract: 185 words (3.46% of main text)
Main text: 5341 words
3 tables, 4 figures, 1 table in appendix.

Alvarez, N., Romero Napoles, J., Anton, K.-W., Benrey, B. & Hossaert-McKey, M. (2005). Phylogenetic relationships in the Neotropical bruchid genus *Acanthoscelides* (Bruchinae, Bruchidae, Coleoptera). *Zoologica Scripta*, 00, 000-000.

Abstract

Adaptation to host-plant defenses through key innovations is a driving force of evolution in phytophagous insects. Species of the neotropical bruchid genus *Acanthoscelides* Schilsky are known to be associated with specific host plants. The speciation processes involved in such specialization pattern that have produced these specific associations may reflect radiations linked to particular kinds of host plants. By studying host-plant associations in closely related bruchid species, we have shown that adaptation to a particular host-plant (e. g., with a certain type of secondary compounds) could generally lead to a radiation of bruchid species at the level of terminal branches. However, in some cases of recent host shifts, there is no congruence between genetic proximity of bruchid species, and taxonomic similarity of host plants. At deeper branches in the phylogeny, vicariance or long-distance colonization events seem to be responsible for genetic divergence between well-marked clades, rather than adaptation to host plants. Our study also suggests that the few species of *Acanthoscelides* described from the Old World, as well as Neotropical species feeding on Mimosoideae, are misclassified, and are more closely related to the sister genus *Bruchidius*.

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Introduction

Secondary metabolites in plants are known to play an important role in defense against herbivores (*e. g.* Herms & Mattson 1992; McKey 1979). In legumes, the diversity of such compounds seems to be even larger than in other plant families, and new secondary metabolites continue to be discovered (see Hegnauer 1994; Hegnauer & Hegnauer 1996, 2001). Based on the tendency of related species to possess similar metabolites, several studies have addressed the use of secondary metabolites as chemical markers in legume taxonomy (e. g., Evans *et al.* 1994; Kite & Lewis 1994; Wink *et al.* 1995). For phytophagous insects, these compounds represent defenses to overcome. However, once an adaptation permitting this has appeared (e. g., by sequestration or detoxification of the toxic compound), the insect can also exploit chemically similar (and usually closely related) plant species. In two examples concerning legumes, *Macrosiphum albifrons* is the only known species of aphid able to develop on the alkaloid-rich varieties of lupin (Wink & Römer 1986); and the bruchid *Caryedes brasiliensis* develops on host plants whose seeds contain high concentrations of arginase and canavanine (Bleiler *et al.* 1988; Rosenthal 1990; Rosenthal & Janzen 1983). Adaptation to a secondary metabolite (or class of similar metabolites) characteristic of a group of closely related plant species may allow a lineage of phytophagous insects to radiate adaptively onto several host plants of this group (Ehrlich & Raven 1964).

Bruchid beetles, with about 1700 known species (Borowiec 1987), are one of the most diverse groups of phytophagous beetles. Larvae of bruchids feed only inside seeds during their development, and most species are associated with legumes. Bruchids have countered the mechanical protection of hard-seeded angiosperm species and have subsequently been able to use hard seed coats as a shield for their developing larvae. This adaptation has constrained bruchids to specialize particularly on legumes, but has allowed them to undergo radiations in other hard-seeded and stone-fruited families (Borowiec 1987), such as Malvaceae *sensu lato* (see Bayer *et al.* 1999) and Arecaceae. *Acanthoscelides* Schilsky (Bruchinae, Bruchidae, Coleoptera) is the largest Neotropical bruchid genus (Johnson 1981). Currently, about 300 species have been described (see

Appendix 1), and many still likely await discovery, especially in poorly studied parts of South America, such as Amazonia and southern South America (Kergoat *et al.* submitted). Most of the species described are oligophagous or monophagous. Among the species for which a valid host plant has been described (Johnson 1983, 1989, 1990), about one hundred species develop on Faboideae, 35 species develop on Mimosoideae, and 6 species develop on Caesalpinioideae. A minority of the described species feed on non-legumes, such as Malvaceae *sensu lato* [Malvoideae (30 species), Grewioideae (8 species), Byttnerioideae (2 species)], Onagraceae (1 species), Rhamnaceae (1 species) and Cistaceae (1 species). Using morphological and ecological criteria, Johnson (1983, 1990) defined 15 groups of species of *Acanthoscelides*, all neotropical. Finally, about nine Palearctic species apparently restricted to seeds of herbaceous species of the faboid tribe Galegeae, such as *Astragalus* spp., were treated as *Acanthoscelides* by Lukjanovitsch & Ter-Minassian (1957), but their status as members of *Acanthoscelides* has been questioned (Borowiec 1987). One of these species was placed in *Bruchidius* by Egorov & Ter-Minassian (1981), and four were placed in a new genus, *Palaeobruchidius*, by Egorov (1990).

Acanthoscelides represents a very good model to examine adaptive radiation of phytophagous insects in legumes, and other hard-seeded families. A recent study of *Bruchidius* Schilsky, the Old-World sister genus of *Acanthoscelides*, has shown the role played by key innovations in the adaptive radiation of several groups of *Bruchidius* species on closely related host plants (Kergoat *et al.* 2004). Another study focusing on European species of *Bruchidius* has demonstrated several cases of ecological specialization in some beetles that were able to feed only on specific host plant species. (Jermy & Szentesi 2003). In the present study, we compare host plant associations of different, apparently monophyletic, groups of *Acanthoscelides* species. Toward this goal, we analyzed relationships in a sample of 26 species of *Acanthoscelides*, including mostly ones specialized on the faboid tribe Phaseoleae, using phylogenetic methods applied to mitochondrial gene sequences. Our goal was to test the role of host-plant identity in the radiation of *Acanthoscelides*. We also included some other Old World and New World Bruchinae as outgroups, to confirm the monophyly of *Acanthoscelides* and of groups of species within it, and to explore the status of the Palearctic species that have been treated as *Acanthoscelides*.

Materials and Methods

Establishing species groups of Acanthoscelides *for studying evolution of host-plant association*

Morphological identity in male genitalia (considered the morphological criterion the most indicative of evolutionary relationships in bruchids [Borowiec 1987]) is not strict in the fifteen species groups of neotropical *Acanthoscelides* described by Johnson (1983, 1990). We therefore tried to determine which groups presented consistently similar male genitalia level and thus were most likely to represent monophyletic groups. Based on illustrations by Johnson (1983, 1990), we examined for each species five qualitative traits of male genitalia that describe the characteristics of the virga (the ventral valve at the apex of the median lobe), the median lobe, and the lateral lobes:

(i) shape of the apical surface of the virga (rounded vs. sharp);

(ii) shape of the lateral edges of the virga (straight vs. concave vs. convex);

(iii) ratio between height and width of the virga (height smaller than half the base vs. height greater than half the base);

(iv) shape of sclerified parts in the lateral edge of the median lobe (straight vs. curved);

(v) proportion of length of lateral lobes fused to each other (less than 1/3 vs. between 1/3 and 2/3 vs. more than 2/3).

We also included a sixth variable corresponding to the biogeographic range of the species (distributed no further south than Panama [N] vs. distributed south of Panama [S] vs. distributed both north and south of Panama [N+S]). In organisms with limited dispersal, closely related species are expected to show biogeographic correspondence at the regional level.

We considered only groups containing five or more species (N=10 groups). Therefore, we examined the *aequalis*, *albopygus*, *blanchardi*, *flavescens*, *megacornis*, *mexicanus*, *obtectus*, *pertinax*, *puellus* and *quadridentatus* groups (Johnson 1983, 1990). We then constructed a multiple correspondence analysis, considering the species group as a supplementary variable, using SAS (1999). We then conducted a

discriminant analysis based on the coordinates of each species in the best represented groups for the nine first dimensions using S-Plus (2001). In this analysis, we tested if well represented groups were different from each other, by a discriminant analysis and by paired comparisons (Hotelling's T Squared for Differences in Means) using S-Plus (2001).

Sampling

Sampling of Bruchinae included 26 species of *Acanthoscelides*, four species of *Bruchidius*, *Merobruchus placidus*, and *Palaeoacanthoscelides gilvus.* As outgroups, we used *Zabrotes planifrons* Horn (subfamily Amblycerinae). Material available for this study was mostly dried, pin-mounted specimens from the personal collections of J. Romero Napoles, K. W. Anton, and C. D. Johnson, collected from 08 Nov. 1983 to 21 Aug. 2002. In addition to these specimens from collections, specimens of *Acanthoscelides obtectus, A. obvelatus, A. argillaceus and Merobruchus placidus* were collected in 2002 by N. Alvarez. Table 1 summarizes information on sampling specimen and associated host plants and gives taxonomic authorities for all names appearing in the paper.

Dna extraction, amplification and sequencing

Total genomic DNA was extracted using DNeasy™ kit (Qiagen). Qiagen protocol for animal tissues was modified to increase yield, due to the fact that most of our dried specimens, some up to 20 years old, contained low amounts of DNA. In particular, the lysis steps lasted 24 hours instead of three; particular attention was given to tissue crushing; final elution lasted 2 hours rather than 1 minute, and was done in 30µL final volume instead of 100µL. PCR amplifications for three mitochondrial genes – *cytb* (primers CB1 & CB2), *COI* (primers C1-J-2183 & TL2-N-3014), and *12S rRNA* (primers 12sai & 12sbi) – were performed (Simon *et al.* 1994). Final volume was10 µL, and contained 1 to 5 µL of extracted DNA, 1 µL of 25 mM MgCl2, 0.1 µL of 10mM dNTPs, 1 µL of PCR buffer (Eurogentec), 1 unit of Taq DNA polymerase (Eurogentec Red Goldstar™), 0.5 µL of forward primer, and 0.5 µL of reverse primer. PCRs were

performed separately for each primer pair on a PTC-200™ thermocycler using the following cycling conditions: initial denaturation at 92 °C (1 min 30 s); 30 to 40 cycles of 92 °C (30 s), annealing at 55°C (45 s), 72 °C (1 min 30 s); final elongation at 72 °C (10 min). Sequencing reactions were carried out using Applied Biosystems BygDye™ protocol. Products of the sequencing reactions were then analyzed on an ABI Prism 310 sequencer.

Phylogenetic analyses

Chromatograms were manually corrected using Chromas 2.23 (Technelysium Pty. Ltd., Helensvale, Australia) and further aligned using ClustalW 1.83 (Thompson *et al.* 1994). Parsimony analysis and maximum likelihood analysis were carried out using PAUP* 4.0b10 (Swofford 2003) on an Intel Pentium IV 2.4 Ghz processor. Analysis were performed using heuristic search and tree-bisection-reconnection (TBR) branch-swapping-algorithm. For maximum likelihood analysis, we used a general time reversible (GTR) model with eight evolutionary rate categories. Gamma shape parameter, proportion of invariable sites, base frequencies and probabilities of substitution were estimated during heuristic search. Bootstrap values were calculated on 100 replicates, both for parsimony analysis and for maximum likelihood analysis. Bayesian inferences were determined using MrBayes version 3.0b4 (Huelsenbeck & Ronquist 2001) on an Apple G5 1.8 Ghz. We used Modeltest 3.06 (Posada & Crandall 1998) to assess the best-fit substitution model, through hierarchical likelihood ratio tests. The asymptote of the fluctuating likelihood values of the Bayesian trees (or burnin period) was determined through preliminary runs. We ran four Metropolis-coupled chains in one run of 20000000 generations, and sampled one tree every 10000 once cycles after the burnin period had passed. The sampled trees were used to generate a majority rule tree showing all compatible partitions and the support for the nodes of this tree was given by posterior probability estimates for each clade. Character tracing of host plant genera (or host plants tribes or subfamilies) corresponding to each studied bruchid species was carried out on the majority rule tree obtained through Bayesian methods, using MacClade 4.06 (Maddison & Maddison 2004).

Results

Multiple correspondence analysis and discriminant analysis of species groups of Acanthoscelides

The nine dimensions of the MCA on morphological and biogeographical characters explained respectively 19.89%, 15.34%, 13.17%, 11.61%, 10.40%, 8.94%, 8.11%, 7.23% and 5.31% (graphs not shown). The discriminant analysis using the coordinates of each species in the ten groups demonstrated highly significant differences among species groups (Hotelling-Lawley Trace: $p=6*10^{-15}$). Pairwise comparisons demonstrated that 33 of 45 pairs of these 10 groups were significantly discriminated (Table 2). Among these groups, five (*aequalis*, *albopygus*, *blanchardi*, *pertinax*, *puellus*) showed significant differences with seven or more other groups (*i. e.* each of these five groups was different from more than 75% of all other groups). The host-plant associations for species of these five groups are represented in Table 3. Each group appears to be associated with a different taxonomic group of host plants. In group *aequalis*, 26 species are associated with species of Malvaceae *sensu lato*. In group *blanchardi*, the 6 species whose host-plant has been described also feed on Malvaceae. The other groups are associated with different legume groups, all faboids, except for group *albopygus*, of which all species with known host plants (4) feed on the mimosoid tribe Mimoseae. In group *pertinax*, most of the species (9) develop on Desmodieae, the others developing on Phaseoleae (2), Amorpheae (1), and on Aeschynomeneae/Amorpheae/Desmodieae (1). In group *puellus*, most of the species feed on Phaseoleae (12), and the others feed on Indigofereae (4), on Galegeae/Loteae (1), and on Phaseoleae/Millettieae (1). In addition, one species of this group feeds on species the non-legume family Rhamnaceae.

Phylogenetic reconstruction

Since most of the specimens were collected several (up to 20) years before the study, and had been preserved dried in insect collections, DNA was in most cases considerably

degraded. Therefore, we could not obtain usable sequences for *COI* and *Cytb*. However, we obtained very good results with primers 12sai and 12sbi, and we could therefore sequence 384 nucleotides for the *12s rRNA* gene, in all the studied species (see accession numbers in Table 1). Although the total number of analyzed nucleotides may seem relatively low, the phylogenetic signal of the *12s rRNA* was not saturated, since both transitions and transversions are linearily proportional to the genetic distances (Kimura 2-parameters model) between species (see Figure 1). We reconstructed parsimony and maximum-likelihood phylogenetic trees (each with 100 bootstraps) that we obtained after 1126 hours of simulation. Parameters estimated in the maximum likelihood analysis were as follows : Gamma = 0.404344, proportion of invariable sites = 0.163879. Empirical bases frequencies were used as follows: A = 0.38002, C = 0.07098, G = 0.13872, T = 0.41028. Substitution probabilities were estimated as follows: A-C = 0.12602, A-G = 5.55604, A-T = 1.74627, C-G = $1.73*10^{-10}$, C-T = 2.76787. Bootstrap values are represented on each node, both for maximum likelihood and parsimony methods. Values less than 10% were not represented. We computed Bayesian inferences using the following prior probabilities parameters determined by Modeltest: GTR model of substitution, Gamma = 0.45, proportion of invariable sites = 0.1409. Bases frequencies were estimated as follows: A = 0.4349, C = 0.0497, G = 0.1151, T = 0.4002. Substitution probabilities were estimated as follows: A-C = 0.1347, A-G = 3.5899, A-T = 1.0650, C-G = 0.2239, C-T = 2.8091. The burnin period was estimated to 100000 cycles. One thousand and nine hundreds ninety trees were sampled and the majority rule tree with posterior probability estimates was reconstructed after 11 hours of simulation in total.

Reconstructions obtained through parsimony and maximum likelihood analysis were considerably different, with only 19 of 33 nodes in common. Oppositely, reconstructions obtained through maximum likelihood and Bayesian inferences were slightly similar, with 29 of 33 taxa being placed identically. The maximum likelihood tree with corresponding bootstrap values is represented in Figure 2, whereas the tree obtained by Bayesian inferences with corresponding posterior probabilities is represented in Figure 3. Due to the major discrepancy between the pattern obtained for parsimony tree and the two other trees – probably because of the lack of parsimony-informative sites in the 384-nucleotide *12s rRNA* sequence – we decided not to take into

account information obtained through parsimony means for further discussion. However, bootstrap values for nodes that are common to the maximum likelihood tree are represented in Figure 2.

The 32 Bruchini species analyzed in this study are represented in two different clades : a first clade containing 22 of the 26 *Acanthoscelides* species studied, and a second containing all Palearctic species plus four Neotropical species, *Acanthoscelides macrophthalamus, A. oblongoguttatus, A. mexicanus* and *Merobruchus placidus*, all of them feeding on Mimosoideae. Globally, *Acanthoscelides* seems thus to be a good genus, with only the species feeding on Mimosoideae (i. e., *A. macrophthalamus, A. mexicanus* and *A. oblongoguttatus*) and the Old-World species *A. plagiatus* being misplaced, actually belonging to the *Bruchidius* clade. In the "true" *Acanthoscelides* (i. e., the 22 species branching together in a single clade), a strong tendency to radiation on similar host-plants is shown, particularly for species feeding on the two Phaseoleae, *Phaseolus* and *Rhynchosia*, on the Desmodieae *Desmodium*, and those on Malvaceae *sensu lato* (except *A. sanblas* [megacornis group], which develops on grewioid species and is unrelated to the other Malvaceae-feeders). However, in some cases, there is evidence of recent host shifts, for example in the case of *A. puellus* (developing on *Calopogonium* sp.), a species closely related to *Desmodium*-feeders.
Robustness (in terms of monophyly) of the morphological groups defined by Johnson was variable. Whereas species from groups *obtectus* (*A. obtectus, A. obvelatus* and *A. argillaceus*) and a*equalis* (*A. anoditus, A. guazumae* and *A. malvastrumicis*) clustered strictly together, groups *flavescens* (*A. flavescens* and *A. isla*), *pertinax* (*A. biustulus, A. cuernavaca, A. desmodicola, A. desmoditus, A. mazatlan, A. stylifer*, and *A. zonensis*) and *puellus* (*A. clandestinus, A. palmasola, A. puellus, A. sanfordi* and *A. taboga*) were not monophyletic. Concerning groups *megacornis, mexicanus, mundulus* and *oblongoguttatus*, we were unable to test monophyly since we analyzed only one species per group (*A. sanblas* for group *megacornis, A. mexicanus* for group *mexicanus, A. mundulus* for group *mundulus, A. oblongoguttatus* for group *oblongoguttatus*).

Discussion

Use of molecular techniques on pin-mounted dry specimens

Because of the poor-preservation of DNA of the studied specimens, we were able to amplify and sequence a sufficiently long portion of only one of the genes tested, about 400 bp of the mitochondrial *12s rRNA*. To our knowledge, most molecular phylogenetic studies of insects have been done on fresh material or material conserved in alcohol (or acetone, or other fluids). This study demonstrates that it is possible to use relatively old dried specimens when no fresh material is available. The primer pair *12Sai* & *12bi* appears capable of annealing onto DNA present in very low concentrations, compared to the *CytB* and *COI* universal primers, with which we could not obtain clean sequences long enough to be informative. Despite the fact that we were able to work with only a single gene, and that bootstrap values of deep nodes were relatively low, the good congruence between results obtained by maximum likelihood and Bayesian inferences methods argues for a relative robustness of our phylogenetic reconstruction.

Host-plant association

In each of the five groups (*aequalis*, *albopygus*, *blanchardi*, *pertinax*, and *puellus*) well defined on the basis of morphology of the male genitalia, there was a very strong tendency for species of the same group to be associated with closely related host plants. This tendency was especially marked for species of the groups whose species develop on Malvaceae (i. e., groups *aequalis* and *blanchardi*) and Mimosoideae (i. e., group *albopygus*). The tendency was less strongly marked for species of groups *puellus* and *pertinax*, which in addition were demonstrated by the phylogenetic analysis to be paraphyletic.

On the basis of the phylogenetic tree obtained from *12s rRNA* sequences, the role of host plants in driving fine-scale patterns of radiation is generally confirmed. Four clades attest to radiation after adaptation to particular kinds of host plant. These are three *Acanthoscelides* species on *Phaseolus*, four species on *Rhynchosia*, four species on *Desmodium* and three species on Malvaceae. This result suggests that when a

lineage of bruchids becomes adapted to a certain kind of host-plant, it may undergo evolutionary radiation onto other closely related plants. Adaptation to the particular secondary metabolites of a group of plants is a likely candidate for such a key innovation. A fifth clade, the group with *A. macrophthalamus, A. oblongoguttatus* and *Merobruchus placidus* – the three species feeding on Mimosoideae – also demonstrate an association between phylogenetic proximity and host-plant categories. This particular case will be discussed later in this study.

Particular attention must be given to the proximity between the clade of species feeding on *Phaseolus* and the clade of species feeding on *Desmodium.* Although Phaseoleae and Desmodieae were long considered not particularly closely related, recent phylogenies indicate that the two tribes can be grouped in a monophyletic clade (Wink & Mohamed 2003). Our results suggest that this phylogenetic relatedness is probably accompanied by some chemical similarity constraining host-plant association in the *Acanthoscelides* on *Phaseolus* and *Desmodium*. This is a good example of how the evolutionary history of phytophagous insects can give insights on the evolution of host plants. However, at least two cases of host shifts at terminal branches attests a more complex dynamics of speciation, since key innovations in herbivores may allow a lineage to colonize newly and chemically-different host plants.

Nature and origin of the genus Acanthoscelides

Our data reveal that *Acanthoscelides* is monophyletic, if the species on Mimosoideae and the Palearctic species questionably attached to the genus (e.g., *A. plagiatus* in this study) are removed. Our study shows that *A. plagiatus* should be placed in *Bruchidius* as previously argued by Borowiec (1987). We consider it highly likely that this result could be generalized to the other Palearctic species described or treated as *Acanthoscelides* by Lukjanovitsch & Ter-Minassian (1957).

Concerning *Acanthoscelides* species specialized on Mimosoideae, along with the other Neotropical bruchid genus studied here – i. e. *Merobruchus placidus* – they are clearly more closely related to the old world genus *Bruchidius* Schilsky (the sister genus of *Acanthoscelides*), than to the main *Acanthoscelides* clade.

The two main clades of Bruchini studied here are therefore the *Bruchidius* clade (including the species discussed above that are incorrectly assigned to *Acanthoscelides*) and *Acanthoscelides* (with these species excluded), respectively. Since most of the species of the *Bruchidius* clade are from the Old World, and all the species of the *Acanthoscelides* clade are from the New World, this dichotomy could be explained by a Gondwanan vicariance origin 90 Mya, or more recently by the disconnection of the early Beringian Bridges between the Eastern Paleartic and the Western Nearctic (35 Mya) (Scotese 2004). However, the position of the small New World clade, represented by *A. oblongoguttatus*, *A. macrophthalamus* and *Merobruchus placidus*, along with *A. mexicanus*, all branching inside the *Bruchidius* clade, could be explained either by vicariance events or by a more recent colonization to the New World by one or more members of the Old World *Bruchidius* clade.

Gondwanan vicariance hypothesis for the origin of the New World individuals branching inside Bruchidius

Because several New World species branch in the *Bruchidius* clade, we cannot eliminate the hypothesis of a Gondwanan vicariance to explain this pattern. We could easily imagine that lineages of all species studied have a New World origin, and that a process of speciation anterior to the separation of Gondwana occurred between what represents now the main *Acanthoscelides* clade, and the clade with the other species of Bruchinae examined here. Subsequent to this divergence and the Gondwanan separation, ancestors of this latter clade could have engendered both Old World *Bruchidius* and species of the small New World clade incorrectly assigned to *Acanthoscelides*.

Colonization hypothesis for the origin of the New World individuals branching inside Bruchidius

We could also imagine that *A. macrophthalamus*, *A. oblongoguttatus*, *A. mexicanus*, and *Merobruchus placidus* are descendants of one or more members of Paleotropical *Bruchidius* ancestors that colonized the New World. This colonization could have been

effected by migrants issued from the *Bruchidius* clade posterior to the Gondwanian or Beringian separation, possibly developing on Mimosoideae, as is consistent with the fact that *A. oblongoguttatus, A. mexicanus, A. macrophthalamus* and *Merobruchus placidus* are the only New World species in our study that feed on Mimosoideae. Colonization of the New World unambiguously posterior to the breakup of Gondwana has been suggested, on the basis of molecular evidence, for a rainforest tree with amphi-Atlantic distribution, *Symphonia globulifera* (Clusiaceae), which may have reached America through marine dispersal of trunks or roots [Dick *et al.* 2003]), and for caviomorph rodents via "stepping stone" islands, and rafts carried by tropical rivers into the ocean (Huchon & Douzery 2001). For a more general review about oceanic dispersal, see Queiroz (2005).

In the case of bruchids, several plausible hypotheses can be formulated. First, since most hurricanes that reach the Atlantic coast of America arise off the coast of Africa, bruchids could have been able to cross the ocean. Insects do occasionally disperse long distances, moved by storms. American Monarch butterflies (*Danaus plexippus*), for example, have been able to reach and establish colonies on the coast of western Europe, as well as on Pacific islands, probably through cyclonic winds or hurricanes (Zalucki & Clarke 2004). Besides, insects are known to be able to cover hundreds, or even thousands, of kilometers when they are carried away in ascending air currents (Compton 2001). However, taking into account the seminivorous biology of bruchids, transcontinental colonization by arrival on floating seeds, or seeds carried in rafts, could also be plausible. Seeds of several African species of legume trees have been found on the Atlantic and Caribbean coasts of America, among them species of *Cassia* or *Caesalpinia* (Gunn *et al.* 1976).

Conclusion

Despite the morphological and ecological diversity among species of the genus *Acanthoscelides* (long considered paraphyletic by several authors [e. g. Borowiec 1987]), the majority of the species described as *Acanthoscelides* constitute a monophyletic group. Exceptions to this are Palearctic species, and Neotropical species developing on Mimosoideae. Whereas deep and intermediate nodes are the result of

either geological vicariance or long-distance colonization, the role of host plant seems globally determinant in driving radiation in the terminal branches, although several host-shift processes have also been addressed. As suggested by Kergoat *et al.* (2004), chemical compounds could be the principal host-plant traits driving these radiations. Testing this hypothesis, already demonstrated in several other phytophagous groups of beetles (Becerra 2003; Termonia *et al.* 2002), will represent the next step of this study.

Acknowledgements

The authors thank C. D. Johnson, A. Delgado-Salinas, T. Jermy, F. Kjellberg, G. Kunstler, D. McKey and X. Morin, for their very helpful comments. They also thank J. Contreras, C. Macias, H. Drummond, R. Torres, E. Avila, V. Souza, L. Eguiarte and A. Valera for providing logistical help in Mexico. The first author wish to thank particularly G. Kergoat, for helping him to improve his knowledge on phylogenetics. This work was financially supported by the Swiss National Science Foundation (project N°3100.064821.01) and the Centre d'Ecologie Fonctionnelle et Evolutive.

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Table 1. List of sampled species, with information about the author, the site of sampling, the sampling date, the name of the collector, the host plant, the morphological group (in Neotropical *Acanthoscelides*, as defined by Johnson [1983, 1990], and the accession number corresponding to the *12s rRNA* sequence deposited in Genbank. Sampling countries were abbreviated as follows: Alg.=Algeria, Aze.=Azerbaijan, Col.=Colombia, Ecu.=Ecuador, Nic.=Nicaragua, Pan.=Panama, Tad.=Tadjikistan, Tur.=Turkey, Ven.=Venezuela, Vie.=Vietnam, Yem.=Yemen. An $^{(*)}$ indicates that collected species were obtained from unknown host plants, and that we assigned the host-plant most commonly associated with the species (from Johnson [1983, 1990] and Udayagari & Wadhi [1989]).

Table 2. Differences revealed by discriminant analysis between the species groups defined on morphological grounds. Pairs of groups were compared using Hotelling's T Squared statistics based on axis values of the multivariate correspondence analysis. *** : $p < 10^{-3}$; **: $p < 10^{-2}$; * : $p < 0.05$; NS : non significant. Groups in bold show significant differences from at least seven of the nine other groups.

Table 3. Host-plant associations for the species groups *aequalis* (*aeq.*), *albopygus* (*alb.*), *blanchardi* (*bla.*), *puellus* (*pue.*), and *pertinax* (*per.*). Names of host-plant groups were abbreviated as follows: Faboideae (Fab.), Aeschynomeneae (Aesch.), Amorpheae (Amor.), Desmodieae (Des.), Galegeae (Gal.), Indigofereae (Ind.), Loteae (Lot.), Millettieae (Mil.), Phaseoleae (Phas.), Mimosoideae (Mim.), Malvaceae *sensu lato* (Mal.), Rhamnaceae (Rha.).

Figure 1. Representation of the number of transitions and transversions as a function of the Kimura 2-parameters distance, for all pairs of species sequenced.

Figure 2. Maximum likelihood consensus phylogenetic tree obtained after 100 bootstraps for 33 species of Bruchidae. At each node, the first number corresponds to the bootstrap value obtained by maximum likelihood methods after 100 bootstraps. The second number at each node corresponds to the bootstrap value obtained by parsimony methods after 100 bootstraps (tree not shown). Absence of this second number

(indicated by "-") signifies non-congruency of the consensus trees obtained by maximum likelihood and parsimony methods.

Figure 3. Tree from the Bayesian inferences analysis. At each node, the number indicates the Bayesian posterior probabilities.

Figure 4. Consensus tree from the Bayesian inferences analysis. On the cladogram is represented (with different branch colors) the host-plant genus – or tribe or subfamily – on which a considered bruchid species develops. On the right side of the tree is figured the biogeographic origin of the species (New World *vs.* Old World).

Appendix. List of all described species of *Acanthoscelides*, according to Johnson (1983, 1990), Udayagari & Wadhi (1989), Egorov 1990, and Egorov & Ter-Minassian (1981). Ranges are coded as follows: N=North America; H=North and South America; S= northern South America; eS=southern South America; A=Amazonian South America; EA=Eurasia. Particular attention must be given to species described by Pic, due to probable synonymies (Boroviec [1987] described this author: "Famous for his maniacal obsession with describing new taxa and for his lack of precise diagnostic notes, M. Pic halted investigations for many years. He simply made the proper interpretation of species described by himself impossible to other researchers.")

Table 1.

Genus	Species	Author	Site of sampling	Samp. date	Collector	Host plant	Morph. group	accession
Zabrotes	*planifrons*	Horn	Mex. Huautla	16/IV/2000	Figueroa de la R. I	ND	-	AY945992
Acanthoscelides	*anoditus*	Johnson	Mex. Irapuato	16/X/2000	ND	*Anoda cristata* (*)	Aequalis	AY945996
Acanthoscelides	*argillaceus*	Sharp	Mex. Playa Azul	01/II/2001	Aebi A	*Phaseolus lunatus*	Obtectus	AY945967
Acanthoscelides	*biustulus*	Fall	Mex. Amealco	11/X/2002	Romero N. J	*Desmodium* sp. (*)	Pertinax	AY945968
Acanthoscelides	*clandestinus*	Motschoulsky	Mex. C. Carmen	17/II/1996	Ramírez DR	*Vigna adenantha*	Puellus	AY945969
Acanthoscelides	*cuernavaca*	Johnson	Mex. Huautla	4/II/2000	Romero N. J	*Desmodium* sp.(*)	Pertinax	AY945970
Acanthoscelides	*desmodicola*	Johnson	Mex. Huautla	5/II/2000	Figueroa de la R. I	*Desmodium* sp. (*)	Pertinax	AY945971
Acanthoscelides	*desmoditus*	Johnson	Ven. Barquisimeto	17/VII/1984	Johnson CD	*Desmodium tortuosum*	Pertinax	AY945972
Acanthoscelides	*flavescens*	Fahraeus	Mex. El Maruqes	22/II/1998	Luna Cozar J	*Rhynchosia minima* (*)	Flavescens	AY945997
Acanthoscelides	*guazumae*	Johnson & Kingsolver	Mex. Huautla	3/XI/1996	Romero N. J	*Guazuma tomentosa*	Aequalis	AY945974
Acanthoscelides	*isla*	Johnson	Ecu. Guayaquil	3/VII/1984	Johnson CD	*Rhynchosia minima*	Flavescens	AY945975
Acanthoscelides	*macrophthalamus*	Schaeffer	Vie. Saïgon	ND	Delobel H	*Leucanea leucocephala*	Mexicanus	AY945976
Acanthoscelides	*malvastrumicis*	Johnson	Mex. El Cielo	28/VII/1998	Niño S & Hernández J	*Malvastrum americanum* (*)	Aequalis	AY945977
Acanthoscelides	*mazatlan*	Johnson	Mex. Huautla	16/IV/2000	Romero N. J	*Desmodium* sp. (*)	Pertinax	AY945978
Acanthoscelides	*mexicanus*	Sharp	Mex. Coxcatlan	15/XII/2002	Alvarez N & Ciao V	*Mimosa* sp.	Mexicanus	AY945979
Acanthoscelides	*mundulus*	Sharp	Mex. Jalcomulco	18/II/1996	Romero N. J	*Nissolia fruticosa*	Mundulus	AY945980
Acanthoscelides	*oblongoguttatus*	Fahraeus	Mex. Cotaxtla	28/VII/2000	Morse GE & Romero N. J	*Acacia cornigera*	Oblongoguttatus	AY945981
Acanthoscelides	*obtectus*	Say	Mex. Tepoztlan	15/I/2002	Alvarez N & Aebi A	*Phaseolus vulgaris*	Obtectus	AY945998
Acanthoscelides	*obvelatus*	Bridwell	Mex. Tepoztlan	15/I/2002	Alvarez N & Aebi A	*Phaseolus vulgaris*	Obtectus	AY945983
Acanthoscelides	*palmasola*	Johnson	Mex. Tenabo	1/I/1979	Johnson CD	*Rhynchosia longeracemosa*	Puellus	AY945984
Acanthoscelides	*plagiatus*	Reichle & Saulcy	Tur. Van Gölü	29/VI/1993	ND	*Astragalus* sp.	-	AY945999
Acanthoscelides	*puellus*	Sharp	Nic. El Progreso	15/IV/1998	Maes JM	*Calopogonium mucumoides*	Puellus	AY946000
Acanthoscelides	*sanblas*	Johnson	Mex. Cordoba	1/III/1996	Romero N. J	*Triumfetta lappula*	Megacornis	AY945986
Acanthoscelides	*sanfordi*	Johnson	Mex. Huautla	4/XI/2000	Romero N. J	*Pachyrhizus erosus*	Puellus	AY945987
Acanthoscelides	*stylifer*	Sharp	Mex. Ixmiquilpan	21/VIII/2002	Romero N. J	*Desmodium* sp. (*)	Pertinax	AY945988
Acanthoscelides	*taboga*	Johnson	Pan. Chepo	2/IV/1980	Johnson CD	*Calopogonium caeruleum*	Puellus	AY945989
Acanthoscelides	*zonensis*	Johnson	Col. Palmira	8/XI/1983	Johnson CD	*Teramnus uncinatus*	Pertinax	AY945990
Bruchidius	*foveolatus*	Gyllenhal	Alg. Amouchas	02/VI/1986	Warchalowski A	*Cytisus* sp. (*)	-	AY946001
Bruchidius	*quinqueguttatus*	Olivier	Tur. Anamurium	01/V/2001	Anton KW	*Vicia* sp. (*)	-	AY945961
Bruchidius	*raddianae*	Anton & Delobel	Yem. Lahj	1/IX/2001	Sallam A	*Acacia tortilis*	-	AY625297
Bruchidius	*tuberculatus*	Hochhut	Aze. Talysh	01/V/1993	Alexeevka V	Unknown Faboideae (*)	-	AY946002
Palaeoacanthoscelides	*gilvus*	Gyllenhal	Tad. Oktynbrskaya	18/V/1991	Dangara S	Unknown Faboideae	-	AY946004
Merobruchus	*placidus*	Horn	Mex. Coxcatlan	20/XII/2002	Alvarez N & Ciao V	*Acacia* sp.	-	AY945965

Table 2.

	albopygus	*blanchardi*	*flavescens*	*megacornis*	*mexicanus*	*obtectus*	*pertinax*	*puellus*	*quadridentatus*
aequalis	***	***	*	NS	*	*	***	***	NS
albopygus		**	**	***	NS	*	***	***	**
blanchardi			NS	***	**	*	***	***	***
flavescens				**	NS	NS	***	***	**
megacornis					NS	*	***	*	NS
mexicanus						NS	***	*	*
obtectus							NS	NS	*
pertinax								**	***
puellus									NS

Table 3.

group	Species	Associated host-plants
aequalis (aeq.)	*Aequalis*	*Abutilon* (Mal.), *Pseudabutilon* (Mal.), *Wissadula* (Mal.)
	altocaura (aeq.)	?
	anoditus (aeq.)	*Anoda* (Mal.)
	apicalis (aeq.)	*Malachra* (Mal.)
	aragua (aeq.)	*Wissadula* (Mal.)
	bechyneorum (aeq.)	?
	bogot (aeq.)*a*	?
	bolivar (aeq.)	?
	brevipes (aeq.)	*Malvastrum* (Mal.), *Sida* (Mal.)
	colombiano (aeq.)	?
	Coro (aeq.)	*Malvastrum* (Mal.), *Sida* (Mal.)
	elkinsae (aeq.)	*Hibiscus* (Mal.)
	falcon (aeq.)	*Abutilon* (Mal.)
	guaibacoa (aeq.)	*Abutilon* (Mal.)
	guazumae (aeq.)	*Guazuma* (Ste.)
	guerrero (aeq.)	*Herissantia* (Mal.), *Malvastrum* (Mal.)
	guiana (aeq.)	*Abutilon* (Mal.), *Hibiscus* (Mal.)
	herissantitus (aeq.)	*Herissantia* (Mal.), *Malvastrum* (Mal.)
	Johni (aeq.)	*Herissantia* (Mal.)
	machiques (aeq.)	*Pavonia* (Mal.)
	malvastrumicis (aeq.)	*Malvastrum* (Mal.)
	malvitus (aeq.)	*Abutilon* (Mal.), *Malva* (Mal.)
	maturin (aeq.)	*Hibiscus* (Mal.)
	merida (aeq.)	*Abutilon* (Mal.)
	monagas (aeq.)	*Hibiscus* (Mal.)
	pyramididos (aeq.)	*Sida* (Mal.)
	santarosa (aeq.)	*Herissantia* (Mal.)
	sleeperi (aeq.)	*Abutilon* (Mal.)
	subaequalis (aeq.)	*Abutilon* (Mal.)
	Tepic (aeq.)	*Abutilon* (Mal.)
	univittatus (aeq.)	*Guazuma* (Mal.)
albopygus (alb.)	*albopygus* (alb.)	?
	buenaventura (alb.)	legume tree (Fab. Mim.)
	caripe (alb.)	?
	cesari (alb.)	legume tree (Fab. Mim.)
	elevatus (alb.)	?
	elvalle (alb.)	?
	lituratus (alb.)	?
	petalopygus (alb.)	*Acacia* (Fab. Mim.)
	sousai (alb.)	*Acacia* (Fab. Mim.)
	*subliturat*us (alb.)	?
	tinalandia (alb.)	?
blanchardi (bla.)	*blanchardi* (bla.)	*Kosteletzkya* (Mal.)
	fryxelli (bla.)	*Kosteletzkya* (Mal.), *Malachra* (Mal.)
	hibiscicola (bla.)	*Hibiscus* (Mal.)
	orlandi (bla.)	*Kosteletzkya* (Mal.), *Malachra* (Mal.)
	pavoniestes (bla.)	*Pavonia* (Mal.)
	santander (bla.)	?
	vexatus (bla.)	?
	wicki (bla.)	?
pertinax (per.)	*argutus* (per.)	*Teramnus* (Fab. Phas.)
	biustulus (per.)	*Desmodium* (Fab. Des.)
	cuernavaca (per.)	*Desmodium* (Fab. Des.)
	desmodicola (per.)	*Desmodium* (Fab. Des.)
	desmoditus (per.)	*Desmodium* (Fab. Des.)
	howdenorum (per.)	*Desmodium* (Fab. Des.)
	lichenicola (per.)	?
	mazatlan (per.)	*Desmodium* (Fab. Des.)
	oaxaca (per.)	?
	pedicularius (per.)	*Petalostemum* (Fab. Amor.)
	pertinax (per.)	*Aeschynomene* (Fab. Aesch.), *Desmodium* (Fab. Des.), *Dalea* (Fab. Amor.), *Stylosanthes* (Fab. Aesch.)
	puelliopsis (per.)	*Desmodium* (Fab. Des.)
	schubertae (per.)	*Desmodium* (Fab. Des.)
	stylifer (per.)	*Desmodium* (Fab. Des.)
	zonensis (per.)	*Teramnus* (Fab. Phas.)
puellus (pue.)	*amabilis* (pue.)	*Rhynchosia* (Fab. Phas.)
	aureolus (pue.)	*Acmispon* (Fab. Lot.), *Astragalus* (Fab. Gal.), *Glycyrrhiza* (Fab. Gal.), *Hosackia* (Fab. Lot.), *Ottleya* (Fab. Lot.), *Oxytropis* (Fab. Gal.), *Syrmatium* (Fab. Lot.)
	barneby (pue.)	?
	barrocolorado (pue.)	?
	caroni (pue.)	*Indigofera* (Fab. Ind)
	chiapas (pue.)	?
	clandestinus (pue.)	*Phaseolus* (Fab. Phas.)
	colombia (pue.)	?
	dominicana (pue.)	*Calopogonium* (Fab. Phas.)
	donckieropsis (pue.)	?
	fernandezi (pue.)	?
	griseolus (pue.)	*Calopogonium* (Fab. Phas.)
	guarico (pue.)	*Rhynchosia* (Fab. Phas.)
	indigoforestes (pue.)	*Indigofera* (Fab. Ind)
	jardin (pue.)	?
	kingsolveri (pue.)	*Indigofera* (Fab. Ind)
	leisneri (pue.)	?
	luteus (pue.)	?
	palmasola (pue.)	*Rhynchosia* (Fab. Phas.)
	prosopoides (pue.)	*Ziziphus* (Rha.)
	puellus (pue.)	*Calopogonium* (Fab. Phas.)
	rhynchosiestes (pue.)	*Rhynchosia* (Fab. Phas.)
	ruficoxis (pue.)	*Indigofera* (Fab. Ind)
	rufovittatus (pue.)	*Galactia* (Fab. Phas.), *Tephrosia* (Fab. Mill.)
	sanfordi (pue.)	*Pachyrhizus* (Fab. Phas.), *Rhynchosia* (Fab. Phas.)
	schaefferi (pue.)	?
	suaveolus (pue.)	*Vigna* (Fab. Phas.)
	surrufus (pue.)	*Rhynchosia* (Fab. Phas.)
	taboga (pue.)	*Calopogonium* (Fab. Phas.), *Pachyrhizus* (Fab. Phas.)
	yecora (pue.)	?

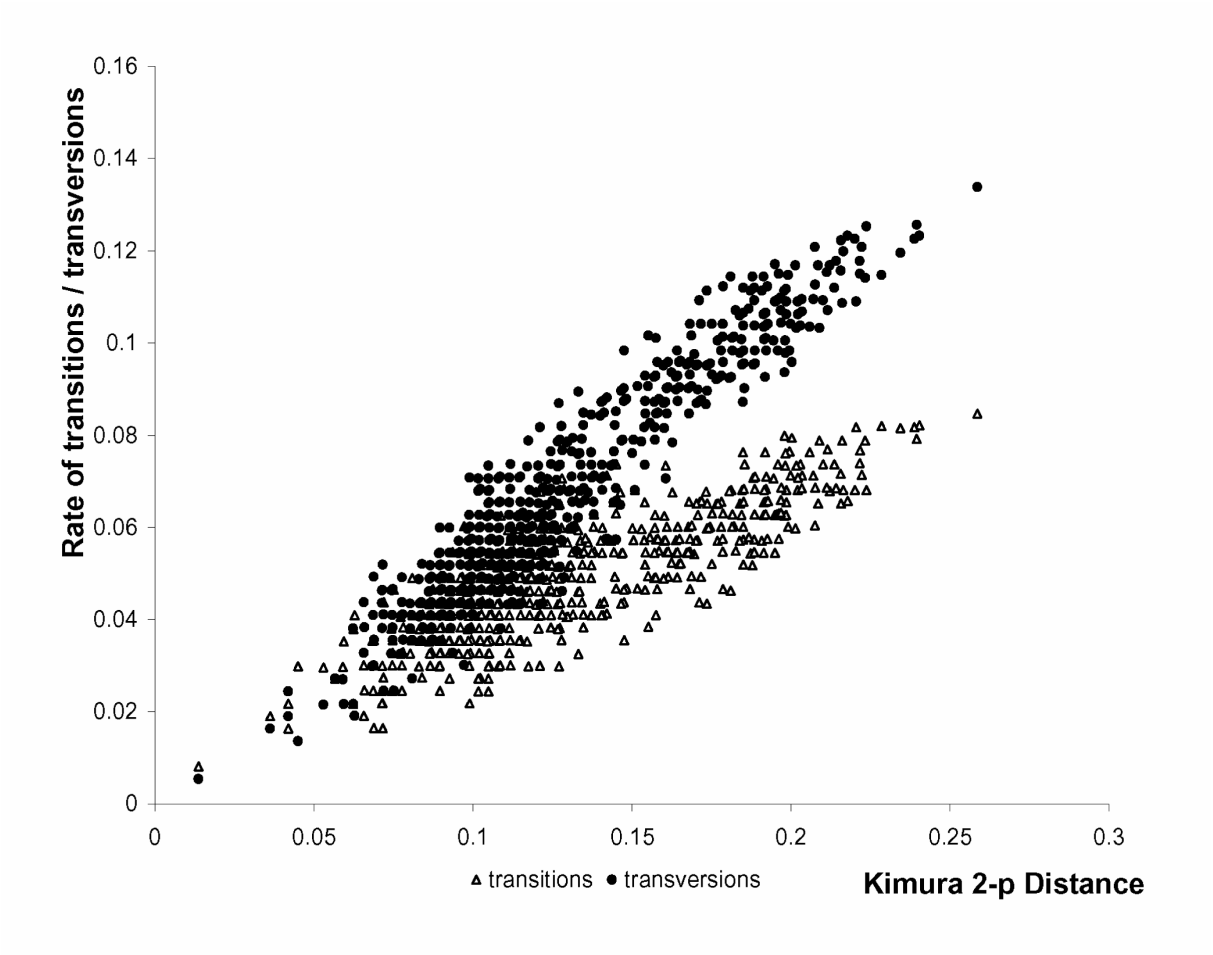
Rate of transitions / transversions
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0
0.05
0.1
0.15
0.2
0.25
0.3
transitions
transversions
Kimura 2-p Distance

Figure 1

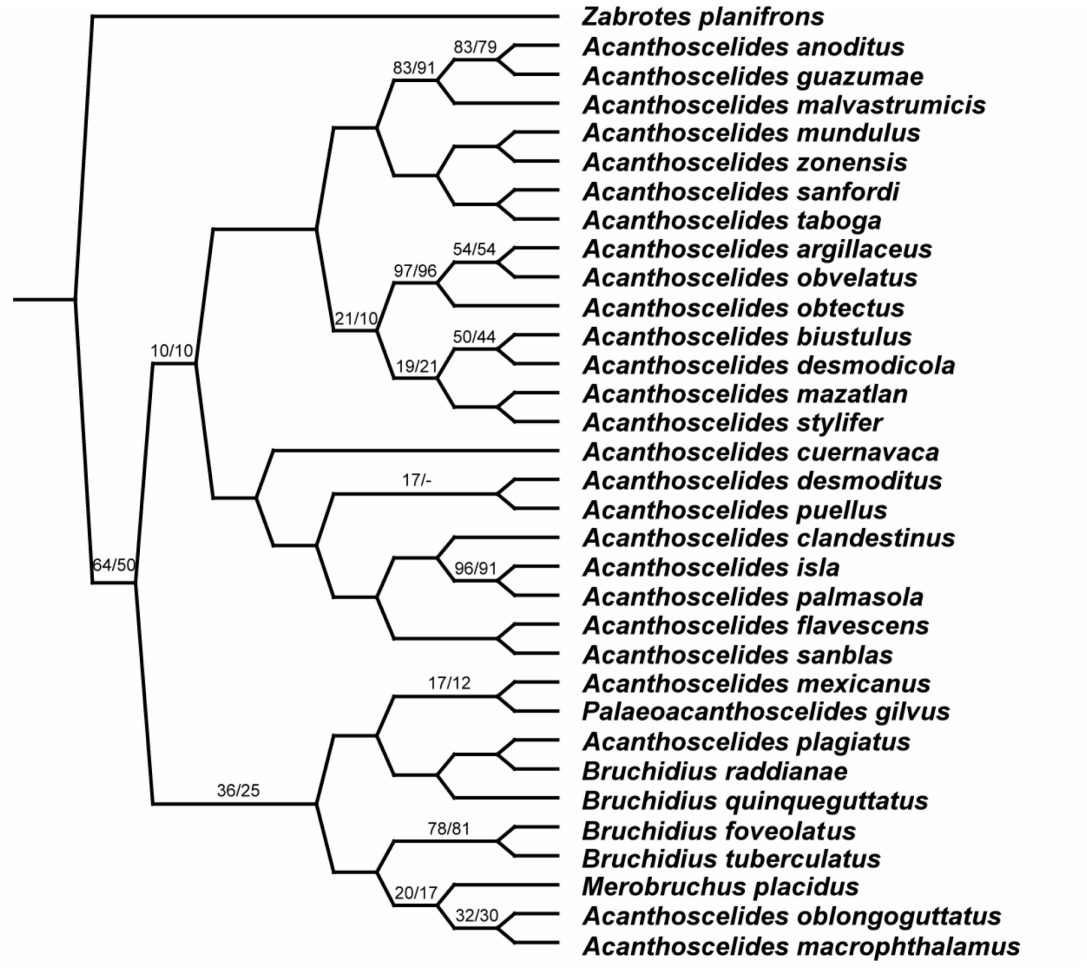
Zabrotes planifrons
Acanthoscelides anoditus
Acanthoscelides guazumae
Acanthoscelides malvastrumicis
Acanthoscelides mundulus
Acanthoscelides zonensis
Acanthoscelides sanfordi
Acanthoscelides taboga
Acanthoscelides argillaceus
Acanthoscelides obvelatus
Acanthoscelides obtectus
Acanthoscelides biustulus
Acanthoscelides desmodicola
Acanthoscelides mazatlan
Acanthoscelides stylifer
Acanthoscelides cuernavaca
Acanthoscelides desmoditus
Acanthoscelides puellus
Acanthoscelides clandestinus
Acanthoscelides isla
Acanthoscelides palmasola
Acanthoscelides flavescens
Acanthoscelides sanblas
Acanthoscelides mexicanus
Palaeoacanthoscelides gilvus
Acanthoscelides plagiatus
Bruchidius raddianae
Bruchidius quinqueguttatus
Bruchidius foveolatus
Bruchidius tuberculatus
Merobruchus placidus
Acanthoscelides oblongoguttatus
Acanthoscelides macrophthalamus
83/79
83/91
54/54
97/96
21/10
50/44
19/21
10/10
64/50
17/-
96/91
17/12
36/25
78/81
20/17
32/30

