Montpellier SupAgro

THÈSE

pour obtenir le grade de

DOCTEUR DE MONTPELLIER SUPAGRO

Discipline: Biologie des organismes et des populations
Formation doctorale: Évolution, Écologie, Ressources génétiques, Paléontologie
Ecole doctorale: Systèmes Intégrés en Biologie, Agronomie, Géosciences, Hydrosciences et Environnement

Systèmes de reproduction et scénarios d'invasion chez la petite fourmi de feu, *Wasmannia auropunctata*

Présentée et soutenue publiquement par

Olivier Rey

Le 16 décembre 2011

Jury

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Claudie Doums	Maître de conférences, EPHE, Paris	Rapporteur
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TABLES DES MATIÈRES

CHAPITRE I

INTRODUTION GÉNÉRALE

I. Les invasions biologiques : contexte général

I.1. Les étapes du processus d'invasion

Le terme d'invasion biologique est défini de plusieurs manières dans la littérature scientifique (e.g. Elton 1958; Williamson 1996; Richardson *et al.* 2000; Valery *et al.* 2008). Les différences majeures entre ces définitions concernent principalement deux points. Certains considèrent qu'une invasion implique nécessairement une étape de dispersion sur une distance supérieure à la capacité naturelle de dispersion des organismes (Elton 1958; Falk-Peterson *et al.* 2006; Richardson *et al.* 2000) alors que pour d'autres, cette distance de dispersion peut être faible et seul un changement de milieu est important (Davis & Thompson 2000). L'intensité de l'impact des populations envahissantes sur le milieu d'accueil et/ou sur les activités humaines est également un critère sur lequel les définitions sont discordantes. Pour certains auteurs, il faut nécessairement que les populations aient un impact important sur la communauté envahie (Davis & Thompson 2000). Cependant, du fait de la difficulté de quantifier ces impacts plusieurs auteurs s'accordent à dire que ce critère de définition est difficile à prendre en considération (Richardson *et al.* 2000; Valéry *et al.* 2008). Dans cette thèse, une invasion biologique est définie comme étant un processus lors duquel certaines populations s'établissent dans un écosystème dans lequel l'espèce n'était pas présente, et deviennent abondantes par rapport aux populations de l'habitat principal de l'espèce.

Toutes les définitions s'accordent cependant sur le fait qu'une invasion est un processus qui implique trois étapes successives, la dispersion initiale, l'établissement dans un nouveau milieu et la propagation des populations nouvellement établies. Ces trois étapes constituent une série de barrières difficiles à franchir ne permettant qu'à une petite fraction de populations de devenir envahissantes (Blackburn *et al.* 2011). Le succès d'une invasion dépend principalement de la capacité de survie, de reproduction et d'adaptation du groupe d'individus initialement introduits (i.e. propagules) mais également du milieu d'accueil. Ces propagules et la néo-population qui s'ensuit pendant au moins plusieurs générations, ne contiennent souvent qu'un nombre limité d'individus ainsi qu'une fraction de la diversité génétique des populations de l'aire native (i.e. effet de fondation suivi d'un goulot d'étranglement). Elles sont de ce fait confrontées à de fortes contraintes démographiques et génétiques associées aux effets de fondation et aux petites populations. Ces contraintes se font

d'autant plus ressentir lorsque le milieu dans lequel les propagules s'établissent présente des caractéristiques environnementales différentes de celles de l'habitat principal de l'espèce.

I.2. Contraintes démographiques et adaptatives des populations envahissantes

D'un point de vue démographique, les petites populations sont sensibles à l'extinction. En effet, le taux d'accroissement d'une population est corrélé positivement à sa taille (i.e. effet Allee; Allee 1931). Cet effet résulte notamment du fait que les petites populations sont plus sensibles à la stochasticité démographique et/ou environnementale et à une probabilité moindre de trouver un partenaire sexuel (Courchamp *et al.* 1999). Ce phénomène est accentué dans les espèces dépourvues de comportements grégaires ou d'attraction tels que les chants ou le relâchement de phéromones d'attraction (Gascoigne *et al.* 2009). D'un point de vue évolutif, les populations de petites tailles sont sensibles aux effets de dérive génétique (i.e. fluctuation aléatoire des fréquences alléliques à chaque génération) qui peut entrainer une perte de diversité importante et/ou la fixation d'allèles délétères dans la population (Wright 1931). Une faible diversité génétique est généralement associée à un potentiel évolutif réduit (Fisher 1958), la diversité génétique (notamment la variance génétique additive) constituant le support sur lequel la sélection naturelle agit. Les petites populations peuvent également souffrir d'effets liés à la dépression de consanguinité (Nei *et al.* 1975) pouvant mener jusqu'à l'extinction des populations (Frankham & Ralls 1998). Ce phénomène est basé sur le fait que la reproduction entre individus apparentés augmente l'homozygotie dans le génome de la descendance, entraînant ainsi l'expression d'allèles récessifs délétères au sein de la descendance. L'intensité de la dépression de consanguinité dépend du nombre d'allèles délétères dans le génome des individus reproducteurs et donc du fond génétique des populations (Charlesworth & Charlesworth 1999). Ces contraintes évolutives expliquent, au moins en partie, le fait que de nombreuses propagules colonisatrices sont vouées à l'échec et que seules quelques unes d'entres elles deviennent envahissantes. De nombreuses études ont été menées pour résoudre le paradoxe apparent des populations qui s'établissent et envahissent avec succès de nouvelles aires géographiques en dépit de ces contraintes (Sax & Brown 2000).