Figure 2

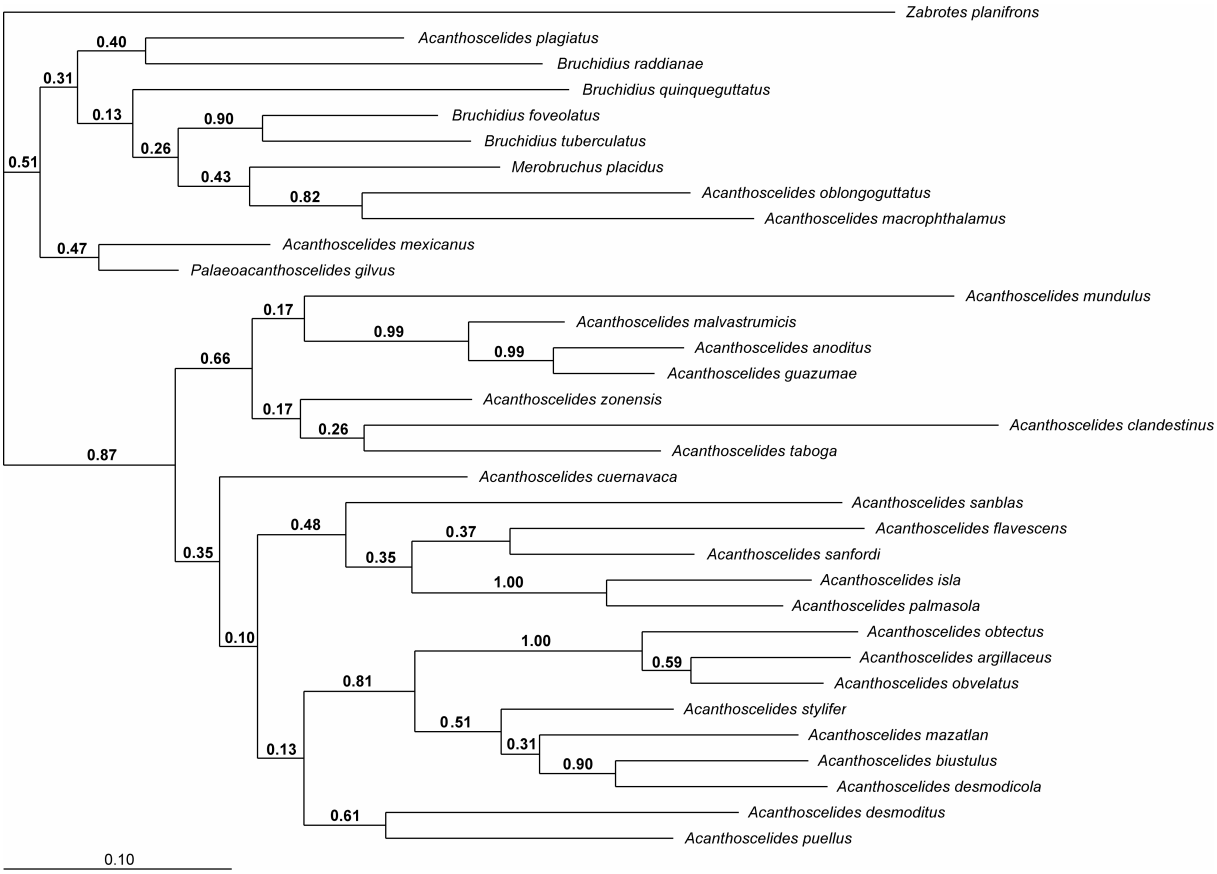
Zabrotes planifrons
Acanthoscelides plagiatus
Bruchidius raddianae
Bruchidius quinqueguttatus
Bruchidius foveolatus
Bruchidius tuberculatus
Merobruchus placidus
Acanthoscelides oblongoguttatus
Acanthoscelides macrophthalamus
Acanthoscelides mexicanus
Palaeoacanthoscelides gilvus
Acanthoscelides mundulus
Acanthoscelides malvastrumicis
Acanthoscelides anoditus
Acanthoscelides guazumae
Acanthoscelides zonensis
Acanthoscelides clandestinus
Acanthoscelides taboga
Acanthoscelides cuernavaca
Acanthoscelides sanblas
Acanthoscelides flavescens
Acanthoscelides sanfordi
Acanthoscelides isla
Acanthoscelides palmasola
Acanthoscelides obtectus
Acanthoscelides argillaceus
Acanthoscelides obvelatus
Acanthoscelides stylifer
Acanthoscelides mazatlan
Acanthoscelides biustulus
Acanthoscelides desmodicola
Acanthoscelides desmoditus
Acanthoscelides puellus
0.40
0.31
0.13
0.90
0.26
0.51
0.43
0.82
0.47
0.17
0.99
0.99
0.66
0.17
0.26
0.87
0.48
0.37
0.35
0.35
1.00
0.10
1.00
0.59
0.81
0.51
0.31
0.90
0.13
0.61
0.10

Figure 3

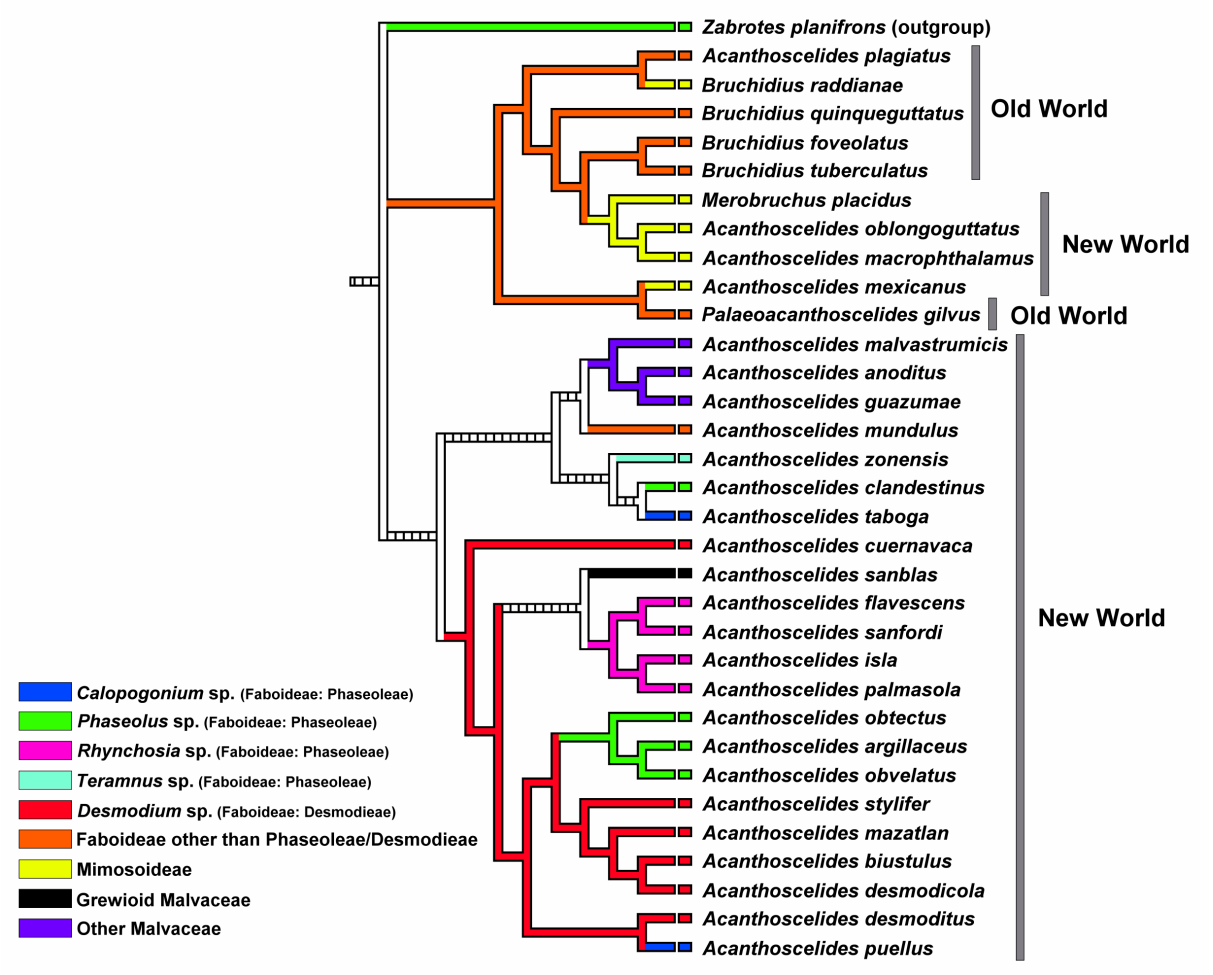
Zabrotes planifrons (outgroup)
Acanthoscelides plagiatus
Bruchidius raddianae
Bruchidius quinqueguttatus
Bruchidius foveolatus
Bruchidius tuberculatus
Old World
Merobruchus placidus
Acanthoscelides oblongoguttatus
Acanthoscelides macrophthalamus
Acanthoscelides mexicanus
New World
Palaeoacanthoscelides gilvus
Old World
Acanthoscelides malvastrumicis
Acanthoscelides anoditus
Acanthoscelides guazumae
Acanthoscelides mundulus
Acanthoscelides zonensis
Acanthoscelides clandestinus
Acanthoscelides taboga
Acanthoscelides cuernavaca
Acanthoscelides sanblas
Acanthoscelides flavescens
Acanthoscelides sanfordi
Acanthoscelides isla
Acanthoscelides palmasola
Acanthoscelides obtectus
Acanthoscelides argillaceus
Acanthoscelides obvelatus
Acanthoscelides stylifer
Acanthoscelides mazatlan
Acanthoscelides biustulus
Acanthoscelides desmodicola
Acanthoscelides desmoditus
Acanthoscelides puellus
New World
Calopogonium sp. (Faboideae: Phaseoleae)
Phaseolus sp. (Faboideae: Phaseoleae)
Rhynchosia sp. (Faboideae: Phaseoleae)
Teramnus sp. (Faboideae: Phaseoleae)
Desmodium sp. (Faboideae: Desmodieae)
Faboideae other than Phaseoleae/Desmodieae
Mimosoideae
Grewioid Malvaceae
Other Malvaceae

Figure 4

Appendix

Species	Author	Range
Aculeatus	Motschoulsky	eS
Aequalis	Sharp	N
Aequinoctialis	Fahraeus	A
Akanthodes	Johnson	S
Albopygus	Johnson	H
alboscutellatus	Horn	N
Alboscutus	Pic	A
Albovittatus	Pic	A
Aldanai	Johnson	S
Altocaura	Johnson	S
Amabilis	Johnson	N
amazonicus	Pic	A
amplilobus	Johnson	S
anoditus	Johnson	N
apicalis	Sharp	H
aragua	Johnson	S
argentinus	Pic	eS
argillaceus	Sharp	H
argutus	Sharp	H
atomus	Fall	N
atrocephalus	Pic	eS
atronotatus	Pic	A
atrosignatus	Pic	N
atrosignatus	Pic	N
attelaboides	Drapiez	A
aureolus	Horn	N
aureomicans	Pic	S
baboquivari	Johnson	N
bahianus	Pic	A
barnebyi	Johnson	H
barrocolorado	Johnson	H
batesi	Jekel	A
bechyneorum	Johnson	S
bellus	Johnson	N
bicolor	Philippi	eS
bicoloriceps	Pic	A
bicoloripes	Pic	A
bicoloritarsis	Pic	eS
bimutatus	Pic	eS
biplagiatus	Gyllenhal	A
bisignatipennis	Pic	eS
bisignatus	Horn	N
biustulus	Fall	N
blanchardi	Johnson	N
bogota	Johnson	S
bolivar	Johnson	S
boneti	Johnson	N
bosci	Fahraeus	S
bosqi	Pic	eS
brevipes	Sharp	H
buenaventura	Johnson	S
burkei	Johnson	N
caliginosus	Baudi	unknown

species	author	Range
callanganus	Pic	S
calvus	Horn	N
campeche	Johnson	N
canar	Johnson	S
capsincola	Fabricius	A
caracallae	Kingsolver	eS
caripe	Johnson	S
carneofasciatus	Pic	H
caroni	Johnson	S
catamarcanus	Pic	eS
cesari	Johnson	S
chiapas	Johnson	N
chilensis	Schilsky	eS
chiricahuae	Fall	N
cingulatus	Motschoulsky	eS
clandestinus	Motschoulsky	H
clitellarius	Fahraeus	H
colombia	Johnson	S
colombiano	Johnson	S
compressicornis	Schaeffer	N
comptus	Kingsolver	eS
conspurcatus	Blanchard	eS
cordifer	Sharp	N
cornis	Johnson	N
coro	Johnson	S
corumbanus	Pic	A
crassulus	Sharp	N
cruciatus	Hummel	A
cuernavaca	Johnson	N
curimagua	Johnson	S
daleae	Johnson	N
darlingtoni	Johnson	S
debilicornis	Sharp	N
derifieldi	Johnson	N
desmanthi	Johnson	H
desmodicola	Johnson	N
desmoditus	Johnson	H
devriesi	Kingsolver	H
difficilis	Sharp	H
diosanus	Pic	S
distinguendus	Horn	N
diversicollis	Pic	A
dominicana	Johnson	S
donckieri	Pic	A
donckieriopsis	Johnson	S
edmundi	Pic	A
egenus	Philippi	eS
elevatus	Sharp	H
elkinsae	Johnson	H
elongatus	Pic	N
elvalle	Johnson	S
ephippiatus	Steinheil	eS
equivocada	Johnson	S

species	author	Range
eriosemicola	Johnson	N
falcon	Johnson	S
fernandezi	Johnson	S
ferrugineipennis	Blanchard	eS
ferrugineus	Olivier	A
filarius	Sharp	N
flavescens	Fahraeus	H
flaviventris	Sharp	N
floridae	Horn	N
fraterculus	Horn	N
fryxelli	Johnson	N
fumatus	Schaeffer	N
funebris	Boheman	A
fuscomaculatus	Blair	S
fuscosparsus	Motschoulsky	A
gilvoides	Lukjanovitsch&Ter-Minassian	EA
glycinae	Fahraeus	A
gregorioi	Pic	A
griseolus	Fall	H
grossoensis	Pic	A
guadeloupensis	Pic	G
guaibacoa	Johnson	S
guanare	Johnson	S
guarico	Johnson	S
guazumae	Johnson&Kingsolver	H
guerrero	Johnson	H
guiana	Johnson	S
gussakovskii	Lukjanovitsch&Ter-Minassian	EA
hectori	Kingsolver	H
helianthemum	Bottimer	N
herissantitus	Johnson	N
hibiscicola	Johnson	N
howdenorum	Johnson	N
indigoferestes	Johnson	H
ingae	Fahraeus	N
ingeborgae	Johnson	S
inquisitus	Fall	N
isla	Johnson	H
jardin	Johnson	N
johni	Johnson	N
johnsoni	Kingsolver	H
jolyi	Johnson	S
kingsolveri	Johnson	H
klagesi	Johnson	S
laicus	Fahraeus	S
lapsanae	Motschoulsky	H
leisneri	Johnson	N
leucaenicola	Johnson	N
leucopygius	Perty	A
lineaticollis	Sharp	N
lituratus	Sharp	H
livens	Suffrian	C
lobatus	Fall	N
lojaensis	Pic	S

species	author	Range
longescutus	Pic	S
longistilus	Horn	N
luteus	Johnson	H
machala	Johnson	S
macheta	Johnson	S
machiques	Johnson	S
macrophthalmus	Schaeffer	N
maculicollis	Gyllenhal	A
malvastrumicis	Johnson	N
malvitus	Johnson	N
manducus	Johnson	H
mankinsi	Johnson	H
manleyi	Johnson	S
mapiriensis	Pic	N
maturin	Johnson	S
mazatlan	Johnson	N
mediolineatus	Pic	A
megacornis	Kingsolver	H
merida	Johnson	N
meridanus	Pic	S
mexicanus	Sharp	H
mimosicola	Johnson	N
mixtus	Horn	N
modestus	Sharp	H
monagas	Johnson	S
mucrofer	Bottimer	N
multialbonotatus	Pic	A
multilineatus	Pic	eS
mundulus	Sharp	N
mutatus	Pic	eS
napensis	Johnson	N
nescius	Fahraeus	A
nigriceps	Steinheil	eS
nigronotaticeps	Pic	eS
notatopygus	Pic	A
notulatus	Fahraeus	S
oaxaca	Johnson	N
oblongoguttatus	Fahraeus	N
obrienorum	Johnson	N
obsoletus	Say	N
obtectus	Say	H
obtusus	Boheman	eS
obvelatus	Bridwell	H
oculatus	Say	N
ophthalmicus	Sharp	N
optatus	Sharp	N
oregonensis	Johnson	N
orlandi	Johnson	H
paleatus	Jekel	A
pallidipennis	Motschoulsky	N+EA
palmasola	Johnson	N
pantherinus	Suffrian	C
patagonicus	Pic	eS
pauperculus	LeConte	N
pavoniestes	Johnson	N

species	author	Range
pectoralis	Horn	N
pedicularius	Sharp	N
perforatus	Horn	N
pertinax	Sharp	H
peruvianus	Pic	S
petalopygus	Kingsolver	N
philippi	Pic	eS
piceoapicalis	Pic	H
pigricola	Kingsolver	H
plagiatus	Reiche&Saulcy	EA
poverus	Blanchard	eS
praecanus	Motschoulsky	eS
prosopoides	Schaeffer	N
puelliopsis	Johnson	H
puellus	Sharp	H
pullatus	Sharp	N
pulloides	Fall	N
pullus	Fall	N
puncticollis	Fahraeus	A
puniceus	Johnson	N
pusillimus	Sharp	H
pygidiolineatus	Pic	A
pygidionotatus	Pic	S
pyramididos	Johnson	H
pyrrhomelas	Philippi	eS
quadratus	Suffrian	C
quadridentatus	Schaeffer	H
ramirezi	Johnson	S
reductelineatus	Pic	eS
reductus	Pic	A
restrictus	Sharp	N
rhynchosiestes	Johnson	N
ruficollis	Motschoulsky	eS
ruficoxis	Sharp	N
rufoplagiatus	Fahraeus	A
rufosignatus	Motschoulsky	A
rufovittatus	Schaeffer	H
sanblas	Johnson	N
sanfordi	Johnson	N
santander	Johnson	S
santarosa	Johnson	S
schaefferi	Pic	N
schaumi	Pic	A
schrankiae	Horn	H
schubertae	Johnson	N
scutellaris	Philippi	eS
scutulatus	Motschoulsky	A
semiannulatus	Pic	A
seminulum	Horn	N
senex	Motschoulsky	A
sennicola	Johnson	S
serenus	Sharp	N
serraticornis	Fabricius	N
sexnotatus	Steinheil	eS

species	author	Range
silvestrii	Pic	eS
sleeperi	Johnson	N
soijae	Gyllenhal	S
sousai	Johnson	N
speciosus	Schaeffer	N
spinosus	Fabricius	H
stylifer	Sharp	H
suaveolus	Sharp	H
subaenescens	Pic	A
subaequalis	Johnson	N
subimmaculatus	Pic	A
sublituratus	Johnson	N
submuticus	Sharp	N
subroseus	Motschoulsky	A
suramerica	Johnson	S
surrufus	Johnson	N
suturalis	Boheman	S
tabidus	Erichson	S
taboga	Johnson	H
tantillus	Motschoulsky	N
tenuis	Bottimer	N
tepic	Johnson	H
testaceopygus	Pic	A
tibiospinalis	Johnson	S
tinalandia	Johnson	S
transversesignatus	Fahraeus	A
triangularis	Say	N
tridenticulatus	Bottimer	N
trinotatus	Pic	A
triumfettae	Kingsolver	N
tucumanus	Pic	eS
univittatus	Pic	S
ventralis	Fahraeus	A
vestitus	Fahraeus	S
vexatus	Sharp	N
vianai	Pic	eS
villicus	Fahraeus	A
virgiliae	Motschoulsky	S
vittatus	Fabricius	S
wicki	Johnson	N
x-signatus	Pic	N
yecora	Johnson	N
yepezi	Johnson	S
zebratus	Kingsolver	N
zeteki	Kingsolver	H
zonensis	Johnson	N
zulia	Johnson	S

MANUSCRIT II

Parallels in the evolution of the two largest seed-beetle genera (Coleoptera : Bruchidae).

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* Ces auteurs ont collaboré de façon égale et sont considérés comme co-premiers auteurs.

(soumis à *Molecular Ecology*)

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4. Date of receipt :

5. Keywords : adaptive radiation; bruchidae; key innovation; host-plant associations; parallel evolution; phytophagous insects

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7. Running title : Parallel evolution in seed-beetles

Abstract

This study provided the first phylogenetic analysis for a large sample of the two largest genera of seed-beetles (Coleoptera, Bruchidae), *Acanthoscelides* Schilsky and *Bruchidius* Schilsky, which mostly feed on legumes (Fabaceae). The goal of this study was to investigate evolutionary patterns in relation to biogeography and host-plant associations. We used three mitochondrial molecular markers and parsimony and Bayesian inference methods to reconstruct the phylogeny of 76 species. We were thus able to examine the evolution of host-plant use by using character optimizations under maximum-likelihood. In addition, we critically reviewed host-plant records of the literature for these two bruchid genera which present a disjunct distribution (New World and Old World). Our results demonstrated the existence of two major clades, consistent with biogeographic regions, and also suggested the paraphyletic nature of both genera. We also highlighted a strong trend toward specialization (with a high taxonomic conservatism in host-plant use) exhibited by the two studied genera. However, we showed the existence of several host shifts during the evolution of this group of bruchids. The analysis of host shifts also demonstrates that the subfamily Papilionoideae is the ancestral host-plant group of *Acanthoscelides* and *Bruchidius* at least for the sampled species. Both our phylogenetic hypotheses and our evaluation of host-plant associations suggest that the two genera seem to have undergone parallel evolution, as they have independently colonized similar host-plants, and independently developed similar mechanisms of detoxification of secondary compounds in seeds, in their respective areas of distribution. Our estimation of divergence times indicated a more ancient origin than that suggested by the fossil records. Interestingly, the suggested timing of diversification is consistent with the hypothesis of an adaptive radiation which could have possibly occurred contemporaneously with the diversification of their legume hosts.

1. Introduction

The evolution of the species-rich superfamily Chrysomeloidea is a fascinating example of insect radiation on angiosperms. During their diversification, they successfully used almost all flowering plant parts (e.g., leaves, roots, seeds, stems and wood) as larval food resources (Johnson 1981). In Curculionoidea, the sister group of Chrysomeloidea, a similar trend is observed (Anderson 1995; Marvaldi 2002), and many authors have introduced the concept of adaptive radiation (Simpson 1953; Schulter 2000) to explain the enhanced rate of diversification of phytophagous Coleoptera on angiosperms (Mitter *et al.* 1988; Farrell 1998; Marvaldi *et al.* 2002; Farrell & Sequeira 2004). According to these authors (see also Futuyma 1986), this diversification is likely to be linked either to the frequent availability of new ecological niches (for which the insect is pre-adapted) or to the development of 'key innovations' (Simpson 1953) which enables the use of new food resources (in effect, creating new potential niches). In the coevolutionary model of Ehrlich & Raven (1964), evolutionary novelties enable insects to circumvent the defenses of plants, particularly toxic secondary compounds. The insects able to bypass these defenses are then likely to undergo a successful diversification on these hitherto unexploited resources. Adaptation to chemical defenses which then constrain host-plant use can account for two major patterns in phytophagous insect host-plant associations. First, the strong trend of taxonomic conservatism in host-use (e.g., Lopez-Vaamonde *et al.* 2003) can be explained by the fact that related plants often share similar chemical defenses. Secondly, convergent similarity in host-plant chemistry can explain host-shifts among taxonomically unrelated plants observed in groups such as Chrysomelidae (Becerra, 1997, 1999, 2003; Termonia *et al.* 2002, 2003). Similar patterns have been found in other groups of phytophagous insects, particularly in Lepidoptera (Wahlberg 2001; Nishida 2002).

The study of the evolutionary processes driving the diversification of Chrysomeloideae has been particularly intensive in the seed-beetles and is well summarized in Johnson's (1990a) review.

Recent changes in taxonomy and thus nomenclature seem to favor the use of the subfamily name Bruchinae rather than Bruchidae (C.D. Johnson, pers. comm.), but for convenience (when using nomenclature ranks below the family level) we have used Bruchidae in this study. Bruchids constitute a homogeneous group of about 1,700 described species in about 60 genera (Southgate 1979; Johnson 1994), most of which are associated with the family Fabaceae (Johnson 1981). Most bruchid species are specialized on a narrow range of host-plants (oligophagy or monophagy) and their larval stages develop exclusively inside seeds (Borowiec 1987). Indeed, the mechanical protection of hard-seeded legumes has been countered by specific adaptations in bruchids, which have thereby been able to use hard-coated seeds as a shield for their developing larvae. This adaptation has led bruchids to specialize on legumes, but has likely allowed them to undergo numerous parallel radiations, notably on different legume groups but also on other taxa with hard-coated seeds (or stony fruits). Moreover, the family Bruchidae is of particular interest as it contains several pest species of economic importance with worldwide distributions (Borowiec 1987; Delobel & Tran 1993). Bruchid taxonomy, biology and ecology have been intensively investigated by many authors (e.g., Johnson 1981; Borowiec 1987), but only recently have molecular phylogenetic methods been applied to evolutionary studies of this group (Silvain & Delobel 1998; Kergoat *et al.* 2004, 2005; Morse & Farrell 2005).

In this study, we have focused on the two largest seed-beetle genera, *Acanthoscelides* SCHILSKY and *Bruchidius* SCHILSKY, which represent about half of the known bruchid species. As currently circumscribed, *Acanthoscelides* is restricted to the New World and *Bruchidius* to the Old World. Working with distinct groups that have radiated independently in different geographic areas will allow us to test hypotheses concerning adaptive radiation in bruchids in the sense of Schluter (2000) who defines it as ‘the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage’. Indeed, these two largest bruchid genera are relevant models to examine general patterns

in adaptive radiation of specialized phytophagous insects, especially concerning the role of host-plant association.

We will also study possible parallel patterns between these two genera by comparing the diversification they have undergone on a large number of host-plant species (Johnson 1981; Borowiec 1987) in their respective areas of distribution, in which they seem to occupy similar ecological niches (Borowiec 1987). Our approach is based on the hypothesis that each of the two genera constitutes a distinct taxonomic group (i.e., monophyletic groups). However, the two genera are morphologically very similar and can only be distinguished by the number of femoral spines on the hind leg. Nonetheless, this usually diagnostic character fails to separate some *Bruchidius* species from *Acanthoscelides* species (A. Delobel, pers. comm.). This disturbing observation could be explained if the genera as currently circumscribed were in some respects artificial. Considering the lack of clear synapomorphies supporting their respective monophyly, several authors have considered these genera as paraphyletic (Johnson 1981; Borowiec 1987; A. Delobel, pers. comm.). Therefore, we will have to test the monophyly of the two genera and possibly to define one or several new monophyletic taxonomic groups among the *Acanthoscelides-Bruchidius* group.

In order to test the previous phylogenetic hypotheses and to discuss the evolution of the two genera a phylogenetic framework was necessary. Consequently we have investigated a large sample of *Acanthoscelides* and *Bruchidius* species, and other representatives of tribe Acanthoscelidini by using sequences for three different molecular genes (12S rRNA, cytochrome *b*, and cytochrome *c* oxidase subunit I).

2. Materials and methods

Taxon sampling and DNA sequencing

The species analyzed, their geographical provenance, collectors and host-records are listed in Table 1. Although the species included in this study comprise fewer than 20 % of known species for the two genera, our sample can be considered as representative of the diversity of host-plant associations. For instance, we have sampled species associated with 16 of the 19 tribes from the family Fabaceae which are known to be larval hosts of *Acanthoscelides* and *Bruchidius*. Sampled individuals were generally obtained by rearing larvae from seeds collected in the field with the exception of 4 species as figured in Table 1. As outgroups, in addition to 11 other species of the tribe Acanthoscelidini, a representative of the supposed primitive subfamily Pachymerinae (Borowiec 1987) was used. Both dry and ethanol-preserved specimens were used for DNA extraction. Total DNA was obtained by using extraction columns (QIAGEN GmbH, Germany). Partial sequences for the three mitochondrial genes were amplified using primers listed by Simon *et al.* (1994) and by Monteiro & Pierce (2001). Standard cycling conditions were 5 min at 94 °C followed by 35-40 cycles of 1 min at 94 °C, 1 min at 45-50 °C (depending on the primers used), 1 min at 72 °C, and a final step at 72 °C for 10 min. Partial sequences for the12S rRNA gene were successfully amplified for all specimens, but for some dry specimens it was not possible to obtain PCR products for the cytochrome *b* and cytochrome *c* oxidase subunit I fragments. PCR products were purified by using QIAquick PCR purification kits (QIAGEN GmbH, Germany). Both strands of the PCR products were sequenced by the Sanger dideoxy method and sequence data were obtained by analyzing samples on ABI 373 and ABI 3100 automated sequencers (Applied Biosystems). The new sequences generated in this study were deposited in GenBank (accession numbers are provided in Table 1). Specimens corresponding to this study are kept in collection of the "Institut de Recherche pour le Développement (IRD)" (Muséum National d’Histoire Naturelle,

153 Paris, France) and in the “Centre d’Ecologie Fonctionnelle et Evolutive (CEFE)” (Montpellier,
154 France).
155
156 *Phylogenetic analyses*
157 The alignment of coding sequences (i.e., cytochrome *b* and cytochrome *c* oxidase subunit I
158 fragments) was trivial as no gap event was detected. Minor differences in the length of 12S rRNA
159 sequences were observed, and their alignments were performed by using ClustalX (Thompson *et al.*
160 1987) with default settings. After alignment, the combined sequence data set was 2,216 bp in length
161 : (i) the sequenced cytochrome *b* region contained 782 characters, 305 of which were parsimony-
162 informative; (ii) the sequenced cytochrome *c* oxidase I region contained 1,018 characters, 375 of
163 which were parsimony-informative; (iii) the sequenced 12s rRNA region contained 416 characters,
164 171 of which were parsimony-informative (gaps were treated as fifth character). To estimate
165 phylogenetic relationships among taxa, we have carried out parsimony analyses, using PAUP*
166 version 4.0b10 (Swofford 2003), and Bayesian inferences, using MrBayes version 3.0b4
167 (Huelsenbeck & Ronquist 2001).
168
169 All parsimony analyses were performed using heuristic search option (TBR, random sequence
170 addition, MaxTrees set to 500) with 1,000 random-addition replicates. We have first conducted
171 separate analyses of the data sets. Congruence between the three gene data sets was assessed by
172 using the incongruence length difference (ILD) test (Farris *et al.* 1994), as implemented in PAUP*,
173 with all invariant characters excluded (Cunningham 1997). Although several sequences were
174 missing for some specimens (only the 12s rRNA data set was complete) we have analyzed the
175 combined data set following Wiens (1998). The latter author has demonstrated, by performing
176 multiple analyses of simulated data sets, that the addition of an incomplete data set to an already
177 complete data set is more likely to increase than decrease the phylogenetic accuracy. The robustness
178 of topologies was assessed by bootstrap procedures (1,000 replicates) and the estimation of decay

indexes (Bremer 1994) using TreeRot 2.0 (Sorenson 1999). Preliminary analyses were also conducted to estimate partitioned Bremer support (PBS) values (Baker & DeSalle 1997; Baker *et al.* 1998) for each data set (with TreeRot). However their estimation was somewhat biased by the incompleteness of the cytochrome *b* and cytochrome *c* oxidase I data sets and resulted in underestimated values. As a result, decay indexes and summed PBS values exhibited major discrepancies (e.g., summed PBS values were negative for some nodes) so that we have chosen to only present decay indexes on the tree corresponding to the combined data set analysis under parsimony.

For Bayesian inference we have performed partitioned Bayesian analyses (Nylander *et al.* 2004; Jordal & Hewitt 2004) on the combined data set. Under this approach, the computational efficiency of the Bayesian MCMC method allows the use of more realistic and complex evolutionary models for each data partition (Nylander *et al.* 2004). For each defined partition (one partition per gene sequenced) the best-fit substitution model was determined by Modeltest 3.06 (Posada & Crandall 1998) through hierarchical likelihood ratio tests (LRTs). We subsequently used four Metropolis-coupled chains with incremental heating in four distinct runs of 2,000,000 generations. Distinct parameters were estimated for each partition defined under the appropriate best-fit substitution models previously determined. During the run process, trees were saved to a file every 100 generations (20,000 trees were thus saved). Overall model likelihood scores were further plotted against generations of the chains in order to determine the burn-in period. The results were presented in the form of a 50 % majority-rule consensus tree (in which trees corresponding to the burn-in period are discarded) and the support for the nodes of this tree was given by posterior probability estimates for each clades.

Character optimizations and hypotheses testing

To study the evolutionary history of several characters of interest we have used the program Mesquite 1.05 (Maddison & Maddison 2004) which allows the reconstruction of ancestral character states. Character histories were mapped under maximum-likelihood on the phylogenetic hypothesis resulting from the Bayesian analyses. In comparison with parsimony optimizations the likelihood reconstruction method has the advantage of providing, for each node, a probability estimate for each ancestral character state (Jordal & Hewitt 2004). This state assignment maximizes the probability of arriving at the observed states in the terminal taxa (for a given model of evolution) and it allows the states at all other nodes to vary (Maddison & Maddison 2004). Two independent optimizations were performed using likelihood reconstruction : (i) character optimization of host-plant associations at the subfamily level (i.e., Caesalpinioideae, Malvoideae, Mimosoideae, Papilionoideae and Tilioideae); (ii) character optimization of host-plant associations at the tribal level for the legume feeders. Owing to the high level of host-plant specificity of the sampled bruchid species (all the species studied were associated with only one botanical family, subfamily and tribe) the character coding of host-plant associations was facilitated and no polymorphous characters were included in the analyses.

In order to test several competing evolutionary hypotheses we have generally used a likelihood-based statistical test : the Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa 1999; Goldman *et al.* 2000), as implemented in PAUP* (RELL method; 1,000 replicates). This test was used to see if the difference between an optimal tree (resulting from an unconstrained analysis under likelihood) and a constrained tree (e.g., a tree in which some species were constrained to be monophyletic) was significant. SH tests were used to test hypotheses regarding biogeography and evolution of host-plant association and to evaluate the monophyly of genera *Acanthoscelides* and *Bruchidius*. In addition, Wilcoxon signed-ranks tests (Templeton 1983), as implemented in PAUP*, were conducted to test the possible paraphyly of both genera under parsimony.

Estimation of divergence times

To estimate node ages we have first tested the hypothesis of a molecular clock for the combined data set by implementing a LRT that compares the likelihood score of the Bayesian phylogenetic hypothesis with (L1) and without (L0) the molecular clock enforced (the best-fit model of evolution selected by Modeltest was hereby used). As the LRT rejected the hypothesis of overall rate homogeneity (-2 (ln L1 - ln L0) = 188.68, df = 74, P = 95.081), we have further estimated an ultrametric tree using the non-parametring rate smoothing (NPRS) method of Sanderson (1997), as implemented in TreeEdit v1.0 (Rambault & Charleston; http://evolve.zoo.ox.ac.uk). This penalized likelihood method is appropriate for data sets that depart from a molecular clock as it smoothes the rapidity of rate change among lineages (in the same way that smoothing techniques in regression analyses). For the timescale estimation, we have used the standard rate estimate (2 % per Myr) for mitochondrial DNA evolution in arthropods (Brower 1994).

Evaluation of host-plant associations

Associations were determined both by sampling seeds of potential host-plants in the field with subsequent monitoring of adult emergences (e.g., Janzen 1980; Gillon *et al.* 1992; Silvain & Delobel 1998; Jermy & Sventesi 2003; Kergoat *et al.* 2004), and by critical examination of the available literature. A summary of known host-plant associations (based on literature and field data) for the genera *Acanthoscelides* and *Bruchidius* is provided in Table 2. Host-plant records for *Acanthoscelides* were intensively and rigorously investigated by Johnson (e.g., see Johnson 1989). He published major studies on North and Central American species (Johnson 1970, 1983) and on species of northern South America (Johnson 1990b). However, there is still a gap in knowledge concerning southern South American species. For *Bruchidius*, many studies on specific groups of species and local and regional faunas are available (e.g., Hoffmann 1945; Lukjanovitsch & Ter-Minassian 1957; De Luca 1967; Arora 1977), but must be used cautiously if they are not explicitly based on rearing. A major review on the family Bruchidae (Udayagiri & Wadhi 1989) which

includes extensive host-plant information was used as a major source of information for *Bruchidius* species, but in a very conservative way so as to avoid many unreliable and unverified records (Delobel & Delobel 2003; Jermy & Szentesi 2003; Johnson *et al*. 2004). Indeed, numerous host-plant records in this study were doubtful, and names for many bruchids and host-plant names were outdated. We have followed Janz *et al.* (2001) in only including questionable host-plant records if they have been reported by at least two independent sources. Data from 13 other studies were also included in our evaluation of host-plant associations (Nongionerma 1978; Janzen 1980; Borowiec 1988; Johnson 1990c; Morimoto 1990; Gillon *et al*. 1992; Delobel & Tran 1993; Johnson & Siemens 1995; Anton 1998; Anton & Delobel 2003; Delobel & Anton 2003; Delobel & Delobel 2003; Delobel *et al.* 2004). Supplementary information on host-plant biogeography and specific host-use in some bruchid taxonomic groups were also provided. The ILDIS (International Legume Database and Information Services; http://*www.ildis.org*) database was used to update host-plant names from the literature and as a source of information on host-plant biogeography and species richness. Host-plant records of species now excluded from the genus *Bruchidius* (Borowiec 1987) were discarded *a priori* (i.e., species belonging to the genera *Conicobruchus, Decellebruchus, Kingsolverius, Megabruchidius*, *Salviabruchus, Sulcobruchus* and *Tuberculobruchus*). However, since the phylogenetic relationships of some of these genera to the genus *Bruchidius* are unclear, we have included host-records of some genera (i.e., *Decellebruchus, Tuberculobruchus*) on the basis of previous molecular analyses and morphological evidence (Kergoat & Silvain 2004).

3. Results

Phylogenetic hypotheses

Separate analyses performed under parsimony yielded poorly resolved topologies (not shown). The ILD test was not significant ($P = 0.674$) and thus supported the combination of the three gene data sets. The analysis of the combined data set yields a single most-parsimonious tree (7375 steps; CI = 0.236; RI = 0.360) which is presented in Fig. 1. On overall, this topology is not well supported as only 20 of 73 nodes are supported by bootstrap values greater than 70 % and only 29 of 73 nodes are supported by decay indexes greater than 5.

For Bayesian analyses, the same model of evolution was selected by the likelihood ratio tests for the three defined partitions, namely the general time reversible model with a proportion of invariable sites and a gamma distribution (Lanave *et al.* 1984; Yang 1994; Gu *et al.* 1995). After four distinct runs of 2,000,000 generations, a burn-in period of 100,000 generations was identified, by plotting graphically likelihood values for each generation. The 1,000 trees corresponding to this burn-in period were subsequently not retained in the 50 % majority-rule consensus trees. Three of the four analyses yielded similar consensus trees (Fig. 2) but one of the four run resulted in a slightly alternative topology (not shown; SH test not significant). Basal and terminal nodes are well supported in this phylogenetic hypothesis but some internal nodes are only weakly supported (less than 50 %), which is probably due to the missing sequences of the dried specimens.

Both genera *Acanthoscelides* and *Bruchidius* appear paraphyletic in all analyses, in agreement with the views of several authors (Johnson 1981; Borowiec 1987; A. Delobel, pers. comm.). Within *Acanthoscelides,* two well-supported clades (posterior probabilities greater than 80 %) are recovered under Bayesian inference, whereas parsimony analyses suggested a more scattered pattern. Within *Bruchidius*, the majority of the sampled species are included in a major clade which

also includes other representatives from the tribe Acanthoscelidini. In all analyses, the two sampled members of *Tuberculobruchus* are found paraphyletic within the same clade of morphologically related *Bruchidius* species, which present similar genitalia (Kergoat & Silvain 2004). Interestingly, one *Bruchidius* species, *Bruchidius lineatopygus*, is found grouped, with strong support (posterior probability of 100 %; bootstrap of 89 %; decay index of 9), with the representative of the genus *Conicobruchus* in a very basal position. Although, to a large extent, Bayesian and parsimony analyses recovered similar relationships some major discrepancies are found between them, as indicated by a significant SH test (P = 0.016). For instance, under Bayesian inference, *Decellebruchus atrolineatus* is related to a *Bruchidius* species whereas he is related to an *Acanthoscelides* species under parsimony. In comparison with the tree obtained with Bayesian analyses, the phylogenetic hypothesis from the combined analysis under parsimony appears less resolved, as fewer nodes are well supported. The parsimony tree also shows greater conflicts with the systematic propositions (i.e., taxonomic groups) based on morphological data (Borowiec 1987; Anton & Delobel 2003). It thus appears that the results of Bayesian analyses provide a clearer view of the phylogeny of the tribe Acanthoscelidini and a clearer circumscription of the genera *Acanthoscelides* and *Bruchidius*.

Character optimization and hypotheses testing

Since the results of character optimizations are highly dependent on the robustness of the phylogenetic hypotheses available, we have chosen to only perform character optimizations on the tree resulting from the Bayesian analyses. In Fig. 3, character histories of host-plant preferences are mapped onto the mirror-image cladogram established. On the left cladogram, the character history of host-plant preferences at the subfamily level indicates a relative conservative pattern of host-plant use. Our results show two unambiguous independent host shifts onto Malvaceae (each to distinct subfamilies). Regarding the species associated with the Fabaceae, only five unambiguous independent host shift colonizations have occurred at the subfamily level. Interestingly, the latter

events have always involved a shift from the subfamily Papilionoideae toward subfamilies Caesalpinioideae or Mimosoideae. Thus, the ancestral host-plants for the lineage represented by the sampled species of *Acanthoscelides* and *Bruchidius* appear to have been members of the subfamily Papilionoideae. The latter assumption is strongly supported by a probability of 97 % under likelihood ancestral state reconstruction. On the right cladogram, the character history of host-plant preferences at the tribe level (for the studied legume feeders) is figured and suggests a dynamic pattern. This is well illustrated for the species associated with the tribe Phaseoleae, in which character optimization indicates three independent colonizations but also three secondary losses of plant use. Several gain and loss events have also occurred for the species associated with tribes Desmodieae and Trifolieae, but to a lesser extent (at least according to our sampling).

In addition to the previous results, the phylogenetic framework allows us to investigate the biogeographical patterns of the sampled species. Most of the sampled species group into two large clades, each of which is restricted either to the New World or the Old World (see Fig. 4). The first clade includes *Algarobius prosopis* and all *Acanthoscelides* species with the exception of the *Acanthoscelides* species associated with Mimosoideae. The second clade groups all *Bruchidius* species (with the exception of *Bruchidius lineatopygus*), but also the sampled members of genera *Callosobruchus*, *Decellebruchus* and *Tuberculobruchus*. The results of the character optimization of the geographical distribution of species under likelihood suggests that the observed pattern is resulting from two distinct vicariant events (represented by letters A and B in Fig. 4.). However, the hypothesis of a single vicariant event cannot be excluded as the result of a SH test in which Old World species are constrained to form a monophyletic group is not significant ($P = 0.165$).

Regarding the hypothesis testing of the monophyly of genera *Acanthoscelides* and *Bruchidius*, both SH and Wilcoxon signed-ranks tests were significant for the genus *Bruchidius* ($P = 0.001$ and $P < 0.0001$ respectively). For the genus *Acanthoscelides*, unlike the Wilcoxon signed-ranks test ($P =$

0.0472) the SH test was not significant ($P = 0.105$). As a consequence, though the paraphyly of this genus is strongly suggested, the hypothesis of a possible monophyly for this genus cannot be totally excluded.

Estimation of divergence times

We present in Fig. 4. a time-scaled NPRS ultrametric tree estimated under TreeEdit, using the topology estimated through Bayesian analyses. Our dating indicates an older origin for bruchids than the one suggested by the extant fossil record, as the oldest known bruchid fossils (assigned to tribe Pachymerinae) only date from early Eocene (Archibald & Mathewes 2000). This finding is consistent with the estimation of bruchid divergence times from previous molecular studies (Farrell 1998; Farrell & Sequeira 2004). This old origin is particularly interessant from an evolutionay perspective, as the diversification of seed-beetles could have occurred nearly at the same time than that of their legume host-plants (Herendeen *et al.* 1992).

Evaluation of host-plant associations

Our results indicate that the two genera share a similar pattern in their host-plant use. Host-plant records indicate that most species of *Acanthoscelides* and *Bruchidius* are specialists which feed on a limited number of host-plants. At the plant family level, each of the *Acanthoscelides* and *Bruchidius* species samples exhibited a strong specificity, feeding exclusively on seeds of species from a single plant family. They are also mostly associated with the family Fabaceae, like most bruchids. This trend is stronger for the genus *Bruchidius*, in which only four species are known to feed outside the Fabaceae. For *Acanthoscelides*, the known host-range is wider as valid host-plant records are known for six other plant families, most frequently Malvaceae.

Regarding species associated with the family Fabaceae, a closer look at the subfamily level reveals once again a strong specificity, for species of both genera. In both, each species feeds exclusively

on a single subfamily, with the exception of *Acanthoscelides compressicornis*, which is known to feed on both *Desmanthus* (Mimosoideae) and *Hoffmanseggia* (Caesalpinioideae). Fewer than ten species of *Acanthoscelides* and *Bruchidius* are known to feed on members of the subfamily Caesalpinioideae, whereas dozens of species are associated with subfamilies Mimosoideae or Papilionoideae. For these latter subfamilies, important differences in host use pattern are noticeable. Indeed *Acanthoscelides* and *Bruchidius* associated with Mimosoideae predominantly feed on three genera, namely *Acacia, Albizia* and *Mimosa*, whereas species associated with Papilionoideae do not exhibit such strong preferences and are associated with 65 distinct genera of host-plants.

At the tribal level, the previously observed taxonomic conservatism in host-plant use is still present, and most species feed on plant species belonging to a single botanical tribe. A total of 14 papilionoid tribes are exploited by *Acanthoscelides* and *Bruchidius* species, and 12 of these 14 tribes are used by both genera. Tribes Amorpheae and Cytiseae are each used by only one of the genera, but this is accounted for their respective natural distributions, either restricted to the New World (Amorpheae) or to the Old World (Cytiseae). Careful examination of the pattern of host-plant distribution shows that when a host-plant genus occurs in both the New World and Old World, it is generally attacked by both *Bruchidius* (Old World) and *Acanthoscelides* (New World). This finding is consistent with the suggestion of Borowiec (1987) that the two genera seem to occupy similar ecological niches in their respective areas of distribution.

4. Discussion

Phylogeny and taxonomy

Although this study focuses only on 76 species, our results provide valuable information on bruchid phylogeny and taxonomy. Our molecular analyses support the paraphyly of genus *Bruchidius* and, to a large extent, the paraphyly of genus *Acanthoscelides* (given the result of the SH test). In addition, our study raises several questions about the status of some other genera belonging to the same tribe. Not only does the genus *Tuberculobruchus* appear paraphyletic in our analyses, but its members are also morphologically indistinguishable from those *Bruchidius* species shown by our analyses to be closely related to them. Within the clade which groups the majority of *Bruchidius* species, species of *Callosobruchus* and *Decellebruchus* are also found. These two genera are morphologically very similar to certain *Bruchidius* species, which appear, according to our analyses, to be closely related to them. They are distinguishable only by the structure of the hind femur (for *Callosobruchus*) and of the male antenna (for *Decellebruchus*). All these results suggest the utility of either redefining a larger genus *Bruchidius* or further splitting this genus into smaller monophyletic genera. The status of the basal *Bruchidius lineatopygus* is thus questionable and we advocate its inclusion within a larger redefined genus *Conicobruchus*. Regarding *Acanthoscelides* species, two groups seem to be well differentiated. Interestingly the position of *Merobruchus placidus* as the sister taxon of one of the two clades of *Acanthoscelides* is consistent with morphological evidence (Alvarez *et al.* in prep; J. Romero pers. comm.). Further studies should investigate larger samples of *Acanthoscelides* to clarify relationships within the genus. The boundaries of some extant taxonomic groups should also be investigated in view of our results. For example, the inclusion of *Bruchidius grandemaculatus* and *Bruchidius uberatus* within the group *centromaculatus* should be studied closely from a morphological viewpoint.

Evolution of host-plant use and host-plant chemistry

Our mapping of host-plant preferences onto bruchid phylogeny underlines the strong taxonomic conservatism of host use, which is shared by both of the genera studied. Within the species specialized on Fabaceae, only a few species, such as *Acanthoscelides compressicornis*, are known to develop on host-plants belonging to more than one tribe or subfamily. For these species, the extant patterns of host-plant use may be better explained by an expansion of host-plant range (C.D. Johnson, pers. comm.). Most host shifts (which correspond to long term evolutionary changes from one host to another) have occurred between related host-plants and the majority of species are specialized on a given host-plant genus or tribe. However, a few host shifts between distantly related plants, followed by successful diversifications, have occurred in the evolutionary history of the two genera. This quite dynamic pattern underlines the fact that there is no evidence for the generality of processes of cospeciation between either *Acanthoscelides* or *Bruchidius* and their respective host-plants. Thus, for our sample, at least two major host shifts between distinct plant families, and five major host shifts between distinct subfamilies (for the Fabaceae) are revealed. Moreover, character optimizations of plant families and subfamilies (for the Fabaceae) suggest that the the subfamily Papilionoideae is the ancestral host-plant group of *Acanthoscelides* and *Bruchidius*, at least for the sampled species. This pattern also argues strongly against cospeciation, or even any broad temporal congruence in speciation events in bruchids and their hosts, because it seems clear that Papilionoideae evolved from caesalpinioid ancestors (Doyle et al. 1997; Wink & Mohamed 2003). This suggests that these bruchids radiated onto host-plant groups that were already diversified, rather than that there was any broad process of coevolution involved. Nonetheless, hasty generalization of this finding is unwarranted, since our sample represents fewer than 20 % of the known species of these two bruchid genera and because some internal nodes in the phylogeny are weakly supported.

The integration of reliable host-plant records for a large number of species of *Acanthoscelides* and *Bruchidius* permits discussion of the evolution of host-plant use at a larger scale. In view of our

464 evaluation of host-plant associations, the trend which is suggested by the mapping of host-plant
465 preferences onto bruchid phylogeny is likely to fit with all *Acanthoscelides* and *Bruchidius* as well.
466 Indeed, in both genera species belonging to the same taxonomic group (Johnson 1983, 1990b;
467 Borowiec 1988; Anton & Delobel 2003; A. Delobel pers. comm.) are generally associated with
468 host-plants of the same tribe (if they develop on Fabaceae) or family (if they develop on hosts other
469 than the Fabaceae) as figured in Table 3. Furthermore, in view of our phylogenetic hypothesis and
470 in accord with the results of Kergoat *et al*. (2004), species belonging to the same taxonomic group
471 are in fact closely related. We can thus assume that conservatism in host use and specialization has
472 strongly influenced the evolution of the two genera. Our molecular analyses also suggest that a total
473 of at least four lineages of *Acanthoscelides* and *Bruchidius* have radiated independently on various
474 host-plant groups. Yet our sampling certainly does not encompass the full extent of these successful
475 diversifications, particularly for the clade which is represented only by *Bruchidius lineatopygus* and
476 *Conicobruchus strangulatus*. These independent radiations have occurred on similar host-plants
477 (belonging to the same taxonomic groups) in the New World and the Old World and thus suggest
478 that *Acanthoscelides* and *Bruchidius* have undergone some kind of parallel evolution in their
479 history, successfully colonizing the same niches (i.e., new food resources) in their respective areas
480 of distribution. The occurrence of multiple independent host shifts between distinct plant families,
481 and between distinct subfamilies (for the Fabaceae), is suggested not only by our character
482 optimization but also by many host-plant records from the literature. We can suppose that these
483 host-shifts between distantly related host-plants have played an important role in the successful
484 diversification of these seed-beetles.
485
486 Host-plant chemistry is likely to be important in the evolution of host-plant use. Many secondary
487 compounds (alkaloids, non-protein amino acids, cyanogenic glycosides, lectins, proteinase
488 inhibitors) are frequently found in seeds, and their toxicity against bruchids has been demonstrated
489 by previous studies (Janzen *et al.* 1977; Janzen 1981; Birch *et al.* 1986; Gatehouse *et al.* 1990;

Rosenthal 1990). Consequently the strong specificity and taxonomic conservatism in host use exhibited by most bruchids suggests that they are somewhat constrained to feed on chemically similar host-plants. However since host-plant chemistry is generally correlated with host-plant phylogeny we can hardly exclude other factors that may constrain bruchid evolution (but see Kergoat *et al.* 2005). Insects feeding inside plant organs (e.g., leaf miners, stem borers, and larvae developing inside seeds) must be finely adapted to the morphology, the physiology and the chemistry of the host, and generally tend to be more constrained in host-plant choice than are externally feeding phytophagous insects. Furthermore, our results also suggest that some species have undergone host shifts to chemically dissimilar host-plants (Wink & Mohamed 2003) (e.g., between plants belonging to the subfamilies Papilionoideae and Mimosoideae), a phenomenon that likely has involved the development of 'key innovations' in the form of detoxification mechanisms. Members of both *Acanthoscelides* and *Bruchidius* have independently developed abilities to feed on many toxic seeds. A striking example is the adaptation of many *Acanthoscelides* and *Bruchidius* species to the non-protein amino acid *L*-canavanine, widespread in Papilionoideae (Bell *et al.* 1978). The mechanisms of detoxification of canavanine and other compounds (e.g., proteinases) are now well understood (Bleiler *et al.* 1988; Rosenthal 1990; Oliveira *et al.* 2002) and suggest either widespread preadaptation to many toxic compounds or the multiple development of similar 'key innovations'.