I.3. Facteurs facilitant l'établissement et l'adaptation des populations envahissantes

Plusieurs facteurs favorisant le succès d'invasion des populations ont été identifiés. En premier lieu, l'établissement d'une population dans un nouvel environnement peut être associé à un relâchement de la pression liée aux interactions biotiques telles que la prédation ou le parasitisme (Wolfe 2002; Torchin *et al.* 2003). Ce relâchement de la pression biotique peut conférer un avantage aux populations introduites par rapport aux espèces compétitrices locales qui, elles, sont en interaction spécifique avec leurs «ennemis» locaux (hypothèse du «*enemy release*»; ERH; Keane and Crawley 2002). Ce relâchement peut également permettre aux populations introduites de réallouer leurs ressources sur des traits phénotypiques importants tels que la croissance ou la reproduction au dépend des mécanismes de défense (hypothèse d'«*evolution of increased competitive ability*»; EICA; Blossey and Notzold 1995).

D'autre part, la pression de propagule (ou effort d'introduction), qui correspond à une mesure du nombre et de la fréquence à laquelle des individus sont introduits dans le nouvel environnement, joue un rôle considérable dans le succès d'invasion (Lockwood *et al.* 2005; Simberloff 2009). En effet, même si les propagules introduites sont de petite taille, un apport fréquent de nouvelles propagules peut augmenter la diversité génétique globale des populations dans l'aire d'introduction. Cette augmentation est d'autant plus importante que le nombre de propagules est élevé et que les populations natives à l'origine de chacune de ces propagules sont génétiquement distinctes. Dans certains cas, la diversité génétique dans les populations introduites surpasse celle des populations de l'aire native (e.g. Kolbe *et al.* 2004). Cet accroissement de la diversité génétique peut permettre à la sélection d'agir plus efficacement et permettre ainsi des changements adaptatifs locaux. A cet égard de nombreuses études basées sur la comparaison de traits d'histoire de vie associés au potentiel de reproduction et/ou de dispersion ont illustré le fait que les populations introduites peuvent subir des changements adaptatifs importants leur permettant une propagation plus rapide dans le nouvel habitat envahi (e.g. Bohn *et al.* 2004; Phillips *et al.* 2006). L'apport de propagules issues de populations natives isolées génétiquement peut également conduire à de la vigueur hybride, c'est à dire à une augmentation de la valeur sélective dans la descendance (Hartl & Clark 1997). Cette vigueur hybride (ou hétérosis) peut résulter d'une atténuation d'un lourd fardeau mutationnel présent dans les lignées parentales (Keller & Waller 2002) ou d'éventuels effets épistatiques entre les génomes parentaux hérités dans la descendance hybride. Ce phénomène a été mis en évidence lors de l'invasion de l'escargot *Melanoides tuberculata* en

Martinique par plusieurs populations différenciées génétiquement (Facon *et al.* 2005; 2008). Au cours du processus d'invasion, des événements rares de reproduction sexuée entre ces lignées majoritairement parthénogénétiques ont produit de nouvelles lignées hybrides qui ont rapidement supplanté les lignées parentales. Le succès d'invasion et de domination des lignées hybrides est associé à une augmentation de la capacité de reproduction et du taux de croissance des individus hybrides résultant d'un effet d'hétérosis (Facon *et al.* 2005).

L'augmentation de la diversité génétique et/ou du nombre d'individus favorise considérablement le potentiel évolutif des populations établies mais ne représente pas une condition sine qua non pour assurer un succès d'invasion. Des études empiriques ont en effet montré que même des propagules de petite taille et caractérisées par une diversité génétique réduite peuvent s'établir, s'adapter et envahir avec succès de nouveaux habitats (e.g. Lindholm *et al.* 2005; Dlugosch & Parker 2008a). L'invasion du millepertuis des Canaries (*Hypericum canariense*) sur la côte Ouest des Etats-Unis et l'île Maui (Hawai) illustre bien ce phénomène (Dlugosh & Parker 2008a). Lors des événements d'introduction, ces populations ont subi une perte d'environ 50 % de la diversité génétique par rapport aux populations natives mais se sont malgré tout adaptées aux conditions environnementales locales. Cette capacité d'adaptation malgré une réduction de variabilité génétique peut résulter du fait qu'une baisse de la diversité génétique lors d'événements de goulots d'étranglement (associés aux événements de fondation) peut être associée à la conversion d'une partie de la variance non-additive (i.e. dominance; épistasie) en variance additive et ainsi temporairement augmenter la variance additive disponible dans la population (Goodnight 1988; Cheverud *et al.* 1999; Lindholm *et al.* 2005). D'autre part, des études théoriques suggèrent que des goulots d'étranglement d'intensité intermédiaire (i.e. réduction d'intensité moyenne de la taille de la population) sont susceptibles de conduire à la purge d'allèles délétères responsables des effets de la dépression de consanguinité (Glémin 2003). Une réduction du fardeau génétique permettrait aux petites populations de se maintenir malgré des événements de reproduction très probables entre individus apparentés. Dans le cadre des invasions biologiques, ce phénomène est fortement pressenti dans les populations envahissantes de la coccinelle asiatique *Harmonia axyridis* (Facon *et al.* 2011).

I.4. Le rôle de l'Homme

Un nombre considérable d'études ont montré que l'Homme joue un rôle important

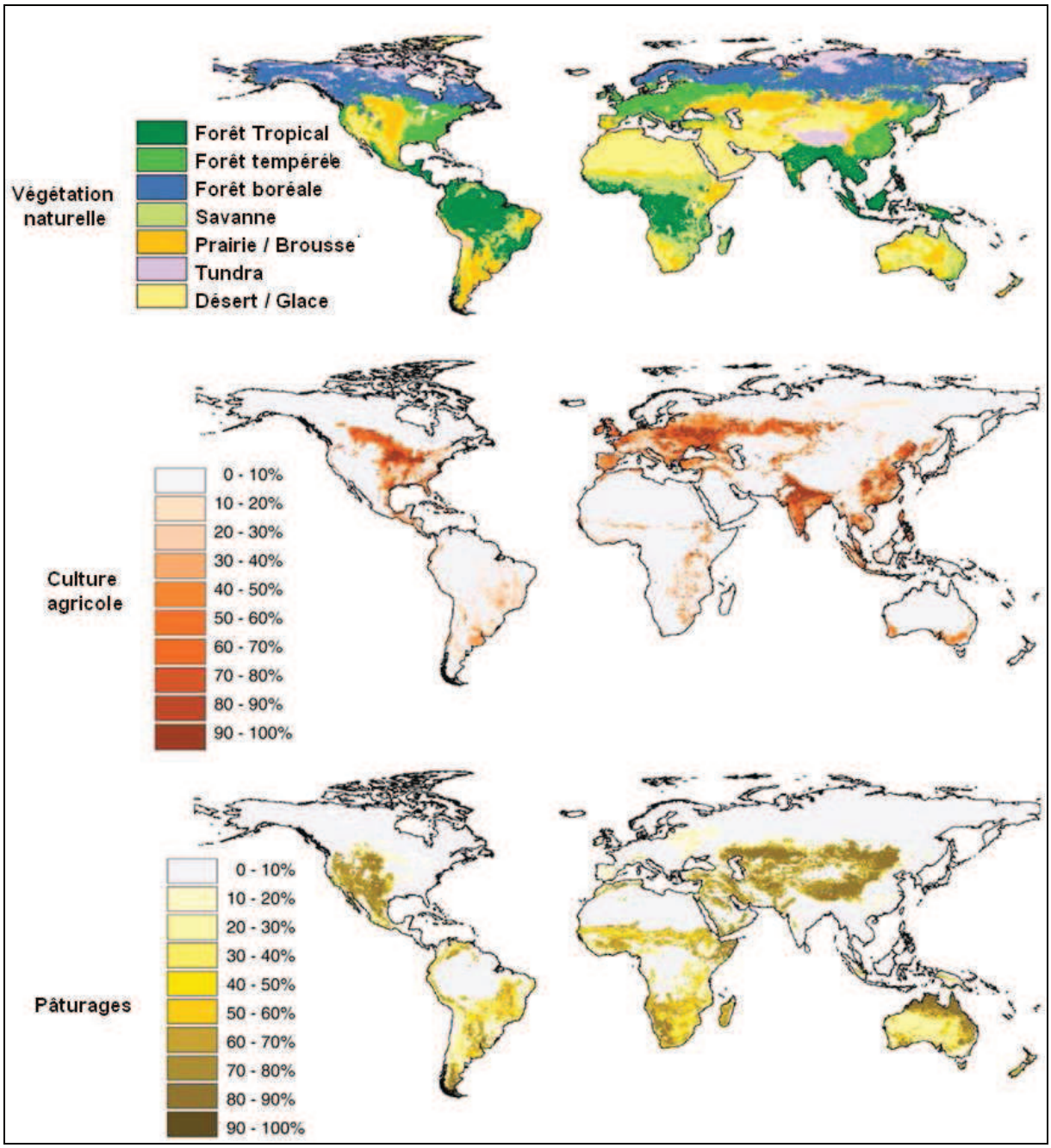


Figure I.1. : Utilisation des sols par les activités humaines (Figure tirée de Foley *et al.* 2005). L'utilisation accrue des sols participe à l'homogénéisation des habitats sur l'ensemble de la planète et à la création d'habitats riches en ressources et pauvres en biodiversité. Ces conditions réunies favorisent l'émergence, l'établissement et la propagation d'espèces envahissantes à l'échelle mondiale.

dans les invasions biologiques (e.g. Jeschke & Strayer 2005; Le prieur *et al.* 2008; Pysek *et al.* 2010) et ce, en intervenant sur les trois étapes du processus d'invasion. L'expansion des échanges commerciaux internationaux brise des barrières à la dispersion et permet ainsi à de nombreuses espèces d'envahir des milieux jusqu'alors inaccessibles (Jeschke & Strayer 2005). En effet, des études ont montré que le nombre d'espèces de vertébrés introduites

depuis le 15ème siècle, et en particulier depuis les 200 dernières années, en Europe et en Amérique du Nord est fortement corrélé à l'immigration humaine entre ces deux continents (Jeschke & Strayer 2005). A cet égard, pour certains auteurs, la terre connait actuellement un nouvel épisode de Pangée au cours duquel les continents sont connectés par des réseaux de transports de plus en plus intenses par lesquels de nombreuses espèces dispersent (McKinney 2005).