Biogeography

Our analyses point to the existence of two large clades, each strictly New World or Old World in distribution. Using the timescale presented in Fig. 4, we can suppose that the separation between Old World and New World species occurred early in the history of the tribe Acanthoscelidini, some 65-60 Myr ago. The suggested Cretaceous-early Tertiary period is consistent with biogeographical evidence (Sanmartin *et al.* 2001) which indicate that the observed basal splits could be accounted for the progressive closure of the Tulean land bridge which have connected Europ and North

America (this landbridge was definitely closed some 50 Myr ago). We can thus exclude the alternative hypothesis of an older vicariant event (Becerra 2003), namely the breakup of West Gondwana (between Africa and South America) some 100 Myr ago, to explain the extant disjunct pattern of bruchid distribution. Our timing cannot exclude possible bruchid faunal interchanges through the early Beringian Bridge, which has connected the Eastern Paleartic with the Western Nearctic until his closure some 35 Myr ago (Sanmartin *et al.* 2001). Yet, prior to his closure, this land bridge was under the influence of major climate changes (from warm climates to colder ones) that have certainly barren the migration of non-cold tolerant organisms, and therefore seed-beetles.

Evidence for adaptive radiation in bruchids?

Schluter (2000) has linked the concept of adaptive radiation to the following four major features : common ancestry, trait utility, phenotype-environment correlation and rapid speciation. At least two of these features are exhibited by the bruchids we have studied. Common ancestry is assessed by our phylogenetic analyses, whereas trait utility is supported by the multiple 'key innovations' which are involved in detoxifying many toxic seed compounds (Bleiler *et al.* 1988; Rosenthal 1990; Oliveira *et al.* 2002). In the absence of strong morphological differentiation within bruchids, it is difficult to demonstrate a phenotype-environment correlation, even if the latter seems to be quite intuitive when examining larval structures involved in seed boring. Estimation of the rapidity of speciation requires a properly calibrated molecular clock to estimate the timing of the diversification of *Acanthoscelides* and *Bruchidius*. The hypothesis of a rapid speciation is not fully supported by our estimation of divergence times (Fig. 4.) as branch lengths between ancestors are not short on the average. Precise information on more recent vicariant events are here required to better constrain the age of nodes under NPRS optimization so as to precise our conclusions.

Conclusions

This study has produced the first phylogenetic reconstruction for a large sample of the two largest genera of seed-beetles, allowing investigation of patterns in their evolution. Despite some limitations due to sample size, we have clarified phylogenetic relationships within the tribe Acanthoscelidini and circumscribed more clearly the genera *Acanthoscelides* and *Bruchidius*. We also showed a strong trend towards a high taxonomic conservatism in host use, despite the existence of several host shifts during the evolution of these two genera. Our phylogenetic analysis and our evaluation of host-plant associations both suggest that the two genera have undergone parallel evolution, as they have independently colonized similar host-plants, and probably developed similar mechanisms of detoxification, in their different areas of distribution. The ancient origin of seed-beetles which is suggested by our estimation of divergence times is consistent with the hypothesis of an adaptive radiation which could have possibly occurred contemporaneously with the diversification of their legume host-plants. However, adaptive radiation cannot yet be fully demonstrated for because we lack sufficient evidence of a rapid speciation.

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Acknowledgments

We are greatly indebted to Alex Delobel (IRD, Paris, France), Clarence Dan Johnson (northern Arizona University, Flagstaff, USA) and Doyle McKey (CEFE-CNRS, Montpellier, France) for comments and corrections on the manuscript. We are also grateful to all the collectors (listed in Table 1) who provide inestimable specimens.

819 **Figure legends**

820

821 **Fig. 1** Most parsimonious tree (7375 steps; CI = 0.236; RI = 0.360) from the unweighted parsimony
822 analysis of the combined data set (heuristic search option with 1,000 random-addition replicates).
823 Numbers adjacents to nodes give bootstrap support values greater than 50 % calculated for 1,000
824 replicates and decay indexes.

825

826 **Fig. 2** Single tree from the partitioned Bayesian inference analysis of the combined data set
827 (topology identical to the 50 % majority-rule consensus tree), using Bayesian estimates of branch
828 lengths corresponding to the latest saved tree (tree number 20,000). Numbers adjacent to nodes give
829 Bayesian posterior probabilities of nodes greater than 50 % and decay indexes. In addition, on the
830 right extant taxonomic groups are figured.

831

832 **Fig. 3** Mirror-image of the 50 % majority-rule consensus tree from the partitioned Bayesian
833 inference analysis of the combined data set. On the left cladogram the character history of host-
834 plant preferences at the subfamily level is figured. On the right cladogram the character history of
835 host-plant preferences at the tribe level is figured. Ancestral character states were reconstructed
836 under Mesquite using likelihood reconstruction (probabilities of character states are figured at the
837 nodes with colored pie diagrams). Detailed legends for both character optimizations are provided on
838 the top of the figure. In addition, between trees vertical bars with the corresponding patterns show
839 the actual host-plant associations.

840

841 **Fig. 4** Time-calibrated NPRS ultrametric tree estimated under TreeEdit, using the topology
842 estimated through partitioned Bayesian inference analyses. On the right vertical bars show the
843 distribution of the species. Hypothetical vicariant events are also figured (A and B).

844

Table 1 : Material examined in this study

Taxon	Locality*	Collector	Host-plant†	
Subfamily Bruchinae Latreille 1802				
Tribe Acanthoscelidini Bridwell 1946				
Acanthoscelides Schilsky 1905				
anoditus Johnson, 1983	Mex. El Copal	(unknown)	*Anoda cristata*	Malvaceae
argillaceus (Sharp 1885)	Mex. Playa azul	N Alvarez	*Phaseolus lunatus*	Fab. Phaseoleae
biustulus (Fall 1910)	Mex. Amealco	J Romero N	*Desmodium* sp.	Fab. Desmodieae
clandestinus (Motschulsky 1874)	Mex. C. Carmen	R Ramírez D	*Vigna adenantha*	Fab. Phaseoleae
cuernavaca Johnson, 1983	Mex. S. Huautla	J Romero N	*Desmodium* sp.	Fab. Desmodieae
desmodicola Johnson, 1983	Mex. S. Huautla	I Figueroa R	*Desmodium* sp.	Fab. Desmodieae
desmoditus Johnson, 1983	Ven. Barquisimeto	CD Johnson	*Desmodium tortuosum*	Fab. Desmodieae
flavescens (Fähraeus 1839)	Mex. Presa Diablo	J Luna Cozar	*Rhynchosia minima*	Fab. Phaseoleae
guazumae Johnson & Kingsolver 1971	Mex. S. Huautla	J Romero N	*Guazuma tomentosa*	Malvaceae
isla Johnson 1983	Ecu. Guayaquil	CD Johnson	*Rhynchosia minima*	Fab. Phaseoleae
macrophthalmus (Schaeffer 1907)	Vie. Saïgon (I)	H Delobel	*Leucanea leucocephala*	Fab. Mimoseae
malvastrumicis Johnson, 1983	Mex. El Cielo	S Niño	*Malvastrum americanum*	Malvaceae
mazatlan Johnson, 1983	Mex. S. Huautla	J Romero N	*Desmodium* sp.	Fab. Desmodieae
mexicanus (Sharp 1885)	Mex. Coxcatlan	N Alvarez	*Mimosa* sp.	Fab. Mimoseae
mundulus (Sharp 1885)	Mex. Jalcomulco	J Romero N	*Nissolia fruticosa*	Fab. Aeschynom.
oblongoguttatus (Fähraeus 1839)	Mex. Cotaxtla	J Romero N	*Acacia cornigera*	Fab. Mimoseae
obtectus (Say 1831)	Egy. Giza (I)	G Fédière	*Phaseolus vulgaris*	Fab. Phaseoleae
obvelatus Bridwell, 1942	Mex. Tepoztlan	N Alvarez	*Phaseolus vulgaris*	Fab. Phaseoleae
palmasola Johnson, 1983	Mex. Tenabo	CD Johnson	*Rhynchosia longeracemosa*	Fab. Phaseoleae
puellus (Sharp 1885)	Nic. Mombacho	JM Maes	*Calopogonium mucunoides*	Fab. Phaseoleae
sanblas Johnson, 1983	Mex. Córdoba	J Romero N	*Triumfetta lappula*	Malvaceae
sanfordi Johnson, 1983	Mex. S. Huautla	J Romero N	*Pachyrhizus erosus*	Fab. Phaseoleae
stylifer (Sharp 1885)	Mex. Ixmiquilpan	J Romero N	*Desmodium* sp.	Fab. Desmodieae
taboga (Johnson 1983)	Pan. Chepo	CD Johnson	*Calopogonium caeruleum*	Fab. Phaseoleae
zonensis Johnson, 1983	Col. Palmira	CD Johnson	*Teramnus uncinatus*	Fab. Phaseoleae
Algarobius Bridwell 1946				
prosopis (LeConte 1858)	Egy. El Tur (I)	G Fédière	*Prosopis glandulosa*	Fab. Mimoseae
Bruchidius Schilsky 1905				
auratopubens Decelle *in litt.*	Sen. Fatick	M Sembène	*Faidherbia albida*	Fab. Ingeae
aurivillii (Blanc 1889)	Sen. Fleuve	M Sembène	*Acacia tortilis*	Fab. Acacieae
bimaculatus (Olivier 1795)	Fra. Corse	A Delobel	*Medicago marina*	Fab. Trifolieae
bernardi Delobel & Anton 2004	Ita. Basilicata	A Delobel	*Astragalus depressus*	Fab. Galegeae
cadei Decelle *in litt.*	Sen. Thies	H Delobel	*Faidherbia albida*	Fab. Ingeae
campylacanthae Decelle *in litt.*	Sen. Thies	H Delobel	*Acacia polyacantha*	Fab. Acacieae
caninus (Kraatz 1869)	Fra. Corse	A Delobel	*Astragalus hamosus*	Fab. Galegeae
centromaculatus (Allard 1868)	Sen. Fleuve	MT Gueye	*Acacia nilotica*	Fab. Acacieae
chloroticus (Dalm. 1833)	Sen. Fatick	H Delobel	*Sesbania pachycarpa*	Fab. Robinieae
dichrostachydis Delobel & Anton 2003	Sen. Fatick	H Delobel	*Dichrostachys cinerea*	Fab. Mimoseae
dispar (Gyllenhall 1833)	Fra. Montfuron	A Delobel	*Trifolium repens*	Fab. Trifolieae
dialii Decelle 1973	Sen. Ziguinchor	A Delobel	*Dialium guineense*	Fab. Cassieae
elnairensis (Pic 1921)	Ken. Kabarnet	B Le Rü	*Acacia dolichocephala*	Fab. Acacieae
fulvicornis (Motschulsky 1874)	Fra. Corse	A Delobel	*Trifolium vesiculosum*	Fab. Trifolieae
grandemaculatus (Pic 1933)	Ken. Tsavo	B Le Rü	*Acacia* prope *nilotica*	Fab. Acacieae
incarnatus (Boheman 1833)	Egy. Baharîya (I)	G Fédière	*Vicia faba*	Fab. Vicieae
jocosus (Gyllenhall 1833)	Fra.	A Delobel	(not based on rearing)	
lineatopygus (Pic 1924)	Sen. Louga	H Delobel	*Indigofera tinctoria*	Fab. Indigofereae
lividimanus (Gyllenhall 1833)	Fra. Monsols	A Delobel	*Cytisus scorparius*	Fab. Genisteae
marginalis (Fabricius 1776)	Fra. Montfuron	A Delobel	*Astrag. monspessulanus*	Fab. Galegeae
nanus (Germar 1824)	Ita. Basilicata	A Delobel	*Medicago orbicularis*	Fab. Trifolieae
niokolobaensis (Decelle 1969)	Sen. Thies	H Delobel	*Tephrosia bracteolata*	Fab. Milletieae
pauper (Boheman 1829)	Fra. Corse	A Delobel	*Ornithopus compressus*	Fab. Loteae

picipes (Germar 1824)	Fra. Corse	A Delobel	*Trifolium angustifolium*	Fab. Trifolieae
poecilus (Germar 1824)	Ita. Basilicata	A Delobel	*Astrag. contortuplicatus*	Fab. Galegeae
pusillus (Germar 1924)	Fra. Gard	A Delobel	*Hippocrepis emerus*	Fab. Loteae
pygidiopictus Decelle *in litt.*	Sen. Louga	M Sembène	*Faidherbia albida*	Fab. Ingeae
pygmaeus (Boheman 1833)	Fra. Corse	A Delobel	*Trifolium angustifolium*	Fab. Trifolieae
quinqueguttatus (Olivier 1795)	Tur.	K-W. Anton	(not based on rearing)	
raddianae Anton & Delobel 2003	Sen. Fleuve	M Sembène	*Acacia tortilis*	Fab. Acacieae
rubicundus (Fahraeus 1839)	Ken. Taveta	B Le Rü	*Acacia laeta*	Fab. Acacieae
seminarius (Linnaeus 1767)	Fra. Séderon	A Delobel	*Lotus maritimus*	Fab. Loteae
sericatus (Germar 1824)	Fra. Corse	A Delobel	*Trifolium angustifolium*	Fab. Trifolieae
submaculatus (Fahraeus 1839)	Sen. Fleuve	M Sembène	*Acacia senegal*	Fab. Acacieae
trifolii (Motschulsky 1874)	Egy. Baharîya (I)	G Fédière	*Trifolium alexandrinum*	Fab. Trifolieae
tuberculatus (Hochhuth 1847)	Kyr.	K-W. Anton	(not based on rearing)	
uberatus (Fahraeus 1895)	Sen. Fleuve	MT Gueye	*Acacia nilotica*	Fab. Acacieae
varipictus (Motschulsky 1874)	Fra. Corse	A Delobel	*Medicago murex*	Fab. Trifolieae
varius (Olivier 1795)	Fra. Corse	A Delobel	*Trifolium angustifolium*	Fab. Trifolieae
villosus (Fabricius 1792)	Fra. Saclas	A Delobel	*Laburnum anagyroides*	Fab. Genisteae
Callosobruchus Pic 1902				
chinensis (Linnaeus 1758)	Egy. Giza (I)	G Fédière	*Cajanus cajan*	Fab. Phaseoleae
maculatus (Fabricius 1775)	Vie. Saïgon (I)	H Delobel	*Vigna unguiculata*	Fab. Phaseoleae
phaseoli (Gyllenhall 1833)	Egy. Baharîya (I)	G Fédière	*Lablab purpureus*	Fab. Phaseoleae
subinnotatus (Pic 1914)	Sen. Cap Vert (I)	H Delobel	*Vigna subterranea*	Fab. Phaseoleae
Conicobruchus Decelle 1951				
strangulatus (Fahraeus 1839)	Sen. Cap Vert	H Delobel	*Crotalaria podocarpa*	Fab. Crotalarieae
Decellebruchus Borowiec 1987				
atrolineatus (Pic 1921)	Sen. Cap Vert	H Delobel	*Vigna unguiculata*	Fab. Phaseoleae
Gibbobruchus Pic 1913				
sp.	French Guyana	G Couturier	(not based on rearing)	
Merobruchus Bridwell 1946				
placidus (Horn 1873)	Mex. Coxcatlan	N Alvarez	*Leucaena leucocephala*	Fab. Mimoseae
Tuberculobruchus Decelle 1951				
albizziarum (Decelle 1958)	Sen. Cap Vert (I)	H Delobel	*Albizia lebbeck*	Fab. Ingeae
natalensis (Pic 1903)	Sen. Thies	H Delobel	*Acacia sieberiana*	Fab. Acacieae
Subfamily Pachymerinae Bridwell 1929				
Tribe Pachymerini Bridwell 1929				
Pachymerus Thunberg 1805				
cardo (Fahraeus 1839)	French Guyana (I)	G Couturier	*Elaeis guineensis*	Arecaceae

* Colombia (Col.), Ecuador (Ecu.), Egypt (Egy.), France (Fra.), Italy (Ita.), Kenya (Ken.), Kyrgistan (Kyr.), Mexico (Mex.), Nicaragua (Nic.), Panama (Pan.), Senegal (Sen.), Turkey (Tur.),Venezuela (Ven.), Vietnam (Vie.); (I) introduced host-plant

† Host-plants systematic was abbreviated as follows: Aeschynomeneae (Aeschynom.), Fabaceae (Fab.); (I) introduced

Table 2 : Host-plant use for the *Acanthoscelides* and *Bruchidius genera**

Host-plant systematic	Host-plant genus†	Host-plant distribution (by genus)	Estimated number of bruchid species‡ *Ac*. spp.	*Br*. spp.
Family Apiaceae			0	1
Family Cistaceae			1	3
Family Fabaceae				
Subfamily Caesalpinioideae				
Tribe Caesalpinieae				
	Delonix (3)	Old World	0	1
	Hoffmannseggia (26)	New World – Old World	1	0
Tribe Cassieae				
	Apuleia (1)	New World	3	0
	Cassia (73)	New World – Old World	1	1
	Dialium (30)	Old World	0	3
	Senna (234)	New World – Old World	5	4
Subfamily Mimosoideae				
Tribe Acacieae	*Acacia* (431)	New World – Old World	3	45
Tribe Ingeae				
	Albizia (70)	New World – Old World	0	22
	Faidherbia (1)	Old World	0	4
Tribe Mimoseae				
	Desmanthus (24)	New World	5	0
	Dichrostachys (5)	Old World	0	3
	Leucaena (25)	New World	4	0
	Mimosa (523)	New World – Old World	19	1
	Piptadenia (32)	New World	2	0
	Prosopis (45)	New World – Old World	1	1 (I)
Tribe Parkieae	*Parkia* (31)	New World – Old World	7	0
Subfamily Papilionoideae				
Tribe Aeschynomeneae				
	Aeschynomene (170)	New World – Old World	4	2
	Chaetocalyx (13)	New World	1	0
	Nissolia (14)	New World	2	0
	Stylonsanthes (41)	New World – Old World	1	0
Tribe Amorpheae				
	Amorpha (16)	New World	3	0
	Dalea (170)	New World	7	0
	Errazurizia (4)	New World	1	0
	Parryella (1)	New World	1	0
Tribe Cicereae	*Cicer* (31)	Old World	1 (I)	4
Tribe Cytiseae				
	Adenocarpus (15)	Old World	0	2
	Argyrocytisus (1)	Old World	0	1
	Calicotome (4)	Old World	0	2
	Cytisophyllum (1)	Old World	0	2
	Cytisus (62)	Old World	0	8
	Genista (114)	Old World	0	4
	Laburnum (2)	Old World	0	4
	Petteria (1)	Old World	0	2
	Spartium (1)	Old World	0	4
Tribe Desmodieae				
	Desmodium (268)	New World – Old World	13	7
	Lespedeza (29)	New World – Old World	1	1
	Pseudarthria (5)	Old World	0	1
Tribe Galegeae				
	Astragalus (1399)	New World – Old World	7	13
	Alhagi (4)	Old World	0	3
	Galega (6)	Old World	0	1

		Glycyrrhiza (17)	New World – Old World	2	5
		Halimodendron (1)	Old World	0	1
		Oxytropis (173)	New World – Old World	1	2
		Sphaerophysa (2)	Old World	0	1
	Tribe Hedysareae				
		Hedysarum (69)	New World – Old World	1	2
		Onobrychis (117)	Old World	0	7
	Tribe Indigofereae				
		Indigofera (554)	New World – Old World	5	11
	Tribe Loteae				
		Acmispon (8)	New World	1	0
		Anthyllis (22)	Old World	0	2
		Coronilla (9)	Old World	0	3
		Dorycnium (8)	Old World	0	1
		Hippocrepis (29)	Old World	0	1
		Hosackia (11)	New World	1	0
		Hymenocarpos (1)	Old World	0	1
		Lotus (116)	Old World	0	2
		Ornithopus (6)	New World – Old World	0	2
		Ottleya (12)	New World	1	0
		Scorpiurus (3)	Old World	0	1
		Securigera (13)	Old World	0	3
		Syrmatium (14)	New World	1	0
	Tribe Milletieae	*Tephrosia* (307)	New World – Old World	3	3
	Tribe Phaseoleae				
		Cajanus (17)	New World	1 (I)	0
		Calopogonium (9)	New World	6	0
		Eriosema (146)	New World – Old World	2	0
		Flemingia (13)	New World	1 (I)	0
		Galactia (98)	New World	2	0
		Lablab (2)	Old World	1 (I)	2
		Macroptilium (14)	New World	1	0
		Pachyrhizus (4)	New World	2	0
		Phaseolus (36)	New World – Old World	4	0
		Rynchosia (221)	New World – Old World	12	0
		Teramnus (8)	New World – Old World	2	1
		Vigna (86)	New World – Old World	5	1
	Tribe Robinieae	*Sesbania* (52)	New World – Old World	2	6
	Tribe Trifolieae				
		Medicago (74)	Old World	0	5
		Trifolium (227)	New World – Old World	3	10
		Trigonella (72)	Old World	0	2
	Tribe Vicieae				
		Lathyrus (127)	New World – Old World	1 (I)	1
		Lens (4)	Old World	1 (I)	2
		Pisum (3)	Old World	1 (I)	3
		Vicia (193)	New World – Old World	1 (I)	5
Family Lythraceae				1	0
Family Malvaceae				40	0
Family Onagraceae				1	0
Family Rhamnaceae				1	0
Family Verbenaceae				1	0

* including *Decellebruchus* spp. and *Tuberculobruchus* spp.

† the estimated numbers of species for each plant genus is given into parentheses

‡ (I) : introduced host-plant. Although the corresponding plant genus originates from the other area of distribution (New World or Old World), a bruchid species has managed to develop upon it

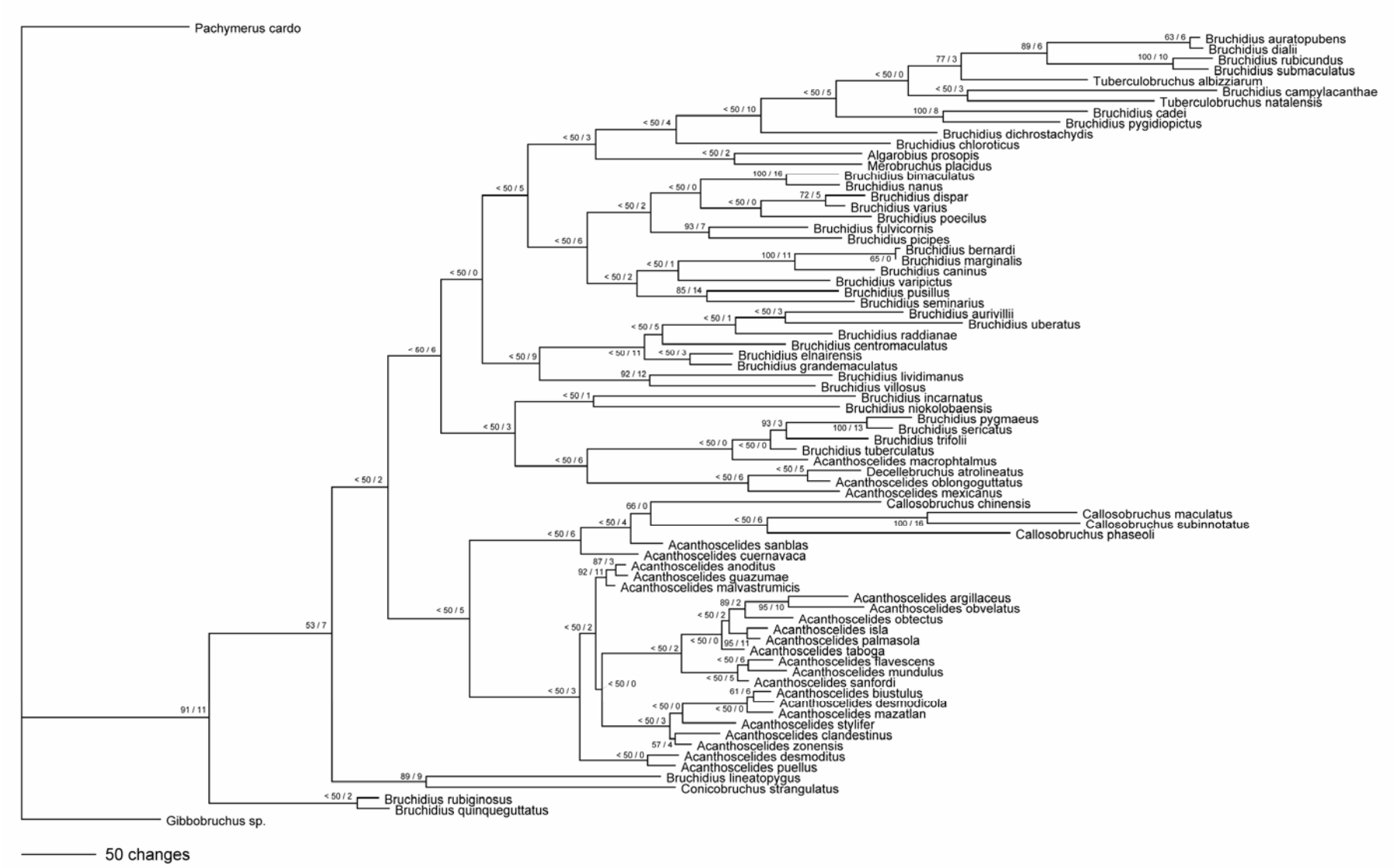
Table 3 : Host-plant preference for existing *Acanthoscelides* and *Bruchidius* taxonomic groups

Taxonomic groups *	References †	Host-plant preference ‡	
(*Acanthoscelides*)			
aequalis	Johnson 1983	non-Tilioid Malvaceae	(exclusively)
albopygus	Johnson 1983	tribe Acacieae	(mainly)
blanchardi	Johnson 1983	non-Tilioid Malvaceae	(mainly)
chiricahuae	Johnson 1983	tribe Mimoseae	(exclusively)
flavescens	Johnson 1983	tribe Phaseoleae	(mainly)
megacornis	Johnson 1983	Tilioid Malvaceae	(mainly)
mexicanus	Johnson 1983	tribe Mimoseae	(mainly)
mundulus	Johnson 1983	tribe Aeschynomeneae	(exclusively)
obtectus	Johnson 1983	tribe Phaseoleae	(exclusively)
pertinax	Johnson 1983	tribe Desmodieae	(mainly)
puellus	Johnson 1983	tribe Phaseoleae	(mainly)
quadridentatus	Johnson 1983	tribe Mimoseae	(mainly)
(*Bruchidius*)			
astragali	Borowiec 1988; Delobel *et al.* 2004	tribe Galegeae	(exclusively)
bimaculatus	Borowiec 1988	tribe Trifolieae	(exclusively)
centromaculatus	Anton & Delobel 2003	tribe Acacieae	(exclusively)
foveolatus	Borowiec 1988	tribe Trifolieae	(mainly)
glycyrrhizae	Borowiec 1988	tribe Galegeae	(exclusively)
murinus	Borowiec 1988	tribe Trifolieae	(mainly)
seminarius	Borowiec 1988; Anton 1998	various tribes	
tibialis	Borowiec 1988	tribe Trifolieae	(exclusively)
unicolor	Borowiec 1988; A. Delobel pers. comm.	tribe Hedysareae	(mainly)
varius	Borowiec 1988	tribe Trifolieae	(mainly)

* only taxonomic groups with more than one species and those for which host plants are known for at least two species were studied

† sources used to circumscribe the taxonomic groups

‡ (mainly) indicates that more than 50 % of the species were associated with a specific plant group;
host-plant data were missing for about 20 % of the species belonging to the studied taxonomic groups



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1113 Figure 1

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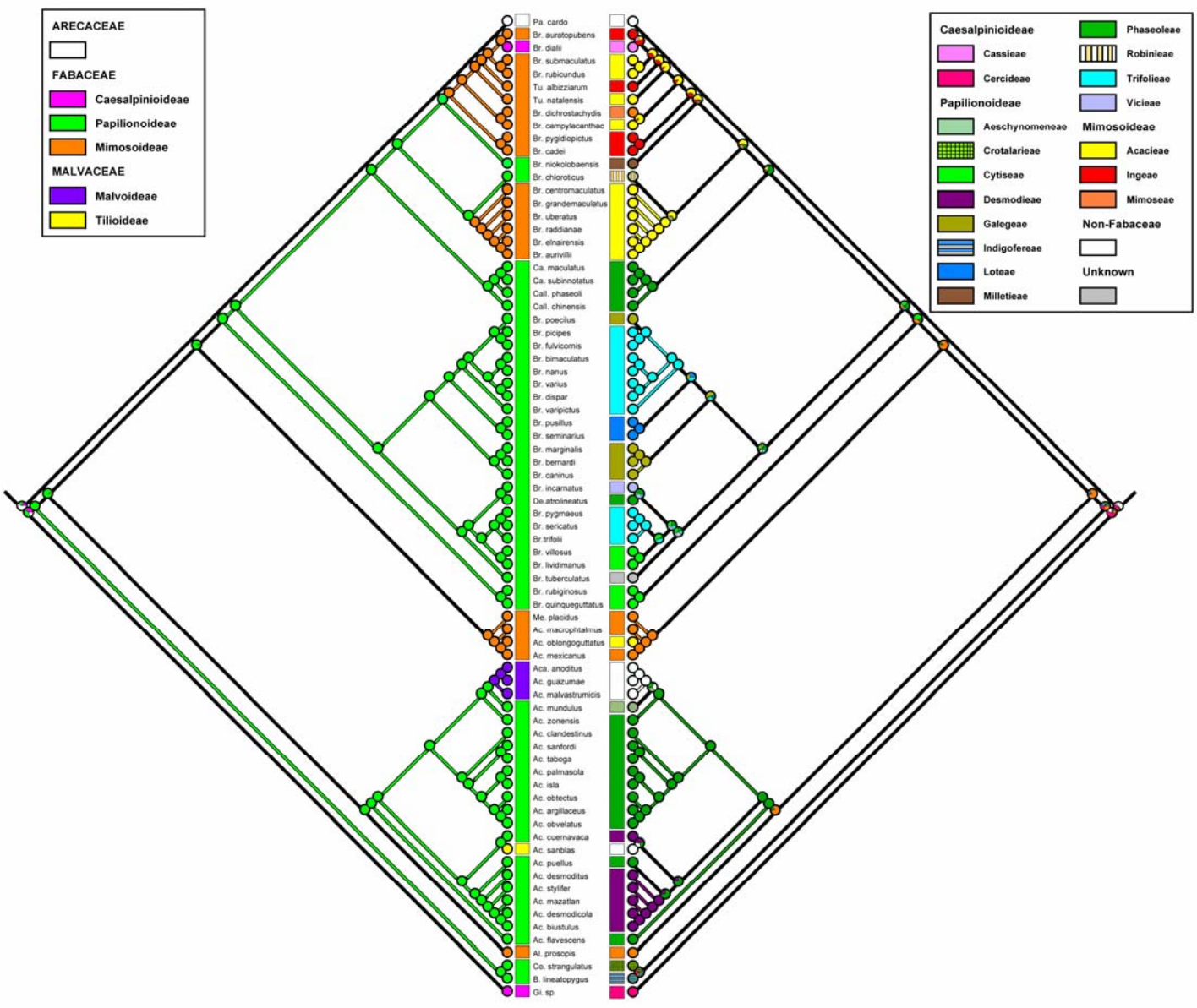
1115 Figure 2



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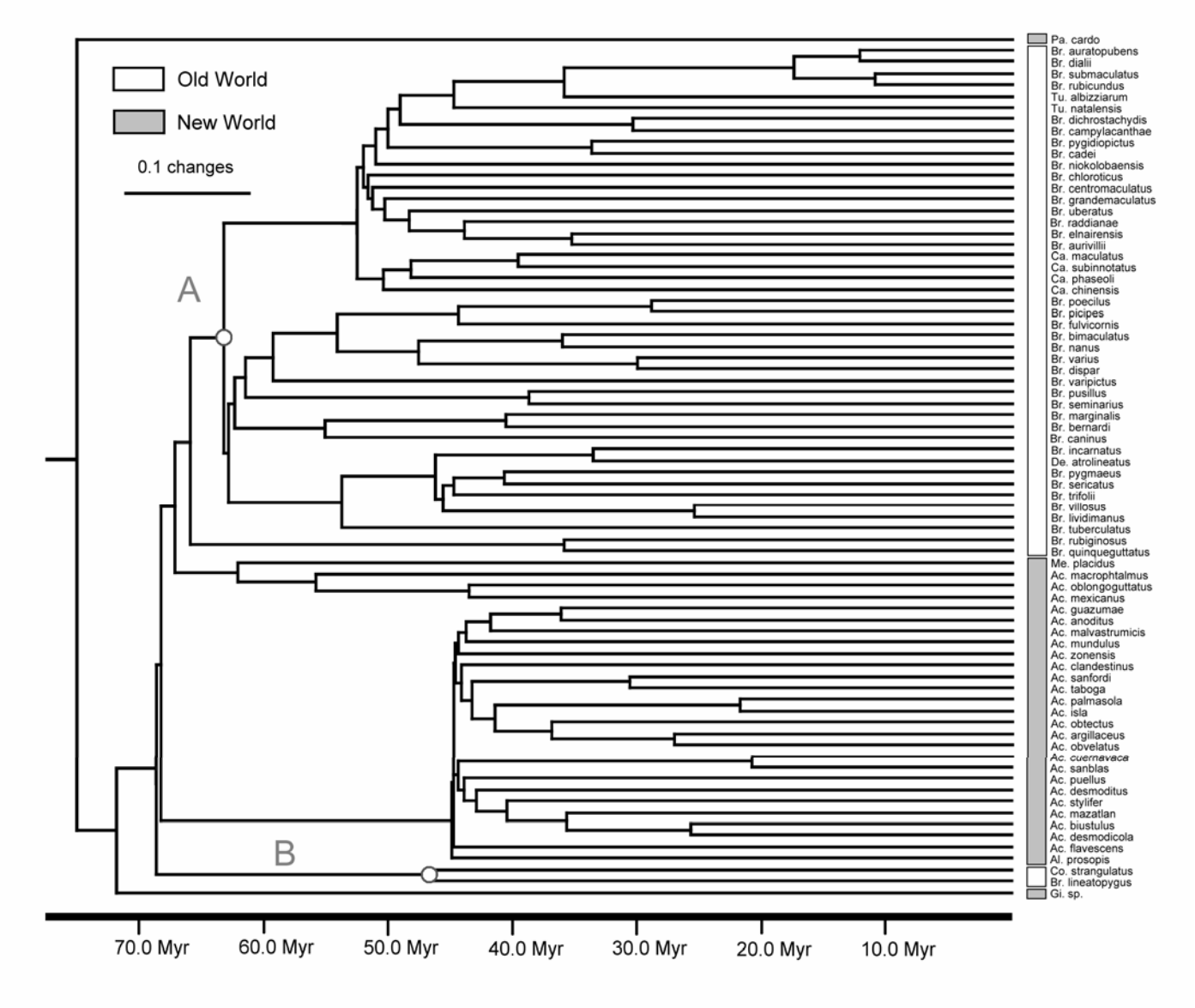
1117 Figure 3



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1119 Figure 4

MANUSCRIT III

Sibling species of bean bruchids: a morphological and phylogenetic study of *Acanthoscelides obtectus* Say and *A. obvelatus* Bridwell.

ALVAREZ, N., HOSSAERT-MCKEY, M., RASPLUS, J.-Y., MCKEY, D., MERCIER, L., SOLDATI, L., AEBI, A., SHANI, T., ET BENREY, B.

Journal of Zoological Systematics and Evolutionary Research (**2005**) **43**, 29–37

Accepted on 18 August 2004
JZS doi: 10.1111/j.1439-0469.2004.00286.x

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Sibling species of bean bruchids: a morphological and phylogenetic study of *Acanthoscelides obtectus* Say and *Acanthoscelides obvelatus* Bridwell

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Abstract

Acanthoscelides Schilsky is a large genus of neotropical bruchid beetles, in which most species show host plant specialization. *Acanthoscelides obtectus* and *Acanthoscelides obvelatus* are two sibling species specialized on *Phaseolus* beans, and are therefore considered pests. Up to now, the status of these two taxa has remained unclear, the few studies conducted having failed to elucidate whether these are two differentiated species or a single morphologically variable species. In addition, *A. obvelatus* has not been taken into account in the great majority of studies of bean bruchids. In this morphological and genetic study, we show that *A. obtectus* and *A. obvelatus* are two 'true' non-hybridizing species, which diverged about 22 Mya. Although the two species demonstrate only few morphological differences, we point out some diagnostic characters that enable their identification in the field. We also address a genetic method of differentiation of the two species, based on species-specific microsatellite loci. The strong morphological resemblance of these two species, despite their ancient divergence, may be the result of evolutionary stasis, which could be the consequence of stabilizing selection. Niche differentiation could enable the two species to coexist indefinitely.

Key words: Sibling species – bruchids – coleoptera – phylogenetic studies – evolutionary stasis

Introduction

The neotropical genus *Acanthoscelides* Schilsky (Coleoptera: Bruchidae) comprises about 300 species of seed-eating beetles (Johnson 1989). It is one of the most diverse bruchid genera. Its species are mostly specific to a narrow range of host plants, which are extremely diverse among *Acanthoscelides* species. Most feed on legumes. Among these are a group of three morphologically similar species specialized on beans of the genus *Phaseolus* (Johnson 1983): *Acanthoscelides argillaceus* Sharp, *Acanthoscelides obtectus* Say and *Acanthoscelides obvelatus* Bridwell. As suggested by recent phylogenetic studies (N. Alvarez et al., unpublished data), the three species constitute a monophyletic group within *Acanthoscelides*. However, *A. argillaceus* differs from the two other species in its specialization on species of the *P. lunatus* group, which exhibit high concentrations of cyanogenic compounds. In addition to this difference, adults of *A. argillaceus* are bright orange. In contrast, *A. obtectus* and *A. obvelatus* are specialized on beans of the *P. vulgaris* group, and exhibit darker colours. These two last species are morphologically similar (Fig. 1). Whereas *A. obtectus* was described in 1859, *A. obvelatus* remained cryptic until 1942. However, ecological and evolutionary studies usually confound the two species, which were regularly considered as one sole entity until the last decade. In fact, only very few morphological characters – essentially coloration – appear to differentiate the two species. Kingsolver (1968) described as main differences the colour of the pygidium, femur, and apical antennal segment, which are orange in *A. obtectus*, but brown-black in *A. obvelatus*. Furthermore, Kingsolver described the shape of antennae as a good discriminant character: longer and thinner segments for *A. obvelatus* and shorter and broader segments in *A. obtectus* (Fig. 2a). Among all the criteria, according to Kingsolver (1968), the most reliable character is only found in males and concerns the shape of lateral lobes of the aedeagus: smooth and thin in *A. obtectus*, sclerified and thick in *A. obvelatus* (Fig. 2b). Similarly reliable criteria have not been described in females.

The two species have overlapping environments and a single bean pod can be attacked by both species. The only marked ecological difference between the two species is their voltinism. Whereas *A. obtectus* is multivoltine and can reproduce as long as resources are available, *A. obvelatus* is univoltine and can only reproduce once a year. As a result of this difference, *A. obtectus* is now distributed worldwide, while *A. obvelatus* is still restricted to Mexico, Central America, and northern Colombia. The status of these two taxa thus remains quite mysterious. Are *A. obtectus* and *A. obvelatus* two distinct species (i.e. Is the speciation process completed?) or do their traits correspond to two extremes within a continuously varying species? Biémont et al. (1986), who found differentiation for isozyme alleles, were the first to suggest that these two sister taxa could be considered as two differentiated sibling species. However, under laboratory conditions, these authors were able to obtain a few cross-specific hybrids of *A. obvelatus* males with *A. obtectus* females after treatment of *A. obvelatus* with chemicals analogous to juvenile hormone. Nevertheless, hybridization success was poor: only half of the *A. obtectus* females laid eggs, and only 15% of eggs produced adults. Among these adults, several showed developmental abnormalities, such as antennal deformations. The authors concluded that introgression was thus possible between the two species, and postulated that such events could occur *in natura*. The discovery of several individuals with antennal deformations in the field was, according to them, a strong argument for natural genetic introgression between the two species. More recently, Gonzalez-Rodriguez et al. (2000) again conducted electrophoretic studies of isozymes on individuals of both species, and found that populations of *A. obtectus* and *A. obvelatus* presented different allele frequencies at different loci, but only

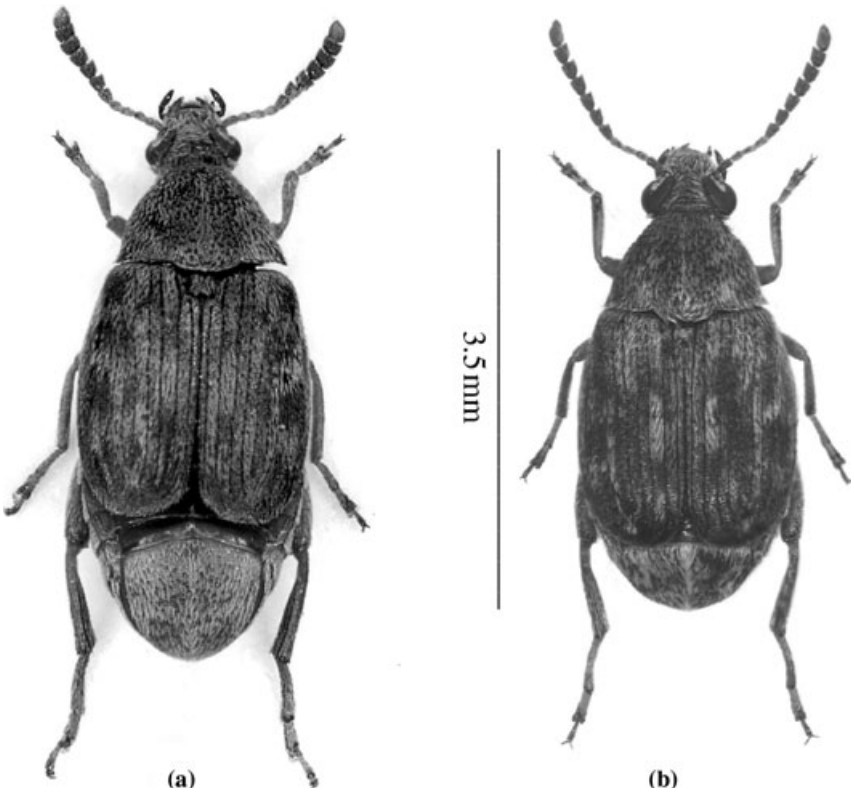

Fig. 1. Habitus (a) *A. obtectus*; (b) *A. obvelatus*. *Note*: The gap between elytra and pygidium in *A. obtectus* is artefactual and must not be taken into account

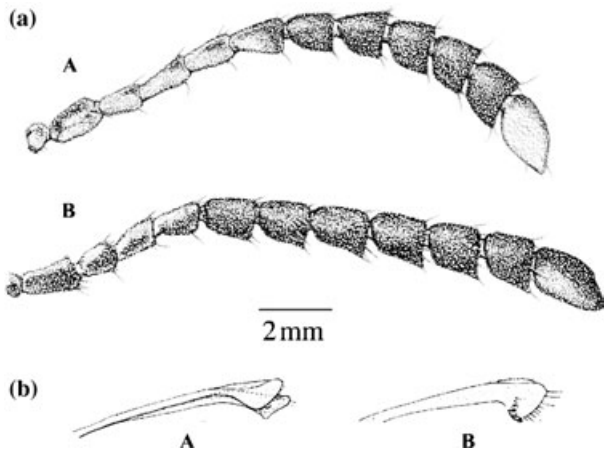

Fig. 2. (a) Antenna (A) *A. obtectus*; (B) *A. obvelatus*. (b) Lateral lobes of aedeagus. (A) *A. obtectus*; (B) *A. obvelatus*

very few private alleles. However, based on high values of Nei's genetic distance between populations of the two species, these authors concluded that the two species were differentiated, and did not interbreed *in natura*. To improve our understanding of the status of *A. obtectus* and *A. obvelatus*, we carried out morphological and DNA analyses on several populations of both species to test whether their reproductive isolation is complete. Because these beetles are economically important pests of beans, better understanding of the differences and relationships between them is required to advance research in various fields, including biological control. Indeed, most studies focused on *Acanthoscelides* associated with *Phaseolus* in Mesoamerica have up to now only considered the presence of *A. obtectus*, ignoring *A. obvelatus*. In this study, we combine morphological and genetic analysis to give a firm taxonomic footing for fundamental and applied research on these bean bruchids.

Materials and methods

Studied sites

Acanthoscelides were sampled in 21 sites between December 2001 and February 2003. Of these sites, 10 were in populations of wild beans [all sites sampled in Mexico (TLP, SJS, SAG, TEP, MAL, YAU, TLA, VDB, HUI, COP)], and 11 in populations of cultivated beans [nine sites from Mexico (SJC, SPT, YOH, OCU, SIL, STL, TZI, XOC, TEQ), one from Cameroon, and one from Switzerland] (Fig. 3 and Table 1). Emerging individuals were assigned to species on the basis of morphological characters (Kingsolver 1968).

Morphological analysis

All sampled individuals were determined using Kingsolver's criteria: (i) coloration and shape of antennae; (ii) coloration of pygidium; (iii) coloration of femur. We also analysed genitalia of all sampled males, determining each to species using this trait. Furthermore, in order to see if we could find discriminant traits in female genitalia, we dissected several females of each species, and observed the different reproductive organs.

DNA sequence analysis

We sequenced one mitochondrial gene, *COI*, and one nuclear gene, *28s rRNA*, in two individuals per species and per population. As outgroup, we used one *A. argillaceus* individual. Primer sequences were defined according to Simon et al. (1994): *C1-J-2183* and modified *TL2-N-3014* (TCCATTGCACTAATCTGCCATATTA) for *COI*; 28ee & 28 mm for *28s rRNA*. To confirm results obtained by *COI*, we sequenced another mitochondrial gene, *12S rRNA* (primers 12Sai and 12Sbi) for 10 individuals per species. Total genomic DNA was extracted using DNeasy™ kit (Qiagen, Hilden, Germany). PCR amplifications were performed in a final volume of 10 µl, which contained 1 µl of extracted DNA, 1 µl of 25 mM $MgCl_2$, 0.1 µl of

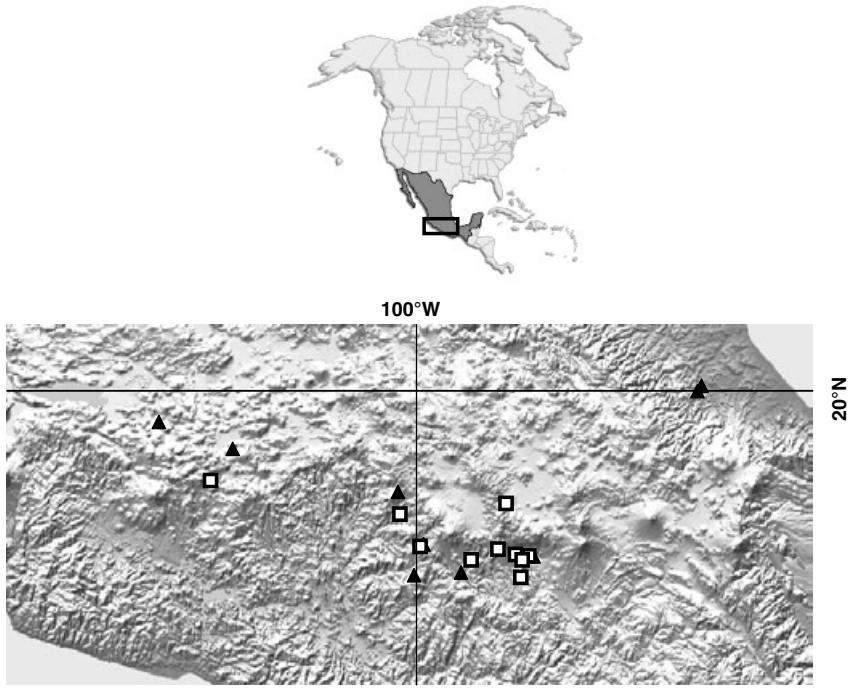

Fig. 3. Distribution of sampled populations in central Mexico. ▲, cultivated bean populations; wild bean populations

Table 1. Coordinates and altitude of all sampled populations

Abbreviations	Site name	Type of bean population	Longitude (West)	Latitude (North)	Altitude (m)
OCU	Ocumicho	c	102°13′11.8″	19°47′46.1″	2045
SIL	San Ildefonso	c	100°08′56.9″	19°22′19.8″	2400
SJC	San Jose de los Laureles pueblo	c	98°58′20.0″	18°58′40.3″	1730
SPT	San Pablo de Tejalpa	c	99°36′00.3″	18°52′59.8″	1750
STL	Santa Lucia	c	100°00′03.7″	18°52′12.5″	1790
TEQ	Tequesquipan	c	99°56′33.1″	19°03′09.2″	2300
TZI	Tzintzuntzan	c	101°34′41.5″	19°37′43.7″	1980
XOC	Xocoyolo	c	97°32′47.0″	19°58′40.0″	1550
YOH	Yohualichan	c	97°30′55.9″	20°00′56.0″	1400
COP	Copandaro	w	101°45′35.5″	19°26′24.6″	2087
HUI	Huitzilac	w	99°16′23.3″	19°01′24.4″	2544
MAL	Malinalco	w	99°30′08.9″	18°57′13.2″	1935
SAG	San Andres de los Gabeles	w	99°57′01.5″	19°02′19.5″	2280
SJS	San Jose de los Laureles campo	w	99°00′05.0″	18°58′49.7″	1855
TEP	Tepoztlan	w	99°07′15.7″	18°59′36.3″	1931
TLA	Tlayecapan	w	99°03′24.4″	18°57′20.0″	1750
TLP	Tlalpan	w	99°12′04.3″	19°17′50.3″	2403
VDB	Valle de Bravo	w	100°07′05.1″	19°13′56.8″	1918
YAU	Yautepec	w	99°01′24.0″	18°45′31.9″	1700
	Cameroon	c	Yaoundé, Province du Centre		
	Switzerland	c	Chambrelien, Canton de Neuchâtel		

c, Cultivated; w, wild.