Les activités humaines, facilitent également l'établissement et la propagation des populations en modifiant considérablement les écosystèmes. Les habitats naturels sont remplacés peu à peu par des habitats anthropisés plus ou moins homogènes d'un point de vue environnemental dans lesquels la biodiversité est réduite et les ressources souvent en abondance (Williamson 1996; Foley *et al.* 2005; Figure I.1.). Les zones urbaines par exemple sont occupées par des espèces végétales constituées à 50 % d'espèces non locales (Pysek 1998). Dans le même ordre d'idée, la proportion d'espèces non locales de plantes, d'oiseaux, d'insectes ou de mammifères augmente des régions périphériques vers les villes (McKinney 2002). Ainsi, de nombreuses espèces sont vouées à disparaître du fait de la réduction de leurs habitats naturels par les activités humaine (i.e. espèces «perdantes») et à être remplacées par un petit nombre d'espèces qui prospèrent dans les environnements modifiés par l'homme (i.e. espèces «gagnantes»; McKinney & Lookwood 1999).

II. Le rôle des systèmes de reproduction dans le succès d'invasion des populations

II.1. L'uniparentalité et l'asexualité dans le cadre des invasions biologiques

Le système de reproduction est une caractéristique biologique qui influence grandement les paramètres génétiques intrinsèques des populations (e.g. effectif efficace, flux de gènes, partitionnement de la diversité génétique au sein des populations et entre populations; Barrett *et al.* 2008). Le rôle du système de reproduction dans le cadre des invasions biologiques a initialement été soulevé par Baker (1955), qui stipule que les plantes autofécondantes (i.e. uniparentales) ont un potentiel de colonisation supérieur aux plantes auto-incompatibles du fait de leur capacité à se reproduire seules et indépendamment d'agents pollinisateurs. Cet avantage qualifié d'assurance de reproduction est étendu par Baker lui-même à d'autres organismes du règne animal capable de se reproduire seuls par voie asexuée (Baker 1965). Les populations autofécondantes bénéficient en plus d'un taux de croissance deux fois plus

élevé que celui des populations sexuées contraintes de produire deux individus de sexe différent pour assurer la descendance (i.e. coût des mâles ; Maynard Smith 1976; Figure I.2.). Cette faculté, également attribuable aux populations asexuées peut cependant parfois être contrecarrée dans ces dernières par une réduction du nombre de descendants viables produits par rapport à leurs congénères sexuées (revue dans Lynch 1984).

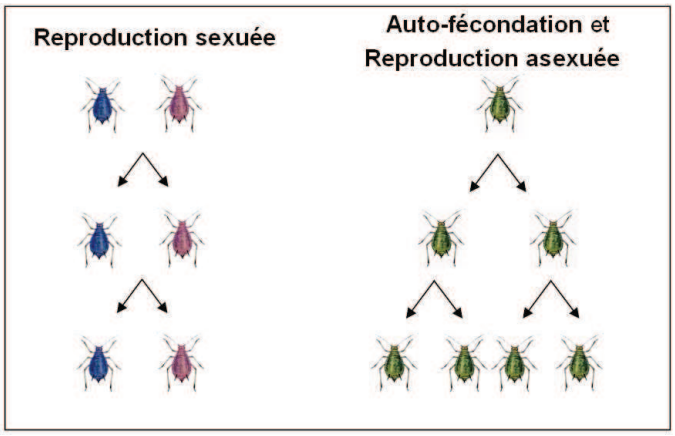


Figure I.2. Schéma du phénomène du coût de deux. Ce coût est associé à la production de mâles dans les populations sexuées. Chaque individu d'une lignée uniparentale ou asexuée se reproduit seul alors qu'il faut nécessairement deux individus pour produire au moins un descendant dans une lignée sexuée.

Les lignées asexuées présentent, par rapport aux lignées autofécondantes, d'autres avantages d'un point de vue évolutif. L'asexualité permet notamment l'évitement des effets de la dépression de consanguinité. Cette caractéristique présente un intérêt particulier dans le cadre des invasions biologiques lors desquelles les propagules initialement dispersées sont souvent de petite taille et de ce fait sensibles aux effets de la dépression de consanguinité. De plus, la transition d'un système de reproduction sexuée à un système asexué, qui implique une réduction de la recombinaison génétique, permet de fixer des combinaisons alléliques coadaptées, de convertir la variance non additive en variance additive (Nieman & Linksvayer 2006) et/ou dans le cas d'introductions multiples de propagules génétiquement distinctes, de fixer la vigueur hybride (Facon *et al.* 2005). La reproduction asexuée permet de maintenir une valeur sélective élevée et constante au fil du temps dans la descendance pour des conditions environnementales données. Cet avantage est d'autant plus important que les traits phénotypiques sélectionnés sont sous un déterminisme complexe (épistasie; Otto & Lenormand 2002) et que le milieu dans lequel la lignée asexuée a émergé est stable (Burger 1999). Enfin, plusieurs études indiquent que les lignées asexuées sont constituées de génotypes sélectionnés pour tolérer de larges gammes de conditions environnementales («general purpose hypothesis»; Baker 1965; Lynch 1984). En effet, la sélection agit sur l'ensemble du génome asexué et favorise les lignées asexuées ayant la valeur sélective maximale dans l'environnement contemporain. Lors de changements environnementaux dans

le milieu, la plupart des lignées sont vouées à l'extinction mais les rares lignées clonales capables de supporter des gammes de conditions environnementales importantes sont sélectionnées (Lynch 1984). L'absence de recombinaison dans ces lignées permet de maintenir les combinaisons génétiques intactes (aux événements de mutations prêts) et la tolérance à ces gammes environnementales est conservée dans la lignée. A l'inverse, une lignée sexuée à l'origine sélectionnée pour tolérer un spectre aussi large, produit quant à elle une descendance dont le spectre de tolérance sera en moyenne plus étroit. En effet, des individus sexués qui présentent des valeurs extrêmes pour des traits phénotypiques dont le déterminisme génétique est complexe, ont tendance à produire une descendance dont la valeur pour ces traits régresse vers la moyenne populationnelle (Falconner 1981). Dans le cadre des invasions biologiques, cette capacité à tolérer de larges gammes de conditions environnementales confère aux lignées asexuées un avantage conséquent pour s'établir mais aussi pour se propager dans le milieu d'accueil.

A long terme cependant, ces lignées asexuées souffrent d'un potentiel adaptatif limité qui les rend particulièrement sensibles aux fluctuations environnementales. En effet, alors que les populations sexuées peuvent mettre en commun des allèles favorables apparaissant dans des lignées différentes via les événements de recombinaison génétique, les lignées asexuées ne peuvent accumuler les allèles favorables que par mutations répétées au sein d'une même lignée et donc moins rapidement (Fisher 1930; Muller 1932; Figure I.3.). Cette différence notable entre les lignées sexuées et clonales est cependant moins évidente dans des populations de petite taille (Crow & Kimura 1965). Dans le cadre des invasions, lors desquelles les populations sont confrontées à de nouvelles conditions environnementales, ce potentiel adaptatif limité représente une véritable contrainte. L'absence de recombinaison entraine également l'accumulation irréversible d'allèles délétères dans les lignées asexuées sous l'effet du cliquet de Müller (Muller 1932 ; Figure I.4.). En effet, la perte par dérive de génotypes sains (i.e. dépourvus de mutations délétères) dans une lignée asexuée est irréversible puisque une fois éliminé, un génotype sain ne pourra réapparaitre que sous l'action de mutations inverses (extrêmement rare). Müller compare ce mécanisme à un cliquet qui avance d'un cran (sans retour en arrière possible) chaque fois que la classe des individus porteurs du nombre minimal de mutations délétères est éliminée sous l'effet de la dérive, entraînant une accumulation de mutations dans la population. L'effet du cliquet de Müller sur la valeur sélective moyenne de la population est particulièrement intense si chaque allèle a un effet indépendant sur la valeur sélective. Cependant un effet épistatique entre allèles délétères

peut limiter voir inverser l'effet de ce phénomène (Kondrashov 1994).

Du fait d'un potentiel évolutif réduit et de l'accumulation d'allèles délétères les lignées asexuées sont souvent considérées comme des culs-de-sac évolutifs (Lynch & Gabriel 1990). Ce constat est d'ailleurs illustré par le fait que les espèces asexuées sont le plus fréquemment sur les branches terminales des arbres phylogénétiques (Rice 2002), bien que de rares lignées asexuées semblent se maintenir depuis des millions d'années (Judson & Normark 1996).

II.2. L'asexualité sous diverses formes

L'asexualité peut être définie comme un processus reproductif aboutissant au développement d'un organisme ayant reçu du matériel génétique d'un seul sexe. D'un point de vue évolutif cependant, l'asexualité, sous cette définition englobe un nombre considérable de mode de reproduction dont les conséquences évolutives diffèrent de manière importante. Il convient donc de distinguer spécifiquement les différents cas d'asexualité. Cette distinction peut se faire à deux niveaux. Lorsque l'asexualité n'implique pas de cellules ou de tissus reproducteurs différenciés, on parle de *reproduction végétative*, lors de laquelle un individu se développe directement à partir d'un autre individu par bourgeonnement (i.e. mitose) sans qu'il y ait formation d'un embryon (de Meeus *et al.* 2007). Ce mode de reproduction ne concerne que les organismes dont les cellules sont totipotentes, c'est-à-dire capables de se différencier en tous les types cellulaires. A l'inverse de la reproduction végétative, la *parthénogenèse* (*Parthénos*: vierge; *Genesis*: naissance – développement) est un mode de reproduction assuré par des femelles vierges impliquant la production d'œufs ou de graines dans des tissus reproducteurs spécifiques. Dans la majorité des cas, la parthénogenèse est thélytoque, c'est à dire que la descendance est uniquement constituée de femelles.

On distingue deux types de parthénogenèse en fonction de l'implication ou non des deux phénomènes majeurs qui caractérisent la sexualité, c'est-à-dire la méiose et la syngamie. Cette distinction est primordiale d'un point de vue des conséquences évolutives qu'engendre chacun de ces systèmes de reproduction. Ainsi on distingue la parthénogenèse apomictique (i.e. sans méiose ni syngamie) de la parthénogenèse automictique (avec méiose). La parthénogenèse apomictique, peut être considérée, au même titre que la reproduction végétative, comme de l'asexualité «vraie». En effet dans ces deux cas, la descendance

produite présente un génome strictement identique au génome parental (aux événements de mutations prêts). La seule différence entre ces deux modes de reproduction est la présence ou non de tissus reproducteurs.