10 mM dNTPs, 1 µl of PCR buffer (Eurogentec, Seraing, Belgium), one unit of Taq DNA polymerase (Eurogentec Red Goldstar^{TM}), 0.5 µl of forward primer, and 0.5 µl of reverse primer. PCRs were performed separately for each primer pair on a PTC-200^{TM} thermocycler using the following cycling conditions: initial denaturation at 92°C (1 min, 30 s); 30 cycles of 92°C (30 s), 55°C (45 s), 72°C (1 min, 30 s); final elongation at 72°C (10 min). The method of Sanger (1981) was carried out using Applied Biosystems BygDye^{TM} (Applied Biosystems, Foster City, CA, USA) protocol, and the sequences of 758 (*COI*), 384 (*12s rRNA*) and 553 (*28s rRNA*) nucleotides were obtained for each individual. Products of the sequencing reactions were then analysed on an ABI Prism 310 sequencer (Applied Biosystems, Foster City, CA, USA). Chromatograms were manually corrected using Chromas 2.23 (Technelysium Pty Ltd, Helensvale, Australia), and sequences were aligned using ClustalW 1.83 (Thompson et al. 1994). Phylogenetic trees were reconstructed by likelihood methods using Gamma nucleotide distance models under PAUP* (Swofford 2002), according to the best phylogenetic method suggested by Modeltest 3.06 (Posada and Crandall 1998). *COI* molecular clock was tested using likelihood ratio tests, comparing likelihoods obtained by molecular clock constrained and non-constrained heuristic searches under PAUP*, using tree bisection-reconnection branch-swapping algorithm and Rambaut's parametrization of the clock. To infer a divergence time, we used a previously calibrated beetle mitochondrial clock (Gomez-Zurita et al. 2000).

Specific analysis using DNA microsatellite loci

Using microsatellite markers developed by Alvarez et al. (2003), each extracted individual [20 per site and per species (morphologically determined)] was subjected to a PCR reaction with loci C09 (216–266 bp, specific to *A. obvelatus*) and D06 (316–366 bp, conserved locus cross-amplifying in *A. obtectus*, *A. obvelatus*, and *A. argillaceus*). Furthermore, every individual was also analysed for a third and new marker, F09 (Alvarez et al., 2004), specific to *A. obtectus* (150–170 bp). This combination of markers permitted us to assess the taxonomic status of each sampled individual of every population through one PCR reaction with three primer pairs. D06 was the *Acanthoscelides*-specific extraction control, C09 amplified only *A. obvelatus* individuals, while F09 amplified only *A. obtectus* individuals. The method theoretically permits identification of hybrids, when three distinct bands are amplified in the same individual. PCR products were revealed in 1.5% agarose gels. Non-hybrid individuals amplified two bands: D06 and C09 for *A. obvelatus* individuals, D06 and F09 for *A. obtectus* individuals. PCR amplifications were performed in a final volume of 16 µl, which contained 1.6 µl of extracted DNA, 1.04 µl of 25 mM $MgCl_2$, 1.6 µl of 10 mM dNTPs, 1.6 µl of PCR buffer (Eurogentec), 1.5 unit of Taq DNA polymerase (Eurogentec Red Goldstar^{TM}), 0.6 µl of 0.01 µM primer (for each locus, 0.6 µl forward and 0.6 µl reverse). PCRs were performed on a PTC-100^{TM} thermocycler (MJ Research, Las Vegas, NV, USA) using the following cycling conditions: initial denaturation at 92°C (1 min); seven 'touchdown' cycles: 92°C (30 s), 1°C drop per cycle to a final annealing temperature of 53°C (45 s) (Table 1), 72°C (40 s); 22 cycles of: 92°C (30 s), 53°C (45 s), 72°C (40 s); final elongation at 72°C (10 min). PCR products were separated by electrophoresis on a 1.5% agarose gel containing 0.5X TBE buffer and 0.002% ethidium bromide. Results were displayed under ultraviolet light, using water as the negative control.

Statistical analysis

Morphological and genetic results were compared using multiple correspondence analysis under SAS v. 8.02 (SAS 1999). In the analysis, pygidium coloration, femur coloration, coloration of apical segment of antenna, and antenna shape were used as explanatory variables, while the species assignment by genetic methods (i.e. the microsatellite pattern of each individual, using primer pairs D06, C09, and F09) was treated as a supplementary variable.

Results

Morphological observations

Examination of individuals from the 20 sampled sites revealed that both *A. obtectus* and *A. obvelatus* were present in six sites (TLP, SJS, SJC, TEP, MAL, SPT), while seven (SAG, YOH, OCU, YAU, TLA, Cameroon, Switzerland) and eight (SIL, STL, TZI, VDB, HUI, XOC, TEQ, COP) sites included populations of only *A. obtectus* or only *A. obvelatus*, respectively. Two individuals from XOC and MAL presented *A. obvelatus* phenotypes, but with deformed antennae. In males, inspection of lateral lobes of the aedeagus led always to an unambiguous categorization. In females, dissections of genitalia of the two species did not detect any difference. *A. obvelatus* female genitalia are indistinguishable from those of *A. obtectus* described by Huignard (1968) (Fig. 4).

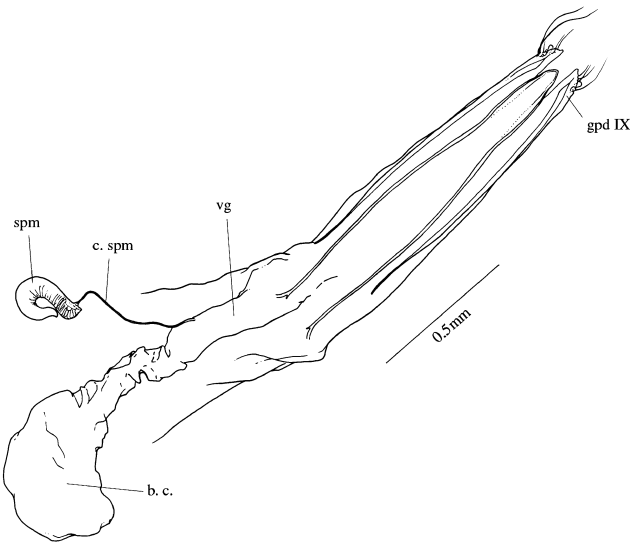

Fig. 4. Female genitalia of *A. obvelatus*. spm, spermatheca; c.spm, spermatheca canal; vg, vagina; b. c., bursa copulatrix; gpd IX, gonopode IX.

DNA sequence analysis

Topologies of phylogenetic trees were congruent for *28s rRNA* and *COI*, and showed each species as a clearly monophyletic group (Fig. 5). For *COI*, 14 and 13 haplotypes were found for *A. obvelatus* and *A. obtectus*, respectively (accession numbers AY676621–AY676675). Modeltest 3.06 suggested after a hierarchical likelihood ratio test that the best phylogenetic model was HKY85 with the following parameters: no invariant sites, gamma shape parameters = 0.2835, ts/tv ratio: 1.8576, estimated base frequencies (freqA = 0.3015; freqC = 0.1747; freqG = 0.1442; freqT = 0.3795). Within-species mean distances were 0.011 and 0.005 for *A. obvelatus* and *A. obtectus*, respectively, while the between-species mean distance was 0.167. This result was confirmed by *12S rRNA* sequences, from which two well-differentiated clades and a mean distance between the two species of 0.055 were obtained (accession numbers AY676676–AY676678; phylogenetic tree not shown). For *28S rRNA*, little genetic differentiation was found, and only two haplotypes – one for *A. obtectus* and one for *A. obvelatus* – were identified (accession numbers AY676679–AY676680). Distance (using any distance method) between the two species for this gene was 0.007. Topologies obtained for *COI*, enforcing/not enforcing molecular clock were identical for all major clades, and values for ln(likelihood) were respectively −2221.98 and −2271.76. The likelihood ratio test is marginally not passed and the molecular clock should be rejected ($p = 0.031$). However, as this probability is not highly significant, we will infer a molecular clock with the goal of obtaining a broad estimate of the divergence time between these two species. Following the clock developed on the beetle genus *Timarcha* (Coleoptera: Chrysomelidae) by Gomez-Zurita et al. (2000), the *COI* distance between *A. obtectus* and *A. obvelatus* corresponds to a divergence time of about 22 Mya.

Concerning the two individuals with deformed antennae (which phenotypically resembled *A. obvelatus*), their sequences showed unambiguously that they belonged to *A. obvelatus*, since the chromatogram signal was unique and because the sequence is clearly closely related to those of other *A. obvelatus* individuals.

Specific analysis using DNA microsatellite loci

Each of the 540 typed individuals (260 *A. obtectus* and 280 *A. obvelatus*) showed an unambiguous pattern, always with

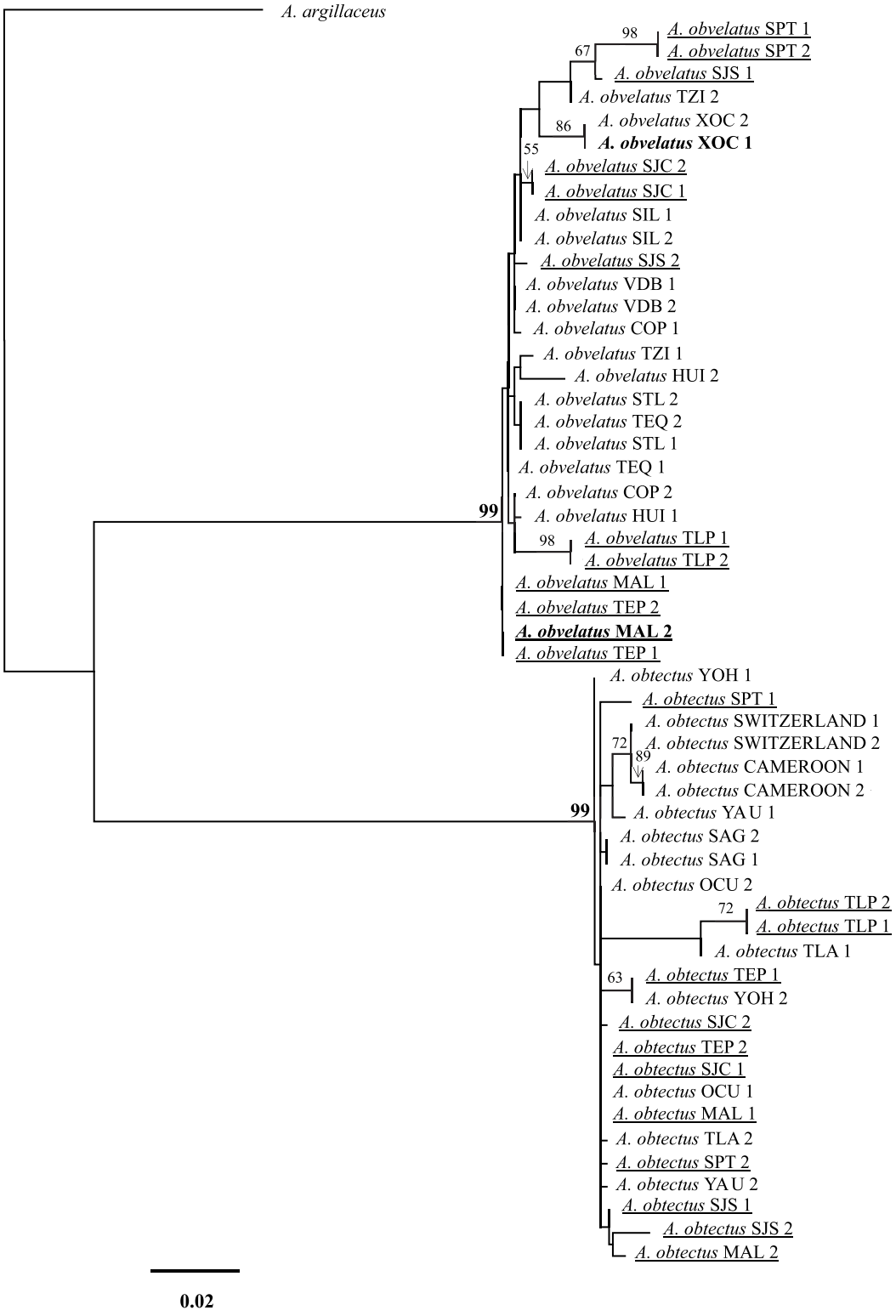

Fig. 5. Phylogenetic tree of 26 *A. obtectus* and 28 *A. obvelatus* individuals for the *COI* gene, reconstructed by maximum likelihood method. *A. argillaceus* is used as outgroup. *A. obvelatus* samples in bold correspond to individuals with deformed antennae. Underlined samples correspond to individuals from bean populations where both *A. obtectus* and *A. obvelatus* were present

two bands, either D06 and F09 for *A. obtectus* individuals, or D06 and C09 for *A. obvelatus* individuals. We found no potential hybrid, that would have borne three bands. Again, the two individuals with deformed antennae were unambiguously *A. obvelatus* individuals. Concerning the third closely related species *A. argillaceus*, only one band (for D06) was amplified. Bruchids other than *Acanthoscelides*, such as *Zabrotes subfasciatus*, seem unable to amplify any of the

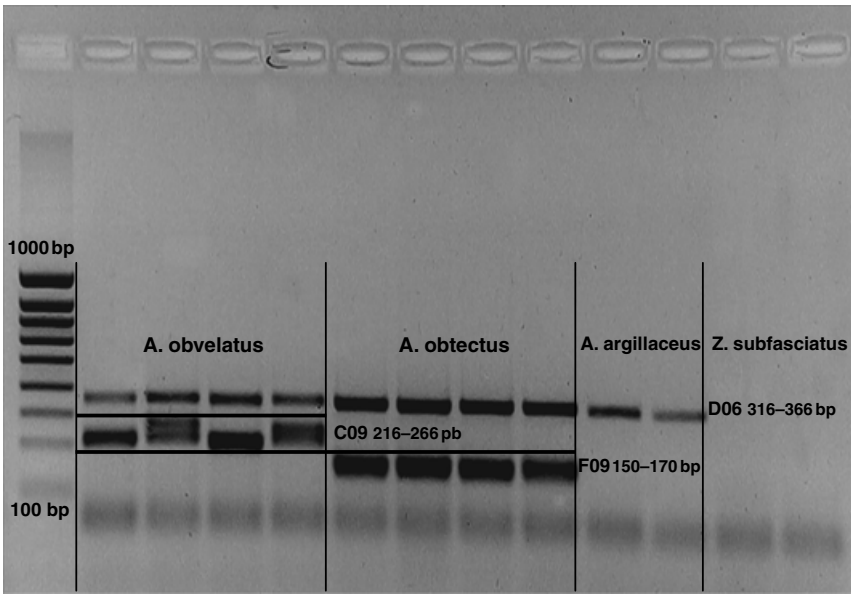

Fig. 6. Molecular diagnostic test with microsatellite loci C09, D06 and F09: *A. obvelatus* individuals amplify D06 and C09, *A. obtectus* individuals amplify D06 and F09, *A. argillaceus* individuals amplify D06 only. Individuals from other bruchid genera (e.g. *Zabrotes subfasciatus*) seem not to amplify any of the loci

specific *Acanthoscelides* microsatellite loci. Due to the fact that the three loci D06, C09, and F09 exhibit different non-overlapping ranges, the diagnostic differences are very reliable, allowing easy and unequivocal genetic determination (Fig. 6). All the results obtained with this diagnostic method were congruent with sequences revealed by *COI*, *12S*, and *28S*.

Statistical analysis

The colour and shape of antennae were by far the best morphological criteria – with the exception of male genitalia – to distinguish between *A. obvelatus* and *A. obtectus* (Fig. 7). However, in one *A. obvelatus* and four *A. obtectus* individuals the morphological (excepting genitalia in males) and genetic

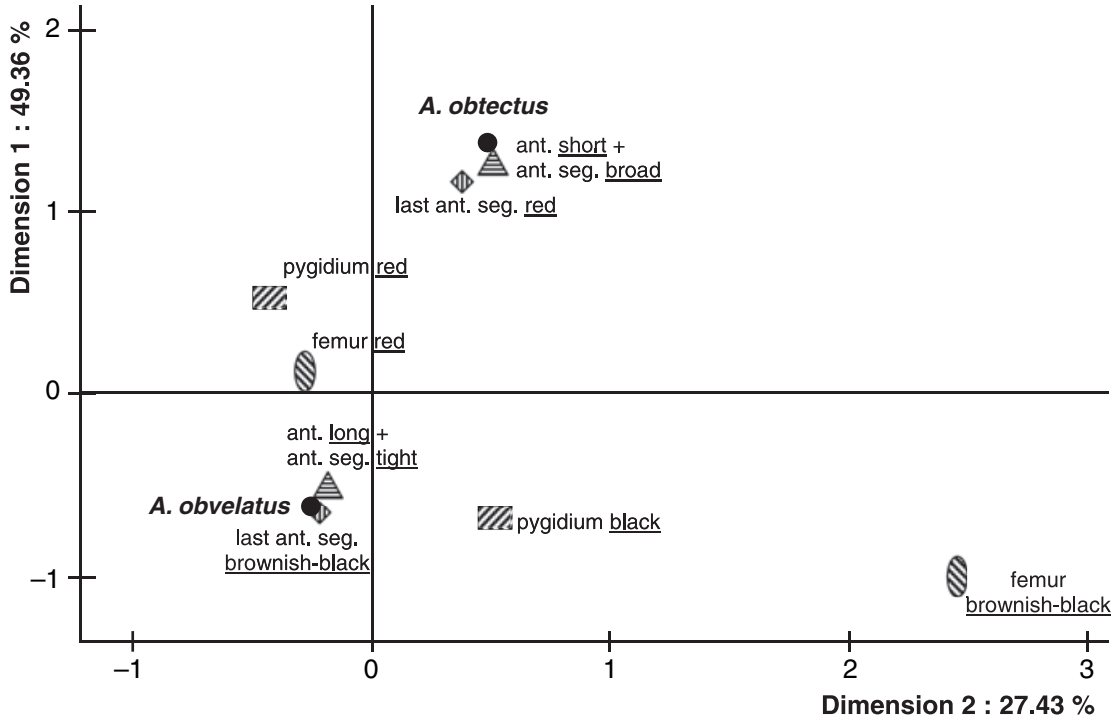

Fig. 7. Multiple correspondence analysis of morphological traits, using the species assignment by genetic methods (●) as a supplementary variable. Explanatory variables are as follows: ▲, shape of antenna; ◊, colour of last antennal segment; ▨, colour of pygidium; ▨, colour of femur

diagnoses were not congruent. Thus, fewer than 1% of individuals (five individuals among 540 typed) could not be reliably diagnosed by morphological traits other than genitalia. In contrast, pygidium and femur colour did not allow reliable diagnosis of the species. In males, the genitalia criterion was diagnostic in 100% of cases. However, in females (in which no differences were found in genitalia), the shape and colour of the last antennal segment was the only diagnostic descriptor.

Discussion

Ancient divergence between *A. obtectus* and *A. obvelatus*

As shown by our DNA analysis, hybridization between *A. obtectus* and *A. obvelatus* does not seem to occur *in natura*. The high estimated divergence time between *A. obtectus* and *A. obvelatus* (about 22 Mya) appears to attest to a strong reproductive barrier, despite great morphological similarity. However, the use of a molecular clock calibrated for another group of species – even if that group (*Timarcha*, Chrysomelidae) is relatively closely related to Bruchidae – must be done with prudence, since the biogeographic events used by Gomez-Zurita et al. (2000) to calibrate the *Timarcha* clock are not common to *Acanthoscelides*. Furthermore, in light of the current debate on the application of molecular clocks to supposed isochronous sequences (Drummond et al. 2003), it is not easy to predict, for example, how differences in voltinism will affect the rate of neutral substitution. Nevertheless, it seems obvious that the lineages of the two species have diverged at least several million years ago. Despite the fact that the two species share exactly the same habitats in many areas of Mesoamerica, they have apparently remained two distinct non-hybridizing entities. Since none of the 54 sequenced individuals, or of the 540 genotyped individuals, yielded any indication of having issued from an interspecific cross, it is likely that the few *in vitro* hybrids obtained by Biémont et al. (1986) were the result of experimental conditions (e.g. artificially-induced hormonal manipulation), that could have modified pre- and post-zygotic barriers. Our genetic data indicate that the presence of individuals with deformed antennae *in natura* – which these authors regarded as an indication of the existence of naturally cross-specific hybrids – does not seem to be necessarily related to any introgression event.

Morphological similarity

Of all the traits studied, only the morphology of the lateral lobes of the aedeagus in males was strictly diagnostic. Female genitalia traits yielded no discriminating trait. Antennal traits (shape and colour) were also very useful and gave the right diagnosis in more than 99% of the analysed individuals, both in males and females. Other criteria, such as colours of the pygidium and femur, were not reliably diagnostic. Taking into account the ancient age of the two species – which diverged about 22 Mya – as well as their inability to interbreed, a less great morphological resemblance between *A. obtectus* and *A. obvelatus* was expected. Morphological similarity seems to be common in insects, particularly in species that diverged recently (e.g. Sharpe et al. 2000). However, some studies on arthropods have shown relatively long divergence times for pairs of sibling species, such as 5 Mya in Diplopoda (Bond and Sierwald 2002). In the last 5 years, studies have identified several groups of cryptic species in insects. To our knowledge, most studies have found congruence between the « genetic » species and their associated morphology (e.g. Sharpe et al. 2000; Sebastiani et al. 2001; Kerdelhué et al. 2002).

In the case of these two *Acanthoscelides* species, the divergence between *A. obtectus* and *A. obvelatus* seems to be more ancient than in these previous examples of sibling species of insects (presuming the applicability of the *Timarcha* molecular clock to *Acanthoscelides* species). We can postulate that, in the light of the ancient divergence time between the two species, such morphological similarity could be the consequence of evolutionary stasis, perhaps because of stabilizing selection and the negligible effect of drift. Such stasis has been reported in several species groups of vertebrates, such as stickleback fishes (Schluter and McPhail 1993) or rainforest lizards (e.g. Schneider et al. 1999), for which stabilizing selection has been proved to produce long-term morphological stability, despite very ancient divergence times. In the case of *A. obtectus* and *A. obvelatus*, a relative stability in developmental conditions of larvae in bean seeds (e.g. constraints due to seed size and to secondary chemical compounds), as well as high densities of host plant populations, may have allowed such stabilizing selection to occur. Furthermore, since metapopulations of bean bruchids seem to be fairly stable (i.e. constant large population sizes and frequent migrations between populations), drift may not have had a very strong effect as an evolutionary pressure in populations of these two species. This could explain why *A. obvelatus* and *A. obtectus* show mostly symplesiomorphic characters, despite their relatively ancient divergence. In a recent study (N. Alvarez et al., unpublished data), we showed that *A. obtectus* and *A. obvelatus* probably speciated in allopatry. According to a scenario consistent with biogeography, *A. obvelatus* originated in Mesoamerica on seasonally fruiting wild beans, where univoltinism and diapause abilities were adaptive, whereas *A. obtectus* originated in Andean South America, where it evolved adaptations to non-seasonal wild bean populations, by losing its ability to diapause and evolving multivoltinism. However, both species – even if demonstrating several ecological differences – develop on beans of the *Phaseolus vulgaris* group (common beans), whose seeds and pods are marbled and dark. Thus, the two species, which probably have evolved under similar pressures of predation and parasitism, would have benefited from traits reducing the ability of predators to detect them, and have therefore evolved similar dark colorations, mimetic with bean seeds and pods. However, since at early and intermediate stages of maturity – when females usually start laying eggs – pods are not dark enough to render beetles cryptic, the significance of their similarity in colour is difficult to evaluate. Evolutionary stasis can be invoked again, to explain the global similarity – not only in colour traits – of the two species. Indeed, examination of Johnson's keys to *Acanthoscelides* species (Johnson 1983, 1990) shows that morphological similarity of closely related species is quite common in the genus; the case of *A. obtectus* and *A. obvelatus* is not unusual.

In the same previous study (N. Alvarez et al., unpublished data), we also showed that *A. obtectus* probably reached Mesoamerica posterior to bean domestication (~7000 years ago), through human-mediated migrations. Consequently, the two sister species became sympatric only recently, compared with their time of divergence. Strong pre-zygotic and post-

zygotic barriers have therefore probably appeared over millions of years, and prevent today any genetic exchange between the two species.

The ecological differences that may permit coexistence of *A. obtectus* and *A. obvelatus* are of great interest. Although the necessity of differentiated niches for durable coexistence is still questioned (Chesson 2000; Hubbell 2001), ecological divergence should at least facilitate coexistence. Both *A. obtectus* and *A. obvelatus* are able to develop both on wild and cultivated common beans. However, the univoltinism of *A. obvelatus* makes it more adapted to the phenology of wild beans, whereas the multivoltinism of *A. obtectus* is one of the main causes of its current cosmopolitan distribution, and of the ability of the species to develop exponentially in granaries (N. Alvarez et al., unpublished data). Nevertheless, the ecological segregation of the two species is not absolute. Several wild bean populations harboured *A. obtectus* only, whereas in some cultivated bean populations, *A. obvelatus* was strongly predominant. The presence of one or another species in a certain geographical zone is nonetheless strongly correlated with the proportion of wild versus cultivated common beans. Predicting whether one or the other *Acanthoscelides* species might be globally more competitive in Mesoamerica, perhaps even replacing the other species, is as yet impossible. Because wild and cultivated bean populations are both common in Mesoamerica, the two species could coexist indefinitely. The fact that these two morphologically similar non-hybridizing species can persist in the same habitats is most likely explained by the coexistence of wild and cultivated beans, allowing niche differentiation of the two species.

Acknowledgements

The authors wish to thank P. Jarne, C. Debain, P. Sourrouille and M. Held for their helpful technical assistance, and A. Delgado-Salinas, E. Jousselin and J. Romero Napoles for discussion. They also thank the publishers of the Proceedings of the Entomological Society of Washington for permission to use the illustrations reproduced in Fig. 2b. This work was financially supported by the Swiss National Science Foundation (project no. 3100.064821.01).

Zusammenfassung

Schwester Arten der Bohnen Bruchiden: eine morphologische und phylogenetische Studie über Acanthoscelides obtectus und A. obvelatus

Die Acanthosceliden (*Acanthoscelides* Schilsky) sind eine grosse neotropische Gattung innerhalb der Bruchiden (Bruchidae, Coleoptera), von denen die meisten Arten eine hohe Wirtsspezifität aufweisen. *Acanthoscelides obtectus* und *A. obvelatus* sind zwei nahe verwandte Taxa, die auf *Phaseolus* Bohnen spezialisiert sind und deshalb als Schädlinge eingestuft werden. Der taxonomische Status dieser beiden Arten ist nach wie vor unklar; die wenigen Untersuchungen, die durchgeführt wurden, konnten nicht klären, ob es sich um getrennte Arten oder nur um eine, morphologisch variable Art handelt. Ausserdem wurde *A. obvelatus* in den meisten Arbeiten über Bohnen-Bruchiden nicht berücksichtigt. In der vorliegenden morphologischen und genetischen Studie zeigen wir, dass *A. obtectus* und *A. obvelatus* zwei nicht hybridisierende Taxa sind, die sich vor ca. 22 Millionen Jahren aufgespalten haben. Trotz der geringen morphologischen Unterschiede dieser beider Arten) zeigen wir einige diagnostische Parameter, die eine Identifikation im Gelände ermöglichen. Zusätzlich beschreiben wir eine genetische Methode zur Unterscheidung der beiden Arten, die auf artspezifischen Mikrosatelliten-Loci beruht. Die hohe morphologische Ähnlichkeit, die diese beiden Arten trotz der langen Zeit seit der Aufspaltung aufweisen, kann auf eine evolutionäre Stasis in Folge einer stabilisierenden Selektion zurückgeführt werden. Nischendifferenzierung könnte zu einer unbegrenzten Koexistenz dieser Arten führen.

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MANUSCRIT IV

Horizontal gene transfer between sympatric bruchid species.

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(soumis à *Heredity*)

Horizontal gene transfer between sympatric bruchid species

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Keywords: ecological context of HGT, bruchid beetles, *Acanthoscelides*, *Zabrotes*, Mexican Altiplano.

Running title: Horizontal gene transfer between beetles.

Word Count: 3119.

Abstract

We report on the horizontal transfer of a mitochondrial gene, *cytb*, between three species of bruchid beetles, *Acanthoscelides obtectus*, *A. obvelatus and Zabrotes subfasciatus*, in a zone where these species are sympatric. The high degree of incongruence of the *cytb* tree with patterns for other genes (either with another mitochondrial gene [*COI*] or with a nuclear gene [*28S rRNA*]) and with traditional taxonomy is discussed in the light of three hypotheses: experimental contamination, hybridization, and pseudogenisation. However, none of these explains the patterns observed. A fourth hypothesis, involving recent horizontal gene transfer (HGT) between *Acanthoscelides obtectus* and *A. obvelatus*, and from one of these species to *Zabrotes subfasciatus* in the Mexican Altiplano, seems the most plausible explanation. We show that the transferred gene is under selection, and expressed in a mitochondrion. This case study offers the first clues for the ecological context that might favour horizontal gene transfer (HGT). The HGT between our study species seems to have occurred recently, and only in a zone where the three beetles are sympatric and share common host plants. This suggests that transfer could have been effected by some external vector such as an eukaryotic parasite.

Introduction

The traditional view of evolution supports that DNA is transferred vertically from parent to offspring. Hybridization and genetic transfer between different species is usually strongly limited. However, exceptions to this rule, i.e. the horizontal transmission of genetic material between distantly related organisms (HGT) are increasingly recognized as an important process of evolution in prokaryotes (Syvanen, 1994; Doolittle, 1999; Jain *et al*, 2002; Rivera and Lake, 2004). In eukaryotes, however, reported cases of between-species genetic exchanges not involving hybridization have been essentially limited to noncoding or parasitic transposons or virus sequences. Exceptions to this are a few instances of horizontal transfer of selected genes between prokaryotes and eurkaryotes (e. g., Andersson, 2003; Mita *et al*, 2003; Goldsmith *et al*, 2005) and two reports suggesting lateral transfer of mitochondrial genes between genetically distant land plant groups (Bergthorsson *et al*, 2003, 2004). No instance of HGT in animals has been reported up to now. In a recent paper, Martin (2005) discusses the relevance of the HGT hypothesis in eukaryotes, focusing on land plants, and argues for the need of corroborating studies in other eukaryote groups. However, he addresses the necessity of a careful interpretation of HGT data,

and points to the requisite of first discussing such data in the light of standard theories of vertical inheritance of genes.

Our study investigates the phylogenetic relationships between four Neotropical bruchid beetles (Coleoptera, Bruchidae), namely *Acanthoscelides obtectus* Say, *A. obvelatus* Bridwell, *A. argillaceus* Sharp, and *Zabrotes subfasciatus* Boheman. These beetles develop on seeds of several bean species (genus *Phaseolus*): *Acanthoscelides obtectus* and *A. obvelatus* feed on the common bean (*P. vulgaris* L. group), *A. argillaceus* on the Lima bean (*P. lunatus* L. group), and *Z. subfasciatus* on both. As *A. obtectus*, *A. obvelatus* and *Z. subfasciatus* can develop on beans of the same species, they are often sympatric in wild or cultivated common bean populations. We sampled individuals from these three species in the southern part of the Mexican Altiplano, one such zone of sympatric occurrence of *A. obtectus*, *A. obvelatus* and *Z. subfasciatus* (Figure 1). Although the geographic range of *A. argillaceus* overlaps with those of its two congeners, it is rarely found in the same habitat, because of its distinct host plant. *Acanthoscelides argillaceus* and the generalist *Z. subfasciatus*, however, commonly co-occur in wild Lima bean populations (Delgado-Salinas, 1988). This is the case in the Pacific coast of Mexico (Figure 1), where individuals from these two species were sampled.

The genera *Acanthoscelides* and *Zabrotes* are morphologically very different (Borowiec, 1987), and thought to have diverged during the Palaeocene or the Eocene (Farrell, 1988), whereas the three *Acanthoscelides* species studied here belong to the same morphologically defined group within the genus and seem to have diverged during the Miocene (Johnson, 1983; Alvarez *et al*, 2005). To confirm the phylogenetic position of these four species in the context of host plant adaptation (see Alvarez *et al*, submitted), we performed amplification and sequencing of two mitochondrial genes and one nuclear gene.

Material and Methods

Individuals of *A. obtectus*, *A. obvelatus*, *A. argillaceus*, and *Z. subfasciatus* were sampled in the southern Mexican Altiplano. Coordinates of sampled sites are given in Table 1. We amplified and sequenced bi-directionally two mitochondrial genes – cytochrome oxidase I (*COI*) and cytochrome b (*cytb)* – and one nuclear gene – 28S ribosomal rRNA (*28S rRNA*) – from 6 to 30 individuals in each species, using universal primers (*28S rRNA*: 28ee and 28mm; *COI*: C1-J-2183 and TL2-N-3014; *cytb*: CB-J-10933 and CB-N-11367 [Simon *et al*, 1994]). Total genomic DNA was extracted using DNeasy™ 96-wells kit (QIAGEN). PCR amplifications were

performed in a final volume of 10 µL, which contained 1 µL of extracted DNA, 1 µL of 25 mM MgCl2, 0.1 µL of 10mM dNTPs, 1 µL of PCR buffer (Eurogentec), 1 unit of Taq DNA polymerase (Eurogentec Red Goldstar™), 0.5 µL of forward primer, and 0.5 µL of reverse primer. PCRs were performed separately for each primer pair on a PTC-200™ thermocycler using the following cycling conditions: initial denaturation at 92 °C (1 min 30 s); 30 cycles of 92 °C (30 s), annealing at 55°C (45 s), 72 °C (1 min 30 s); final elongation at 72 °C (10 min). Sequencing reaction was carried out using Applied Biosystems BygDye™ protocol. Products of the sequencing reactions were then analyzed on an ABI Prism 310 sequencer. Chromatograms were manually corrected using Chromas 2.23 (Technelysium Pty. Ltd., Helensvale, Australia) and further aligned using ClustalW 1.83 (Thompson *et al*, 1994). Phylogenetic trees were reconstructed under Mega 2.1 (Kumar *et al*, 2001) using a Neighbour-Joining method (Kimura 2-parameters; gamma shape parameter = 0.5). Primers specific for *cytb* consensus haplotypes of *A. obtectus* and *A. obvelatus*, and for *cytb* haplotypes of Pacific coast *Z. subfasciatus*, respectively, were designed. Sequences of specific primers to the most common haplotype shared by *A. obtectus* and *A. obvelatus* are: GAGATAATGCAACATTAACA (forward primer) and GGTTTGCGGGCGTAAAATTA (reverse primer). Sequences of

specific primers to Pacific-coast *Z. subfasciatus cytb* are: TTGATAACGCAACCTTAACC (forward primer) and GATTAGCAGGAATGAAGTTG (reverse primer). To test whether the sequenced *cytb* genes were under purifying selection (i.e., they were not the result of a nuclear mitochondrial pseudogene), we compared rates of synonymous and non-synonymous substitutions. Two models of codon evolution (Godman and Yang, 1994) were tested. In model M1, the ratio of non-synonymous to synonymous evolutionary rate (ω) was set to 1, as expected for a pseudogene. In model M2, the ratio was free to vary. A likelihood-ratio test was performed to compare the two models.

Results

Topologies of phylogenetic trees were congruent for *28S rRNA* and *COI*, and showed each species as a monophyletic group, the three *Acanthoscelides* species forming a distinct clade from *Z. subfasciatus* (see Figure 2a) (*28S rRNA* [accession numbers AY881176-AY881195]; *COI* [accession numbers AY881196-AY881214, AY881232]). *COI* genetic distances ranged from 0.273 to 0.324 substitutions per site between *Acanthoscelides* spp. and *Zabrotes subfasciatus*, and from 0.172 to 0.212 substitutions per site among *Acanthoscelides* species. According to a recent calibration of

a beetle mitochondrial molecular clock (Gomez-Zurita *et al*, 2000), such genetic distances correspond to divergence times of 35-40 My between *Acanthoscelides* spp. and *Zabrotes subfasciatus*, and 20-25 My among *Acanthoscelides* species. These divergence times were confirmed by estimates obtained from mitochondrial *12S rRNA* (Alvarez *et al*, submitted), and are slightly similar to the fossil record (Farrell, 1998).

The analysis of *cytb* sequences, in contrast, revealed an unexpected pattern (see Figure 2b) (accession numbers AY422474-AY422479, AY422485-AY422488, AY881215-AY881232). First, individuals of *A. obtectus* and *A. obvelatus* were not separated in two distinct clades, but grouped into a single, little variable monophyletic unit, as would be expected for individuals belonging to the same species. A core clade containing 46 of the 50 sequenced individuals from the two species included 14 closely related haplotypes (maximal distance: 0.0084), some of them shared by the two species, others private to *A. obtectus* or *A. obvelatus*. In contrast, *A. argillaceus* individuals clustered in a separate clade, whose mean distance to the *A. obtectus* - *A. obvelatus* core clade was 0.143. Secondly, *cytb* data revealed a surprising discrepancy among the individuals of *Z. subfasciatus*. Whereas an expected 0.25 nucleotide divergence from *Acanthoscelides* spp. was found for the *Z. subfasciatus* individuals

from the Pacific coast of Mexico, three *Z. subfasciatus* individuals from the Altiplano (coming from three different populations) showed a *cytb* sequence identical to the most common *A. obtectus* - *A. obvelatus* core clade haplotype.

PCR with specific primers and subsequent sequencing revealed that the three Altiplano *Z. subfasciatus* individuals surprisingly carried both *cytb* genes (Figure 2b), their own plus the one demonstrating an *Acanthoscelides* origin. The specific primers were then applied with 25 individuals of a laboratory colony of *Z. subfasciatus*, that had been maintained for 10 months in the Laboratory of Entomology and Animal Ecology at the University of Neuchâtel (Switzerland). The colony was founded using individuals from the Mexican Altiplano, i.e., potentially affected by the HGT. These individuals had not been in contact with *A. obtectus* for about 5-10 generations. Three individuals out of the 25 were revealed to carry both *cytb* genes, while 22 showed only the *Z. subfasciatus* haplotype.

An analysis of *cytb* sequence variation within the *A. obtectus* - *A. obvelatus* core clade revealed no frameshift, and a low (0.106) ratio of non-synonymous to synonymous changes, significantly lower than 1 (likelihood ratio test: 2(ln[Lieklihood(M1)]-ln[Likelihood(M2)])=47.5; χ^2 [1 df]: P < 10-4), indicating that this gene is under selection. Furthermore, these sequences included no mitochondrial stop codon,

but typically five nuclear stop codons, strongly suggesting that this gene is replicated by a mitochondrion, and neither by a nuclear or a bacterial machinery.

Discussion

The *cytb* pattern shown by *A. obtectus*, *A. obvelatus* and three Mexican Altiplano *Z. subfasciatus* individuals is highly incongruent with traditional taxonomy, and with the *COI* and *28S rRNA* pattern. This is even more astonishing, considering that *cytb* and *COI* are supposedly genetically linked in the non-recombining mitochondrial genome.

Four hypotheses can be addressed to explain this unexpected result: (i) experimental contamination, (ii) hybridisation, (iii) pseudogenisation and (iv) horizontal gene transfer.

Contamination hypothesis

All experiments were simultaneously conducted on many bruchid (>200) samples, including museum specimens with low amounts of DNA, by the same researcher in the same laboratory. Only the *cytb* PCR for Mexican Altiplano individuals yielded an additional, unexpected band. In *Acanthoscelides*, no individual from the Pacific Coast *A. argillaceus* carried the transferred gene, although they were

analyzed simultaneously with the *A. obtectus* and *A. obvelatus* samples. A putative laboratory contaminant would not only have affected the samples coming from the Mexican Altiplano but also individuals from other sites and particularly museum specimens, which contain very small amounts of DNA, given the fact that samples were randomized on the 96-wells plates, both during DNA extraction and PCR reactions. Another, hardly revocable argument against the contamination hypothesis is that several individuals of the core clade from both *A. obtectus* and *A. obvelatus* demonstrated private *cytb* haplotypes. These haplotypes can not be the consequence of a contamination, since they were found only once (we sequenced all the amplified *cytb* fragments). In case of a contamination, a single haplotype would be expected to be occur in a large number of random samples, whereas our data show many related but distinct – and sometimes private - haplotypes found in a non-random subset of the samples. Sampling contamination (e.g., the presence of *Acanthoscelides* tissues in *Z. subfasciatus* samples) can be also be excluded, since three *Z. subfasciatus* individuals sampled from the experimental colony showed the additional *cytb* PCR band. The experiments on colony individuals (DNA extraction, PCR) were conducted several weeks after the analysis of the wild samples, making any cross-experiment contamination impossible.

Hybridization hypothesis

Hybridization between *Acanthoscelides* species and *Zabrotes subfasciatus* is very unlikely. First, the split between the two genera dates back to around 40 mya (molecular clock data) to 60 mya (fossil record) ago. Moreover, they demonstrate a very high level of nucleotide divergence (25%). Usually, the degree of genetic divergence in documented cases of interspecific hybridization in insects never exceeds 5%-10% of nucleotide divergence for protein-encoding mitochondrial genes (Orr *et al*, 1994; Orr, 1996; Willett *et al*, 1997), which is much lower than the 25% divergence found between *Acanthoscelides* and *Zabrotes*. Secondly, hybridization appears functionally impossible, given the highly differentiated morphology, especially in the male genitalia . The lateral lobes of the male genitalia – which play the decisive role of pushing aside parts of the female genitalia to allow penetration by the median lobe, and which are known to be part of a specific lock / key system – are spectacularly different between the two genera. Borowiec (1987) points out that the structure of the *aedeagus* is very different in *Zabrotes* and *Acanthoscelides* and highly specialized in both. The lateral lobes are particularly distinct, short and almost entirely fused in *Zabrotes*, but elongate and divided by a deep cleft almost as far as the base in *Acanthoscelides*. In the bruchid family, in which many

cryptic species remain to be described, male genitalia are often the only character to distinguish between for the rest morphologically identical (but never hybridizing) sibling species. This clearly explains why any attempt to produce hybrid offspring between *Acanthoscelides* and *Zabrotes* failed (N. Alvarez, unpublished observations). Moreover, even in the two morphologically similar *Acanthoscelides obtectus* and *A. obvelatus*, no evidence of interspecific hybridization has been detected *in natura*, despite the genetic analysis (by means of diagnostic microsatellite loci) of hundreds of individuals from the two species, among which dozens originating from sympatric populations (Alvarez, 2005).

Pseudogene hypothesis

An additional surprise in our data is that the putatively transferred fragment appears to evolve under purifying selection. The rate of non-synonymous mutations is significantly lower than expected under a model involving a non-selected gene, and there is no insertion or deletion in any *cytb* sequenced fragment. Moreover, the fragment seems to be replicated in a mitochondrion . Given that the gene is under purifying selection, it could hardly have been replicated using the nuclear genetic code, since in that case, five codons would have been translated in stop-codons. This irrevocably argues for an

active gene, rather than a pseudogene. A pseudogene – which by definition can hardly ever be under purifying selection – would not show a bias to synonymous over non-synonymous mutations, and its sequence may most likely show nucleotide insertion or deletion. Furthermore, in the exceptional context of a selected pseudogene (Hirotsune *et al*, 2003), the gene must not contain any stop codon when translated with the nuclear genetic code. This combined evidence suggests that genes from the core clade are (I) translated in a mitochondrion, (II) are under purifying selection and are therefore not pseudogenes.

Horizontal gene transfer hypothesis

Our data clearly argue against any standard explanation for the genetic pattern we observed. A horizontal transfer of the *cytb* gene between *A. obtectus* and *A. obvelatus*, and from one of these species to *Z. subfasciatus*, seems the only plausible hypothesis, even though the probability of such a mitochondrial genetic exchange between distinct eukaryote species is very low (but invoked in a recent study in plants [Bergthorsson, 2003, 2004]).

The transfer of such a gene from one species to another would possibly be carried out by an external vector. Candidate vectors include viruses, prokaryotes such as *Wolbachia* or other

endosymbiotic bacteria – a case of horizontal transfer of genes from *Wolbachia* to the nuclear genome of its host was recently reported in bruchids (Kondo et al, 2003) – or even eukaryotes (e.g., intra- or extra-cellular parasite, endoparasitoïds). The location of the transferred *cytb* in the recipient species remains to be investigated. A parsimonious hypotheses would be, that the transferred *cytb* is still hosted by its vector. A eukaryotic parasite might have "domesticated" a bruchid mitochondrion (or at least some bruchid mitochondrial gene), carrying it on, when switching to a new species. Such an hypothesis could explain the presence of two *cytb* genes in *Z. subfasciatus* individuals from the Altiplano, suggesting that one gene could be carried by a parasite, and the other could belong to the own mitochondria of *Z. subfasciatus*. This could also be the case in *A. obtectus* or *A. obvelatus*, even though we couldn't yet demonstrate the existence of a second *cytb* gene in individuals belonging to the core clade.

If we consider that the vector may belong to an eukaryotic group (I) capable of mitochondrial recombination and (II) showing a genetic code that uses the same mitochondrial stop-codons as those used by the mitochondria of invertebrates – these two conditions are for example filled by most unicellular eukaryotes –, the mitochondria carried by the parasite may have "included" the "bruchid"-*cytb* in its

genome after recombination. Another interesting point is that the transferred gene replicates in *Z. subfasciatus*, since it survived ~5-10 generations in the laboratory.

Ecological context of HGT

As this HGT event has been detected only between individuals from the Altiplano where the three species co-occur and share the same host plant, it gives clues for the ecological context which may favor such a gene exchange. Indeed, the ecologically distinct *A. argillaceus* does not demonstrate a HGT pattern for *cytb*, despite its phylogenetic proximity to *A. obtectus* and *A. obvelatus*. This suggests that the probability of the occurrence of lateral gene transfer is partly controlled by the environment. The occurrence of HGT between bruchid species (i) presenting a high degree of phylogenetic divergence but (ii) sharing some dimensions of their ecological niche (e.g., their host plant) could indicate that also ecology, not only phylogeny, might play a role in the distribution of genetic variation across taxa.

Conclusions

Recent horizontal gene transfer (HGT) between *A. obtectus* and *A. obvelatus*, and from one of these species to *Z. subfasciatus* in the

Mexican Altiplano, seems the only plausible hypothesis to explain the pattern we observed in our data for *cytb*.

Obviously, additional molecular, cellular and tissular characterization would be of great relevance to the understanding of this intriguing system, of the exact length and structure of the apparently transferred fragment, and the nature of the genome hosting it. The recent nature of this putative HGT – it is still polymorphic in *Z. subfasciatus* at least– makes it a promising model for further investigating the mechanisms underlying genetic exchanges between species

Acknowledgements

We thank P. Jarne for his support during this study; E. Desmarais, P. Sourrouille and C. Debain for technical assistance; J. Romero, T. Shani and Y. Borcard for providing several *Z. subfasciatus* individuals; F. Kjellberg and P. David for critical reading of the manuscript; A. Delgado-Salinas, J. Moreau and A. Aebi for discussion. Financial support was provided by the Swiss National Science Foundation (Project 3100.064821.01) and the Swiss Center of Competence in Research “Plant Survival”.

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Titles and legends to figures

Figure 1. Geographic origin of *Acanthoscelides obtectus* (⛰), *A. obvelatus* (▽), *A. argillaceus* (⯁) and *Zabrotes subfasciatus* (◍) individuals sampled.

(a) Pacific coast populations; only one *cytb* gene is present in *Z. subfasciatus* populations (SJB, ELA). **(b)** Altiplano populations; two *cytb* genes are present in the same individual in some *Z. subfasciatus* (OCM, YAU, TLA); **(c)** population of origin of the *Z. subfasciatus* laboratory colony.

Figure 2. Phylogenetic patterns.

a. Phylogenetic relationships between different populations of *Acanthoscelides obtectus* (⛰), *A. obvelatus* (▽), *A. argillaceus* (⯁) and *Zabrotes subfasciatus* (◍) for the mitochondrial *COI* gene. An identical topology is supported by *28S rRNA* (not shown); **b.** Phylogenetic relationships between different populations of *Acanthoscelides obtectus* (⛰), *A. obvelatus* (▽), *A. argillaceus* (⯁) and *Zabrotes subfasciatus* (◍) for the *cytb* gene. Underlined individuals correspond to *Z. subfasciatus* populations from the Mexican Altiplano - some carry two *cytb* haplotypes. Stars (*) indicate *Z. subfasciatus* haplotypes identical to identical to haplotypes from *Acanthoscelides*. "*Z. subfasciatus* CLNY" corresponds to an individual of the laboratory colony affected by HGT.

Tables

Table 1. List of sampled sites.