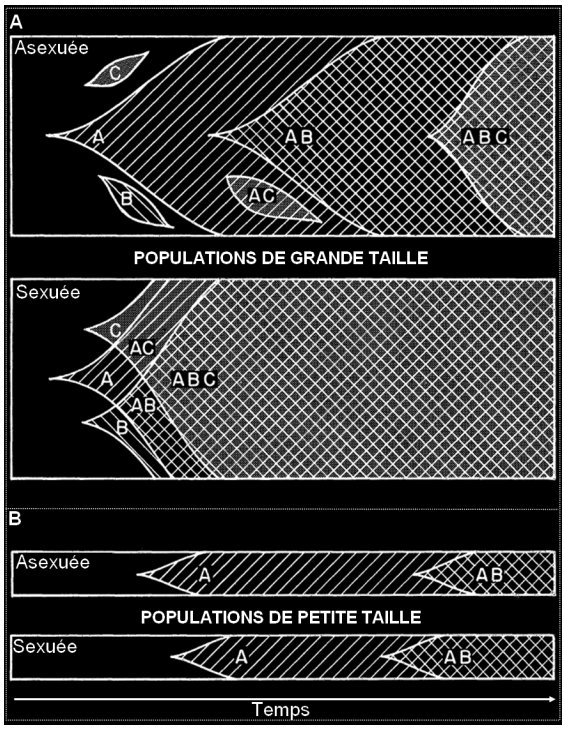


Figure I.3. Modèle de Fisher-Müller. Ce modèle montre l'effet de la recombinaison sur l'évolution de la fréquence de nouvelles mutations émergeant dans des populations asexuées et sexuées dans des populations A) de grande taille et B) de petite taille (Figure tirée de Crow et Kimura 1965).

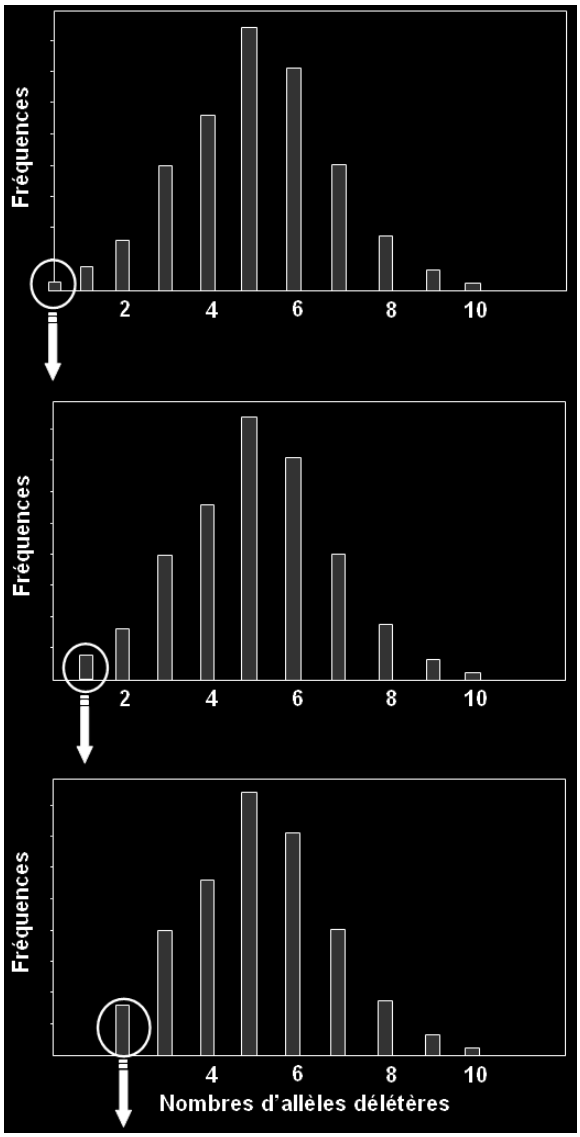


Figure I.4. **Illustration du phénomène du cliquet de Müller**. A chaque cliquet, le génotype possédant le moins d'allèles délétères est irréversiblement perdu. Le nombre d'allèles délétères augmente ainsi au fur et à mesure dans la population.

Contrairement à l'asexualité vraie, la parthénogenèse automictique, implique une méiose aboutissant à la production de gamètes dont le nombre de chromosomes est réduit et implique de ce fait une étape de re-ploïdisation de la cellule œuf. Il existe plusieurs mécanismes cytoplasmiques de parthénogenèse automictique, chacun impliquant des conséquences évolutives particulières (Suomalainen *et al.* 1987; Figure I.5.). D'une manière générale, bien que l'ensemble du génome de la descendance soit issu d'un seul parent, ce type de parthénogenèse aboutit à une descendance dont le génome diffère de celui du parent du fait des événements de recombinaison intra-chromosomique (i.e. *crossing-over*) liés à la méiose et à l'étape de re-ploïdisation. Ces mécanismes entrainent une perte partielle ou totale de l'hétérozygotie dans la descendance par rapport au génome maternel. D'un point de vue évolutif, l'augmentation de l'homozygotie au sein de la descendance peut entrainer l'expression d'allèles récessifs délétères dont l'effet est masqué par des allèles dominants «sains» dans le génome parental mais qui se retrouvent à l'état homozygote dans la descendance. Cet effet s'apparente aux effets de la dépression de consanguinité et réduit la valeur sélective de la descendance. Il faut noter l'existence d'un mécanisme particulier de parthénogenèse automictique qui n'entraine pas de perte d'hétérozygotie indépendamment du taux de recombinaison. Le doublement préméiotique est un mécanisme qui implique un doublement des chromosomes par endomitose avant la méiose. Pendant la première division de la méiose, les chromosomes s'apparient avec leurs homologues qui sont génétiquement identiques, ce qui élimine l'effet de la recombinaison au cours de l'appariement. Il en résulte quatre cellules filles diploïdes toutes identiques à la cellule mère aux événements de mutations prêts (Terhivuo & Saura 2006, Lutes *et al.* 2010).

Dans certains organismes, les mâles peuvent aussi être produits par parthénogenèse dans ce cas appelée parthénogenèse arrhénotoque. Ce type de parthénogenèse est associé au système de reproduction haplo-diploïde dans lequel, les femelles diploïdes sont généralement produites par sexualité alors que les mâles se développent à partir d'un œuf non fécondé (pathénogénétique) et sont haploïdes. Ce mode de reproduction est notamment répandu dans plusieurs ordres d'insectes tels que les hyménoptères, les hémiptères, les coléoptères ou encore les thysanoptères (Normark 2003).

D'autres modes de reproduction sont considérés comme des systèmes asexués mais se distinguent des autres formes précédemment décrites, par le fait qu'ils requièrent l'implication de deux partenaires sexuels. En effet la gynogenèse est un mode de reproduction au cours

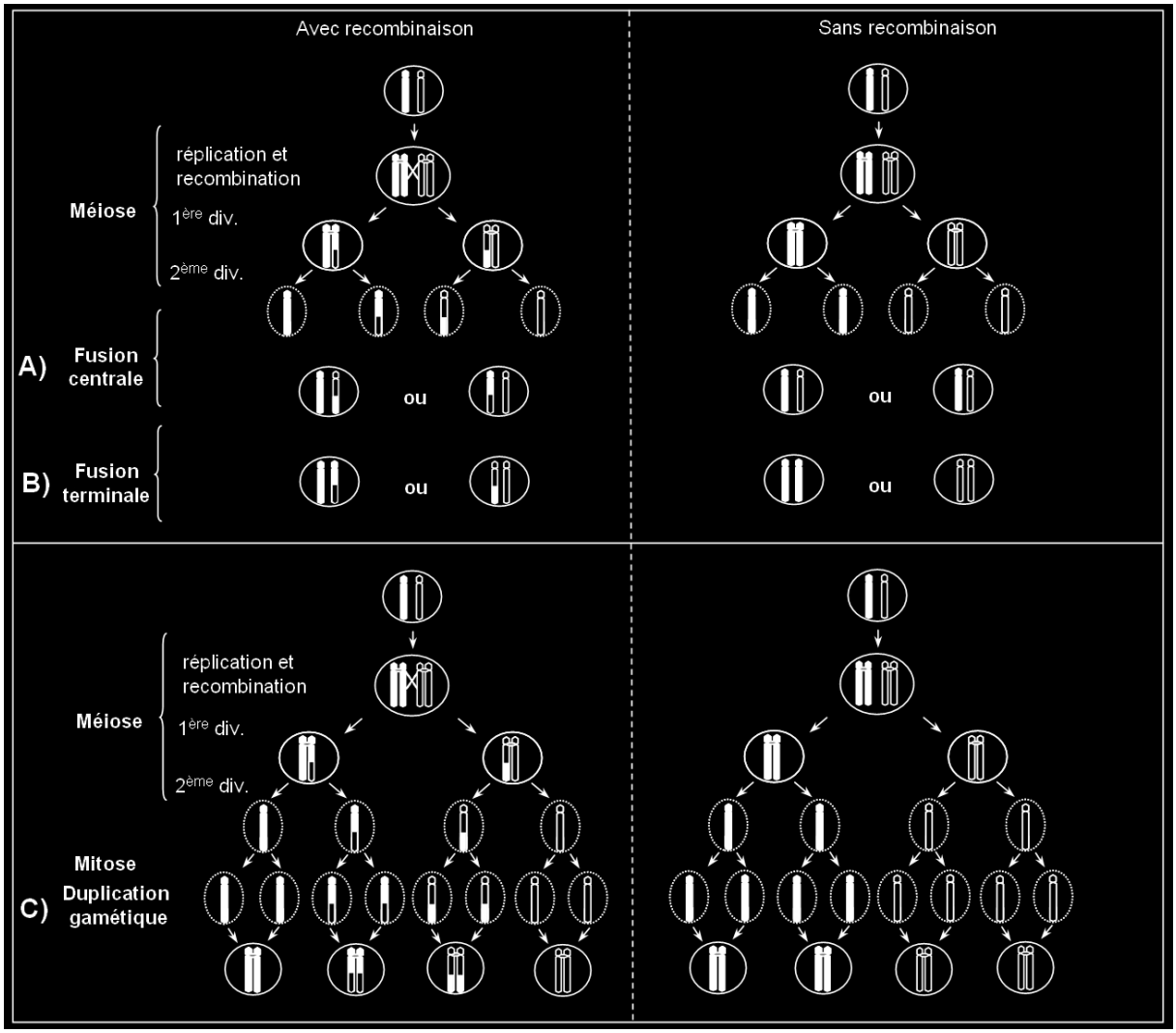


Figure I.5. Schéma des différents modes de parthénogenèse automictique et effet de la recombinaison intrachromosomique (crossing-overs) sur la descendance parthénogène.
A) La fusion centrale est une fusion non aléatoire entre deux oocytes haploïdes qui maintient l'ensemble de l'hétérozygotie dans le génome de la descendance pour les gènes n'étant pas affectés par la recombinaison. L'hétérozygotie est maintenue partiellement ou totalement en fonction du taux de recombinaison. B) La fusion terminale est une fusion non aléatoire entre deux oocytes qui résulte en la production d'un zygote partiellement ou totalement homozygote en fonction du taux de recombinaison. C) La duplication gamétique est un phénomène post-méiotique au cours duquel les gamètes sont dupliqués par mitose et fusionnent entre eux. La descendance est constituée uniquement de génotypes homozygotes.