Code	Site name	Geographic position	Sampled species	Longitude (° West)	Latitude (° North)	Altitude (m)
CLNY	(proceeding from Malinalco)	Altiplano	*Z. subfasciatus*	99°30'08.9''	18°57'13.2''	1935
COP	Copandaro	Altiplano	*A. obvelatus*	101°45'35.5''	19°26'24.6''	2087
ELA	Elabillal	Pacific coast	*A. argillaceus* *Z. subfasciatus*	102°21'44.8''	18°00'27.0''	28
HUI	Huitzilac	Altiplano	*A. obvelatus*	99°16'23.3''	19°01'24.4''	2544
OCM	Tilapa	Altiplano	*Z. subfasciatus*	99°25'12.2''	19°11'24.5''	1300
PAZ	Playa Azul	Pacific coast	*A. argillaceus*	102°21'14.4''	17°59'20.8''	21
SIL	San Ildefonso	Altiplano	*A. obvelatus*	100°08'56.9''	19°22'19.8''	2400
SJB	San Juan Bosco	Pacific coast	*Z. subfasciatus*	102°08'24.9''	18°07'12.4''	150
SJS	San Jose de los Laureles arriba	Altiplano	*A. obtectus* *A. obvelatus*	99°00'05.0''	18°58'49.7''	1855
SJC	San Jose de los Laureles abajo	Altiplano	*A. obtectus*	98°58'20.0''	18°58'40.3''	1730
SPT	San Pablo de Tejalpa	Altiplano	*A. obtectus*	99°36'00.3''	18°52'59.8''	1750
STL	Santa Lucia	Altiplano	*A. obvelatus*	100°00'03.7''	18°52'12.5''	1790
TEP	Tepoztlan	Altiplano	*A. obtectus* *A. obvelatus*	99°07'15.7''	18°59'36.3''	1931
TLA	Tlayecapan	Altiplano	*A. obtectus* *Z. subfasciatus*	99°03'24.4''	18°57'20.0''	1750
XOT	Xochitlan	Altiplano	*A. obtectus*	97°39'02.0''	19°57'59.9''	1450
YAU	Yautepec	Altiplano	*Z. subfasciatus*	99°01'24.0''	18°45'31.9''	1700

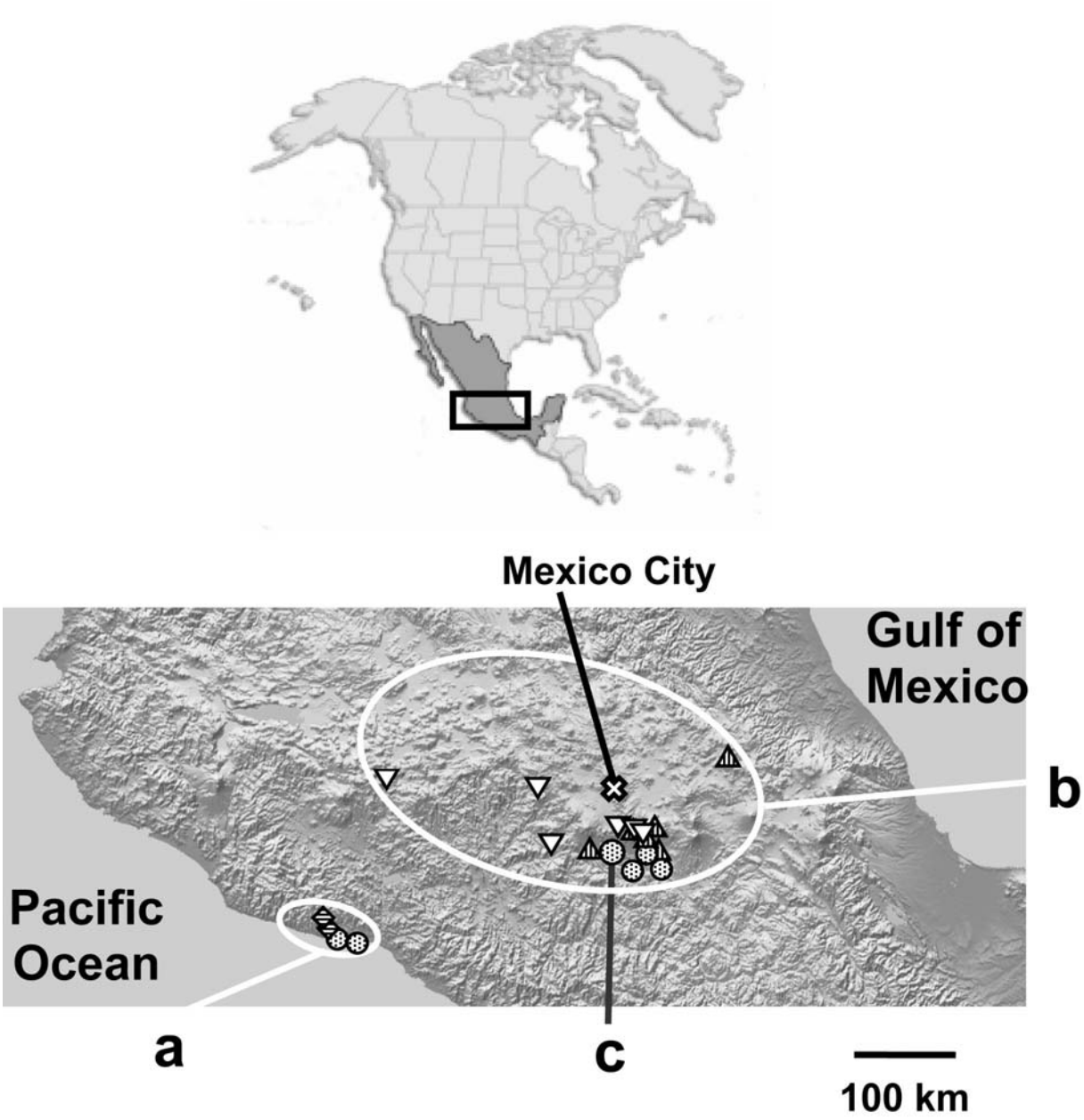
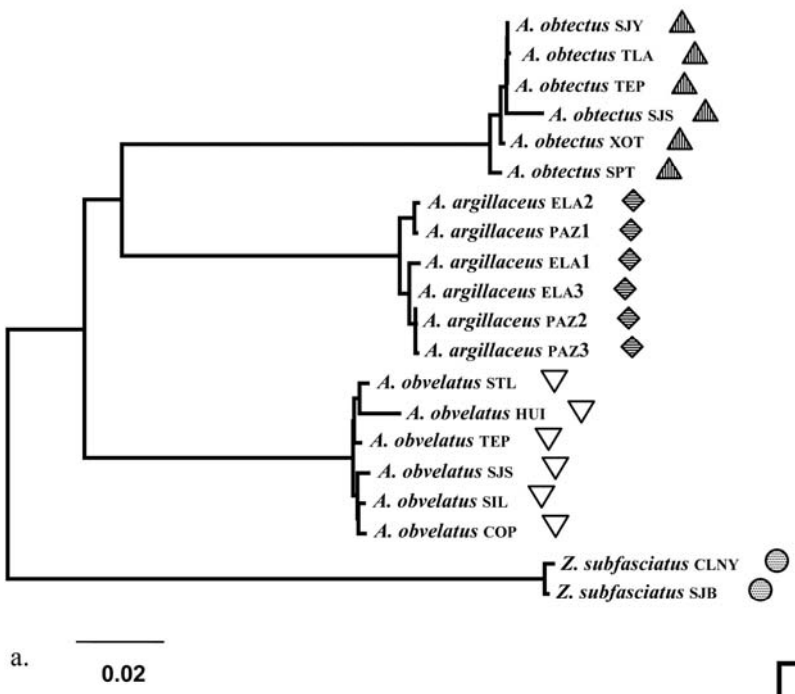


Figure 1


A. obtectus SJY
A. obtectus TLA
A. obtectus TEP
A. obtectus SJS
A. obtectus XOT
A. obtectus SPT
A. argillaceus ELA2
A. argillaceus PAZ1
A. argillaceus ELA1
A. argillaceus ELA3
A. argillaceus PAZ2
A. argillaceus PAZ3
A. obvelatus STL
A. obvelatus HUI
A. obvelatus TEP
A. obvelatus SJS
A. obvelatus SIL
A. obvelatus COP
Z. subfasciatus CLNY
Z. subfasciatus SJB
a.
0.02


A. obtectus TEP
A. obvelatus COP
A. obtectus XOT
Z. subfasciatus YAU*
Z. subfasciatus OCM*
Z. subfasciatus TLA*
A. obtectus SPT
Z. subfasciatus CLNY*
A. obvelatus TEP
A. obtectus SJS
A. obvelatus HUI
A. obvelatus SJS
A. obtectus TLA
A. obvelatus STL
Core clade
A. obtectus SJC
A. obvelatus SIL
A. argillaceus PAZ1
A. argillaceus ELA2
A. argillaceus ELA1
A. argillaceus PAZ3
A. argillaceus PAZ2
A. argillaceus ELA3
Z. subfasciatus YAU
Z. subfasciatus TLA
Z. subfasciatus OCM
Z. subfasciatus CLNY
Z. subfasciatus ELA
Z. subfasciatus SJB
b.
0.02

Figure 2

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Ecological distribution and niche segregation of sibling species: the case of bean beetles, *Acanthoscelides obtectus* Say and *A. obvelatus* Bridwell.

ALVAREZ, N., MERCIER, L., HOSSAERT-MCKEY, M., KUNSTLER, G., AEBI, A., ET BENREY, B.

(soumis à *Oikos*)

Ecological distribution and niche segregation of sibling species: the case of bean beetles, *Acanthoscelides obtectus* Say and *A. obvelatus* Bridwell.

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Abstract

Molecular techniques have greatly added to the number of known sympatric cryptic species in insects. Ecological differences between these newly distinguished species are little explored, but niches often appear to overlap strongly. These cases are good models for exploring new ideas about species coexistence and community structure. *Acanthoscelides obtectus* and *A. obvelatus* are two sister species of Neotropical bean bruchids, which have been confused until the last decade. However, one important ecological difference between them has emerged: *A. obtectus* is multivoltine and now distributed worldwide, whereas *A. obvelatus* is univoltine and restricted to Mesoamerica. Where their ranges overlap in Mesoamerica, the two species share the same host plants, and they can sometimes emerge in the same bean pod. The aim of this study was to examine their ecological differentiation in this zone of sympatry. The analysis of 27622 Mexican individuals of the two species in 2001 and 2002 indicates that their niches overlap, but are differentiated with respect to altitude and the kind of beans (wild vs. domesticated). The principal patterns in their relative abundance in different kinds of habitats, and at different seasons, were constant from one year to the next. Since sympatry of the two species seems to be of relatively recent origin, the observed niche differentiation may not have evolved in response to competition, but could instead be the consequence of physiological differences, evolved independently in each species in allopatry, that preadapted them for different altitudes and kinds of resources. The combination of biological and historical factors thus appears to allow these two sibling species to coexist in sympatry, despite their broadly overlapping ecological niches.

Introduction

Mayr (1942) used the term "sibling species" for the first time to designate species which cannot be diagnosed using a strictly morphological species definition. One of the first models of study of sibling species was the genus *Drosophila* (Diptera: Drosophilidae), in which Dobzhansky (1946) first observed that morphologically almost identical species could coexist without interbreeding. The emergence of comparative (cytogenetic) studies rapidly led to new insights in this genus (e.g. Burla et al. 1949, Dobzhansky et al. 1950, Salzano 1956), in which sibling species often showed differences in arrangement and number of chromosomes. In *Drosophila*, the first attempts were made to examine ecological differentiation between sibling species. Sibling species seemed to occupy different habitats (Dobzhansky and Pavan 1950, Da Cunha et al. 1951, Da Cunha and Dobzhansky 1954, Sokoloff 1955). In the late 1960's, with the advent of the study of enzymatic polymorphism, new insights on the nature of sibling species emerged from work on *Anopheles* (Diptera: Culicidae) (Bianchu 1968), and again in *Drosophila* (Hubby and Throckmorton 1968, Ayala et al.1970, Ayala and Powell 1972). With the emergence of techniques based on DNA analysis in the middle of the 1980's, new molecular tools confirmed the existence of additional sibling species in these two well-studied genera (in *Drosophila*: Stephens and Nei 1985, Satta *et al*. 1987; in *Anopheles*: Cockburn et al. 1988). In the past two decades, numerous increasingly cryptic sibling species have been discovered (see Figure 1) in various invertebrate groups (e. g., Sharpe et al. 2000, Wilding et al. 2000, Romi et al. 2000, Navajas et al. 2000, Manonmani et al. 2001, Hohenlohe and Boulding 2001, Bond and Sierwald 2002, Fettene et al. 2002, Mattiuci et al. 2003, Martin-Sanchez et al. 2003, Maingon et al. 2003, Borghuis et al. 2004, Duncan et al. 2004).

Since the earliest studies on *Drosophila*, ecology and niche segregation of sibling species have been studied in about twenty-five genera of invertebrates. In insects, investigations continued to focus on the two well-studied genera *Anopheles* (e. g., White and Rosen 1973,

Mosha and Petrarca 1983, Schneider et al. 2000) and *Drosophila* (e. g. Yamamoto et al. 1985, Aspi 1996) but broadened to include other Diptera (e. g., *Rhagoletis* [Bush 1969, Linn et al. 2003], *Simulium* [Adler and Kim 1984]), Lepidoptera (e. g., *Lethe* [Shapiro and Carde 1970], *Leptidea* [Benes et al. 2003]) and Hymenoptera (e. g., *Asobara* [Vet et al. 1984]). In other invertebrates, ecological studies have focused on genera of copepods (Bergmans et al. 1988), decapods (Walker 2001), nematodes (Nascetti et al. 1993), polychaetes (Gamenick et al. 1998), and trematodes (Bartoli 1988).

The number of behavioral, physiological, and morphological studies has kept pace with the discovery of new sibling species. However, the number of studies treating the ecology of sibling species during the same period has not increased proportionally (see Figure 1). Because many of the sibling species discovered by new DNA molecular methods are morphologically, behavioral, and physiologically more cryptic than those previously discovered, there is a strong need to study their ecology. Because most of these newly discovered sibling species were for long not distinguished from one another, their ecological differentiation is unstudied. To our knowledge, the few studies concerning the ecology of sibling species (e. g., Vet et al. 1984, Walker 2001, White and Rosen 1973) concern species whose existence was known before the emergence of DNA based molecular techniques.

Niche differentiation needs to be examined in some of the new cases. To some workers (e. g., Zhang et al. 2004), the lack of obvious ecological segregation in recently discovered sibling species suggests that these cases might provide support for Hubbell's (2001) neutral theory of community structure, which predicts that ecologically equivalent species can coexist for a long time, since dynamics of competitive exclusion or coexistence are slow for identical competitors. In an extreme case, such as the neotropical fig wasps in which Molbo et al. (2003) demonstrated several parallel cases of coexistence of supposedly morphologically identical sympatric species of pollinators in different fig species, the concept of niche

74 segregation seems to be violated. However, niche segregation could involve subtle
75 differences, and fine-scale studies are required. Quantifying and understanding ecological
76 differentiation between cryptic species is thus an emerging field of investigation. Are some
77 sibling species ecologically equivalent? In such complex systems, how life-history traits can
78 affect ecological segregation of different species has been little studied. The aim of this study
79 is to compare the ecology of two sibling species of bruchid beetles (Coleoptera: Bruchidae),
80 that are broadly sympatric and feed on the same larval hosts. Our study will examine how a
81 difference in a key life-history trait could lead to ecological segregation despite very broad
82 niche overlap.
83 Bruchids are a well-studied family of seed-eating beetles, relatively homogeneous in
84 terms of external morphological traits. About one-quarter of the species described belong to
85 the neotropical genus *Acanthoscelides*, which comprises about 300 species (Johnson 1983,
86 1990, Udayagiri and Wadhi 1989). Among them, the great majority feed on legumes (Johnson
87 1989). In this genus, many species are very similar in external morphology and can be
88 distinguished only with great difficulty on the basis of the morphological species definition.
89 This is particularly the case for two species associated with the common bean (*Phaseolus*
90 *vulgaris*), *Acanthoscelides obvelatus* Bridwell and *A. obtectus* Say (Kingsolver 1968). These
91 two species belong to the same small clade within the genus, together with *A. argillaceus*
92 Sharp, a Mesoamerican species specialized on beans of the *P. lunatus* L. group (Alvarez et al.
93 submitted). Whereas *A. argillaceus* is morphologically distinct from the two others, *A.*
94 *obtectus* and *A. obvelatus* have long been confused, because the only apparently diagnostic
95 external morphological trait (recently confirmed by Alvarez et al. 2005a) is the color of the
96 last antennal segment. Despite their similarity, *A. obtectus* and *A. obvelatus* are two
97 reproductively isolated species whose gene pools have accumulated substantial differences
98 (Alvarez et al. 2005a), confirming the validity of their separation by Kingsolver (1968).

In Mesoamerica, the two species often co-occur in the same habitats, on wild beans as well on domesticated beans. Leroi et al. (1990) suggested that *A. obtectus* was more adapted to *P.vulgaris* as a host plant and *A. obvelatus* more adapted to *P. coccineus* L., another Mesoamerican species of the *P. vulgaris* group. However, evidence for this hypothesis is weak. The functional trait that best differentiates the two species is their difference in voltinism (Biémont et al. 1986). Whereas *A. obvelatus*, like most related species, is univoltine, *A. obtectus* is multivoltine. While multivoltinism has allowed *A. obtectus* to multiply in bean granaries, to exploit these continuously available resources, and to move with beans during their introduction into new areas, multivoltinism is not an adaptation to bean domestication, because it pre-dated this event and characterizes *A. obtectus* populations on wild beans as well (Alvarez et al. 2005b). How differences in voltinism affect the ecology of these two bruchids in natural populations in Mexico has not been studied.

To define more clearly the niches of these two bruchid species, we analyzed their distribution in relation to several ecological factors, including not only the host plant species identity, but also the altitude and the kind of beans (domesticated vs. wild) in a wide range of Mexican populations. In the light of our results, we also discuss the role of voltinism as the central factor explaining the patterns observed.

Material and Methods

Sampling method

Forty-four sites ranging from latitude 18°49'03.6'' to 19°50'56.6'' N and from longitude 98°59'10.4'' to 102°24'29.0'' W were sampled in the Altiplano of southern Mexico between December 2001 and March 2002. Each site corresponds to a population of *Phaseolus* beans, either domesticated (23 sites), or wild (21 sites). Altitude and coordinates of each site are given in Annex. Among the 23 sites for domesticated beans, 21 were *P. vulgaris* fields, while

two were fields where *P. vulgaris* and *P. coccineus* were cultivated together. Of the 21 sites for wild beans, 12 were in wild *P. vulgaris* populations, 5 in wild *P. coccineus*, and 4 had wild populations of both *P. vulgaris* and *P. coccineus*. The two bean species demonstrate a similar seasonality, fruiting from December to April. For wild bean populations, we collected mature pods (between 100 and 1500, depending on the population size, each pod containing between 4 and 8 seeds), whereas for domesticated bean populations, we asked farmers to give us (or sell us) seeds of their last harvest (between one and two liters), except in rare cases where we were allowed to collect bean pods directly in farmers' fields. Each site was sampled two times (rarely three), to capture the dynamics of the two species throughout the dry season, first at the beginning of the dry season (15 Dec. 2001 - 15 Jan. 2002), and a second time at the end of the dry season (15 Feb. 2002 - 15 Mar. 2002). During the first sampling period, bruchids cannot be detected by external visual examination. *Acanthoscelides* females start laying eggs in bean pods during the last stage of maturation of seeds (i. e., from early December to mid-January, depending on the population, in the Mexican Altiplano). Because *Acanthoscelides* need 30-80 days for development from eggs to adults in natural habitats (Labeyrie 1962), all bruchids collected during the first sampling period were in a larval stage. However, in one single seed collection, different instars were present at the same time (N. Alvarez, pers. obs.). During the second sampling in the case of the multivoltine species *A. obtectus*, larvae of the second generation, issued from eggs recently laid by adults of the first generation, were also present. In each collection site, pods were shelled and seeds placed in ventilated plastic recipients. Each day, emerging *A. obtectus* and *A. obvelatus* were collected in 70% ethanol and the date of emergence was recorded. Adult beetles were collected daily to prevent them from reproducing in the plastic recipients. However, some reproduction may have been possible in the containers, since some emerging adults would have had about 24 hours to copulate and lay eggs. In all sites with both *P. vulgaris* and *P. coccineus*, we

149 separated pods of the two species, and were thus able to record the host plant from which each
150 adult emerged. A third and final sampling was done between December 2002 and March 2003
151 in 16 of the 44 sampled sites, to examine inter-annual variation in the frequencies of the two
152 species. In the first year, 14537 individuals were collected, whereas in the second year, we
153 collected a total of 13085 individuals.
154
155 **Statistical analysis**
156 The effects of host-plant species, altitude, date of emergence and kind of host-plant
157 population (domesticated vs. wild) on the respective proportions of individuals belonging to
158 the two species were tested using a generalized linear model with a binomial distribution and
159 a logit link function (for each observation, an individual belonged either to *A. obvelatus* or to
160 *A. obtectus*), using the "genmod" procedure in SAS (1999). Because *P. coccineus* grows at
161 higher altitudes and is much less frequently cultivated than *P. vulgaris*, the host-plant species
162 could be a confounding factor of both the altitude and the kind of host plant. We therefore
163 checked first the correlation of the host plant species with the altitude and with the kind of
164 host plant [domesticated vs. wild] using the "genmod" procedure in SAS. We tested
165 separately for the effect of host plant, by comparing the ratios of the number of *A. obvelatus*
166 and *A. obtectus* individuals in the six mixed populations (where both *P. vulgaris* and *P.*
167 *coccineus* occurred) by a Wilcoxon signed rank test (based on paired comparisons), using S-
168 Plus (2001). In these six populations, frequencies of *A. obvelatus* on *P. vulgaris* and on *P.*
169 *coccineus* were compared by pairs. We then again tested the effects of altitude, date of
170 emergence and kind of host-plant population (domesticated vs. wild) – without taking into
171 account the effect of host plant species – on the probability that a beetle would be *A.*
172 *obvelatus*, using a generalized linear model. The estimates of the probability to be *A.*
173 *obvelatus* for each of the modalities/variables were recorded for the three tested variables, and

174 the probability distribution surface was drawn using S-Plus. The inter-annual variation was
175 tested by a Wilcoxon signed rank test, using S-Plus, in which the frequencies of *A. obvelatus*
176 in each site were compared for the 16 populations sampled in both years.
177

178 ## Results

179 The host-plant species (*P. vulgaris* vs. *P. coccineus*) (χ^2= 10.45, P = 0.0012), the kind
180 (domesticated vs. wild) of host-plant (χ^2= 938.00, $P<10^{-4}$), the altitude (χ^2= 665.60, $P<10^{-4}$)
181 and the date of emergence (χ^2= 55.70, $P<10^{-4}$) all had effects on the relative frequencies of the
182 two sibling species. However, it seemed that host plant species could be a confounding factor,
183 since frequencies of the two host plant species were strongly correlated with both the kind of
184 beans (domesticated vs. wild) (χ^2= 1258.37, $P<10^{-4}$) and the altitude (χ^2= 4151.28, $P<10^{-4}$). In
185 agreement with this interpretation, frequencies of *A. obtectus* and *A. obvelatus* on the two
186 bean species in the mixed *Phaseolus* populations were not significantly different (P = 0.5625;
187 see Figure 2). Therefore, in a second analysis, we discarded the host-plant species as a factor
188 that might influence the frequencies of the two bruchids. In this analysis, we examined
189 frequencies of the two bruchids in all populations, taking into account only the kind of host
190 plant (domesticated vs. wild), the altitude, and the date of emergence. The data did not show
191 over-dispersion (scaled deviance = 0.7306). Again, each of the three factors showed a
192 significant relationship with the frequencies of the two species: *A. obvelatus* was more
193 frequent on wild than on domesticated beans (χ^2= 1042.84, $P<10^{-4}$); it was more frequent at
194 higher altitudes (χ^2= 739.86, $P<10^{-4}$); and it became less frequent over the course of the
195 reproductive season, from December to April (χ^2= 62.53, $P<10^{-4}$). The probability surface of a
196 sampled beetle being *A. obvelatus* (obtained from the "genmod" estimates for each modality /
197 factor) is represented in Figure 3. In wild bean populations, *A. obvelatus* was never less
198 frequent and almost always significantly more frequent than *A. obtectus*. Frequencies of *A.*

obvelatus on wild beans ranged between 0.5 at low altitudes and 1.0 at high altitudes. On domesticated beans, *A. obvelatus* was more frequent than *A. obtectus* at higher altitudes, but less frequent at lower altitudes. Frequencies of *A. obvelatus* ranged between 0.15 (at low altitudes) and 1.0 (at high altitudes). The limit between low altitude populations, where *A. obtectus* was more frequent, and high altitude populations, where *A. obvelatus* was more frequent, was at about 1800m. Finally, the relative frequency of *A. obtectus* gradually increased between 15 December and 15 April. The comparison between 2002 and 2003 showed no significant differences in the proportions of the two bruchid species between years ($P = 0.4474$; see Figure 4).

Discussion

Niche segregation

Our results show that although the niches of *A. obtectus* and *A. obvelatus* overlap broadly, they are statistically differentiated. *Acanthoscelides obvelatus* was more frequent at higher altitudes, and on wild bean populations, whereas *A. obtectus* was more common at lower altitudes and on domesticated beans. These two species are thus quite distinct ecologically. In these insects, there is no reason why morphological similarity should be associated with a lack of ecological divergence. Merilä et al. (2001) have suggested that real-time microevolution is rarely observed in the wild, invoking, for example, temporally and spatially fluctuating selection. Other studies invoke the role of stabilizing selection (e. g., Colborn et al. 2001). Fluctuating or stabilizing selection could be invoked to explain the observed pattern in these two bruchids, in which rates of microevolution may be different between morphological and ecological traits. More attention should be given to the fact that cryptic taxa may demonstrate different evolutionary histories and different adaptations, and therefore also

223 exhibit different ecological niches. In the case of *A. obtectus* and *A. obvelatus*, applying the
224 theory of neutral diversity (Hubbell 2001) to explain coexistence appears unnecessary.
225 Our study shows that the effect of altitude on the respective frequencies of the two
226 *Acanthoscelides* sibling species depended on the kind (wild vs. domesticated) of host plant.
227 Whereas on wild bean populations, the probability of occurrence of *A. obvelatus* decreased
228 slowly with decreasing altitude, there was a much steeper decline in the frequency of *A.*
229 *obvelatus* with decreasing elevation on domesticated beans. This result indicates an
230 interaction between the respective physiological aptitudes of the two species to evolve in
231 cold/dry and warm/wet environments, and their respective reproductive biologies. Because *A.*
232 *obvelatus* only produces one generation per year, it is rapidly overtaken in numbers by *A.*
233 *obtectus* in low-altitude granaries. Conversely, the impossibility for *A. obtectus* to enter
234 reproductive diapause imposes severe limitations to its survival during the unfavorable season
235 at higher altitudes. At such altitudes, the reproductive diapause of *A. obvelatus* (which is
236 probably associated with a general reduction in metabolic activity) confers higher
237 survivorship, compensating for its ability to produce only a single generation per year.
238 Diapause in tropical insects imposes a severe energetic cost because high temperature during
239 the unfavourable season leads to high rates of respiration in heterothermous organisms such
240 as insects, as well as high rates of water loss (Janzen 1973). The cost of reproductive diapause
241 should be lower at higher elevation, where temperatures in the unfavourable season are cooler
242 than at low elevation. This factor could contribute to explain the restriction of *A. obvelatus* to
243 higher elevations.
244 In populations of wild beans, *A. obvelatus* was always more frequent than *A. obtectus.*
245 This could be explained if wild beans always represent sink populations for *A. obtectus*. Its
246 short adult lifetime compared to *A. obvelatus* (Pichard et al. 1991) coupled with the strong

247 fruiting seasonality of wild *Phaseolus* beans in Mexico, probably often prevents its survival
248 from one year to another.
249
250 **Host-plant species effect**
251 In opposition to the hypothesis of Leroi et al. (1990), our data from mixed bean populations
252 show that the host plant species (*P. vulgaris* vs. *P. coccineus*) did not affect the respective
253 frequencies of the two sibling species of *Acanthoscelides*. The host plant species is merely a
254 factor that is confounded with the two other variables examined here, since in the Mexican
255 Altiplano, *P. coccineus* grows at higher altitudes (1800-2700m) than does *P. vulgaris* (0-
256 2100m), and domesticated populations account for a low proportion of all populations of *P.*
257 *coccineus*, in contrast to the situation for *P. vulgaris*.
258
259 **Inter-annual stability of respective frequencies**
260 Despite differences in the densities of populations of the two species from one year to
261 another, the relative frequencies of *A. obvelatus* and *A. obtectus* remained similar in almost all
262 populations, demonstrating the stability of their niche segregation. If niches were not well
263 defined, a lottery situation would occur each year, leading to changes in relative frequencies
264 from one year to another in the different populations. While this result needs to be confirmed
265 by longer-term studies, the observed stability over two successive years in virtually all
266 populations is highly suggestive.
267
268 **Insights on the origin of the two species**
269 The higher densities of populations of *A. obvelatus*, especially on wild beans, are consistent
270 with the hypothesis of a Mesoamerican origin of this species. It is possible that the arrival of
271 *A. obtectus* in Mexico was very recent (as suggested by Alvarez et al. 2005b). Therefore the

observed niche segregation between the two species may not be the "ghost" of past competition between them. For *A. obvelatus*, whose history in Mesoamerica could be very long, competition with bruchids other than *A. obtectus* may have led to its adaptation to high altitudes. Likely important competitors are *Zabrotes subfasciatus* and *Z. sylvestris*, which also develop on *Phaseolus* beans, but at altitudes lower than *A. obvelatus* (from sea level to 1500-1800m). In this scenario, past competition with *Zabrotes* could have driven *A. obvelatus* to specialize on high altitudes, while *Zabrotes* spp. adapted to low altitudes. In such a circumstance, recently arriving *A. obtectus* would have overlapped on niches of both *Zabrotes* spp. and *A. obvelatus.* This could be the reason why pure *A. obtectus* populations are very rare, since *A. obvelatus* is able to develop in all populations higher than 1500-1800m and *Zabrotes* spp. develops below 1500-1800m. In contrast, pure *A. obvelatus* populations are quite frequent in the Mexican Altiplano, especially at high altitudes (2100m and higher).

Role of voltinism

The effect of the date of emergence on the relative frequency of the two species demonstrates the role played by voltinism. Whereas in *A. obvelatus* cohort overlapping is impossible, *A. obtectus* can produce multiple generations throughout the year as long as resources are present. Consequently, the relative frequency of *A. obtectus* gradually increased between 15 December and 15 April. Nevertheless, the higher frequency of *A. obvelatus* at the beginning of the season compared to its frequency four months later indicates a lower survival of *A. obtectus* during the rest of the year, since inter-annual frequencies are stable. This is possibly due to a higher mortality rate of *A. obtectus* during the rest of the season, especially in populations on wild beans, in which once the last bean pods have matured, resources are no longer available, and no further opportunities for oviposition occur within the lifetime of individuals. The highest longevity recorded for *A. obtectus* adults *in natura* is about 80 days

297 (Labeyrie 1962). Therefore, populations on wild beans would appear to have a high
298 probability of becoming locally extinct.
299 Even though multivoltinism has allowed *A. obtectus* to colonize granaries more easily
300 than *A. obvelatus*, this trait is not the only one required for a bruchid to become a pest species.
301 Indeed, both biological traits and historical accident can moderate the ability of a multivoltine
302 bruchid species to develop in seed stocks. It is true that multivoltinism has allowed *A.*
303 *obtectus* to become worldwide. But it has not resulted in *A. obtectus* becoming the most
304 frequent species in Mesoamerica. Furthermore, the adaptation of *A. obvelatus* to altitude (*i. e.*
305 its obligatory reproductive diapause, probably associated with a slower general metabolism)
306 allows it to colonize bean populations where *A. obtectus* could not develop, independently of
307 their nature (domesticated vs. wild), and to be the only present pest species on domesticated
308 beans at such altitudes. The combination of biological and historical factors will thus allow
309 these two sibling species to coexist in sympatry, despite their ecological simlarity.
310

311 ## Acknowledgements

312 The authors wish to thank Doyle McKey for critical reading of this manuscript, and
313 Constantino Macias, Roxana Torres and Hugh Drummond for their helpful assistance. We
314 also wish to thank all the staff from the Laboratorio de Conducta Animal (Instituto de
315 Ecologia, UNAM, Mexico) for providing logistic help in Mexico. This work was financially
316 supported by the Swiss National Science Foundation (project N°3100.064821.01). Collecting
317 and exporting licenses for all studied individuals were obtained at the SEMARNAT (Mexico).
318
319

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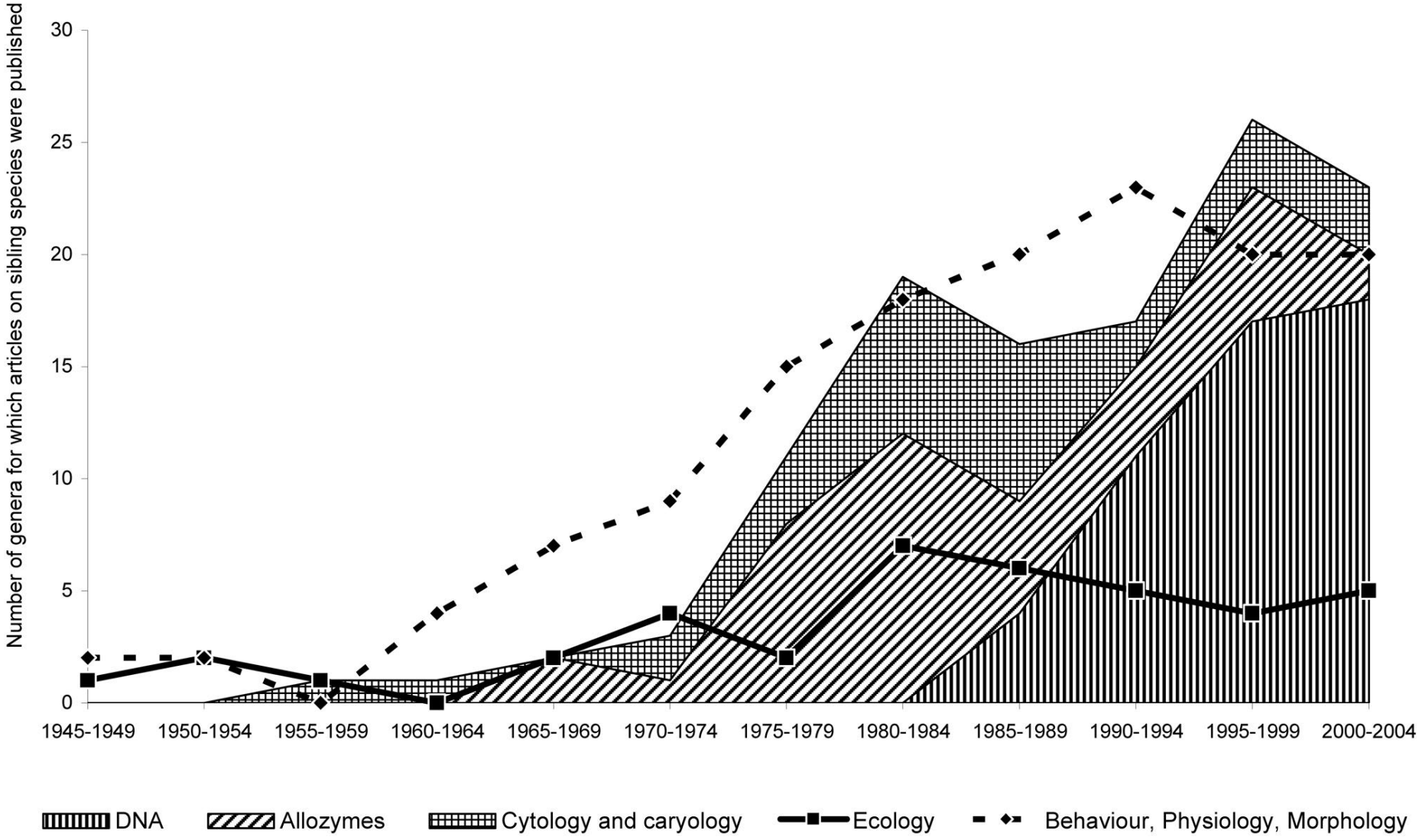
Figure 1. Studies treating different aspects (ecology; DNA; cytology and caryology; allozymes; behaviour, physiology and morphology) of sibling species, based on 298 publications on sibling species of invertebrates from 1945 to 2004 (source: Web of Science). The number of genera studied has been calculated for each period of time. Eighty-eight publications considered the genus *Drosophila* whereas 70 treated *Anopheles*.

Figure 2. Proportions of *A. obtectus* and *A. obvelatus* on *Phaseolus vulgaris* and *P. coccineus*, in six mixed populations where both bruchids were present. A "⋆" indicates a population of bruchids issued from domesticated beans. N is the number of emerging insects per population.

Figure 3. Surface of the probability of a sampled beetle being *A. obvelatus*, as a function of altitude (from 1500m to 2700m) and the date of emergence (from 15 Dec to 15 Apr), for *Acanthoscelides obtectus* and *A. obvelatus* populations on (a) wild beans and (b) domesticated beans.

Figure 4. Frequencies of *A. obvelatus* in years 2002 and 2003, for 16 populations of *Acanthoscelides obtectus* and *A. obvelatus*, in wild (11 populations) and domesticated beans (5 populations). A "⋆" indicates a population of bruchids issued from domesticated beans. N is the number of emerging insects per population.



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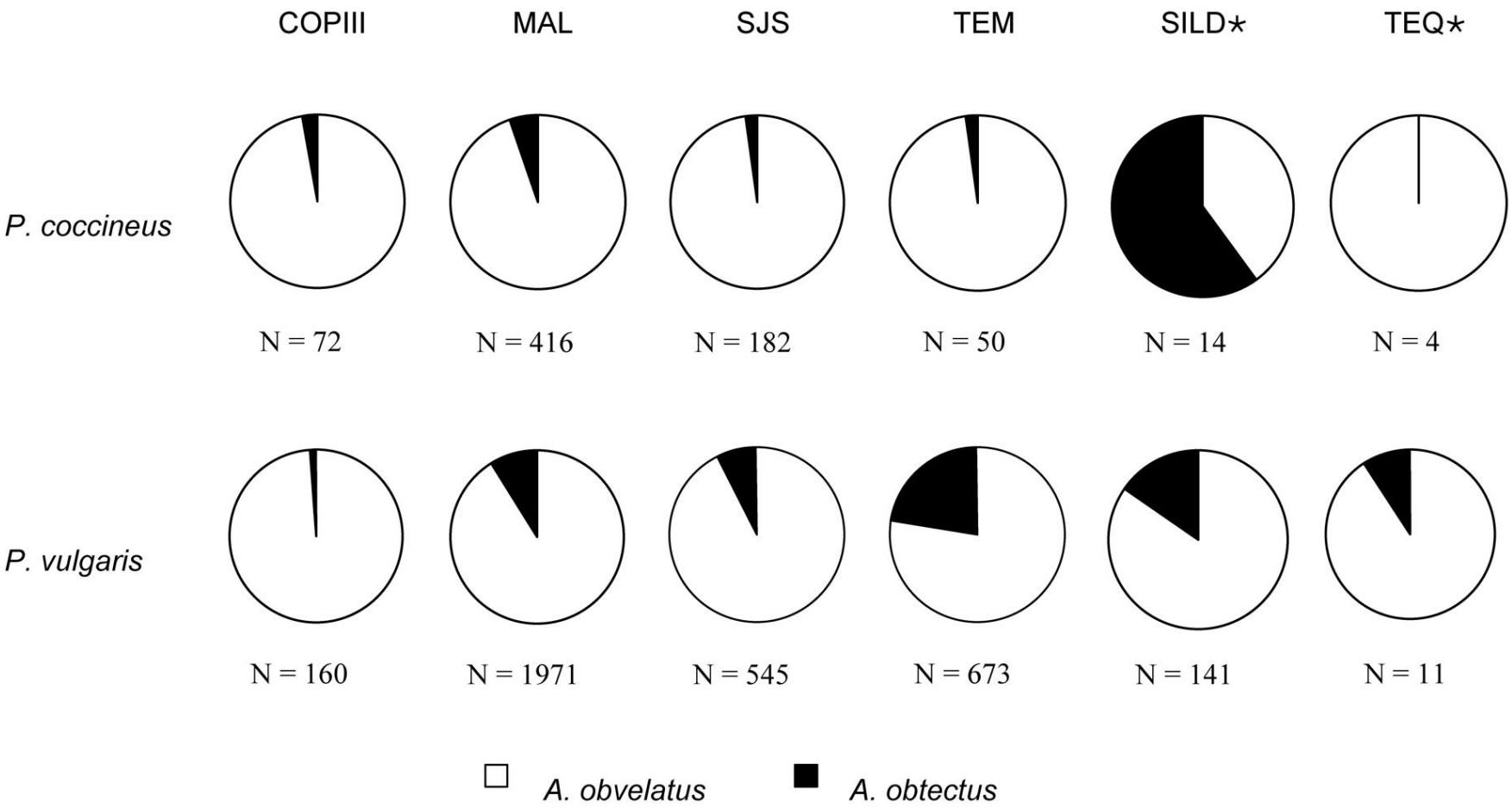
506 Figure 1



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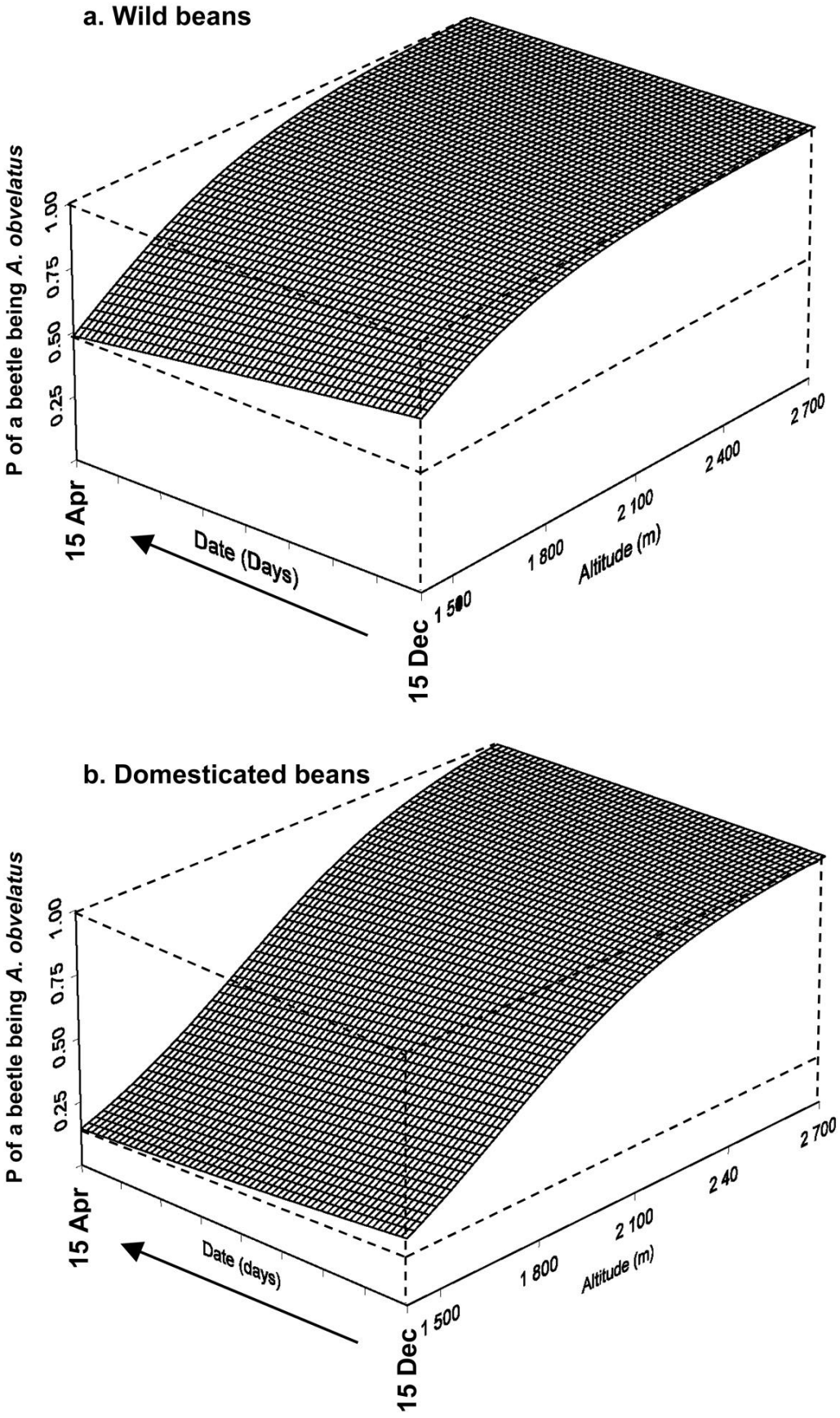
508 Figure 2



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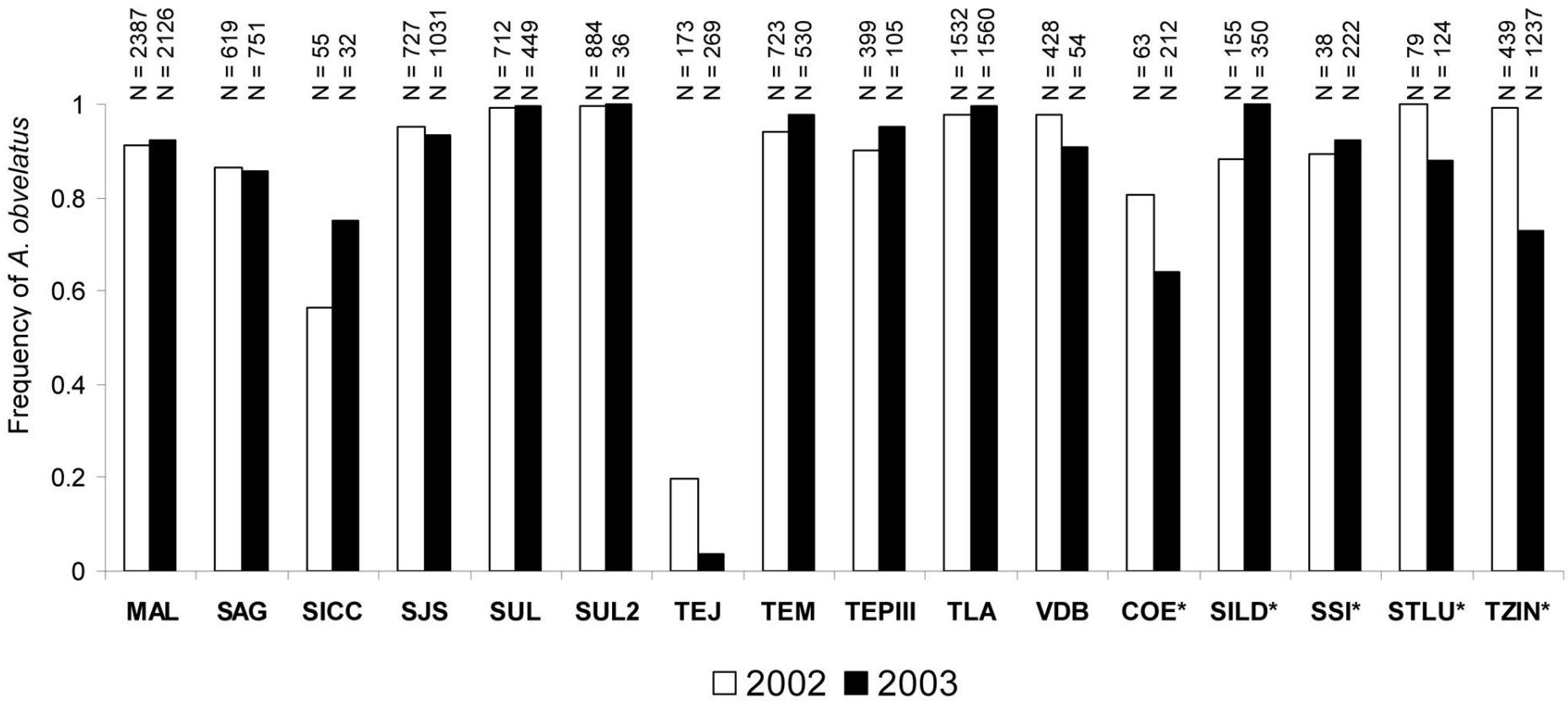
510 Figure 3



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512 Figure 4

Annex. List of sampled sites. A "✶" besides the code indicates a population of bruchids issued from domesticated beans. The host plant species is referred as "v" for *P. vulgaris* and as "c" for *P. coccineus*.

Name	code	Host-plant species	longitude (°W)	latitude (°N)	Altitude (m)
Arocutin	ARO✶	v	101°41'37.7''	19°33'22.5''	2060
Coajomulco	COA	c	99°12'00.3''	19°02'05.3''	2664
Coatepec Harinas	COAT✶	v	99°46'00.1''	18°52.59.9''	2100
Coeneo	COE✶	v	101°34'59.3''	19°49'13.9''	2100
Coinzio	COI✶	v	101°16'28.2''	19°38'25.0''	1905
Copandaro - campo	COP✶	v	101°46'27.3''	19°26'36.2''	2120
Copandaro I	COPI	v	101°45'35.5''	19°26'24.1''	2087
Copandaro III	COPIII	v, c	101°46'11.8''	19°26'26.1''	2117
Erongaricuaro	ERO	v	101°42'32.8''	19°35'56.3''	2072
Huitzilac	HUI	c	99°16'23.3''	19°01'24.0''	2544
Malinalco	MAL	v, c	99°30'08.9''	18°57'13.2''	1935
Napizarro	NAP✶	v	101°41'33.6''	19°35'50.5''	2060
Ocumicho	OCU✶	v	102°13'11.8''	19°47'46.1''	2045
San Andres de los Gabeles	SAG	v	99°57'01.5''	19°02'19.5''	2280
San Antonio	SAT✶	v	101°17'33.1''	19°38'49.9''	1930
San Bartolo	SBO	c	100°03'20.8''	19°14'31.7''	2320
San Bartolo - campo	SBO✶	v	100°03'34.8''	19°14'29.8''	2310
San Francisco Periban	SFP	v	102°24'29.0''	19°32'31.4''	1620
San Francisco Periban - campo I	SFPI✶	v	102°24'27.5''	19°32'33.5''	1850
San Francisco Periban - campo II	SFPII✶	v	102°24'28.4''	19°32'32.4''	1800
San Gabriel	SGA✶	v	100°07'28.1''	19°15'35.8''	2292
San Isidro cerca Coeneo	SICC	v	101°34'23.9''	19°50'56.6''	2040
San Ildefonso	SILD✶	v, c	100°08'56.9''	19°22'19.8''	2400
San Jose de los Laureles	SJS	v, c	99°00'05.0''	18°58'49.7''	1855
San Jose de los Laureles - campo I	SJS✶	v	98°59'35.0''	18°58'48.1''	1800
San Jose de los Laureles - campo II	SJSCY✶	v	98°59'10.4''	18°58'40.3''	1730
San Lorenzo	SLO✶	v	102°06'43.4''	19°31'33.3''	2125
San Pedro de Tejalpa	SPTJ✶	v	99°36'00.0''	18°52'59.9''	1750
San Simon	SSI✶	v	100°00'25.8''	19°01'27.4''	2135
Santa Lucia	STLU✶	v	100°00'03.7''	18°52'12.5''	1790
Santa Maria	STM✶	v	99°33'42.7''	18°49'03.6''	2000
Sultepec I	SUL	v	99°59'20.9''	18°51'07.8''	2164
Sultepec II	SUL2	v	99°58'04.1''	18°50'44.0''	2200
Tejupilco	TEJ	v	100°09'00.1''	18°55'51.2''	1400
Tejupilco - campo	TEJ✶	v	100°09'00.3''	18°55'50.0''	1400
Temascaltepec	TEM	v, c	100°02'44.2''	19°02'35.9''	1734
Tenango	TEN	c	99°36'07.1''	19°06'38.7''	2768
Tepoztlan I	TEPI	v	99°07'15.7''	18°59'36.3''	1931
Tepoztlan III	TEPIII	v	99°07'19.2''	18°58'14.9''	1700
Tequesquipan	TEQ✶	v, c	99°56'33.1''	19°03'09.2''	2300
Tlalpan	TLA	c	99°12'04.3''	19°17'50.3''	2403
Tlayecapan	TLAY	v	99°03'24.4''	18°57'20.0''	1750
Tzintzuntzan	TZIN✶	v	101°34'41.5''	19°37'43.9''	1980
Valle de Bravo	VDB	v	100°07'05.1''	19°13'56.8''	1918

MANUSCRIT VI

Role of host-plant on the genetic structure of phytophagous insects: the absence of evidence for the two bean bruchids *Acanthoscelides obtectus* and *A. obvelatus*.

ALVAREZ, N., RESTOUX, G., BORN, C., BENREY, B., ET HOSSAERT-MCKEY, M.

(en préparation)

ROLE OF HOST-PLANT ON THE GENETIC STRUCTURE OF PHYTOPHAGOUS INSECTS : THE ABSENCE OF EVIDENCE FOR THE TWO BEAN BRUCHIDS *ACANTHOSCELIDES OBTECTUS* AND *A. OBVELATUS*

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Short title: Genetic structure of bean bruchids

Abstract

Genetic differentiation is a consequence of restriction in gene flow between populations, due to barriers to dispersal, or to selection against individuals resulting from inter-population matings. In phytophagous insects, local adaptation to different kinds of host plants can sometimes lead to genetic structuring, or even to speciation. *Acanthoscelides obtectus* Say and *A. obvelatus* Bridwell are two bean bruchids specialized on beans of the *Phaseolus vulgaris* group, attacking both wild and domesticated forms of *P. vulgaris* and *P. coccineus*. In this study, we show that genetic structure in populations of these two bruchids is explained mainly by their geographical position, and that neither species appears to show any local adaptation to one or the other plant species, or to a particular kind (wild or domesticated) of bean. The absence of evidence of genetic structure in function of the host plants contrasts with results obtained for some parasitoid species that attack both bruchids which demonstrate differentiation in association with the species of host plants.

Keywords: phytophagous insects, bruchid beetles, *Acanthoscelides*, genetic structure,

Introduction

Genetic differentiation takes place when gene flow between populations is restricted, due to geographic barriers to dispersal, or to counter-selection of individuals resulting from inter-population matings. In the last decade, numerous studies have examined restricted gene flow in phytophagous insects in natural populations on wild plants, as well as for pest species attacking wild (e. g., Costa and Ross 1994, Keyghobadi *et al.* 1999, Peterson *et al.* 2001, Brouat *et al.* 2003) or domesticated plants, (e. g., Sembene and Delobel 1998), Kerdelhué *et al.* 2002, Cognato *et al.* 2003, Nahrung and Allen 2003). However, few studies have compared the genetic structure of phytophagous insects attacking both domesticated and wild populations of host-plants. Plant domestication has led to important modifications, in many plant traits, including morphology as well as the nature and quantity of both nutrients and defensive chemical compounds (e. g., Smartt [1988] in beans, Benrey *et al.* [1998] in *Brassica* and *Phaseolus*). Divergence of domesticated populations from their wild ancestors could drive local adaptation in populations of associated phytophagous insects, with some populations being preferentially adapted to these new characteristics of domesticated host plants (Mopper 1996).

Bruchid beetles on beans are an appropriate model to test local adaptation on wild and domesticated host plants since they are usually oligophagous or monophagous, demonstrating a very tight relation with seeds of their hosts. They should therefore be very sensitive to modifications in secondary compounds and in the morphology of seeds, modifications that are frequent in the domestication of grain legumes (Smartt 1988). The genus *Acanthoscelides* is one of the largest bruchid genera comprising about 300 species, all neotropical (Alvarez *et al.* submitted#1). Among them, two non-hybridizing sibling species, *A. obtectus* Say (multivoltine and distributed worldwide) and *A. obvelatus* Bridwell (univoltine and restricted to Mesoamerica) are specialized on beans of the *Phaseolus vulgaris* L. group (Alvarez *et al.* 2004a). These two

species demonstrate differences in their ecological niches, particularly with respect to elevation. *Acanthoscelides obtectus* is adapted to lowlands and *A. obvelatus* is found at higher elevations on the Mexican Altiplano (Alvarez *et al.* submitted#2). However, altitudinal ranges overlap, and the two species sometimes occur in the same habitats at intermediate elevations where both species can be found emerging from the same pod, or even from the same seed. Larvae of both species develop on both wild and domesticated forms of the two *Phaseolus* species most frequently encountered at such intermediate elevations, *P. vulgaris* and *P. coccineus*. These two bean species also demonstrate niche differentiation with respect to elevation, *P. vulgaris* being most adapted to low elevations, whereas *P. coccineus* occurs most commonly at higher elevations (Delgado-Salinas 1988). Nevertheless, at the intermediate elevations where the two species co-occur, seeds of both wild and domesticated forms of both species are attacked by both *Acanthoscelides* species without distinction. Comparative studies on chemical compounds of bean species have shown numerous differences between *P. vulgaris* and *P. coccineus*, especially concerning the concentration of cyanogenic compounds and of toxic proteins (Calderon *et al.* 1992). We hypothesized that differences in chemical compounds between populations of host plants, either as a function of the species or of the kind (wild vs. domesticated) of beans, could exert a sufficiently strong selection pressure to produce structuring in bruchid populations.

We used microsatellite markers, to examine whether the identity of host plant species or the kind (wild vs. domesticated) of beans is related to genetic structuring among populations of *A. obtectus* and *A. obvelatus.* Gonzalez-Rodriguez *et al.* (2000) examined genetic structure of populations of *A. obtectus* using allozyme markers, and found no structuring, either as a function of the host plant identity or a function of the kind of beans (wild vs. domesticated). They also tried to assess the genetic structure of *A. obvelatus*, but were hampered by the lack of variability.

We return to these questions with much more variable microsatellite markers whose high mutation rate could provide more information on the evolution of recent local adaptation.

Material and methods

Sampling of A. obtectus

One hundred and sixty-seven *A. obtectus* individuals were collected from 14 populations in Mexico, Peru and Europe (see Fig. 1b and Table 1), between December 2001 and March 2003. Although only *P. vulgaris* is grown in most places in Europe, we included several populations to compare host-plant structuring and geographic structuring. All Peruvian individuals (proceeding from one population [MAM*]) and all European individuals (proceeding from four populations [PRA*, BEL*, CHA*, NEU*]) fed on seeds of domesticated *P. vulgaris*, whereas in Mexico, four populations fed on domesticated *P. vulgaris* (COE*, SPT*, STM*, TEJ*), two on wild *P. vulgaris* (SAG, TEP), one on wild *P. coccineus* (TLA), and one on mixed bean populations with both *P. vulgaris* and *P. coccineus* (MAL). Again, we considered in a first approach that the mixed bean population harbored two distinct populations of *A. obtectus*. Since *P. coccineus* grows at relatively high altitudes, we were able to find only two sites – all on wild individuals – were it was fed on by *A. obtectus*.

Sampling of A. obvelatus

Five hundred and eighty-one individuals proceeding were collected from 21 *A. obvelatus* populations between December 2001 and March 2002 in the Mexican Altiplano, in the states of Michoacan, Morelos and Mexico, within a range of 500 km. Among these populations, 13 were sampled on wild beans (three on *P. coccineus* [SBO, HUI, TLA], six on *P. vulgaris* [ERO, SICC, VDB, SAG, SUL, TEPI], and 4 on mixed wild bean populations with both *P. vulgaris* and *P.*

coccineus [COPIII, MAL, SJS, TEM], whereas seven were collected from domesticated beans (all on *P. vulgaris* [COP*, SLO*, TZIN*, STLU*, SJS*, SFPII*, NAP*]) (see Fig. 1a and Table 1). In every population from mixed bean populations, sampled individuals were segregated as a function of host plant identity (*P. coccineus* [« c »] and *P. vulgaris* [« v »]). We therefore considered initially that each mixed bean population was attacked by two distinct populations of *A. obvelatus*. Because *P. coccineus* is only cultivated in remote places and at very low densities, probably to low to permit the maintenance of bruchid populations, we were unable to find any site where domesticated *P. coccineus* were attacked by bruchids.

Genetic analysis

DNA was extracted using a DNEasy Tissue Kit (Qiagen). Seven (AcobC09, AcobC10, AcobD04, AcobD06, AcobF11, AcobG09, and AcobH01) and five (AcobtC12, AcobtE01, AcobtE07, AcobtF09, and AcobtG08) microsatellite loci were amplified for *A. obvelatus* (Alvarez *et al.* 2003, Alvarez *et al.* submitted#3) and *A. obtectus* respectively (Alvarez *et al.* 2004b). Genotyping was done using an ABI PRISM 310 sequence analyzer (Applied Biosystems). Results were analyzed with Genescan 3.1.2 and Genotyper 2.5 (Applied Biosystems) softwares, and exact values of allele sizes were rounded to integers after plotting exact allele sizes and determining the mode of each allele.

Genotypic disequilibrium between loci and deviation from Hardy-Weinberg equilibrium were tested using Genepop 3.4 (Raymond and Rousset 1995). A factorial analysis of correspondence considering the frequency of each allele in the populations was conducted using Genetix 4.05 (Belkhir 2004). Values of F_{ST} between populations were calculated using Genepop 3.4, whereas F_{ST} values between groups of populations were determined using Fstat 2.9.3 (Goudet 1995). Determination of F-statistics was done considering the Infinite Alleles Model

(IAM) for microsatellite evolution since most of the microsatellite markers used in the study were composed of complex motifs (i. e., the probability of mutation from one allele to another is not a function of the relative size of alleles) (Jarne and Lagoda 1996).

Results

Genetic structure of A. obtectus *populations*

Four of the five microsatellite loci examined (AcobtC12, AcobtE07, AcobtF09, and AcobtG08) amplified in all 177 individuals, and were polymorphic. However, locus E01 did not amplify consistently in all populations, and we decided to discard it in further analysis. Genotypic disequilibrium was not significant between any pair of loci, after Bonferoni correction. As shown by a factorial correspondence analysis, populations were structured geographically (see Fig. 1a). Points representing coordinates for each individual on the 3 main dimensions were generally grouped together and close to the barycentre of the considered population (not shown on the figure).

The spatial representation of the three first dimensions of the correspondence analysis (45% of contribution) clustered European populations together, whereas the Peruvian population was isolated from all other populations, particularly on the first dimension. Although Mexican populations were mostly grouped together, they were much more dispersed. The two *A. obtectus* populations on *P. coccineus* (MALc, TLA) were well separated from populations on *P. vulgaris*. However, these two populations were not clustered together, and were on the opposite extremes along the first dimension of the factorial analysis. Moreover, these two populations were highly differentiated from each other (F_{ST} = 0.318). This estimate must be treated with caution, however, considering the very small number of insects emerging from our samples in both populations (six and eight individuals analyzed in MALc and TLA respectively). Furthermore, whereas the TLA

population is different from the other Mexican populations on all the three represented dimensions (which together explained 45.0% of the variability), MALc is only different from the other Mexican populations along the second dimension (which explained 15.6% of the variability). MALc is however differentiated from the MALv population, which originates from the same site (F_{ST} = 0.106). Populations on wild beans and those on domesticated beans were not differentiated, clustering together in the correspondence analysis, when populations on *P. coccineus* were discarded. F_{ST} between the two groups of populations was not significant (F_{ST} = 0.0015).

Genetic structure of A. obvelatus *populations*

Six of the seven examined microsatellite loci (AcobC09, AcobD04, AcobD06, AcobF11, AcobG09, and AcobH01) amplified in most of the 581 individuals, and were polymorphic. Locus AcobC10 gave much poorer results, with only a few individuals demonstrating exploitable data. We therefore decided to discard it. As in *A. obtectus,* genotypic disequilibrium was not significant between any pair of loci, after Bonferoni correction. On the spatial representation of the three first dimensions of the correspondence analysis (which explained 27% of the variability), individuals were generally grouped together and close to the barycentre of a considered population (not shown on the figure). Although we only analyzed Mexican populations, the correspondence analysis showed a trend to geographic differentiation, with the westernmost populations sampled (Estado de Michoacan) being separated from the populations sampled in the center (Estado de Mexico) and the south (Estado de Morelos) of the study area. F_{ST} between western populations and the others was significant (F_{ST} = 0.107). However, two populations on domesticated beans (NAP*, SFP*) sampled from the west part of the study area were grouped with the populations from the center-south. There was no genetic structure either as

a function of the kind of beans (wild vs. domesticated), or as a function of the host-plant species identity. Moreover, "subpopulations" collected on *P. vulgaris* and on *P. coccineus* in the same site, were mostly clustered together on the correspondence analysis, and F_{ST} between these "subpopulations" were particularly low (F_{ST} [COPc-COPv] = 0.0124; F_{ST} (MALc-MALv) = 0.0103; F_{ST} [SJSc-SJSv] = 0.0183; F_{ST} [TEMc-TEMv] = 0.008).

Discussion

Genetic structure of A. obtectus *populations*

Acanthoscelides obtectus populations demonstrated geographic structuring. Mexican populations were clearly separated from European populations, and the Peruvian population was isolated from all other populations. Whereas all European populations are closely related, the case of Mexican populations is more complicated. Mexican populations on *P. coccineus* demonstrate a particular pattern, being genetically distant from the Mexican populations on *P. vulgaris*. However, based on our results, the two populations on *P. coccineus* (MALc and TLA) are also very distant one from the other. This pattern hardly suggests a speciation process, with individuals either adapted to *P. vulgaris* or to *P. coccineus*. In fact, the marginal position of the two populations on *P. coccineus* seems to be the result of short-term processes of counter-selection on individuals feeding on this species, undergoing a very strong effect of drift. Furthermore, whereas MALc is distinct from the other Mexican populations along one dimension of the correspondence analysis, TLA is much more different, being completely opposed to all the other Mexican populations on the first dimension, and being far distant as well on dimensions 2 and 3. This could be due to the particular position of the TLA individuals, which were sampled in a public park in Mexico City, where human-mediated migrations of bean seeds from the whole country may lead to the constitution of a very heterogeneous population, and make the TLA

population more genetically distant to all the other populations. However, urban fragmentation may have isolated this population more than any other, and drift should also be an important factor there. Nevertheless, we cannot completely eliminate the hypothesis of a local adaptation at a small geographic scale in the case of these two populations on *P. coccineus*, particularly in the case of MALc, which is highly differentiated from MALv, despite the fact that the two populations were sampled in the same site (Malinalco). However, this hypothesis on local adaptation of *A. obtectus* seems unlikely, since *A. obtectus* populations are clearly not structured as a function of the kind of bean (wild vs. domesticated) even if wild and domesticated forms of beans are morphologically and chemically different. We prefer therefore the hypothesis of a strong counter-selection against individuals feeding on *P. coccineus*, leading to a strong effect of drift and the survival of only a few lineages (i. e., our sampling probably involved closely related individuals). In the case of the MALc population, individuals sampled may not be related with those sampled in the MALv population, due to a strong and very short-term effect of drift, amplified by the fact that MALv and MALc were separated by a short distance (ca. 250m), because *P. coccineus* and *P. vulgaris* did not grow in the same areas in the Malinalco site. Interestingly, this result contrasts with a previous study which defined the niche of *A. obtectus* as a function of elevation, but not as a function of the identity of the host-plant species (Alvarez *et al.* submitted#2). We suggest that *A. obtectus* populations on *P. coccineus* act as sinks, and that there should be a tendency for *A. obtectus* to specialize on *P. vulgaris*.

Genetic structure of A. obvelatus populations

Similarly to the pattern observed in *A. obtectus*, the genetic structure of *A. obvelatus* populations was primarily structured geographically. Two groups – the first comprising most of the populations proceeding from the western part of the sampling area, and the second including all

populations sampled in the central and southern parts – are clearly distinguished on the basis of the factorial correspondence analysis. Exceptions to this pattern are two populations (NAP*, SFP*) sampled in the western group, but which are genetically closer to the other group of populations. This could be explained by the fact that these two populations proceed from domesticated beans. Individuals from these populations are likely to migrate over much longer distances, through human-mediated dispersion of bean seeds, than are individuals from population associated with wild beans (see Alvarez *et al.* submitted#3).

Comparison between genetic structure of populations at other levels

Our analysis showed that populations of both *A. obtectus* and *A. obvelatus* are geographically structured. However, there was little support from our analysis for the hypothesis of local adaptation on particular species or kind (wild vs. domesticated) of beans. This result contrasts with the considerable differentiation of the host-plant populations. Indeed, populations of *P. vulgaris* and *P. coccineus* are not known to be able to hybridize, and gene flow between wild and domesticated populations of *P. vulgaris* is limited (Gepts 1999). Variation in the bruchids seems to be primarily structured by geographic distance, with the possible exception of *A. obtectus* on *P. coccineus*. This pattern also contrasts with the pattern obtained in one species of parasitoïd associated with these bruchids, the structure of which reflects differentiation among the different bean species (Aebi *et al.* in prep).

Acknowledgements

We are very grateful to Doyle McKey for discussion and for improving our English. We also thank Alfonso Delgado-Salinas for providing information on the location of populations in Mexico and for providing Peruvian specimens. Finally, we thank Alex Aebi, Jorge Contreras, and

the teams of the Instituto de Ecologia (UNAM) in Mexico City for discussion and logistic help. This work was financially supported by the Swiss National Science Foundation (project N°3100.064821.01) and the Centre d'Ecologie Fonctionnelle et Evolutive (CNRS).

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Table 1. List of sampled sites for the two species *A. obtectus* and *A. obvelatus*. The kind of bean is referred to as “d” for domesticated and “w” for wild, whereas the bean species is referred to as “v” for *P. vulgaris* and “c” for *P. coccineus.*

Figure 1. Correspondence analysis based on allelic frequencies in individuals and populations. Each population is referred to by a specific code (see Table 1). Underlined population codes refer to populations on *P. coccineus*, whereas non-underlined population codes refer to those on *P. vulgaris*. Populations issued from domesticated beans are noted with a “∗” besides the code. Subpopulations collected from *P. vulgaris* and *P. coccineus* from the same site are linked by a dotted line. **a.** Correspondence analysis for *A. obtectus* populations: the green circle surrounds populations from Mexico, the red circle surrounds European populations, and the blue circle represents the Peruvian population. **b.** Correspondence analysis for *A. obvelatus* populations. The red circle surrounds populations from the western part (Estado de Michoacan) of the sampling area, whereas the green circle includes the populations both from the central (Estado de Mexico) and southern parts (Estado de Morelos) of the sampling area.

Table 1.

Species	Abbrev.	Site name	Bean species	Kind of bean population ([d]=domestic.; [w]=wild)	Longitude	Latitude	Altitude (m)
A. obtectus	COE	Coeneo, MEXICO	*P. vulgaris*	d	101°34’59.3’’ W	19°49’13.9’’ N	2100
A. obtectus	MALc	Malinalco, MEXICO	*P. coccineus*	w	99°30’08.9’’ W	18°57’13.2’’ N	1935
A. obtectus	MALv	Malinalco, MEXICO	*P. vulgaris*	w	99°30’08.9’’ W	18°57’13.2’’ N	1935
A. obtectus	SAG	San Andres de los Gabeles, MEXICO	*P. vulgaris*	w	99°57’01.5’’ W	19°02’19.5’’ N	2280
A. obtectus	SFP	San Francisco Periban, MEXICO	*P. vulgaris*	d	102°24’28.4’’ W	19°32’32.4’’ N	1800
A. obtectus	STM	Santa Maria, MEXICO	*P. vulgaris*	d	99°33’42.7’’ W	18°49’03.6’’ N	2000
A. obtectus	TEJ	Tejupilco, MEXICO	*P. vulgaris*	d	100°09’00.1’’ W	18°55’51.2’’ N	1400
A. obtectus	TEP	Tepoztlan, MEXICO	*P. vulgaris*	w	99°07’15.7’’ W	18°59’36.3’’ N	1931
A. obtectus	TLA	Tlalpan, MEXICO	*P. coccineus*	w	99°12’04.3’’ W	19°17’50.3’’ N	2403
A. obtectus	MAM	Mamorimamba, PERU	*P. vulgaris*	d	78°48’33.6’’ W	6°30’42.2’’ S	1900
A. obtectus	BEL	Bellevue, FRANCE	*P. vulgaris*	d	Région du Poitou, FRANCE		
A. obtectus	CHA	Chail, FRANCE	*P. vulgaris*	d	Région du Poitou, FRANCE		
A. obtectus	NEU	Chambrelien, SWITZERLAND	*P. vulgaris*	d	Canton de Neuchâtel, SWITZERLAND		
A. obtectus	PRA	Prado, SPAIN	*P. vulgaris*	d	Provincia de León, SPAIN		
A. obvelatus	COP*	Copandaro - campo, MEXICO	*P. vulgaris*	d	101°46’27.3’’	19°26’36.2’’	2120
A. obvelatus	COPc	Copandaro, MEXICO	*P. coccineus*	w	101°46’11.8’’	19°26’26.1’’	2117
A. obvelatus	COPv	Copandaro, MEXICO	*P. vulgaris*	w	101°46’11.8’’	19°26’26.1’’	2117
A. obvelatus	ERO	Erongaricuaro, MEXICO	*P. vulgaris*	w	101°42’32.8’’	19°35’56.3’’	2072
A. obvelatus	HUI	Huitzilac, MEXICO	*P. coccineus*	w	99°16’23.3’’	19°01’24.0’’	2544

Table 1 (continued)

Species	Abbrev.	Site name	Bean species	Kind of bean population ([d]=domestic.; [w]=wild)	Longitude	Latitude	Altitude (m)
A. obvelatus	MALc	Malinalco, MEXICO	*P. coccineus*	w	99°30’08.9’’	18°57’13.2’’	1935
A. obvelatus	MALv	Malinalco, MEXICO	*P. vulgaris*	w	99°30’08.9’’	18°57’13.2’’	1935
A. obvelatus	NAP*	Napizarro, MEXICO	*P. vulgaris*	d	101°41’33.6’’	19°35’50.5’’	2060
A. obvelatus	SAG	San Andres de los Gabeles, MEXICO	*P. vulgaris*	w	99°57’01.5’’	19°02’19.5’’	2280
A. obvelatus	SBO	San Bartolo, MEXICO	*P. vulgaris*	w	100°03’20.8’’	19°14’31.7’’	2320
A. obvelatus	SFP*	San Francisco Periban - campo, MEXICO	*P. vulgaris*	d	102°24’28.4’’	19°32’32.4’’	1800
A. obvelatus	SFP	San Francisco Periban, MEXICO	*P. vulgaris*	w	102°24’28.4’’	19°32’32.4’’	1800
A. obvelatus	SIC	San Isidro cerca Coeneo, MEXICO	*P. vulgaris*	w	101°34’23.9’’	19°50’56.6’’	2040
A. obvelatus	SJS*	San Jose de los Laureles - campo, MEXICO	*P. vulgaris*	d	98°59’35.0’’	18°58’48.1’’	1800
A. obvelatus	SJSc	San Jose de los Laureles, MEXICO	*P. coccineus*	w	99°00’05.0’’	18°58’49.7’’	1855
A. obvelatus	SJSv	San Jose de Los Laureles, MEXICO	*P. vulgaris*	W	99°00’05.0’’	18°58’49.7’’	1855
A. obvelatus	SLO*	San Lorenzo, MEXICO	*P. vulgaris*	d	102°06’43.4’’	19°31’33.3’’	2125
A. obvelatus	STL*	Santa Lucia, MEXICO	*P. vulgaris*	d	100°00’03.7’’	18°52’12.5’’	1790
A. obvelatus	SUL	Sultepec, MEXICO	*P. vulgaris*	w	99°59’20.9’’	18°51’07.8’’	2164
A. obvelatus	TEMc	Temascaltepec, MEXICO	*P. coccineus*	w	100°02’44.2’’	19°02’35.9’’	1734
A. obvelatus	TEMv	Temascaltepec, MEXICO	*P. vulgaris*	w	100°02’44.2’’	19°02’35.9’’	1734
A. obvelatus	TEP	Tepoztlan, MEXICO	*P. vulgaris*	w	99°07’15.7’’	18°59’36.3’’	1931
A. obvelatus	TLA	Tlalpan, MEXICO	*P. coccineus*	w	99°12’04.3’’	19°17’50.3’’	2403
A. obvelatus	TZI*	Tzintzuntzan, MEXICO	*P. vulgaris*	d	101°34’41.5’’	19°37’43.9’’	1980
A. obvelatus	VDB	Valle de Bravo, MEXICO	*P. vulgaris*	w	100°07’05.1’’	19°13’56.8’’	1918

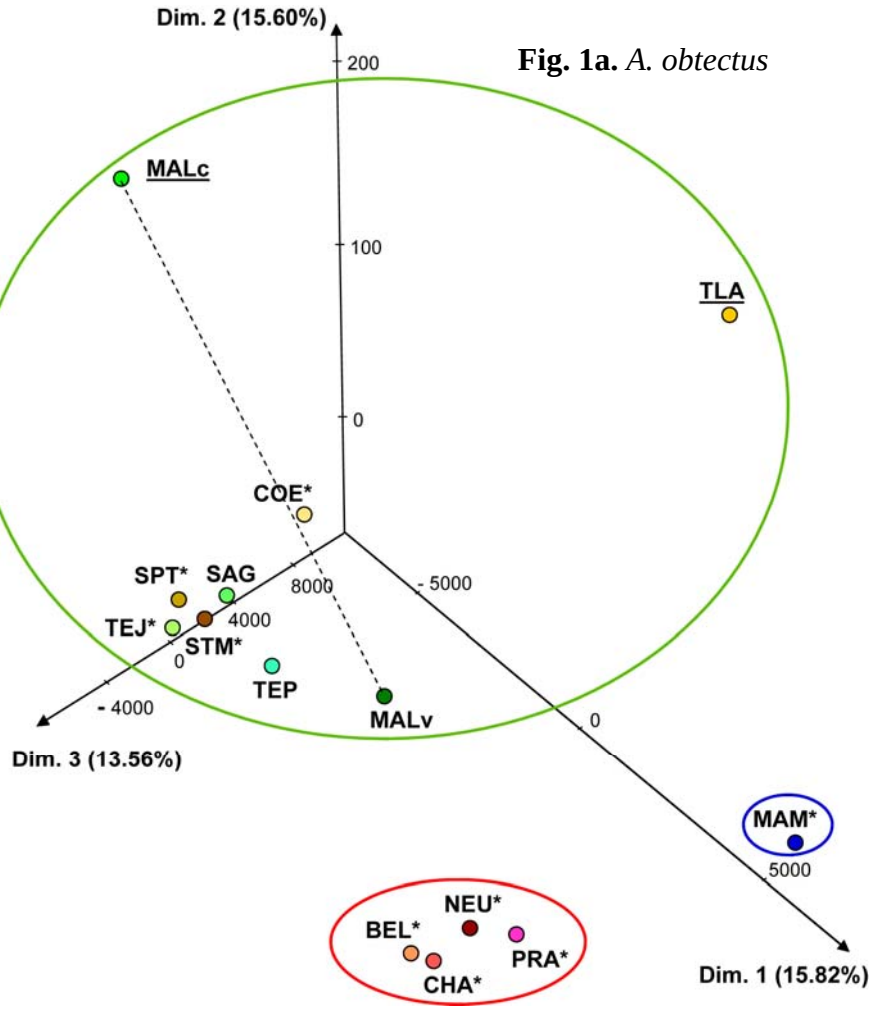

Fig. 1a. *A. obtectus*

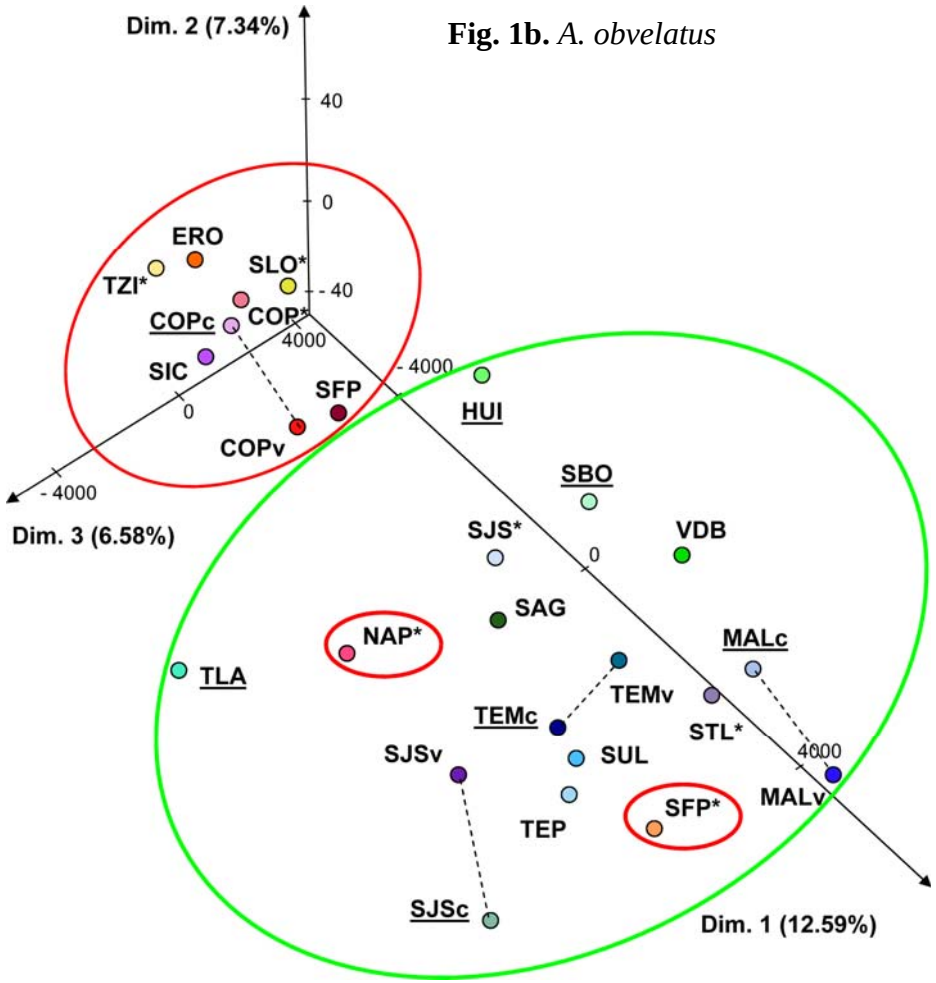

Fig. 1b. *A. obvelatus*

Figure 1

MANUSCRIT VII

Ancient and recent evolutionary history of the bruchid beetle *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans.

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Molecular Ecology (**2005**) **14**, 1015–1024

Molecular Ecology (2005) **14**, 1015–1024 doi: 10.1111/j.1365-294X.2005.02470.x

Ancient and recent evolutionary history of the bruchid beetle, *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans

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Abstract

Acanthoscelides obtectus* Say is a bruchid species of Neotropical origin, and is specialized on beans of the *Phaseolus vulgaris* L. group. Since the domestication and diffusion of beans, *A. obtectus* has become cosmopolitan through human-mediated migrations and is now a major pest in bean granaries. Using phylogeographic methods applied to mitochondrial DNA (mtDNA) and nuclear microsatellite molecular markers, we show that the origin of this species is probably further south than Mesoamerica, as commonly thought. Our results also indicate that *A. obtectus* and its Mesoamerican sister species *Acanthoscelides obvelatus*, two morphologically close species differing principally in voltinism, speciated in allopatry: *A. obtectus* (multivoltine) arising in Andean America and *A. obvelatus* (univoltine) in Mesoamerica. In contrast to Mesoamerica where beans fruit once yearly, wild beans in Andean America fruit year-round, especially in regions showing little or no seasonality. In such habitats where resources are continuously present, multivoltinism is adaptive. According to existing hypotheses, multivoltinism in *A. obtectus* is a new adaptation that evolved after bean domestication. Our data suggest the alternative hypothesis that multivoltinism is an older trait, adapted to exploit the year-round fruiting of wild beans in relatively aseasonal habitats, and allowed *A. obtectus* to become a pest in bean granaries. This trait also permitted this species to disperse through human-mediated migrations associated with diffusion of domesticated beans. We also show that diversity of Old World *A. obtectus* populations can be quite well explained by a single colonization event about 500 BP. Human-mediated migrations appear not to be rare, as our results indicate a second more recent migration event from Andean America to Mexico.

Keywords: *Acanthoscelides obtectus*, Coleoptera, human-mediated migrations, pest species, *Phaseolus*, phylogeography

Received 11 August 2004; revision received 19 November 2004; accepted 10 December 2004

Introduction

Bruchid beetles (Bruchidae, Coleoptera) are seed-eating insects, most species of which feed on legumes. Among the more than 1500 bruchid species described assigned to 58 different genera (Borowiec 1987), a substantial percentage are specialized on economically important plant species. Most of these bruchid species now have worldwide distributions, as a result of movement in stocks of seeds, which have undergone very long distance migrations through human exchanges at the intra- and intercontinental levels.

Acanthoscelides obtectus Say is a bruchid species of Neotropical origin, specialized on beans of the *Phaseolus vulgaris* L. group (see Delgado-Salinas *et al*. 1999). Understanding the native and introduced range of this species and the phylogeographic relationships among populations is of scientific interest in itself, particularly for the study of the consequences of past and present human actions — involving for example migration patterns — on

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the current structure and biology of populations. The role of human-mediated migrations is of particular importance in understanding the biogeographical history of *A. obtectus*, as its dispersal capacity has been artificially increased since the domestication of *Phaseolus* beans about 7000 BP (Kaplan 1971).

Understanding phylogeographic patterns in the species is also of particular agronomic concern, because knowledge of the native range of a species may give new data on the climatic conditions in which the species originally evolved, and can also otherwise guide the search for agents of biological control. *A. obtectus* is now cosmopolitan, and has become a frequent pest in bean granaries. In developing countries, farmers who produce *Phaseolus* beans experience significant losses because of the high reproductive rate of *A. obtectus*, and its ability to reproduce in a broad range of ecological conditions (Labeyrie 1981), including tolerance to large amounts of several kinds of pesticides (N. Alvarez, unpublished). In the last 30 years, *A. obtectus* has been reported on new host plant species, among them several domesticated legumes such as *Cajanus indicus*, *Vigna unguiculata*, *Pisum sativum* and *Vicia faba* (Johnson 1983, 1990). This modern expansion of the geographical and host range of *A. obtectus* thus threatens production of other crops, and there is an urgent need to control bruchid populations by other than chemical means.

Most authors situate the origin of *A. obtectus* in Mesoamerica (e.g. Labeyrie 1990). Based on this assumption, entomologists and agronomists have started scientific programs in Mexico and Central America (south to northern Colombia) to find candidate parasitoid species for biological control (e.g. Schmale *et al*. 2001). However, results have so far been disappointing, and to our knowledge Mesoamerican species of parasitoids have never been able to reduce densities of *A. obtectus* in seed stocks (Schmale *et al*. 2002). The challenge to find an appropriate biological control agent originating in the Neotropical region, and initially adapted to *A. obtectus*, is thus of current interest.

The assumption that *A. obtectus* originates from Mexico is not based on strong arguments. The contemporary range of *A. obtectus* in the New World extends from Chile to the United States (Johnson 1983, 1990), and the original range of the species may have been further south, rather than in Mesoamerica as currently believed. One of the objectives of this study was thus to examine the phylogeography of *A. obtectus* in the Neotropics, to gain insights into its area of origin.

Acanthoscelides obvelatus Bridwell is the sister species of *A. obtectus*, and also feeds on beans of the *P. vulgaris* group. These two species are very similar morphologically. A few anatomical and morphological characters make their differentiation possible (Leroi *et al*. 1990; Alvarez *et al*. 2004a), but confusion between the two species can still easily arise, especially when they occur in sympatry. The most important biological difference between the two species is in their voltinism. Whereas *A. obtectus* can continue to reproduce as long as resources are available, *A. obvelatus* only produces a single generation per year, in synchrony with the fruiting of beans. This trait of *A. obvelatus* precludes long-distance human-mediated migrations like those undertaken by *A. obtectus*, and *A. obvelatus* is today still restricted to Central America and extreme northern South America [the species has been recorded only from Mexico, Guatemala and northern Colombia (Johnson 1983)]. Furthermore, *A. obvelatus* has never been recorded from other species than beans of the *P. vulgaris* group.

The important difference in voltinism has led several authors to suppose that the two species speciated sympatrically (e.g. Pichard *et al*. 1991). According to this hypothesis, *A. obvelatus* has remained adapted to the phenology of wild bean populations, whereas *A. obtectus* has evolved multivoltinism as an adaptation to reproduction in granaries. This hypothesis implies a very recent speciation, since bean domestication occurred around 7000 BP (Kaplan 1971; Kaplan & Lynch 1999). In a recent study, Alvarez *et al*. (2004a) provided strong arguments for a much more ancient separation between the two species. The extent of divergence in *COI* sequences of *A. obtectus* and *A. obvelatus* (16.7%) indicates that the separation between these two species is at least 20 Ma. In this study, we will provide new molecular data that give insights on their origin. This information suggests a new scenario, which points to a decisive role played by human-mediated migrations. An implication of this scenario is that entomologists and agronomists should extend the search for candidate species for use in biological control to South America.

Materials and methods

Specimens of *Acanthoscelides obtectus* were collected between December 2001 and June 2003 from sites in Mexico, Peru, France, Switzerland, Spain, and Cameroon (see Table 1).

Mitochondrial DNA

To examine the phylogeographic pattern of the species, we sequenced three mitochondrial genes [*12s rRNA* (379 bp), *16s rRNA* (465 bp) and *COI* (736 bp)], from 14 sites (one individual per site, with the exception of the Peruvian site with 10 individuals): Mexico (9 sites), Peru (1 site), Cameroon (1 site), Switzerland (1 site), France (1 site), and Spain (1 site). Old World specimens were all collected in cultivated bean fields, whereas specimens from the New World originated either in cultivated bean fields (Peru; Mexico COE, SFP, XOT, YOH), or in wild bean populations (Mexico SAG, SCA, SJS, STO, TLA). Sampled sites in Mexico are situated in the Mesoamerican altiplano, which is considered to be a centre of diversity for

Table 1 List of sampled sites

Abbrev.	Site name	Kind of bean population	Longitude	Latitude	Altitude (m)
COE	Coeneo, Mexico	d	101°34′59.3″W	19°49′13.9″N	2100
MAL	Malinalco, Mexico	w	99°30′08.9″W	18°57′13.2″N	1935
SAG	San Andres de los Gabeles, Mexico	w	99°57′01.5″W	19°02′19.5″N	2280
SCA	San Calletano, Mexico	w	100°05′51.4″W	19°22′09.1″N	2611
SFP	San Francisco Periban, Mexico	d	102°24′28.4″W	19°32′32.4″N	1800
SIC	San Isidro Coeneo, Mexico	d	101°34′23.9″W	19°50′56.6″N	2040
SJS	San Jose de los Laureles, Mexico	w	99°00′05.0″W	18°58′49.7″N	1855
STM	Santa Maria, Mexico	d	99°33′42.7″W	18°49′03.6″N	2000
STO	Santo Tomas, Mexico	w	100°16′30.2″W	19°11′20.0″N	1390
TEJ	Tejupilco, Mexico	w	100°09′00.1″W	18°55′51.2″N	1400
TEP	Tepoztlan, Mexico	w	99°07′15.7″W	18°59′36.3″N	1931
TLA	Tlalpan, Mexico	w	99°12′04.3″W	19°17′50.3″N	2403
XOT	Xochitlan, Mexico	d	97°39′01′2″W	19°57′59.9″N	1450
YOH	Yohualichan, Mexico	d	97°30′55.9″W	20°00′56.0″N	1400
MAM	Mamorimamba, Peru	d	78°48′33.6″W	6°30′42.2″S	1900
YAH	Cameroon	d	Yaoundé, Province du Centre		
BEL	France	d	Bellevue, Poitou		
CHA	France	d	Chail, Poitou		
NEU	Switzerland	d	Chambrelien, Canton de Neuchâtel		
PRA	Spain	d	Prado, Provincia de León		

Kind of bean population: d, domestic; w, wild; altitude (m), meter above sea level.

beans (e.g. Delgado-Salinas *et al*. 1988). The Peruvian site was situated in Mamorimamba, in the Departamento de Cajamarca. Origins of the Old World sampled sites were as follows: Spain, Provincia de Leon; France, Région du Poitou; Switzerland, Canton de Neuchâtel; Cameroon, Province du Centre. *Acanthoscelides argillaceus* Sharp and *Acanthoscelides obvelatus* were used as outgroup species. To compare the genetic diversity of *A. obtectus* in Mexico to that of its sister species *A. obvelatus*, we sequenced the mitochondrial *COI* gene from 16 *A. obtectus* and 18 *A. obvelatus* specimens (originating from 8 and 11 sites, respectively). Primer sequences were defined according to Simon *et al*. (1994): *12Sbi* (AAGAGCGACGGGCGATGTGT) and *12Sai* (AAACTAGGATTAGATACCCTATTAT) for *12s rRNA*; modified *16Sbr* (CCGGTTTGAACTCAGATCATGT) and modified *16Sar* (CGCCTGTTTAACAAAAACAT) for *16s rRNA*; *C1-J-2183* (CAACATTTATTTTGATTTTTTGG) and modified *TL2-N-3014* (TCCATTGCACTAATCTGCCATATTA) for *COI*. Total genomic DNA was extracted using DNeasy Kit (QIAGEN). Polymerase chain reaction (PCR) amplifications were performed in a final volume of 10 μL, which contained 1 μL of extracted DNA, 1 μL of 25 mM $MgCl_2$, 0.1 μL of 10 mM dNTPs, 1 μL of PCR buffer (Eurogentec), 1 unit of *Taq* DNA polymerase (Eurogentec Red Goldstar), 0.5 μL of forward primer, and 0.5 μL of reverse primer. PCRs were performed separately for each primer pair on a PTC-200 thermocycler using the following cycling conditions: initial denaturation at 92 °C for 1 min 30 s; 30 cycles of 92 °C for 30 s, annealing temperature for 45 s, 72 °C for 1 min 30 s; and final elongation at 72 °C for 10 min. Annealing temperatures were, respectively, 55 °C, 45 °C, and 55 °C for *12s rRNA*, *16s rRNA*, and *COI*. The method of Sanger (1981) was carried out using Applied Biosystems BygDye protocol, and the sequence of 1580 nucleotides for each individual was obtained. Products of the sequencing reactions were then analysed on an ABI Prism 310 sequencer. Chromatograms were manually corrected using Chromas 2.23 (Technelysium Pty. Ltd), and sequences were aligned using CLUSTALW 1.83 (Thompson *et al*. 1994). Phylogenetic trees were reconstructed by a tree-bisection-reconnection (TBR) branch-swapping-algorithm, under a general time reversible (GTR) model, using a maximum-likelihood algorithm with the following parameters under PAUP* 4.0.10 (Swofford 2002): eight evolutionary rate categories, gamma shape parameter (alpha) estimated, no invariable sites. Bootstrap values were calculated on 100 replications. Likelihoods of constrained and nonconstrained trees were compared with a Kishino–Hasegawa (RELL bootstrap) test, using PAUP*. The molecular clock was tested by a likelihood ratio test comparing likelihoods obtained by the two PHYLIP 3.6b package programs (Felsenstein 2004), DNAML and DNAMLK (less time-consuming than under PAUP*), using the parameters previously estimated by PAUP*, and eight evolutionary rate categories (including invariant sites). Sequence diversities and distances between individual groups were calculated using gamma nucleotide

(TN93) and gamma amino acid distance models under MEGA 2.1 (Kumar *et al*. 2001).

Microsatellite markers

In order to compare diversities and historical biogeography of *A. obtectus* individuals, we amplified individuals whose mtDNA sequences had been examined earlier, with five microsatellite loci (*C12*, *E01*, *E07*, *F09*, and *G08*) defined by Alvarez *et al*. (2004b). We genotyped other samples (when available) collected in each site, plus samples proceeding from four populations geographically close to SJS (MAL, TEJ, TEP, STM) and one population close to COE (SIC). In all, we genotyped 191 individuals distributed as follows: 102 Mexican individuals [COE (11), MAL (12), SAG (6), SCA (1), SFP (1), SIC (3), SJS (10), STM (16), STO (1), TEJ (6), TEP (14), TLA (3), XOT (17), YOH (1)]; 52 Old World individuals [Cameroon (1), France (21, in two sites within 80 km), Spain (19), Switzerland (11)]; 37 Peruvian individuals, all of them proceeding from Mamorimamba. Total genomic DNA was extracted using DNeasy Kit (QIAGEN). PCR amplifications were performed according to Alvarez *et al*. (2004b). Products of the reactions were then analysed on ABI Prism 310 and 3100 sequencers. Genotypes were analysed using GENESCAN 3.1.2 and GENOTYPER 2.5 software (Applied Biosystems). Exact sizes of alleles were manually corrected to round integer sizes. Allelic richnesses for the three biogeographic groups (Mexico, Peru, and Old World) were calculated using FSTAT 2.9.3 (Goudet 1995). Pairwise genetic differentiation between the 191 genotyped individuals was calculated using GENEPOP 3.4 (Raymond & Rousset 1995) by computing estimates of a genetic parameter analogous to estimates of $F_{ST}/(1 - F_{ST})$ for populations (Rousset 2000). The dendrogram was built from the matrix of genetic distance using the NEIGHBOUR program from the PHYLIP package (Felsenstein 2004).

Results

Mitochondrial DNA

Sequencing of *12s rRNA*, *16s rRNA*, and *COI* revealed polymorphism among individuals. For *12s rRNA*, only two haplotypes were found: one for New World individuals, and the other for Old World individuals plus the Mexican individual originating from the XOT site (Accession nos AY826433–AY826457). For *16s rRNA*, individuals were distributed among four haplotypes (see table 2 Accession nos AY826458–AY826482). The first haplotype was shared by almost all Mexican individuals (except those coming from sites XOT and YOH); a second haplotype was found only in the Peruvian individuals and in one Mexican

Table 2 Distribution of the four *16s rRNA* haplotypes among the sequenced individuals, showing only polymorphic sections, and with variant sites in bold and underlined

Haplotype		10 17 |.....|		21 27 |.....|		186 192 |.....|		307 313 |.....|
1	Peru MAM 2	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 6	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 8	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 12	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 13	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 15	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 16	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 19	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 23	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Peru MAM 29	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
	Mexico YOH	TAT**C**CCCA	*	TCT**A**CAC	*	TTCAATT	*	AATTTTA
2	Mexico SAG 5	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico TLA 1	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico SCA	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico SFP	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico STO	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico COE 2	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico SJS 2	TATTCCCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
3	Spain PRA 1	TATT**T**CCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
	Mexico XOT 3	TATT**T**CCA	*	TCTGCAC	*	TTCAATT	*	AATTTTA
4	Switzerland NEU 1	TATT**T**CCA	*	TCTGCAC	*	TTC**G**ATT	*	AAT**C**TTA
	Cameroon YAH	TATT**T**CCA	*	TCTGCAC	*	TTC**G**ATT	*	AAT**C**TTA
	France CHA 5	TATT**T**CCA	*	TCTGCAC	*	TTC**G**ATT	*	AAT**C**TTA

individual originating from YOH; the third haplotype was present in the Spanish individual and the Mexican individual originating from XOT; and the fourth haplotype was present only in the individuals from Switzerland, Cameroon, and France. For *COI*, almost each individual exhibited a unique haplotype (Accession nos AY826483–AY826507).

The tree reconstructed for *Acanthoscelides obtectus* — anchored by its two closest relatives *Acanthoscelides obvelatus* and *Acanthoscelides argillaceus* — clearly showed the basal position of a small clade comprising the 10 Peruvian individuals and one of the Mexican individuals (YOH) (see Fig. 1a). This position is shown not only after the common analysis of all nucleotides sequenced, but also by the individual analysis of each of the two most variable genes, *16s rRNA* and *COI*. This congruence strengthens the basal phylogeographic position of the Peruvian clade. A Kishino–Hasegawa test comparing the likelihood of this tree with the likelihood of a tree constrained to have the Peruvian clade in nonbasal position, found a trend in the same direction ($P = 0.10$).

From this first clade branches an unresolved cluster — with only Mexican individuals — in which is nested another clade with mostly Old World individuals [but one Mexican individual (XOT)]. The monophyly of this last clade is supported by the individual analysis of each of the three genes. Moreover, constraining the individual originating from XOT to be in the larger group of taxa leads to a tree whose likelihood is significantly lower (Kishino–Hasegawa test, $P = 0.038$). Using the same method, an analogous tendency showing a lower tree likelihood for the constrained topology was also obtained by constraining the individual originating from YOH to be in the larger group of Mexican taxa ($P = 0.09$). Topologies obtained using DNAML and DNAMLK without outgroups were identical for all major clades, and values for ln (likelihood) were, respectively, –2342.37865 and –2325.38982. The likelihood ratio test is passed and the molecular clock cannot be rejected ($P = 0.0655$).

COI haplotype diversities calculated with a nucleotide gamma distance (TN93) were 0.0066 ± 0.0013 and 0.0116 ± 0.0021 for *A. obtectus* and *A. obvelatus*, respectively (ratio = 0.57). Diversities calculated with amino acid gamma distance are 0.0053 ± 0.0025 and 0.0189 ± 0.0051, respectively (ratio = 0.28). Sequence diversity in *A. obtectus* was thus much lower than in *A. obvelatus*, about 43% lower for nucleotides and 72% lower for amino acids.

Microsatellite markers

Four of the five examined microsatellite loci (*C12*, *E07*, *F09*, and *G08*) amplified in all 191 individuals, and were polymorphic. The fifth locus *E01*, however, gave much poorer results — especially in Peruvian individuals in which fewer than 50% of individuals were amplified — and we decided to discard it. An online supplement (see supplementary material) containing the allelic data (PCR product sizes) for loci *C12*, *E07*, *F09*, and *G08* is provided as an Excel file. Allelic richness for the four loci retained, in each site where at least 10 individuals had been genotyped, ranged from 3.5735 and 7.6153 and did not vary significantly among sites ($P = 0.068$). When considering three biogeographical regions (i.e. Old World, Mexico, Peru), allelic richnesses were, respectively, 4.009, 6.879, and 5.215. Whereas allelic richness was not significantly different between Mexico and Peru ($P = 0.2073$), differences appeared significant when comparing the Old World with both Mexico ($P = 0.017$) and Peru ($P = 0.0328$).

The unrooted neighbour-joining (NJ) tree reconstructed from the distance matrix for the 191 genotypes is represented in Fig. 1(b). It shows that most of the Peruvian individuals (20 of 37) are grouped in one well-separated clade (A), which includes only one non-Peruvian individual, one from the Mexican population YOH. Several individuals of this clade demonstrate much longer branch lengths than any other individual from other sites. The other 170 individuals (which include 17 Peruvian individuals) are grouped in three clades and demonstrate much shorter branches. Clade B comprises 14 Peruvian individuals and 16 Mexican individuals. Clade C contains mostly European individuals (51), along with 3 Peruvian and 18 Mexican individuals. Clade D includes 67 Mexican individuals, and one Old World individual from the Spanish population.

Discussion

Although bootstrap values were not exceptionally high in the mtDNA analysis (ranging from 53 to 78 for the three major nodes), all sequenced genes gave congruent information, and our results thus appear robust. The most parsimonious scenario places the Peruvian individuals at a basal position, along with one Mexican individual (YOH). Because the Mexican individual from YOH emerged from an extensive culture of a modern bean variety (the worldwide distributed 'negro mata' variety) in Puebla State, and because the Peruvian individuals come from a traditionally managed field of landraces in a remote valley of the Departamento de Cajamarca where incoming long-distance human-mediated migrations seem improbable, it is more likely that the Mexican individual of this basal clade originated from a recent migrant from South America (through human trade of broadly commercialized beans).

This Andean origin is further supported by the microsatellite analysis, despite the fact that allelic richness was not greater in Peru than in Mexico. What distinguishes several Peruvian individuals is that they are on much longer branches than individuals from Mexico and Europe. Long branches of the Peruvian individuals signify that these individuals are issued from older lineages than the other individuals, all on short branches. Longer branches

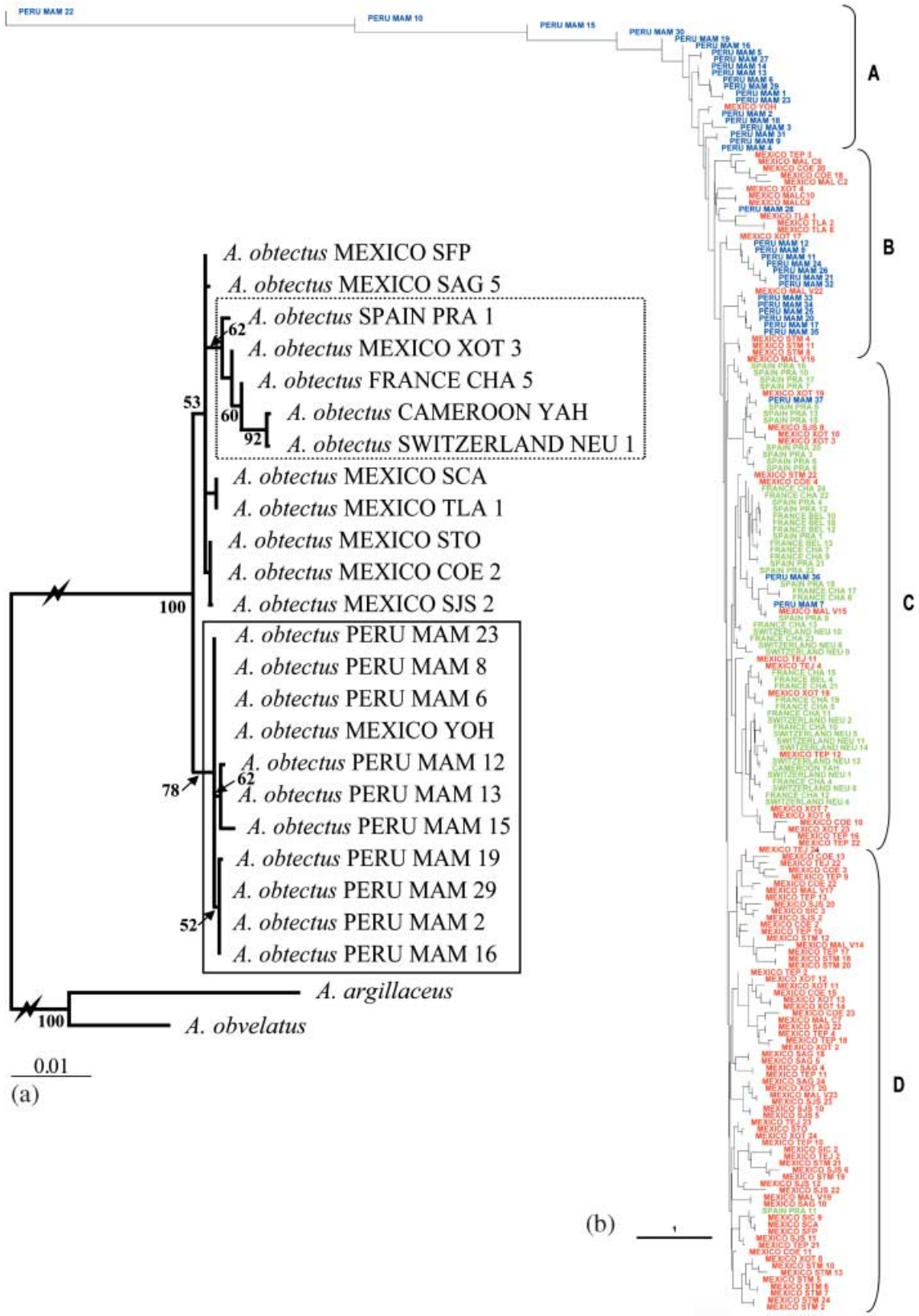

Fig. 1 (a) Phylogenetic reconstruction of individuals sequenced for the three genes *12s rRNA, 16s rRNA,* and *COI*. Numbers at the nodes show bootstrap values. The frame in the continuous line illustrates the basal Peruvian clade. The frame in the dotted line illustrates the Old World clade. The ⚡ indicates that the very long branches linking *Acanthoscelides obtectus* individuals to outgroups are condensed by 0.1. (b) Dendrogram of 191 individuals genotyped for four microsatellite loci. Individuals in blue are from Peru, individuals in red are from Mexico, and individuals in green are from the Old World.

could also be explained by hybridization of *Acanthoscelides obtectus* with another bruchid species in this area. However, the microsatellite loci used in this study are all species-specific, and have not been able to cross-amplify any other species, including those most closely related to *A. obtectus* (*Acanthoscelides argillaceus* and *Acanthoscelides obvelatus*). Furthermore, we have never seen evidence of interspecific hybridization in any bruchis, even between sister species such as *A. obtectus* and *A. obvelatus* (Alvarez *et al*. 2004a). We thus prefer to consider much more likely the hypothesis that long branches in Peru reflect older lineages. Furthermore, despite the fact that a majority of genotyped individuals from Peru branch together on the tree, some Peruvian individuals are found in two other clades. They could thus have included the ancestral members of all clades of the phylogenetic tree. Concerning the YOH individual, which was the sole Mexican individual to group with the Peruvian individuals in the mtDNA analysis, it also branches in the main Peruvian clade in the microsatellite analysis, confirming its proximity to Peruvian individuals. However, because we had sampled only one individual in YOH, we could not examine if other individuals would have branched elsewhere. Nevertheless, as in the mtDNA analysis, the other clades containing Mexican individuals include all five that were found on wild bean populations. The most likely hypothesis to account for our results is that the YOH individual derives from a recent introduction of *A. obtectus* from Peru (or elsewhere in the species' South American range), associated with the intensive cultivation of a widespread commercial bean variety. The alternative hypothesis, that the Mexican individual from the YOH site is more closely related to Mexican individuals than to the Peruvian individual, the latter having originated from a recent migration from Mexico, seems unlikely. Moreover, branch length of the YOH individual in the microsatellite analysis is relatively short, in comparison to several Peruvian individuals.

In the mtDNA analysis, Old World individuals appear to have originated from a limited genetic base and are of Mexican ancestry. The microsatellite analysis, however, does not confirm the monophyly of Old World individuals, probably because of shared ancestral polymorphism in individuals from Mexico and from the Old World, and also because the method used to calculate genetic distances between individuals (Rousset 2000) is more appropriate to assess distance in theoretical continuous populations. However, in this analysis, Old World individuals are more closely related to Mexican individuals than to most Peruvian individuals. The XOT individual, which branches together with Old World individuals in the mtDNA analysis, could have resulted from a human-mediated migration back to Mexico from a population of European origin. Alternatively, it might be close to the Mexican population that gave rise to the migrants that reached the Old World. Examination of microsatellite results provides a clue to the polarity of the migration. Whereas Old World individuals are well segregated [51 individuals of 52 branch into the same clade (C)], individuals from XOT are relatively diverse and are present in three of the four clades (Fig. 1b). A migration from Europe back to Mexico would probably have involved very few individuals, with a very low genetic diversity. It is much more parsimonious to consider that individuals from XOT are closely related to the ancestors of the Old World population, which resulted from migration 500 BP.

Ancient history of A. obtectus

According to current hypotheses, *A. obtectus* lost its capacity for diapause subsequent to domestication of *Phaseolus vulgaris*. The evolution of multivoltinism was adaptive because it allowed continuous reproduction in granaries. However, this hypothesis no longer seems tenable. First, both *A. obtectus* and its sister species *A. obvelatus* occur on both wild and domesticated beans, and only the difference in voltinism is characteristic of the species. Whether attacking wild or domesticated beans, *A. obtectus* is multivoltine and *A. obvelatus* is univoltine (N. Alvarez, unpublished). Second, we have recently shown (Alvarez *et al*. 2004a) that these two species diverged at least several million years ago. Therefore, assuming that the divergence of the voltinism character is as old as the divergence between the species, this is incompatible with a scenario of evolution of multivoltinism in *A. obtectus* after domestication of *P. vulgaris* (*c.* 7000 BP).

Multivoltinism thus appears to have characterized *A. obtectus* prior to bean domestication. Nonetheless, this trait would have made its survival impossible in Mexican wild bean populations, which show annual and strongly seasonal production of a single fruit crop (in the dry season). Wild bean populations in Mexico appear to be sinks for *A. obtectus* (Alvarez *et al*. submitted). However, in other parts of its Neotropical range, *A. obtectus* encounters potential host plants with phenologies that could be effectively exploited by a multivoltine bruchid. For example, spontaneous forms (escapees from cultivation) of domesticated *Phaseolus polyanthus* Greenman at intermediate altitudes (1000–2600 m) in the equatorial Andes are known to fruit throughout the year (A. Delgado-Salinas, personal communication) This character is of real importance, since Andean farmers call this bean species 'frijol de toda la vida', which means 'bean of the whole life'. The distribution of *P. polyanthus* extends from Peru to Mexico; wild forms are known today only from mountains of Guatemala (Schmitt & Debouck 1991), but may have been much more broadly distributed prior to the domestication of this species. If *A. obtectus* were associated with this or another wild *Phaseolus* with continuous fruiting, the capacity to

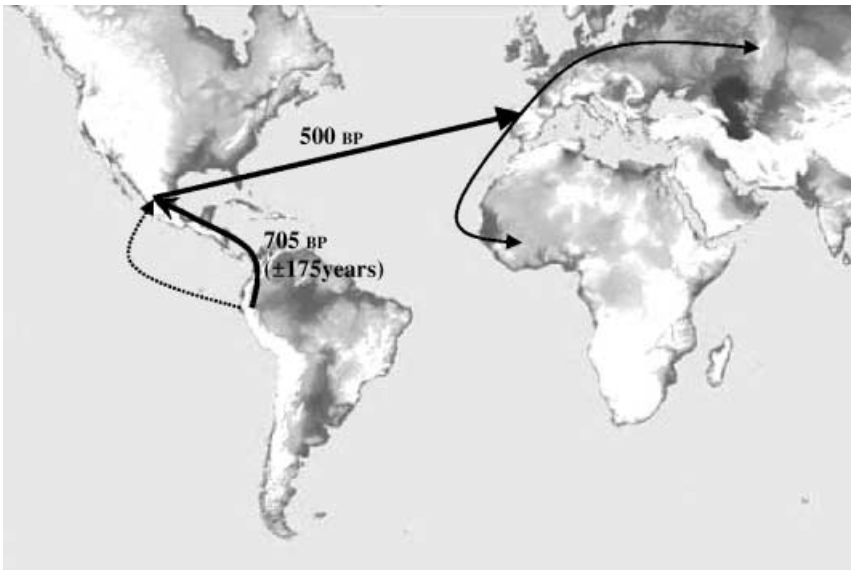

Fig. 2 Hypothetical pathways of the main colonization of *Acanthoscelides obtectus* from Andean America to the rest of the world by human-mediated migrations. Values on the arrows are in years before present. The arrow in dotted line illustrates a second, more recent migration in the same direction, from Peru to Mexico.

produce multiple (5–10) generations per year, rather than one, would have been strongly favoured by selection. Nowadays, *A. obtectus* is known to develop on *P. polyanthus* throughout the range of this bean species.

The trees based on our mtDNA and microsatellite evidence show that this scenario is plausible. The Peruvian individuals occupy the most basal position in the clade in the mtDNA tree, whereas in the microsatellite analysis, branch lengths of Peruvian individuals are much longer than those of other individuals. Peruvian individuals originated from a remote valley of the Departamento de Cajamarca, and it is unlikely that bruchids in such a remote area are recent migrants from Mexico. Moreover, *P. polyanthus* fruits continuously all around the sampling station (A. Delgado-Salinas, personal communication), because valleys of the tropical Andes show little seasonality (Brush 1982).

We propose a new hypothesis that is consistent with biological information and molecular evidence. According to this hypothesis, multivoltinism characterized *A. obtectus* before bean domestication. *A. obtectus* may have been originally associated with wild beans (one or more species) that individually or collectively provided fruits year-round. Such an ecological situation is most likely to be encountered in equatorial regions at mid-altitude with little seasonality. However, the multivoltinism of this species gave it an advantage, compared to univoltine species, in the exploitation of beans stored in granaries, enabling *A. obtectus* and not other species to become a serious pest of domesticated beans. *Phaseolus vulgaris* was domesticated twice independently, in Andean America and Mesoamerica (Gepts & Papa 2002; Islam *et al*. 2002), and we propose that the association of *A. obtectus* with domesticated beans may have originated in the first of these two centres of domestication, followed by the spread of *A. obtectus* to Mesoamerica. Parallel with this hypothesis, we propose that *A. obvelatus* originated in Mesoamerica and became associated with domesticated beans in this second centre of domestication.

Consistent with this scenario are the data obtained by comparing sequence diversities between *A. obtectus* and its sister species *A. obvelatus*, which occupy a very similar niche. Greater genetic diversity in an area may indicate the long presence of a species in the area; diversity should be reduced if populations were initiated from a reduced pool of migrants that colonized a new region. Our results on *COI*, which demonstrate diversities in Mexico ranging from 1.75 to 3.57 times greater in *A. obvelatus* than in *A. obtectus*, suggest that *A. obtectus* may have arrived relatively recently in Mexico. The extent of genetic diversity of *A. obtectus* populations in South America is still unknown.

Recent history of A. obtectus

Based on the introduction of beans into Europe in the early 16th century (Sturtevant 1887), the Old World clade in the mtDNA tree can be considered to have emerged about 500 BP through one sole event from the undifferentiated clade of Mexican individuals in which it is nested. As the mean divergence (calculated with a TN93 NJ model) between the two groups is 0.0051 nucleotide, we can calibrate a molecular clock of $1.02 * 10^{-5}$ mutations/nucleotide/year. Thus, as the divergence between the basal clade and the other clades is 0.0094 ± 0.0021, we can situate the arrival of *A. obtectus* in Mexico at 705 ± 175 BP (Fig. 2). This date appears to be relatively recent. However, such utilization of the molecular clock must be done cautiously because neutral diversity in populations remains a stochastic process, especially when considering closely

related sequences, and because we don't know if the diversity present in Old World individuals is still present in Mexico (i.e. Old World and Mexican populations could share ancestral polymorphism). Nevertheless, we can suppose a quite recent migration of *A. obtectus* from South America to Mexico. This is corroborated by a recent study on the ecology of the two sibling species (Alvarez *et al*. submitted) which showed that *A. obvelatus* is almost always more frequent than *A. obtectus* in about 50 sampled sites of the Mexican altiplano. This suggests that *A. obvelatus* is more adapted to the environmental conditions of the Mexican altiplano than is *A. obtectus*, and that the presence of *A. obvelatus* in Mexico is older than that of *A. obtectus*.

A recent arrival of *A. obtectus* in Mexico could be the consequence of the tropical climate of lowland Central America, which should act as an important barrier to this species. Indeed, even today its densities at low altitudes are much lower than those of other bruchid species, such as *Zabrotes subfasciatus* Boheman. Barriers such as the low-lying Isthmus of Panama could have prevented *A. obtectus* from the Andes from reaching the Central American or Mexican highlands. In such lowlands, *A. obtectus* would probably have been supplanted by other bruchid species (Leroi *et al*. 1990). We postulate that *A. obtectus* arrived in Mexico by recent long-range migration through exchanges of bean seeds, which may have occurred once the web of human communication was sufficiently developed in the Americas. In this context, the date 700 BP — i.e. about 1300 AD — is plausible, given the archaeological records showing agricultural exchanges between Mesoamerican and Andean civilizations, for example between the years 1000 and 1525 (Pacheco 2000). The possibility that more ancient trade between the two civilizations occurred is also suggested by several authors (e.g. Webb 1974; Lorenzo 2000). However, since precaution is required when applying a molecular clock calibrated on a very recent event, we cannot yet discard the hypothesis that *A. obtectus* could have migrated on its own, independently of human-mediated seed movements, long before bean domestication. Indeed, climatic barriers in Central America seem not to have been an obstacle for wild beans such as *P. vulgaris*, which is not particularly adapted to low altitudes.

Nonetheless, our results give credence to the hypothesis that long-distance exchanges occurred between Mesoamerica and Andean America posterior to bean domestication. Movement of beans could have been the way by which *A. obtectus* migrated to Mexico. Consistent with this hypothesis, ancient gene flow between the Mesoamerican and Andean centres of origin of cultivated beans has been shown in several landraces (Gepts 1998).

Another remarkable finding is that the arrival of *A. obtectus* in the Old World seems to have resulted from a single colonization event, probably (based on the mtDNA tree) starting in Spain. The hypothesis of a single colonization is supported by the reciprocal proximity of mtDNA sequences of Old World individuals. Data from analysis of microsatellite markers confirm this probable origin, because the only Old World individual that branches outside clade B is a Spanish individual, which branches along with Mexican individuals of clade D (Fig. 1b). Because all Old World individuals but one branch together in the microsatellite analysis, a sole colonization event from Mexico, rather than a hypothesis of multiple colonization, seems more likely. However, it cannot be excluded that other colonization events have contributed (rarely) to Old World populations. The single Spanish individual branching outside the Old World group in the microsatellite analysis may represent the influence of such secondary, minor colonization, or alternatively, it may represent ancestral polymorphism. This is again consistent with historical evidence of bean diffusion. The capacity to disperse through human-mediated migrations and further adapt to new ecological conditions may explain why *A. obtectus* is today a pest of at least six legume genera (Johnson 1983, 1990). Attention concerning its biology and natural enemies has so far been focused on Mesoamerica. However, we have known for some time that domestication of beans has a complex history, with two independent centres of domestication. This study points to the interest — and the urgent need — of devoting more attention to the Andean American centre, where this bruchid seems to have originated, in particular concerning efforts to find appropriate parasitoid species for biological control. The first step to reach this goal should be increased sampling of South American populations, not only for phylogeographical, but also for ecological studies.

Acknowledgements

The authors thank P. Sourrouille and C. Debain for their helpful technical assistance, and P. Jarne, A. Delgado-Salinas, J.-Y. Rasplus, E. Jousselin, and G. Kunstler for discussion. They also thank A. Delgado-Salinas and A. Aebi for providing specimens at different stages of this research. This work was financially supported by the Swiss National Science Foundation (project N°3100.064821.01) and the Centre d'Ecologie Fonctionnelle et Evolutive.

Supplementary material

The supplementary material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2470/MEC2470sm.htm

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This work is part of a project led by Betty Benrey on the effects of plant domestication on phytophagous insects and their parasitoïds. Nadir Alvarez works on legume/bruchid interactions, focusing on the Neotropical genus *Acanthoscelides* (Coleoptera: Bruchidae). Doyle McKey and Martine Hossaert-McKey are investigating the evolutionary ecology of several plant/insect systems. Céline Born works on population genetics of several animal and plant species. Lény Mercier's interests are centred on ecological interactions between insects and plants.

MANUSCRIT VIII

Anthropogenic effects on population genetics of phytophagous insects associated with domesticated plants.

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(en préparation)

Anthropogenic effects on population genetics of phytophagous insects associated with domesticated plants

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Classification:

Biological Sciences – Population Biology

Manuscript information:

x text pages, 2 tables, 1 figure,

Word and character counts:

Abstract

The hypothesis of isolation by distance (IBD) predicts that genetic differentiation between populations increases with geographic distance. However, gene flow is governed by numerous factors and the correlation between genetic differentiation and geographic distance is never as simple as a linear regression. In this study, we analyze the interaction between the effects on genetic differentiation of geographic distance and those of host plant use in the bean beetle *Acanthoscelides obvelatus* Bridwell. We found that most of the between-population genetic differentiation was explained by geographic distance. However, IBD varied depending on the kind of population pairs for which the correlation between genetic differentiation and geographic distance was examined. Whereas pairs of populations associated with wild beans showed significant IBD, no IBD was found when pairs of populations on domesticated beans were examined. This result can be the consequence of long-distance migrations of bruchids on domesticated plants resulting from human exchanges of bean seeds. Populations associated with wild beans were also significantly more likely than those on domesticated plants to contain rare alleles. However, at the population level, bruchids on cultivated beans were similar in allelic richness to those on wild beans.This analogy in allelic richnesses combined with differences in other aspects of the genetic diversity (*e.g.* IBD, allelic diversity) seems to be explained by strongly contrasting effects of migration and drift. This is the first study showing the role of human practices on population genetics of phytophagous insects associated with domesticated plants.

Introduction

The balance between mutation, drift, selection and migration drives the genetic structure of populations. Whereas local selection and drift promote genetic differentiation, gene flow acts to homogenize it. Gene flow is governed by numerous factors, among them the dispersal capability of individuals and the frequency of extinction and re-colonization events. The most intuitive pattern of variation in gene flow is the global decrease in gene flow with geographic distance. As proposed by Wright (2), the model of genetic isolation by distance (IBD) predicts that genetic differentiation increases with geographic distance. In their review, Peterson and Denno (1) have shown that slopes of IBD decreased with increasing mobility of the species considered. They thus suggested that highly mobile species, such as most insect pests, are characterized by high levels of gene flow compared to species occurring in natural habitats, and postulated that non-equilibrium conditions are frequent in such mobile species. However, in the majority of studies, they observed that there was considerable scatter in the IBD relationship, and attributed it to populations or individuals either more differentiated than expected by distance alone, or less differentiated than expected by distance alone, because of variation in processes such as genetic drift or extinction and recolonization processes.

Factors that mold the genetic structure of phytophagous insect populations have been broadly studied in the last decade – by means of molecular tools – in natural populations (3, 4, 5, 6). Other studies have considered the genetic structure of populations of pest species in agricultural environments or managed woodlands (7, 8, 9, 10). Herbivorous insects that achieve pest status usually continue to exist in natural habitats alongside managed ones. Those in managed habitats may be subject to natural selection for increased natural dispersal rates when their habitats are frequently ploughed or otherwise rendered ephemeral (Southwood 1974). They may also be directly dispersed by humans when they are transported along with domesticated plants. These anthropogenic impacts on dispersal, both direct and

evolutionary, should generate strong differences in population structure between conspecific insects in natural and managed habitats, and also affect geographic patterns of genetic differentiation. Study of this differentiation promises to be both conceptually interesting, in that it makes use of the anthropogenic effects on dispersal as a natural experiment, and practically useful, in that it may help predict insect evolutionary responses to pest control techniques. However, studying IBD in species occurring on both wild and domesticated host plants remains largely unexplored. Up to now, only two studies have compared the populations of pest species both on domesticated host-plants and on wild host-plants. One of these studies, in the aphid *Brevicoryne brassicae* showed substructuring between populations originating from domesticated plants and wild relatives [11]. The second, in the bruchid *Acanthoscelides obtectus* showed no such structuring [12].

Here, we describe a more detailed investigation than previous work, in which we examine the effect of the kind of host plant (wild vs. domesticated) on IBD and patterns of genetic diversity within and among populations. We use a species, the bean bruchid *Acanthoscelides obvelatus* Bridwell, whose intrinsic dispersal capability is augmented by human-mediated migrations. Such bruchids seem unable to disperse more than 2 km (14) by flying, whereas they can travel several hundreds of kilometers through human-mediated migrations of bean seeds (N. Alvarez, pers. obs). This augmentation leads us to expect that IBD should be stronger between pairs of populations feeding on wild host plants than between pairs of populations developing on domesticated host plants. In a recent study, González-Rodríguez *et al.* (12) tried to assess the genetic structure of this species using allozyme markers. Variability in these markers was low, and no differentiation was detected among populations. However, highly variable microsatellite markers are now available for this species (13). In this study, we used these microsatellite markers to compare both the

genetic structure and the genetic diversity of Mexican populations of *Acanthoscelides obvelatus* differing in the kind of host plant they are associated.

Material and Methods

Sampling sites. *Acanthoscelides obvelatus* individuals were obtained from bean seeds collected from 20 Mexican populations, between December 2001 and March 2002 (see Table 1). Mexico is an ideal site to perform such type of study, because it is one of the centers of domestication of *Phaseolus* beans, and domesticated populations and wild populations coexist in sympatry. Among the sampled populations, 13 were from wild populations of bean species of the *Phaseolus vulgaris* group (see Delgado-Salinas 1999), whereas seven populations were from domesticated populations of *P. vulgaris*.

Genetic analysis. Total genomic DNA of 16 to 24 individuals per population was extracted using DNeasy™ kit (Qiagen, Hilden, Germany). Three previously developed microsatellite loci AcobC09, AcobD04, and AcobD06 (13) and three new loci AcobF11, AcobG09, and AcobH01 (see Table 2) were used. PCR amplifications were performed, following the standard protocol of the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany), in a final volume of 10 µl, which contained about 5 ng of extracted DNA, 5 µl of 2x Multiplex PCR Master Mix (1x at final concentration), 1 µl of 5x Q-Solution (Qiagen, Hilden, Germany) (0.5x at final concentration), and 0.2 µM of each multiplexed primer. Products of the PCR reactions were then analyzed on ABI Prism 310 and 3100 sequencers. Genotypes were analyzed using Genscan 3.1.2 and Genotyper 2.5 softwares (Applied Biosystems, Foster City, CA, USA). Exact sizes of alleles were manually corrected to round integer sizes.

Genetic structure and diversity among populations. Genotypic disequilibrium between the different pairs of loci, departure from Hardy-Weinberg equilibrium for all loci, and F-statistics within and between populations (using an infinite alleles model [IAM]) were tested or calculated using Genepop 3.4 (16). A correspondence analysis considering allele identity of each individual for all loci was performed, and centers of gravity for each population were determined using Genetix 4.03 (17). Geographic distances between populations were computed using the software G7towin (Version A.00.174, Ron Henderson 2002). A Mantel test between genetic distance (F_{ST}) and geographic distance matrixes was computed using Genepop 3.4. Further Mantel tests comparing genetic distance (F_{ST}) and geographic distance separately in pairs of populations that were both on wild beans, and in pairs of populations that were both on domesticated beans, were also computed. In these particular pairs of populations, residuals of the Mantel test were compared to values corresponding to the kind of host-bean population (wild bean host was coded as '0' and domesticated bean host was coded as '1' in the matrix) using a Kruskal-Wallis test (SAS, Version 8.0, SAS Institute, Cary, NC, USA). Mean allelic richnesses in populations derived from wild beans and in those from domesticated beans were calculated using Fstat 2.9.3 (18after grouping all individuals collected from wild beans and all individuals from domesticated beans allelic richnesses were calculated after 500 bootstraps with weighted population sizes, using S-Plus (Release 6.0. Insightful Corporation, Seattle, WA, USA). Bottlenecks were determined by comparing Nei's gene diversity and allelic richnesses in each population, by computing signed-rank Wilcoxon tests using Bottleneck 1.02 under IAM hypothesis (Cornuet and Luikart 1996).

Results

Genotypic disequilibrium and departure from Hardy-Weinberg equilibrium. No genotypic disequilibrium between pairs of loci was observed in any population when exact

tests and a correction for multiple tests (Dunn-Sydák method for sequential Bonferroni procedure) were performed. Overall analysis for all populations and individual analysis within each population showed heterozygote deficits that departed significantly from Hardy-Weinberg equilibrium values. F_{IS} values in all populations ranged between 0.117 and 0.410 for the six loci. Such high values of F_{IS}, within the range considered more typical of self-fertilizing hermaphrodites than of animals with separate sexes, indicate the presence of null alleles at all loci considered. However, whereas null alleles have a significant impact on F_{IS}, they have a weak effect on F_{ST} estimates (19). F_{ST} between populations for all loci ranged from -0.006 to 0.121, and there was no genetic differentiation between bruchid populations on wild and domesticated beans ($F_{ST} = 0.0015$ between both groups).

Isolation by distance. Mantel tests showed that geographic distances and F_{ST} were significantly positively correlated overall ($P = 0.0005$), (slope = $4*10^{-5}$; $R^2 = 0.1226$) (not shown). When considering only pairs of bruchid populations on wild beans, the correlation between geographic distance and F_{ST} was stronger ($P < 10^{-4}$) and demonstrated a positive relation with a steepest slope and larger R^2 (slope = 0.0002; $R^2 = 0.2453$) than for all pairs of populations considered together (see Figure 1). However, when only pairs of populations on domesticated beans were considered, there was no correlation between geographic distance and F_{ST} (see Figure 1). Residuals from Mantel tests (in which the variance explained by geographic distance is removed) were also significantly different between pairs of populations on wild beans and pairs of populations on domesticated beans, being greater for pairs of populations on domesticated beans than for pairs of populations on wild beans (Kruskal-Wallis test: $\chi^2_{1df} = 4.3259$; $P = 0.0375$).

Allelic richnesses and diversities. Weighted allelic richnesses in bruchid populations derived from domesticated beans (6.93 alleles per locus and per population) and in populations from wild beans (7.46 alleles per locus and per population) were not significantly different (P = 0.304). However, the weighted overall number of alleles in the entire sample from wild beans (20.09 alleles per locus) was significantly higher than that in the entire sample from domesticated beans (18.40 alleles per locus) after 500 bootstraps (P = 0.002). Furthermore, for each locus, the mean number of alleles present in fewer than 10% of populations for each locus was significantly greater in populations from wild beans than in those from domesticated beans (Wilcoxon Signed Rank test, Z = 1.7691, p = 0.0384). After sequential Bonferroni correction (Dunn-Sydák method), three of seven bruchid populations on domesticated beans (San Francisco Peribán, San José de los Laureles - campo, Santa Lucía) exhibited significant bottlenecks. In contrast, no bottleneck was detected for any of the 13 bruchid populations on wild beans.

Discussion

Our results demonstrate unambiguously a difference in IBD depending on the kind of host plant, and an interesting complementary result on the nature of the allelic diversity between groups of individuals on wild beans and groups of individuals on domesticated beans.

The overall positive relation observed between genetic differentiation and geographic distance is consistent with Wright's IBD theory. However, this correlation is only due to the IBD occurring between populations on wild beans. There is not even a trend towards such a correlation between genetic differentiation and geographic distance when only pairs of populations on domesticated beans are considered. This result is confirmed by the relation arising between the residuals from Mantel test and the kind of host plant: on average, points situated close to the overall regression slope correspond to pairs of populations on wild beans,

whereas points situated far from the slope correspond to pairs of populations on domesticated beans. The absence of IBD in populations originating from domesticated beans is explained by frequent long-distance human-mediated migrations of bean seeds. Indeed, *A. obvelatus* individuals can emerge very far geographically from the bean field in which they originated, if farmers exchange seeds or sell seeds in local or regional markets. Such seed exchanges can be a driving force of migration for beans and associated bruchids, which can thereby travel hundreds of times farther than by natural means. Within the range of our study (500 km), individuals emerging from domesticated beans can potentially interbreed with any conspecific developing on domesticated plants, independently of the geographic distance. Such long-range gene flow through extensive migration appears to homogenize the intra-populational genetic diversity of bruchid populations on domesticated beans. As a result of this homogenization, allelic richnesses in populations on domesticated beans are not different from populations on wild beans, despite the strong drift that must occur in seed granaries. Such drift in populations on domesticated beans seems to be consequent to a strong demographic stochasticity, as attested by the bottlenecks detected in almost half of the analyzed populations on domesticated beans (but not in any of the bruchid populations on wild beans). Bottlenecks may be due to the use of insecticide, human seed consumption and predation during migrations from granaries to fields or wild populations of *Phaseolus*. Without the extensive migration resulting from seed exchanges, this drift should have led to among-population differentiation and loss of genetic diversity.

In contrast, individuals emerging from wild beans can disperse only by natural means and at short distances, and low migration rates lead to differentiation between populations. However, drift is expected to be much less important in such populations because of relatively constant population sizes in populations issued from wild beans comparatively to the frequent bottlenecks occurring in populations issued from domesticated beans.

Similarity in allelic richnesses of populations on wild beans and populations on domesticated beans thus appears to be the consequence of different populational processes. We propose that in demes proceeding from wild beans, large population sizes and low drift maintain allelic diversity, whereas in demes proceeding from cultivated beans, the homogenizing migration counteracts the strong effect of drift (due to the high demographic stochasticity), and maintains diversity.

A remarkable result is that the nature of this diversity is not identical between populations on wild beans and populations on domesticated beans. Indeed, the overall allelic diversity among individuals from all populations of wild beans was significantly higher than among individuals from domesticated beans. This outcome is confirmed by the fact that the number of alleles present in fewer than 10% of populations was significantly greater in populations issued from wild beans than in populations issued from domesticated beans. In other words, populations on wild beans are more likely to maintain rare alleles than are populations on domesticated beans.

This pattern suggests that relatively constant sizes of populations of bruchids on wild beans allow the persistence of rare alleles at the scale of a single deme and results in a larger overall allelic diversity. In contrast, populations on domesticated beans only contain few rare alleles, demographic stochasticity having led to their loss, resulting in an overall lower diversity over all populations on domesticated beans. However, when evaluating each single deme, allelic diversity is not different for populations on wild beans and populations on domesticated beans.

This study shows how human practices have contributed to shape the genetic structure of phytophagous insects associated with domesticated plants. First, large differences in population processes, constant population sizes (*i.e.*, low effect of drift) and low migration rates (*i e.* differentiating migration) in populations on wild beans, and frequent bottlenecks

(*i.e.*, strong effect of drift) and high migration rates (*i.e.* homogenizing migration) in populations on cultivated beans, will have led to the same allelic diversity at the level of a single deme, between the two kinds of populations. However, this diversity is structured differently in the two kinds of demes. Population processes in demes on wild beans allow the persistence of rare alleles and a high overall allelic diversity, whereas strongly contrasting processes in demes on domesticated beans only maintain frequent alleles (*i.e.* most populations on domesticated beans share the same alleles) and a lower overall allelic diversity.

The absence of IBD between populations issued from domesticated beans suggests that individuals originating from domesticated beans can be considered as belonging to one unique population. Therefore, selection would be expected to act on a much greater effective population size, and emergence of an adaptive mutation would be quickly spread in all individuals on domesticated beans. If such a mutation was counter-selected on wild beans, such spread could be followed by the formation of host races (with some individuals being particularly adapted to domesticated beans and the others remaining on wild beans) and further possible speciation. Such a process could also be responsible of a potential quick dissemination of resistance genes – for example to GMO host plants or insecticides – acquired by bruchids. Hence, if an adaptive resistance gene would appear in any bruchid population on domesticated, such a gene would rapidly spread, making non-useful the use of GMO or insecticide.

The role of seed exchange is already recognized as an important factor molding genetic structure of domesticated plants (20, 21). In this study we present evidence that such exchange also affects the structure of populations of a phytophagous insect associated with a domesticated plant. Similar patterns are likely in other organisms associated with both

domesticated plants and their wild relatives, such as other phytophagous insects and their natural ennemies, microbial pathogens and microbial symbionts.

Acknowledgements

The authors thank Mike Singer, Philippe Jarne, and Doyle McKey for discussion and review. They thank Jorge Contreras for help with sampling in Mexico. They also thank Constantino Macias, Roxana Torres, Hugh Drummond, Edgar Avila, Valeria Souza, Luis Eguiarte, Hector Arita and Aldo Valera for providing logistical help in Mexico. This work was financially supported by the Swiss National Science Foundation (project N°3100.064821.01) and the Centre d'Ecologie Fonctionnelle et Evolutive (Montpellier, France).

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Table 1. List of sampled sites. The kind of bean is referred as “d” for domesticated and as “w” for wild.

Name	kind of bean	longitude (°W)	latitude (°N)	Altitude (m)
Copándaro - campo	d	101°46’27.3’’	19°26’36.2’’	2120
Copándaro	w	101°46’11.8’’	19°26’26.1’’	2117
Erongaricuaro	w	101°42’32.8’’	19°35’56.3’’	2072
Huitzilac	w	99°16’23.3’’	19°01’24.0’’	2544
Malinalco	w	99°30’08.9’’	18°57’13.2’’	1935
Napizaro	d	101°41’33.6’’	19°35’50.5’’	2060
San Andrés de los Gabeles	w	99°57’01.5’’	19°02’19.5’’	2280
San Bartolo	w	100°03’20.8’’	19°14’31.7’’	2320
San Francisco Peribán	d	102°24’28.4’’	19°32’32.4’’	1800
San Isidro cerca Coeneo	w	101°34’23.9’’	19°50’56.6’’	2040
San José de los Laureles	w	99°00’05.0’’	18°58’49.7’’	1855
San José de los Laureles - campo	d	98°59’35.0’’	18°58’48.1’’	1800
San Lorenzo	d	102°06’43.4’’	19°31’33.3’’	2125
Santa Lucía	d	100°00’03.7’’	18°52’12.5’’	1790
Sultepec	w	99°59’20.9’’	18°51’07.8’’	2164
Temascaltepec	w	100°02’44.2’’	19°02’35.9’’	1734
Tepoztlán	w	99°07’15.7’’	18°59’36.3’’	1931
Tlalpan	w	99°12’04.3’’	19°17’50.3’’	2403
Tzintzuntzan	d	101°34’41.5’’	19°37’43.9’’	1980
Valle de Bravo	w	100°07’05.1’’	19°13’56.8’’	1918

Table 2. Primer sequences for the three new microsatellite loci in two populations of *Acanthoscelides obvelatus*. Repeat motif is listed 5' to 3' with respect to the forward primer (F). 'Size' refers to the length of the cloned allele.

Locus	Genbank accession number	Primer sequences (5' to 3')	Repeat motif in library	Size (bp)	Size range (bp)
AcobF11	AY730624	F: ACAGGTAAGCAGAGCATC R: GGAAATAATGAAAATACAACTG	$(TG)_{10}$	387	369-447
AcobG09	AY730623	F: GCCGTGCCGTCATATTTG R: TCTGGACGTTGGTGGTAATTTG	$(AC)_8CTACAT(AC)_3GC(AC)_3$ CCGC$(AC)_2GC(AC)_7$	351	314-366
AcobH01	AY730622	F: AGGGTTGCTGTGCGATTC R: TGCGAGCATATTCAAACAAAG	$(GT)_9GCGG(GT)_6$	295	287-303

Figure 1. Genetic differentiation (F_{ST}) between pairs of populations according to geographic distance (km). Blue squares refer to pairs in which the two bruchid populations develop on wild beans, and red triangles correspond to pairs in which the two bruchid populations develop on domesticated beans. The blue line is the regression slope for pairs of populations feeding on wild beans. The red dotted line is the regression slope (non-significant) for pairs of populations feeding on domesticated beans.

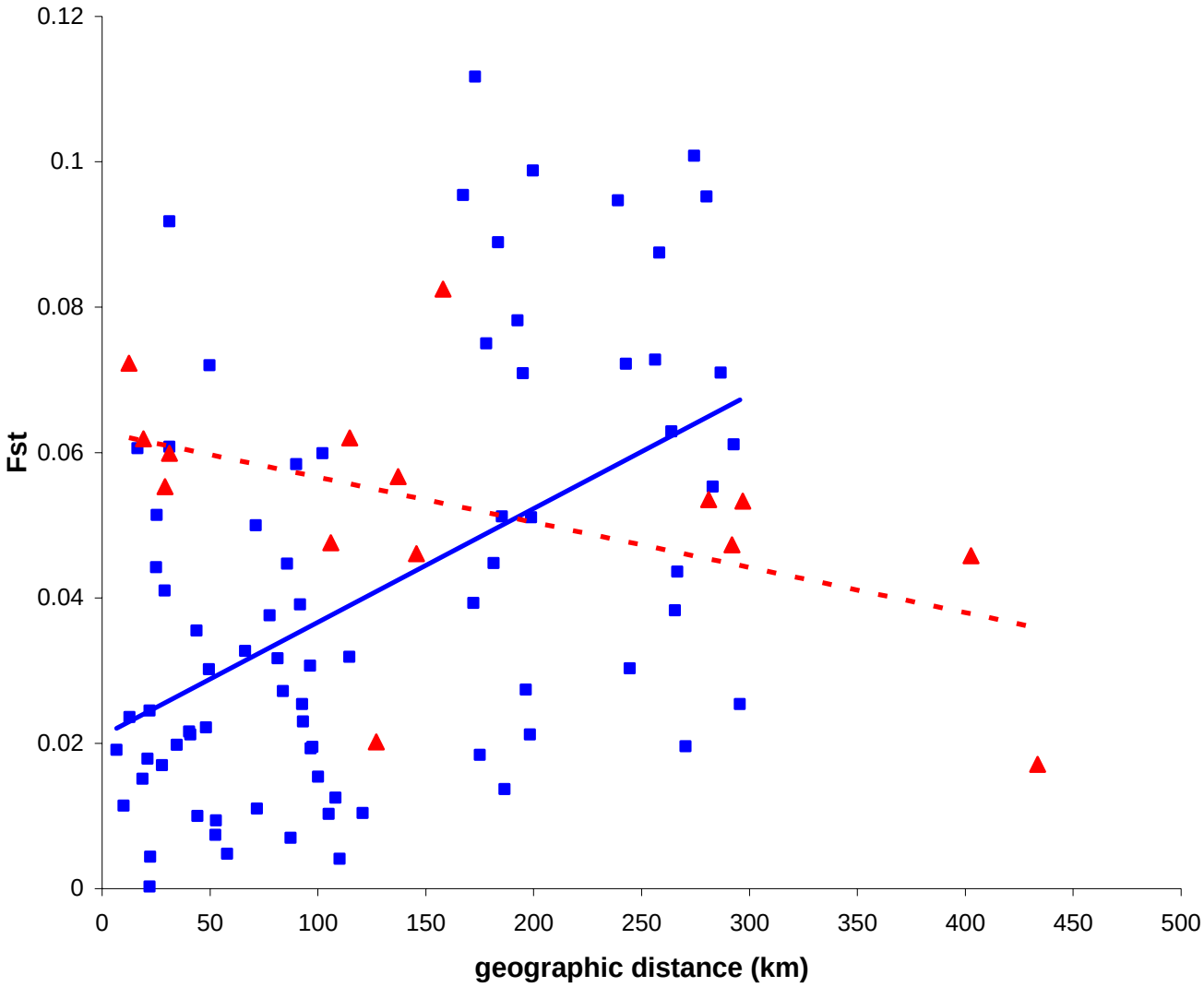
0.12
0.1
0.08
0.06
0.04
0.02
0
Fst
0
50
100
150
200
250
300
350
400
450
500
geographic distance (km)

Figure 1

MANUSCRIT IX

Isolation and characterization of polymorphic microsatellite loci in *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae).

ALVAREZ, N., BORN, C., RISTERUCCI, A.-M., SOURROUILLE, P., BENREY, B., ET HOSSAERT-MCKEY, M.

Molecular Ecology Notes (**2004**) **4**, 683–685

 doi: 10.1046/j.1471-8286 .2003.00335.x

PRIMER NOTE

Isolation and characterization of polymorphic microsatellite loci in *Acanthoscelides obvelatus* Bridwell (Coleoptera: Bruchidae)

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Abstract

Five microsatellite loci were isolated from the bruchid *Acanthoscelides obvelatus* Bridwell (Coleoptera: Bruchidae). Each locus was polymorphic, with the number of alleles ranging from two to 15. We found high levels of within-population variation at most loci, with heterozygosity ranging from 0.182 to 0.900. Cross-species amplification of these loci was tested in two other species of the genus *Acanthoscelides*, *A. obtectus* Say and *A. argillaceus* Sharp.

Keywords: *Acanthoscelides obvelatus*, Bruchidae, Coleoptera, microsatellite, pest species, *Phaseolus*

Received 27 July 2002; revision received 10 September 2002; accepted 10 September 2002

Almost all *Acanthoscelides* species (and more generally Bruchidae species) are predators of legume seeds (Johnson 1989). *Acanthoscelides obvelatus* Bridwell is specialized on seeds of the common bean (*Phaseolus vulgaris* L.). Nevertheless, the species is able to feed on other species of the genus *Phaseolus* such as *P. coccineus* L. and *P. polyanthus* Greenman, and also on other genera of legumes, such as *Vigna* (Delgado-Salinas *et al*. 1988). Its distribution is limited to Mexico, Central America and northern South America. *Acanthocelides obvelatus* represents a major problem in the management of bean stocks, and is considered a major pest of field crops and storage sites. This species belongs to the supraspecific taxonomic group of *A. obtectus* Say (which also includes *A. obtectus* Say and *A. argillaceus* Sharp). A previous study using allozyme markers investigated genetic diversity in *A. obtectus* and *A. obvelatus* (Gonzalez-Rodriguez *et al*. 2000). Sampling involved 14 populations throughout Mexico, which represents the native range of these species, and results revealed differences between taxa, but not at the population level. We isolated microsatellite loci in *A. obvelatus* in order to assess population genetic structure, and to test the role of ecological and human factors on the evolutionary forces acting on the different populations of *A. obvelatus*.

Total genomic DNA was extracted from a pool of 29 individuals using DNeasy™ kit (QIAGEN). Microsatellite-enriched libraries were built following Billotte *et al*. (1999): DNA was digested with *Rsa*I (Eurogentec) and DNA fragments ranging between 500 and 1000 bp were selected after migration on agarose gel and isolated using an extraction kit (Promega). The partial genomic library was then constructed by ligating the DNA fragments into a pGEM-T plasmid. Epicurian-coli XL1-Blue MRF' supercompetent cells (Stratagene) were used to transform the DNA fragments. One hundred and eighty white transformant clones were transferred on Hybon-N^+ nylon membranes (Amersham), and hybridized using inosin/biotin-labelled microsatellite oligoprobe $(CT)_8$ and $(GT)_8$. For 29 of these clones, which gave a satisfactory positive signal, the inserted DNA fragment was sequenced. Eighteen primer pairs were designed using OLIGO Version 3.3 (Rychlik & Rhoads 1989), among which five gave satisfactory amplification patterns [i.e. polymerase chain reaction (PCR) product of the predicted size, and supernumerary bands of low intensity].

PCR amplifications were performed in a final volume of 10 μL, which contained 1.25 μL of extracted DNA, 0.32–0.4 μL of 25 mM $MgCl_2$ (Table 1), 1 μL of 2.5 mM dNTPs, 1 μL of buffer 10× [750 mM Tris–HCl pH 8.8; 200 mM $(NH_4)_2SO_4$; 0.1% (v/v) Tween 20], 1.33 units of *Taq* DNA polymerase (Eurogentec Red Goldstar™), 0.25 μL of 0.01 μM reverse primer labelled with [γ^{33}P]-dATP, and 0.75 μL of 0.01 μM unlabelled forward primer. PCRs were performed on a PTC-100™ thermocycler using the following cycling conditions: initial denaturation at 94 °C (1 min); six (for locus *AcobD04*) or seven (for loci *AcobC09*, *AcobC10*,

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Table 1 Primer sequences, PCR conditions and polymorphism statistics for five microsatellite loci in two populations of *Acanthoscelides obvelatus* Bridwell. Repeat motif is listed 5′ to 3′ with respect to the forward primer (F). 'Size' refers to the length of the cloned allele. T_a: lower annealing temperature. [$MgCl_2$]: concentration of $MgCl_2$ for PCR reactions. *n* is the number of genes analysed. N_a: number of allele size variants observed. H_O: observed proportion of heterozygous individuals. H_E: expected heterozygosity (i.e. gene diversity; Nei 1987)

Locus	GenBank Accession no.	Primer sequences (5′ to 3′)	Repeat motif in library	Size (bp)	T_a (°C) [$MgCl_2$] (mM)	Size range (bp)	Valle de Bravo			Tzintzuntzan		
							n	N_a	H_O/H_E	*n*	N_a	H_O/H_E
AcobC09	AF527375	F: TAGGAAAGGGTGAGAGATGCG R: ATTGGAGACGAGAAAAAGAGGG	$(AG)_{10}GG(AG)_{13}ACGA(AG)_3$	246	59 [0.8]	216–266	20	12	0.800/0.937	20	10	0.900/0.900
AcobC10	AF527376	F: CACCACCCTACCACCTC R: TTCTTATAGGCGATGATGTG	$GC(GT)_8(GC)_2(CA)_5(GTGC)_2GT$	130	53 [1.2]	124–132	20	2	0.200/0.189	20	4	0.400/0.432
AcobD04	AF527377	F: CAGAAACAATTTGCACGAC R: CGGCTGAGACTATGAATCTG	$(AC)_2GC(AC)_8$	103	52 [1.0]	97–105	20	7	0.455/0.532	22	8	0.182/0.333
AcobD06	AF527378	F: ATTTACTGTCCTCGTTGTCG R: ATGCTTATCCGTTCTACTGC	$(TG)_9C(TG)_2$	350	59 [0.8]	316–362	22	11	0.500/0.842	20	9	0.818/0.792
AcobE04	AF527379	F: ACAGTGATGTTAAATAAAAA R: GGTTAGAGATAGGTTGAGTA	$(AT)_5(AC)_2C(CA)_4AAT(AC)_3(AT)_3(AC)_2$ $ATAC(AT)_3AC(AT)_3(AC)_8CCGC(AC)_9$	341	53 [1.0]	265–369	22	15	0.818/0.957	20	11	0.800/0.926

AcobD06 and *AcobE04*) 'touchdown' cycles: 92 °C (25 s), 1 °C drop per cycle to a final annealing temperature of T_a (35 s) (Table 1), 72 °C (30 s); 24 (for locus *AcobD04*) or 23 (for loci *AcobC09*, *AcobC10*, *AcobD06* and *AcobE04*) cycles of: 92 °C (25 s), T_a (35 s), 72 °C (30 s); final elongation at 72 °C (10 min). PCR products were separated by electrophoresis on a 5% denaturing polyacrylamide gel containing urea and 1× TBE buffer. Results were displayed by autoradiography, using water as the negative control.

The DNA of 20 individuals, sampled in two Mexican populations (10 individuals per population), Valle de Bravo (Mexico State) and Tzintzuntzan (Michoacan State), which are 159 km apart, was extracted using DNeasy™ kit (QIAGEN) and genotyped for the five loci. All loci were polymorphic in both populations. The observed number of alleles per population ranged between 2 and 15, and heterozygosities were between 0.182 and 0.900 (Table 1). No significant deviation from Hardy–Weinberg equilibrium and linkage equilibrium between loci was observed in either population when exact tests (GENEPOP Version 3.3 package; Raymond & Rousset 1995) and a correction for multiple tests (Dunn-Sydák method for sequential Bonferroni procedure) were performed.

Cross-species amplifications were tested on individuals of the other species of the *A. obtectus* group (*A. obtectus* and *A. argillaceus*). PCR conditions were identical to those used for *A. obvelatus*. *Aconthocelides argillaceus* feeds on seeds of *P. lunatus* L. (Johnson 1989) and its distribution overlaps broadly with that of *A. obvelatus*, whereas *A. obtectus*, also native to Mexico, is now worldwide in distribution. Despite their taxonomic proximity, *A. argillaceus* amplified successfully for only two of the five microsatellite loci (*AcobD04* and *AcobD06*), whereas *A. obtectus* amplified for three (*AcobD04*, *AcobD06* and *AcobE04*). All cross-amplified loci showed polymorphism (*AcobD04*: two alleles, ranging from 220 to 222 for *A. argillaceus* and from 184 to 186 for *A. obtectus*; AcobD06: three alleles ranging from 344 to 350 for *A. argillaceus* and two alleles ranging from 346 to 350 for *A. obtectus*; *AcobE04*: four alleles ranging from 265 to 321 for *A. obtectus*). Heterozygosities were not calculated as most individuals were collected from different populations.

Acknowledgements

The authors thank P. Jarne, G. Debout, A. Dalecky, D. McKey, P. Sourrouille and C. Debain for their helpful assistance and J.-Y. Rasplus for the identification of *Acanthoscelides* species. This work was financially supported by the Swiss National Science Foundation (project N°3100.064821.01).

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MANUSCRIT X

Isolation and characterization of polymorphic microsatellite loci in *Acanthoscelides obvelatus* Bridwell (Coleoptera : Bruchidae).

ALVAREZ, N., AEBI, A., RISTERUCCI, A.-M., HOSSAERT-MCKEY, M., ET BENREY, B.

Molecular Ecology Notes (**2003**) **3**, 12-14

Molecular Ecology Notes (2004) **4**, 683–685 doi: 10.1111/j.1471-8286.2004.00784.x

PRIMER NOTE

Isolation and characterization of polymorphic microsatellite loci in *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)

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Abstract

Six microsatellite loci were isolated from the bruchid *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Each locus was polymorphic, with the number of alleles ranging from 3 to 18. We found high levels of within-population variation at most loci, with heterozygosities ranging from 0 to 0.75. Cross-species amplification of these loci was tested in two other species of the genus *Acanthoscelides*, *A. obvelatus* Bridwell and *A. argillaceus* Sharp.

Keywords: *Acanthoscelides obtectus*, Bruchidae, Coleoptera, microsatellite, pest species, *Phaseolus*

Received 14 July 2004; revision accepted 3 August 2004

Acanthoscelides obtectus Say is a bruchid species of neotropical origin, whose larvae feed on beans of the *Phaseolus vulgaris* L. group. Since the domestication and diffusion of beans, this species has become cosmopolitan, through human-mediated migration. The consequence of its worldwide status is that *A. obtectus* is now a major problem in the management of bean stocks, and is considered a major pest of field crops and storage sites. In the last 30 years, the species has also been recorded on new host plants, such as *Pisum*, *Lens* and *Vigna (*e.g. Jarry & Bonet 1982). Therefore, there is a strong need to understand the population genetics of this species and to examine the roles of ecological and human factors in the genetics and demography of its populations. However, no useful markers have yet been developed, with the exception of three cross-amplifiying microsatellite loci developed for its sister species, *A. obvelatus* (Alvarez *et al*. 2003). Here we present sequences and preliminary data for six new microsatellite loci in *A. obtectus*.

Total genomic DNA was extracted from a pool of 20 individuals using DNeasy™ kit (Qiagen). Microsatellite-enriched libraries were built following Billotte *et al*. (1999): DNA was digested with *Rsa*I (Eurogentec) and DNA fragments ranging between 500 and 1000 bp were selected after migration on agarose gel and isolated using an extraction kit (Promega). The partial genomic library was then constructed by ligating the DNA fragments into a pGEM-T plasmid (Promega). Epicurian-coli XL1-Blue MRF supercompetent cells (Stratagene) were used for the transformation of the DNA fragments. One hundred white transformant clones were transferred on to Hybond-N+ nylon membranes (Amersham), and hybridized using inosin/biotin-labelled microsatellite oligoprobes $(CT)_8$ and $(GT)_8$. For 14 of these clones, which gave a satisfactory positive signal, the inserted DNA fragment was sequenced using Applied Biosystems BigDye™ protocol, and further analysis using an automated ABI 310 genetic analyser. Eleven primer pairs were designed using OLIGO 3.3 software (Rychlik & Rhoads 1989), of which, six gave satisfactory amplification patterns (i.e. polymerase chain reaction product of the predicted size and supernumerary bands of low intensity).

Polymerase chain reaction (PCR) amplifications were performed following the standard protocol of the Qiagen Multiplex PCR kit, in a final volume of 10 μL, which contained ~5 ng of extracted DNA, 5 μL of 2× Multiplex PCR Master Mix (Qiagen; 1× at final concentration), 1 μL of 5× Q-Solution (Qiagen; 0.5× at final concentration) and 0.2 μM of each multiplexed primer. Primers were multiplexed as follows: *AcobtC12*, *AcobtE07* and *AcobtF01* (multiplex #1); *AcobtE01*, *AcobtF09* and *AcobtG08* (multiplex #2). Reverse primers were labelled with fluorochromes HEX, 6-FAM and NED (Applied Biosystems), as follows: *AcobtC12*, *AcobtE07* and *AcobtF01* (HEX); *AcobtF09* and *AcobtG08* (6-FAM); *AcobtE01* (NED). PCRs were performed on a PTC-100™ thermocycler (MJ Research) using the following

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Table 1 Primer sequences, PCR conditions and polymorphism statistics for six microsatellite loci in two populations of *Acanthoscelides obtectus* Say

Locus	GenBank Accession no.	Primer sequences (5′–3′)	Repeat motif in library	Size (bp)	Size range (bp)	Coeneo			Xochitlan		
						n	N_a	H_O/H_E	*n*	N_a	H_O/H_E
AcobtC12	AY681082	F: GATCCTCTGATGCTACATTTGGTC R: GAGCACGAGCACACGCA	$(TG)_{25}$	311	262–358	34	15	0.294/0.945	34	8	0.118/0.816
AcobtE01	AY681083	F: ATTCACTTAACCACAATACG R: GCTCCTTGAACCTTCTAC	$(AC)_8(AT)_5AC(AT)_3$ $(AC)_2(AC)_3AT$	151	149–161	30	6	0.133/0.623	22	3	0.000/0.329
AcobtE07	AY681084	F: ACACAGTCATGATGACAGC R: AAGTAGAAAATGACGACGAC	$(AG)_{14}$	126	106–140	48	11	0.792/0.764	46	13	0.826/0.865
AcobtF01	AY681085	F: CATAAGGATATTGATTTCGTC R: TGTTCACAATTTCACAGC	$(GT)_8$	236	232–236	46	2	0.087/0.085	42	3	0.238/0.296
AcobtF09	AY681086	F: AGCAGACGACAAGCAGCACAC R: CGAGCCGCATACGCATTG	$(CA)_{11}$	169	158–170	40	7	0.750/0.763	48	4	0.542/0.556
AcobtG08	AY681087	F: GGTGGAGGGACCGCACAC R: CCTTCGGAAATCGTGGATACCC	$(GT)_{14}$	379	366–372	46	4	0.435/0.697	44	3	0.500/0.468

Repeat motif is listed 5′ to 3′ with respect to the forward primer (F). 'Size' refers to the length of the cloned allele. *n* is the number of genes analysed (i.e. two genes per individual). N_a: number of allele size variants observed. H_O: observed proportion of heterozygous individuals. H_E: expected heterozygosity (i.e. gene diversity; Nei 1987).

cycling conditions: initial denaturation at 95 °C (15 min); 30 cycles of: 94 °C (30 s), T_a (1 min 30 s), 72 °C (1 min); final elongation at 72 °C (10 min). The annealing temperature (T_a) was 57 °C for loci *AcobtC12*, *AcobtE07* and *AcobtF01* and 60 °C for loci *AcobtE01*, *AcobtF09* and *AcobtG08*. Fragments were separated on an ABI 310 genetic analyser (Applied Biosystems) with internal size standard ROX500 (Applied Biosystems).

The DNA of 48 individuals, sampled in two Mexican populations (24 individuals per population), Coeneo (Michoacan State) and Xochitlan (Puebla State), which are 412 km apart, was extracted using DNeasy™ kit (Qiagen) and genotyped for the six loci. All loci were polymorphic in both populations. The observed number of alleles per population ranged between 2 and 15, and heterozygosities per population and per locus were between 0 and 0.750 (see Table 1). No genotypic linkage disequilibrium between loci was observed in either population when exact tests (GENEPOP 3.3; Raymond & Rousset 1995) and a correction for multiple tests (Dunn–Sydák method for sequential Bonferroni procedure) were performed. No significant deviation from Hardy–Weinberg equilibrium was observed for loci *AcobtE07*, *AcobtF01*, *AcobtF09* and *AcobtG08* when exact tests (GENEPOP 3.3; Raymond & Rousset 1995) were performed. However, a significant heterozygote deficiency, that would probably be the consequence of null alleles, was found for loci *AcobtC12* and *AcobtE01*. We were unable to design new primer pairs at locus *C12*, because the flanking region in which the forward primer was designed was only 18 nucleotides long, and allowed only the definition of one unique forward primer. Concerning locus *E01*, we designed two other primer pairs, which unfortunately did not allow any amplification, because the flanking region where the forward primer was designed (despite the fact that it was 49 nucleotides long) was composed of several small repeated motifs fewer than 10 nucleotides long that resembled the major motif itself. Other forward primers did not anneal, probably because of interindividual variability in this flanking region.

Cross-species amplifications were tested on individuals of the other species of the *A. obtectus* group (*A. obvelatus* and *A. argillaceus*). PCR conditions were identical to those used for *A. obtectus*. *A. argillaceus* feeds on seeds of *Phaseolus lunatus* L., whereas *A. obvelatus* — the sister species of *A. obtectus* — also develops on seeds of beans from the *P. vulgaris* L. group (Johnson 1989; Alvarez *et al.* in press). The distributions of both species are restricted to Mesoamerica. Whereas *A. obvelatus* amplified none of the six loci, *A. argillaceus* successfully amplified one of the six microsatellite loci (*AcobtF01*: three alleles ranging from 230 to 236). Heterozygosities were not calculated, as most individuals were collected from different populations.

Acknowledgements

The authors thank P. Jarne, D. McKey and C. Debain for their helpful assistance. This work was financially supported by the Swiss National Science Foundation (project N° 3100.064821.01).

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MANUSCRIT XI

Farmers' practices, metapopulation dynamics, and conservation of agricultural biodiversity on-farm: a case study of sorghum among the Duupa in sub-sahelian Cameroon.

ALVAREZ, N., GARINE, E., KHASAH, C., DOUNIAS, E., HOSSAERT-MCKEY, M., ET MCKEY, D.

Biological Conservation (**2005**) **121**, 533–543

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BIOLOGICAL CONSERVATION
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Farmers' practices, metapopulation dynamics, and conservation of agricultural biodiversity on-farm: a case study of sorghum among the Duupa in sub-sahelian Cameroon

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Received 22 May 2004; received in revised form 30 May 2004; accepted 30 May 2004

Abstract

In many traditionally managed agroecosystems, populations of domesticated plants maintain high levels of genetic diversity. The threat of erosion of this diversity is a current conservation concern, motivating studies of how diversity can be maintained by in situ conservation measures. Precisely how the biological traits of plants and the cultural practices of farmers act on fundamental evolutionary forces – drift, migration, selection, and mutation – to create and maintain crop plant diversity has been little investigated in detail. We develop some elements of the framework required for studying such biocultural interactions, focusing on one component of management: farmers' decisions on what to plant, and the structure of germplasm exchange among farmers. We illustrate the approach with a study of Duupa farmers in northern Cameroon. Our results suggest that sorghum populations managed by the Duupa function like source–sink metapopulations. Fields of older farmers, larger and containing a greater number of varieties, act as sources, whereas fields of younger farmers act as sinks, becoming sources as their owners mature. In each field, seeds for sowing are selected from a small number of plants. The frequent exchange of germplasm among fields may counteract the genetic bottlenecks associated with the small number of genitors within each field. Identifying key processes and key individuals should facilitate the design of in situ conservation measures to maintain crop plant diversity against the threat of genetic erosion.

Keywords: Farmers' practices; Population biology; Evolutionary forces; Sorghum; Duupa

1. Introduction

In many traditional agroecosystems, genetic diversity within crop species has important functions. Varieties of the same species not only differ in their cultural roles, e.g., the uses to which they are put, but also in their ecological tolerances. As used here, a "variety" is a category of plants recognized as a separate entity by farmers, based on morphological and other traits (see for example Zeven, 1998).

Farmers cultivate crop populations in environments that are heterogeneous in space and often unpredictable in time, and varieties often differ in their response to such variation. Genetically diverse populations may also be less susceptible to high levels of attack by pathogens and herbivores. Thus, in systems where farmers have limited capacity to control spatial and temporal environmental variability with material inputs, planting a diverse assemblage of genotypes can lower the risk of failure and increase food security (Altieri, 1999). Diversity matters not only within farmers' fields, but also

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doi:10.1016/j.biocon.2004.05.021

at the level of farmer communities. Farmers count on the diversity present in other farms, to get new seed lots when they need them. Seed exchange may even extend as far as cyclic renewal of seed lots (seed change), a frequent feature in traditional agricultural systems (Louette et al., 1997; Zeven, 1999; Louette and Smale, 2000).

The positive valuation of agricultural biodiversity has progressively led to an extraordinary store of genetic diversity. Nevertheless, recent socio-economic changes threaten this diversity. Modern varieties, created to satisfy particular criteria, and environmental modifications that favour these varieties, may leave little place for local varieties. Longer-term adaptation to a fluctuating and heterogeneous environment, or to a rich cultural context, may thus be compromised (Brush, 2000).

There are two broad approaches to conserving agricultural biodiversity. First, the ex situ approach attempts to maintain genetic resources outside of agroecosystems, in germplasm banks. Whereas such preservation may prevent the extinction of abandoned varieties, it stops or greatly alters the evolutionary processes that mould the populations' diversity (Oldfield and Alcorn, 1987; Brush, 1995). Second, in situ approaches (or on-farm conservation) aim to maintain the existing genetic resources on-farm, allowing evolutionary processes to maintain and continue to create diversity (Cleveland et al., 2000; Maxted et al., 2002). The two approaches have complementary advantages and drawbacks (Brush, 2000). However, the scientific basis for in situ dynamic conservation remains weak. All agree that cultural practices of farmers are important in maintaining diversity, but much information is anecdotal (Louette and Smale, 2000). Which practices are important, and how they affect fundamental evolutionary forces to maintain genetic diversity, are poorly understood.

1.1. First understand, then manage

In the past decade, agronomists have carried out studies on how innovations they consider desirable could be propagated in traditional agroecosystems, e.g., by examining the insertion of new varieties into local seed systems (e.g. Cromwell, 1990; Voss, 1992; Almekinders et al., 1994), or by developing participatory management approaches in which plant breeders work with farmers to develop new varieties (e.g. Witcombe et al., 1999). In contrast, too few studies have considered how traditional systems work. The enormous socio-economic and ecological changes facing many farming communities surely modify the functioning of crop populations in many ways. Developing effective on-farm programs to maintain adaptation in the face of such change depends first of all on a solid understanding of how farmers have through ages created and maintained crop diversity and persist in doing so without outside help in large parts of the world. Farmers' plant breeding must be understood in terms of the same theoretical principles that underlie both professional plant breeding (Soleri and Cleveland, 2001) and the functioning of wild plant populations.

Few studies examine how farmer practices affect evolutionary forces acting on crop plant populations, and only a small proportion of the possible interactions have been treated. These studies focus, for example, on farmers' criteria in seed selection and the goals of selection (Louette and Smale, 2000); on choices among varieties (Bellon, 1996; Cleveland et al., 2000); and on spatial arrangement of planting in ways that encourage hybridization between varieties (Cleveland et al., 2000; Perales et al., 2003). As numerous aspects of crop-plant ecology and population genetics remain unexplored, there are large gaps in our understanding of the evolutionary genetics of crop plants in traditional agroecosystems. These gaps limit the effective application of in situ conservation approaches.

1.2. Natural and human factors interact to shape evolutionary forces

Natural factors and human management are inextricably linked and jointly shape the genetic diversity of crop plant populations. Natural factors comprise both environmental pressures and biological traits of the plant that affect its population structure. Human management modifies not only selection pressures but also population structure, thereby affecting drift, migration, and metapopulation dynamics (Jarvis and Hodgkin, 1999). Natural and human factors interact. For example, the breeding system of many crops depends not only on inherited traits of the plant (e.g. self-incompatibility), but also on the number and the disposition of varieties planted in the same field (Cleveland et al., 2000; Perales et al., 2003).

Farmers make many kinds of decisions all through the chain of agricultural operations that affect genetics of crop plants. Research generally focuses on the selection of seeds for planting, but other segments of the chain, e.g. how varieties are associated (or segregated) in space during planting, are often neglected. Even for seed selection, many aspects are poorly studied, such as the proportion of seeds that are planted by the farmer who produced them, the proportion that migrate through farmer exchange, the period and modalities of selection, and the structure of the local system of seed exchange.

1.3. Populations and metapopulations of crop plants

Crop populations must be considered as structured populations. Subpopulations are managed by different farmers in spatially discrete fields, which are submitted to different evolutionary forces and exchange migrants.

Subpopulations sometimes go extinct, as farmers replace seeds from sources other than their own seed (Zeven, 1999; Louette and Smale, 2000). Here, population extinction is immediately followed by massive migration, in a way different from natural structured populations in wild plants. Hence, there is a need to model this structure and examine its consequences for the population genetics of crop plants. Such a model should incorporate the following parameters:

1. Variation among farmers in their practices or knowledge, which may be a function of social categories (age, sex, social status) or other sources of variation (individual preference, familial history). In studies of structured crop populations, the average farmer does not exist.
2. Exchanges among farmers. If certain patterns are predictable, the migration web among subpopulations can be estimated.
3. Impacts of social or economic changes, which may act at a larger scale of time and space. Contemporary patterns and mechanisms at the local scale must be placed into the context of the regional history of diffusion of crop germplasm (for sorghum see Seignobos, 2000), and even the history of initial domestication and diffusion of the crop (Harlan, 1989).

1.4. Applying the approach: a preliminary case study

We conducted a preliminary study among the Duupa, farmers in sub-sahelian northern Cameroon, with the goal of clarifying some of these parameters, and examining how farmer practices related to planting and harvesting can influence crop population structure and the action of selection. We investigated the local seed system with particular emphasis on the structure of exchanges, and on the way the germplasm for planting is selected. We also analysed variations in Duupa farmer practices and longer-term changes, whose impacts on population processes is less predictable. By identifying fundamental questions and the kinds of data needed to answer them, we aim to design more precise studies of the knowledge and practices of farmers, and thereby to plan the population genetic studies required to test the hypotheses generated.

2. Methods

Our study was conducted in the Duupa village of Wanté (8°27′N, 13°18′E). The Duupa comprise 4000 people occupying about 1000 km^2, in the Bénoué plain and mountainous Poli massif. The Duupa are mostly sedentary farmers, cultivating a great diversity of subsistence crops, such as yams, cowpea, groundnuts, okra, and cereals. The latter include pearl millet, eleusine, and most important of all, sorghum (Garine, 1995). Accounting for more acreage than any other crop, sorghum grown by the Duupa is also highly diverse. We found about 25 locally recognized and named 'varieties' in this single village, and perhaps twice this number occur in the region occupied by the Duupa (Garine, 1995). These varieties are planted in polyvarietal mixtures, with a mean of around nine in a single field. Several races of sorghum, including guinea, guinea-caudatum, and kafir-durra, are represented among Duupa varieties, which collectively exhibit great morphological diversity (Garine, 1995).

2.1. Sorghum cultivation among the Duupa

Sorghum (*Sorghum bicolor* [L.] Moench) is at the centre of Duupa diet, and is also an important element of the social system, since all social and ritual occasions, e.g., exchange of services and bouts of communal work (*kombuma*), are articulated around the invitation to drink sorghum beer, called in the Duupa language *buma*.

Wanté village is a relatively isolated settlement, where approximately 20 families share flat to gently rolling lands covering a total surface of almost 10 km^2.

In the Duupa society, every active adult works in his/her own field. However, some women and many children work in the field cultivated by the head of the household. The location of a farmer's field may change from year to year, and the same parcel may be used by different farmers in successive years. Land is collectively owned by the village community as a whole. Agricultural work is performed in different ways. For some tasks, the farmer works alone or with his household. For other tasks, such as harvest, a farmer organises *kombuma*, where his extended family, neighbours, and affines are invited to work. This "employment" is free, except for the rule of reciprocity. Sorghum beer, produced from germinating sorghum seeds, is an indispensable ingredient of every social meeting, including collective work parties like *kombuma* (Garine, 2001).

Such a conservative society provides good conditions to study the impact of farmers' practices on the continuing evolution of crop plants. The Duupa agricultural activities begin with the planting period, from late April–May. This period corresponds to the first rain of the rainy season. During the growing season (April–October), fields are weeded two (or three) times, and sorghum is harvested from early December to late January (Garine, 1995). Threshing of the harvested crop can take place from early February to late March. In March and April, the stocks of seeds are set aside for planting, and can thus be observed. Hence, we conducted the interviews during the months of March and April 2001, a time corresponding both to the period

when seed stocks are present and to the lowest activity in the Duupa agricultural calendar. Nevertheless, we were sometimes unable to examine the granaries, since many Duupa farmers believe that to show seeds to strangers can cause misfortune.

Interviews were conducted with the help of a Duupa interpreter. We interrogated 32 Duupa farmers (19 men and 13 women) belonging to 19 of the 20 households present in Wanté. The interviews were carried out separately with each person, to avoid collective answers that rarely reflect individual strategies. Despite the relatively small sample size, our study is exhaustive in Wanté, including almost all households of the village. Increasing the sample size would have required extension of the study to other villages, which was infeasible given our logistical limitations and the time-consuming nature of the ethnobiological approach. For each farmer, we recorded gender and age, which crops he/she cultivated, and the total area cultivated. During the inquiries, we focused on determining the modalities of several key factors, all partially or completely dependent on farmer behavior, capable of modifying selection pressures and crop plant population structure. These include the *local seed system* (seed exchanges leading to immigration and emigration events), *seed selection practices*, which determine the percentage of plants that play the role of female genitors for the next generation (and thereby influence selection and genetic drift) and the *way crops are propagated*, which influences the plant's breeding system. In the results, we consider only sorghum, which is cultivated by 90% of the farmers we interviewed.

2.2. Local seed system

The structure of the local seed system depends on several factors, most of them based on human social factors. We focused our interviews on the following questions:

1. Do you give seeds away (or sell seeds)?
2. Do you receive seeds from others (or buy seeds)?
3. What was (were) the source(s) of the seeds you used for your most recent planting?

The pertinence of farmers' answers was directly tested during inquiries by asking the same question in different ways. Responses to the third question enabled verification of the concordance of responses to questions 1 and 2. Strictly quantitative answers were neither expected nor obtained. We classified each farmer's answer in categories (*never*, *rarely*, *sometimes*, *at least once a year* for questions 1–2 and in *own seeds used*, *seeds obtained elsewhere*, or *both* for question 3. We then examined the relationship of answers to these questions to the following factors, which present two or three modalities: farmer's gender, farmer's age (distributed over three roughly equally sized groups: *less than 36 years old; between 36 and 56 years old; 56 years old or more*), destination of the last harvest (*farmer's own use only* versus *own use and exchange*), number of varieties cultivated (*less than the mean number of varieties per farmer; more than the mean number of varieties per farmer*), and area cultivated over the previous years 1999–2000 (*less than half the mean area cultivated by all farmers sampled; between half the mean and 1.5 times the mean; more than 1.5 times the mean area cultivated*. "Area cultivated" (see Table 1) was estimated by the farmer him/herself. From inspection we made of parcels cultivated in the previous year, such estimates appeared realistic. However, an imprecision in these estimates arises from the fact that crops are planted in polycultural associations. Sorghum is by far the dominant crop in fields, so estimates of field size for this crop are probably less affected by this bias than those for other crops.

2.3. Seed selection practices

The way farmers select the seeds to plant in the following year is directly linked to the proportion of plant individuals that will play the role of female genitors (i.e., mothers of the next generation) in the field. This proportion depends on several factors, but most importantly on the moment in the crop cycle at which seeds are selected. Duupa farmers choose sorghum seeds before panicles are threshed. They preferably choose infructescences of a few individuals, which provide them with all the seeds required, and the proportion of plants serving as female genitors is low. Consequently a few individuals provide the bulk of seeds for the next generation. We estimated the proportion of plants selected as female genitors in the fields of five Duupa farmers: We first estimated the total number of panicles harvested, by asking each farmer how many baskets -the standard measure used locally – he/she had filled with panicles during harvesting. According to our observations, one full basket contains about 150 panicles. The number of panicles conserved to provide seeds for the next planting was simply counted during interviews with each farmer. As a sorghum plant produces almost always only one panicle, the proportion of plants selected as female genitors can thus be estimated by the number of panicles selected to provide seeds divided by the total number of panicles harvested.

2.4. Propagation of crops

Sorghum is self-compatible, but substantial levels of outcrossing (up to 15%) occur under traditional farming systems (Dje et al., 1999). Mating structure is affected by the spatial patterns in which varieties are planted in fields. We interviewed farmers to obtain descriptions of

Table 1
Frequency of cultivation, number of varieties, and area cultivated for crop plants grown in Wanté village

Crop species	Total area cultivated	Sorghum	Cowpea	Groundnut	Okra	Yam	Cassava	Bambara groundnut	Maize	Finger millet
		Sorghum bicolor	*Vigna unguiculata*	*Arachis hypogaea*	*Abelmoschus esculentus*	*Dioscorea* spp.	*Manihot esculenta*	*Voandzeia subterranea*	*Zea mays*	*Eleusine coracana*
Percentage of farmers cultivating the crop ($N_{tot} = 30$)		90	73.3	63.3	50	33.3	30	13.3	13.3	13.3
Total number of varieties cultivated in the whole village		25	4	4	?[a]	6[b]	3	1	3	1
Mean number of varieties per farmer cultivating the crop		9.1 ± 5.1	2.2 ± 0.9	2.1 ± 0.9	?[a]	3.5 ± 1.3	2.0 ± 0.9	1.0 ± 0	1.4 ± 0.5	1.0 ± 0
Mean area cultivated (ha) per farmer cultivating the crop	0.75 ± 0.59	0.5 ± 0.49	0.08 ± 0.05	0.23 ± 0.08	–	0.11 ± 0.5	0.12 ± 0.1	0.03 ± 0.03	0.08 ± 0.03	0.12 ± 0.1

[a] Only one okra variety was cited by the farmers interviewed, but in previous years many farmers had more than one variety (E. Garine, unpublished field notes). The reason for this discrepancy is unclear.
[b] Previous field work had shown the presence of more than 10 varieties in Duupa villages. This value (6) may therefore be underestimated.

Table 2
Differences related to sex and age of farmers in the proportion of individuals that cultivate different crops, and in the total area cultivated

Cultivated crop		Total area cultivated		Proportion of farmers that cultivate or not cultivate (in %; $N = 30$)									
				Sorghum		Cowpea		Groundnut		Okra		Yam	
		<3/4 ha	⩾ 3/4 ha	Cultivate	Do not cultivate	Cultivate	Do not cultivate	Cultivate	Do not cultivate	Cultivate	Do not cultivate	Cultivate	Do not cultivate
Farmer's sex	♀ ($n = 12$)	66.7	33.3	75.0	25.0	66.7	33.3	91.7	8.3	41.7	58.3	0	100
	♂ ($n = 18$)	33.3	66.7	100	0	77.8	22.2	44.4	56.6	55.6	44.4	55.6	44.4
	P	(0.143)		(0.056)		(0.679)		(0.014)		(0.710)		(0.002)	
		0.603		*0.292*		*0.999*		***0.081***[MNS]		*0.999*		***0.012*** *	
Farmer's age	< 36 years ($n = 14$)	71.4	28.6	85.7	14.3	57.1	42.9	78.6	21.4	64.3	35.7	14.3	85.7
	36–56 years ($n = 10$)	50	50	90	10	90	10	50	50	30	70	50	50
	⩾ 56 years ($n = 6$)	0	100	100	0	83.3	16.7	50	50	50	50	50	50
	P	(0.015)		(1.000)		(0.116)		(0.289)		(0.175)		(0.116)	
		0.089[MNS]		*1.000*		*0.521*		*0.870*		*0.684*		*0.521*	

The number in parentheses corresponds to the *P*-value calculated with a generalised Fisher's exact test (performed with 10^6 iterations). The number in italics refers to the *P* obtained after correction for multiple tests with the method of Dunn–Šydák (Bonferroni procedure). Bold numbers indicate *P* less than 0.1. The '*' sign indicates a significant result ($P < 0.05$ after Bonferroni correction). The '[MNS]' sign indicates a marginally non-significant result ($0.05 < P < 0.1$ after Bonferroni correction).

planting practices that could affect mating structure of sorghum populations. These included how seeds from different varieties were mixed before planting (which could affect their spatial distribution at very local scales) and whether different sets of varieties were planted in different kinds of fields (e.g., large fields vs. home gardens).

3. Results

3.1. Description of the agricultural system: differences related to gender and age of farmers in the cultivation of different crops and in the total area cultivated

Gender and age of farmers were correlated with several traits, including which crops an individual grew. As stated above, Duupa farmers cultivate numerous species of crop plants. Sorghum, cowpea, okra, groundnut, and yam were the crops planted by the highest proportions of farmers. Of these, all except okra are represented by multiple varieties (Table 1). As confirmed by a generalised Fisher's test (Raymond and Rousset, 1995), yam was exclusively cultivated by men in Wanté, whereas groundnut was a women's crop (Table 2).

Age of the farmer seemed not to affect preferences for any crop species. However, there was a relationship (marginally non-significant after Bonferroni correction) between the total area cultivated by a farmer and his/her age: older Duupa farmers cultivated bigger fields of sorghum than did younger ones (see Table 2).

3.2. Proportion of plants serving as female genitors of the next crop generation

Quantitative data concerning sorghum are shown in Table 3. Duupa farmers employ mass selection in choosing seeds for planting. Farmers select entire panicles, either before harvesting of the crop, or between harvesting and threshing. They select large panicles with well-filled grains. For sorghum, we estimated that 1.01% of plants contribute to the next generation. Despite the small number of farmers ($N = 5$) on which this estimate is based, variation among farmers was relatively low (standard deviation = 0.199%), justifying use of this estimate as a broad approximation.

3.3. Local seed system

The relationships between responses to the three initial questions for sorghum and the factors listed were analysed using a generalised Fisher's test. Results are presented in Table 4. Interviews reveal that exchange of seeds selected for planting is never a matter of monetary transaction, whereas some farmers may sell part of their harvest in markets. 'To give seeds away (or to sell part of the harvest)' was significantly associated with the farmer's gender (more frequent in men), age (more frequent for older farmers), destination of harvest (more frequent among farmers whose yield covered need), and surface of fields (frequency increased with surface). Furthermore, 'to give seeds away' showed a strong trend to positive correlation with the number of varieties cultivated (marginally non-significant after Bonferroni correction). 'To receive seeds from others' showed the opposite pattern for all variables examined. Its frequency was significantly negatively correlated with the area cultivated; farmers that gave seeds away – or that sold part of their harvest – tended to be less likely to receive seeds; young farmers were more likely to receive seeds than older farmers; and farmers with few varieties were more likely to receive seeds than those with many varieties. Farmers who reported that they received seeds planted fewer varieties. Source of the seeds for the most recent planting showed a trend to association with field surface: farmers cultivating large fields were more likely to use exclusively their own seeds.

Farmers reporting that they received seeds usually reported also that they did not use exclusively their own seeds in their most recent planting (χ^2 tests before Bonferroni correction, $P = 0.01$). This significant relationship further attests to the internal consistency of the responses to our questionnaires. Nevertheless, farmers that gave away seeds showed no preference in using exclusively their own seeds or not (χ^2 tests before

Table 3
Proportion of plants (percent) selected as female genitors in the sorghum fields of five Duupa farmers

Size of field (ha)	Number of panicles harvested	Number of panicles selected to provide seeds for next planting	Percentage of plants selected as female genitors
0.5	2250	30	1.33
0.75	2250	20	0.89
1	3000	25	0.83
1	3750	40	1.07
2	7500	70	0.93
			Mean = 1.01%

See text for methods used to estimate number of panicles harvested.

Table 4
Correlations between farmer practices relating to seed exchange and several other variables, for sorghum

Variable		Proportion of cultivators (in %; $N = 27$) categorized in classes for several variables, according to their practices relating to seed exchange											
		Farmer's sex		Farmer's age (in years)			Sorghum area cultivated (in ha)			Number of cultivated varieties		Destination of the last harvest	
		♀ ($N = 9$)	♂ ($N = 18$)	< 36 ($N = 12$)	36–56 ($N = 9$)	⩾ 56 ($N = 6$)	⩽ 1/4 ($N = 14$)	1/4–3/4 ($N = 5$)	>3/4 ($N = 8$)	<10 ($N = 17$)	⩾ 10 ($N = 10$)	Only own consumption ($N = 19$)	Consumption and market sales ($N = 8$)
1. Do you give seeds away (or sell seeds)?	Never or rarely	88.9	22.2	75.0	11.1	40.0	71.4	20.0	12.5	52.9	30.0	57.9	12.5
	Sometimes	11.1	22.2	0	55.6	0	21.4	20.0	12.5	29.4	0	26.3	0
	At least once a year	0	55.6	25.0	33.3	60.0	7.2	60.0	75.0	17.7	70.0	15.8	87.5
	P	(0.003) ***0.013****		(0.002) ***0.012****			(0.005) ***0.025****			(0.021) ***0.100***^{MNS}		(0.002) ***0.011****	
Do you receive seeds from others (or buy seeds)?	Never	22.2	22.2	0	22.2	60.0	0	20.0	62.5	11.8	40.0	10.5	50.0
	Rarely	66.7	38.9	50.0	55.6	20.0	50.0	60.0	37.5	41.2	60.0	47.4	50.0
	Sometimes or at least once a year	11.1	38.9	50.0	22.2	0	50.0	20.0	0	47.0	0	42.1	0
	P	(0.322) *0.857*		(0.019) ***0.090***^{MNS}			(0.004) ***0.020****			(0.015) ***0.072***^{MNS}		(0.017) ***0.082***^{MNS}	
What was the source of the seeds you used for your most recent planting?	Seeds obtained elsewhere included	33.3	33.3	50.0	33.3	0	57.1	20.0	0	47.1	10.0	47.4	0
	Exclusively own seeds used	66.7	66.7	50.0	66.7	100	42.9	80.0	100	52.9	90.0	52.6	100
	P	(1.000) *1.000*		(0.104) *0.423*			(0.016) ***0.077***^{MNS}			(0.092) *0.380*		(0.026) *0.122*	

The number in parentheses corresponds to the *P*-value calculated with a generalised Fisher's exact test (performed with 10^6 iterations). The number in italics refers to the *P* obtained after correction for multiple tests with the method of Dunn–Šydák (Bonferroni procedure). Bold numbers indicate *P* less than 0.1. The '*' sign indicates a significant result ($P < 0.05$ after Bonferroni correction). The '^{MNS}' sign indicates a marginally non-significant result ($0.05 < P < 0.1$ after Bonferroni correction).

Bonferroni correction, $P = 0.23$), and there was no correlation between frequency of receiving and frequency of giving (χ^2 tests before Bonferroni correction, $P = 0.16$).

3.4. Planting practices

In Wanté village, the number of varieties of sorghum varied among farmers (mean ± standard error of 9.1 ± 5.1 varieties per farmer; see Table 1). While panicles of different varieties are collected and managed separately, at planting time seeds of all varieties are mixed in a common bowl and sown randomly. Thus seeds of several varieties are commonly mixed in a single planting hole.

In addition to large fields, Duupa sometimes plant small "house gardens" of a few particular sorghum varieties in small plots near dwellings. According to our informants, the varieties planted in these parcels are relatively demanding of nutrients and more susceptible to bird predation. These varieties often flower precociously.

4. Discussion

4.1. Description of the agricultural system

Our study in Wanté shows that Duupa agriculture maintains high diversity, not only at the interspecific level, but also in terms of the number of local varieties of each crop, particularly in the case of sorghum. Our analysis of how the Duupa manage crop plants illustrates the importance of taking into account not only specific farming practices, but also the larger web of social relations within which exchanges of germplasm take place.

4.2. Proportion of plants serving as female genitors of the next crop generation

In contrast with other seed-propagated plants grown by the Duupa, only few sorghum plants serve as female genitors, since farmers obtain all the seeds for the next planting from a limited number of individuals. These are selected either before the harvest or from the harvest pile before threshing. Maize farmers in Mexico also select a small proportion of ears to supply seed. In maize populations in Jalisco, Louette and Smale (2000) estimated that about 1% of ears provided all seeds for the next generation. This is similar to our estimate for sorghum. However, since each maize plant may produce several ears, the proportion of plants serving as female genitors could be less than 1%. Louette and Smale (2000) note that farmers usually select ears for next year's seed from the harvest pile, and point out that this precludes direct selection on traits not observable in ears. It is not known whether Duupa farmers use different criteria for seed selection, depending on whether they choose panicles from plants in the field or from the harvest pile. Some farmers of sorghum select seeds in a way that should have very different population-genetic consequences (Almekinders and Louwaars, 1999).

However, consequences may be more complex. Mass selection in outcrossed crops is thought to favour highly heterozygous individuals. Even though the number of plants that contribute to the next generation is small, the genetic diversity of the seeds produced may be high (Ollitraut et al., 1997). Moreover, the number of fathers (pollen donors) that sired the seeds in a single panicle is unknown. In a wind-pollinated crop such as sorghum, with genetically diverse individuals planted in close spatial proximity, this number could be substantial. As stressed by Louette and Smale (2000) for maize in Mexico, the lack of control of the pollen source in open-pollinated crops could contribute to the maintenance of diversity.

We have shown that sorghum seed exchanges among Duupa farmers are extensive, leading possibly to an equilibrium between migration and drift. This high rate of migration favours the maintenance of the great varietal diversity we observed in Wanté village. Given the low proportion of plants serving as female genitors and the repeated bottlenecks this implies, we assume that any long-term reduction in the scale or intensity of seed exchange could alter the migration/drift equilibrium of sorghum and diminish the genetic diversity of its local populations.

4.3. Local seed system

Our observations provide an image of patterns of exchange of sorghum seeds in a Duupa village at a single point in time, and do not address phenomena acting at greater temporal and spatial scales. The village is not a closed system, and the set of varieties available is not static over time. Exchanges of seeds are attested between Duupa farmers from Wanté and Duupa from other villages, as well as with farmers of other ethnic groups. Even at low frequency, such long-distance exchanges could have long-lasting effects on the structure of crop diversity at the village level.

4.3.1. Variation in farmer practices depending on the farmer's age

Age of the farmer appears to be related to the area the farmer cultivates. In an agrarian civilization where cereal agriculture plays a central role in ethnic identity and the foundations of society (Garine, 2002), several advantages are associated with having a large sorghum field. First, having a larger field means that a house-

hold's food security is increased. This is an important advantage in a region where the growing season is short and where droughts and other events lead to great variation in harvest between years. Secondly, farmers with large fields can more often sell some grain or display it on ritual or social occasions. Third, and not least, owners of large fields play bigger roles in social exchanges, e.g., by their greater capacity to organise collective activities by offering sorghum beer. Farmers thus strive to have large fields, and their ability to accomplish this increases as they become older and acquire experience, resources, and prestige.

4.3.2. Structure of the local seed system

The most important result of our inquiries is that the local seed system appears to be structured like a source–sink metapopulation: older farmers provide seeds, whereas young farmers most often receive seeds. As the size of the cultivated area is correlated with the farmer's age, larger fields (i.e., larger plant sub-populations) tend to play the role of source in the metapopulation, whereas smaller fields (i.e., smaller plant sub-populations) tend to be sinks. As said an old Duupa farmer, "an older farmer will never ask a younger one for seeds; in the field, older people must help younger ones, not the opposite". This view of the social roles appropriate for individuals of different age is not restricted to seed exchange. Older Duupa are reluctant to be indebted in any way to younger individuals.

A corollary inference is that sink populations become source populations over the farmer's lifetime. A further finding is that larger fields, which generally belong to older farmers and function as sources, also contain the largest number of varieties. Thus, sorghum populations managed by the Duupa seem to mimic the functioning of some wild plant populations, in which after initial establishment both population size (through growth and immigration) and genetic diversity (through immigration (e.g. Giles and Goudet, 1997)) often increase over time, until at some point the population declines or disappears.

Similar source–sink dynamics might characterize other situations in which individual farmers vary in the roles they play in seed exchange. Among maize farmers in Jalisco, Mexico, for example, individuals covered the spectrum between those who always used their own seeds and those who almost never did so (Louette et al., 1997). This variation was related to several factors, including the size of the area farmed, as in the Duupa. In other respects, the two cases appear different. For instance, in the study by Louette et al. (1997), there is no indication that "sink" farmers become "source" farmers over time, as in the Duupa.

Source–sink dynamics in Duupa sorghum populations might be strengthened by the apparent rarity of "seed change" in favour of seeds obtained by exchange (Zeven, 1999; Louette and Smale, 2000). Duupa farmers rarely abandon a variety, but a farmer may neglect his own seeds when he considers them too "tired" to provide good yields. During the period of the study, no case was reported in which "tired" seeds were changed. However, we did record a few exceptional situations, e.g., when illness prevented a farmer from cultivating and replenishing his seed stock.

The farmer's gender may also influence his/her role in the local seed system. We have shown that men more often provide seeds to other farmers than do women. Many farmers organize *kombuma* during the sorghum harvest and threshing. Every helping participant of the *kombuma* is allowed to bring back home a reasonable quantity of seeds. This practice is underlain by a strong moral sentiment expressed by the Duupa that access to seeds must remain free to anyone in the community. Because men more frequently organize *kombuma* than do women (a difference anchored in the Duupa social code), men have more occasions to provide seeds to other farmers. Source and sink populations are thus also linked to the gender of farmers.

Our results indicate that seed exchanges can occur at a large spatial scale, and not only between neighbours. Spatial proximity among sorgum metapopulations is thus likely to exert less influence on gene flow than among structured populations of wild plants (Zimmerer, 1998). In addition, seeds from major 'source' farmers (i.e., those who provide seeds; see Table 4) may travel far away from their native site of production, through long-distance social exchanges or regional market stands. Farmers reported acquisitions of seeds of new varieties during trips to Ngaoundéré, 150 km to the south. Such acquisition could contribute signitifcantly to large-scale gene flow.

4.3.3. Will seed systems change?

These findings all indicate that older farmers play key roles in maintaining diversity. In view of recent social and economic changes affecting the region, it is not easy to predict whether young farmers will continue to assume the role of guardians of agrobiodiversity when they become older. The seed system we describe currently depends on a relatively small number of key individuals and might therefore be inherently fragile.

Cash crops such as cotton are being introduced in the region, and along with them new practices – e.g., the use of chemical fertilisers and herbicides – that could also affect the management of subsistence crops. For example, farmers who develop a mixed economic strategy combining the production of cotton and sorghum take advantage of the residual effect of chemical fertilizers spread on cotton to plant sorghum in the same field the next year, creating by the same occasion a new type of crop rotation. This new rotation could affect farmers

preferences for varieties that better respond to increased nutrient levels.

Such large-scale changes could lead not only to the direct loss of diversity, but also to changes in the evolutionary forces, especially migration and drift, affecting crop metapopulations. Changing the sizes of populations and the migration patterns will modify the equilibrium between migration and drift. Crop genetic diversity could thus be threatened not only by abandonment of varieties and modification of the selective environment, but also by reduction of gene flow and increased fixation of genes by drift.

4.4. Planting practices: possible effects on mating structure

Our inquiries indicate that even with one general biological breeding system (i.e. sexual reproduction with frequent allogamy), practices of Duupa farmers could have substantial impacts on the "observed" breeding system of sorghum. This variation should affect the breeding system (e.g., relative proportion of outcrossed and inbred matings), assuming that there is substantial genetic variation among varieties. A striking feature of Duupa sorghum cultivation is that seeds of many different varieties are mixed in a common bowl before sowing. Thus plants of different varieties are found in close proximity in fields. This pattern, in which numerous varieties occur closely mixed in a single field, should lead to extensive gene flow among varieties, which all flower at about the same time (Garine, E., personal observation). How Duupa varieties of this partially outcrossed crop (Dje et al., 1999) are preserved, despite a planting pattern that encourages extensive hybridization between different varieties, remains unclear. Three explanations can be proffered. First, as yet unidentified barriers may minimize the frequency of intervarietal crosses. Second, individuals of a given variety may share a few major genes responsible for the variety's distinctive traits, and exhibit little differentiation at other loci. Third, panicles chosen for planting might be a non-random subset corresponding to distinctive ideotypes, with the population of panicles in the field including individuals with intermediate characters. Louette and Smale (2000) found evidence for such a pattern for maize grown by Mexican farmers.

Another feature of Duupa planting patterns is the segregation of a group of nutrient-demanding, rapidly growing, bird-susceptible varieties of sorghum in home gardens. These varieties should exchange genes more frequently with each other and less with the varieties planted in large fields. By affecting mating structure, planting patterns could thereby facilitate a kind of local adaptation of these ecologically similar varieties. Their precocious flowering could further increase the likelihood of homogamy.

4.5. Limitations of the study

This study is the first step in the examination of effects of farmers' practices on evolutionary forces acting on sorghum. Its principal limitation, aside from the restriction to a single village in our interviews, is the absence thus far of data on molecular and phenotypic diversity of Duupa sorghum varieties, and on the ecology and genetics of these polyvarietal populations. Studies building on this work are currently in progress and should overcome these limitations.

5. Conclusion

In this study, we have shown how Duupa seed selection, seed exchange, and planting patterns could influence genetic processes in the sorghum populations they manage. Like many other groups, the Duupa are faced by changes that could disrupt the social and ecological conditions that underlie their farming practices, including the local seed system. Understanding the consequences of these changes depends on understanding the links between farmers' practices and fundamental evolutionary forces. The role of farmers' practices in the continuing evolution of crop plants must be taken into account to produce models that combine concepts from natural sciences (e.g., dynamics of source–sink metapopulations) and knowledge of the impact of cultural diversity, which together shape the peculiar functioning of populations of domesticated plants.

Acknowledgements

This research was funded by a grant from the "Institut Français de la Biodiversité". We thank the Duupa research assistants who helped in gathering the data. Additional thanks go to the MEADEN (Mission d'Etudes pour l'Aménagement et le Développement de la Province du Nord) and to the authorities of Poli who provided us with logistical help. We are particularly grateful to the Djaoro and all the residents of Wanté village. We also thank Aboubakar Moussa and Nathalie Raufaste for their help at different stages of this research. Finally, we thank H. Joly and A. Barnaud for critical reading of the manuscript.

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2001-2004 PhD position, at the Centre d'Ecologie Fonctionnelle et Evolutive (CNRS, Montpellier, France), and at the Laboratoire d'Ecologie Animale et Entomologie (Univ. Neuchâtel, Switzerland). My thesis is focused on phylogenetic relationships in bruchid beetles (Coleoptera) of the neotropical genus *Acanthoscelides* (see below for a summary of my PhD topic).
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2000-2001 DEA (equivalent of M. Sc. with thesis) in Evolutionary Biology and Ecology, at the Centre d'Ecologie Fonctionnelle et Evolutive (CNRS, Montpellier, France), with high distinction. Research thesis: "Modeling of farmers' practices which act on the population genetics of cultivated plants: the case of the Duupa agricultural system (northern Cameroon)".
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RESEARCH AND TEACHING EXPERIENCE

Field experience

2001-2003: 2 fieldwork seasons (3 months each) in Mexican Altiplano for PhD studies.
2000: 1 fieldwork season (2 month) in Soudano-Sahelian North-Cameroon for M. Sc. thesis
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Laboratory experience

-AFLP and sequencing of chloroplastic markers in several plant species, to assess and compare phylogeographical patterns
- Sequencing of several mitochondrial and nuclear genes in several bruchid beetle species, to assess phylogeographical and phylogenetic studies.
- Use of microsatellite DNA markers for genetic differenciation of two bean bruchid species *Acanthoscelides obtectus* Say and *A. obvelatus* Bidwell.

Student supervision

2003-2004: Supervision of two advanced studies degree students: « Influence of ecological factors on the distribution of two seebling species of bean bruchids » and « Genetic structuration of populations of a Mexican coleopteran species, seed-eater of *Phaseolus* beans: Does a host plant effect exist ? ».

Teaching experience

2002-2004: Teaching assistant at the University of Neuchâtel (Switzerland): lecturer in laboratory practicals on plant-insect interactions (25 hours/year).
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INTERDISCIPLINARY ACTIVITIES

- Collaboration with two PhD students from the University of Montpellier, Ambroise Dalecky and Jeremie Gilles, on the phylogeny and phylogeography of *Petalomyrmex* sp. (Hymenoptera) and *Stomoxys* sp. (Diptera) respectively.

- Participation to the pluridisciplinary project on “Comparative biodiversity histories in three agroecosystems of North-Cameroon: ecological and anthropological approaches”, awarded in 2002 to E Garine by the “Institut Français de la Biodiversité”.

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12. Alvarez, N., Hossaert-McKey, M., Restoux, G., Delgado-Salinas, A., Romero Napoles, J. and Benrey, B. – *in prep* – "Anthropogenic effects on population genetics of phytophagous insects associated with domesticated plants."

11. Kergoat, G.J.[¶], **Alvarez, N.**[¶], Hossaert-McKey, Faure, N. and Silvain J.-F. – submitted to *Molecular Ecology* – "Parallels in the evolution of the two largest seed-beetle genera (Coleoptera : Bruchidae)."
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10. Alvarez, N., Romero Napoles, J., Anton, K.-W., Benrey B. and Hossaert-McKey, M. – submitted to *Zoologica Scripta* – "Phylogenetic relationships in the Neotropical bruchid genus *Acanthoscelides* Schilsky."

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8. Alvarez, N., L. Mercier, B. Benrey, and M. Hossaert-McKey – submitted to *Oikos* – "Ecological distribution and niche segregation of two sibling species of bean beetles, *Acanthoscelides obtectus* Say and *A. obvelatus* Bridwell."

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Résumé

Depuis 400 millions d'années, les plantes-hôtes ont influencé l'évolution de leurs insectes phytophages à différents niveaux, des processus populationnels à la diversification des espèces. Parmi les nombreux groupes d'insectes phytophages, les bruches (Coleoptera : Bruchidae), qui comprennent environ 1700 espèces, sont caractérisées par la phytophagie des semences de certains groupes d'angiospermes. La grande diversité des bruches, ainsi que l'étroite relation que celles-ci entretiennent avec leurs espèces d'angiospermes associées, en font un modèle approprié aux tests d'hypothèses traitant des interactions évolutives entre les insectes phytophages et leurs plantes-hôtes.

Cette thèse, qui s'est focalisée sur les bruches néotropicales du genre *Acanthoscelides* Schilsky, a démontré que l'organisation de la diversité des insectes phytophages est liée aux composés secondaires (ou aux défenses chimiques contre les herbivores), mais également à la capacité de dispersion des plantes-hôtes. Ainsi à partir de la reconstruction des patrons phylogénétiques, nous avons montré que les processus de radiation évolutive présentent le plus souvent un conservatisme taxonomique de la plante-hôte, c'est-à-dire que des insectes phylogénétiquement proches sont associés à des plantes-hôtes taxonomiquement, et donc souvent chimiquement, proches. Au niveau des processus populationnels, la dispersion des plantes-hôtes est quant à elle un facteur-clé de la structuration génétique des bruches associées, comme nous l'avons montré chez les espèces du groupe *A. obtectus* (qui comprend notamment des espèces inféodées au haricot), au travers d'approches écologique, phylogéographique et de génétique des populations. Dans le cas des espèces inféodées aux plantes domestiquées, cette dispersion est également liée, depuis peu, à l'histoire humaine et aux flux des semences.

Mots-clés : phytophagie, bruches, *Acanthoscelides*, radiation évolutive, processus populationnels, dispersion, composés secondaires des plantes-hôtes, facteurs anthropiques.

Summary

For 400 million years, host-plants have influenced the evolution of phytophagous insects at different levels, from population processes to the generation of species diversity. Among the numerous groups of phytophagous insects are bruchid beetles (Coleoptera: Bruchidae), which include 1700 species whose larvae consume the seeds of certain groups of angiosperms. The great ecological diversity of bruchids (and their host plants), and the tight relationship between each bruchid species and its host-plant(s), makes them an appropriate biological model for testing hypotheses about evolutionary interactions between phytophagous insects and host plants.

This thesis, which focused on a group of neotropical bruchids of the genus *Acanthoscelides* Schilsky, showed that the organisation of diversity of these phytophagous insects is tied to the secondary compounds (or to the chemical defences) of host plants, but also to the dispersal capacity of their host plants. Thus, based on the reconstruction of phylogeny of this bruchid group, we showed that processes of adaptive radiation usually exhibit taxonomic conservatism in host-plant association, i.e., related bruchid species usually occur on related (and chemically similar) host plants. At the level of population processes, the dispersal of the host-plants is a key factor in influencing the spatial genetic structure of the associated bruchids, as we showed for the species of the *A. obtectus* group (which includes species associated with *Phaseolus* beans), using an approach combining ecology, phylogeography, and population genetics. In the case of bruchids specifically associated with domesticated plants, dispersal is also tied, during the few thousand years since the beginning of domestication, to human history and the human-mediated movements of seeds.

Keywords: phytophagy, bruchids, *Acanthoscelides*, evolutionary radiation, population processes, dispersal, host-plant secondary compounds, anthropogenic factors.