duquel la descendance est issue d'oocytes diploïdes n'ayant pas subi de méiose et peut être de ce fait considéré comme un mode «d'asexualité vraie». Cependant le développement de ces oocytes requiert une stimulation par un spermatozoïde (Hughes 1989). L'hybridogenèse est un autre mode de reproduction impliquant un deuxième partenaire. Lors de l'oogenèse qui se déroule sans événements de recombinaison, les chromosomes issus de l'un des deux parents (toujours le même) sont perdus. Une partie du génome correspondant aux chromosomes de

l'autre parent se transmet donc de manière clonale de génération en génération. De part ce mécanisme particulier, ce mode de reproduction est également appelé hemiclonalité. L'hybridogènese et la gynogenèse sont deux modes de reproduction qui ont été décrits dans des complexes d'hybrides et notamment chez des espèces de poissons et d'amphibiens (Schultz 1969; Lampert et Scharlt 2010).

II-3. Origine de la parthénogenèse

La transition de la sexualité vers l'asexualité peut avoir trois origines. Premièrement, une transition spontanée vers l'asexualité peut provenir d'événements de mutations sur des gènes impliqués dans la reproduction sexuée (Simon *et al.* 2003). Ces mutations peuvent conduire à l'émergence de lignées asexuées facultatives ou obligatoires. A cet égard, certains auteurs ont suggéré que la transition la plus simple de la sexualité vers l'asexualité implique la conservation des mécanismes liés à la méiose. Ainsi la parthénogenèse automictique serait le mode asexué vers lequel la transition est la plus simple (Bell 1982; Schwander & Crespi 2009). Il faut cependant noter que certains organismes, comme les rotifères ou les pucerons, ont un cycle de vie qui alterne entre une phase de reproduction sexuée et une phase de reproduction parthénogénétique (i.e. apomixie) en fonction des conditions environnementales (Simon *et al.* 2002). Il est probable que des lignées apomictiques obligatoires émergent de ces lignées cycliques du fait que les mécanismes sous-jacents de l'apomixie soient déjà présents dans ces organismes (Maynard Smith 1978).

L'asexualité peut également émerger d'événements d'hybridation interspécifique tant dans le règne végétal (Prentis *et al.* 2008) que dans le règne animal (Judson & Normark 1996). Dans ce cas, le passage à l'asexualité est généralement associé à un changement d'état de ploïdie pouvant conférer d'autres avantages, notamment une augmentation de l'hétérozygotie et une réduction de la dépression de consanguinité (Soltis & Soltis 2000; Lee *et al.* 2002; Prentis *et al.* 2008).

L'asexualité peut finalement être induite par des parasites de reproduction (Huigens *et al.* 2000). Parmi ces parasites, *Wolbachia* est probablement le plus connu et le plus répandu (Jeyaprakash & Hoy 2000; Werren & Windsor 2000). Cette bactérie endosymbiontique, transmise majoritairement verticalement (i.e. d'une génération à l'autre), est capable de manipuler le système de reproduction de ses hôtes sous divers mécanismes pour augmenter sa

propre transmission (Breeuwer 1997; Rousset *et al.* 1992; Hurst *et al.* 1999; Stouthamer *et al.* 1999). L'un de ces mécanismes est l'induction de la parthénogenèse augmentant ainsi la production de femelles au dépend des mâles qui constituent un cul-de-sac évolutif pour les bactéries endosymbiontiques. *Wolbachia* induit la parthénogenèse particulièrement dans les organismes haplo-diploïdes en induisant la duplication gamétique post-méiotique (Stouthamer 1994; Figure I.5.). Adashi-Hagimori *et al.* (2008) ont récemment démontré que *Rickettsia*, une autre bactérie qui se comporte en parasite de reproduction, induit également la parthénogenèse des femelles de *Neochrysocharis formosa*, un hyménoptère, par un mécanisme qui est similaire à la parthénogenèse apomictique et permet donc de maintenir l'hétérozygotie parentale dans la descendance.

Comme nous l'avons vu, l'asexualité au sens strict du terme (i.e. apomixie fonctionnelle) peut conférer un certain avantage, au moins à court terme, aux populations envahissantes notamment du fait de leur importante capacité d'établissement (i.e. assurance de reproduction, croissance rapide et évitement de la dépression de consanguinité). Ces populations sont cependant vouées à une extinction plus ou moins rapide du fait de leur faible potentiel adaptatif et du fait qu'elle soient contraintes d'accumuler les mutations délétères sans possibilité de les purger. Cependant, l'asexualité peut avoir plusieurs origines et existe sous diverses formes pouvant mener à des conséquences évolutives très différentes desquelles vont dépendre leurs rôles potentiels dans le succès d'invasion des espèces. Il est de ce fait primordial d'étudier les systèmes de reproduction des populations envahissantes pour comprendre leur implication potentielle dans le succès d'invasion.

III. Principaux scénarios éco-évolutifs d'invasions

Lors d'une invasion, on considère généralement que les changements adaptatifs associés au succès d'invasion ont lieu lors de l'établissement des propagules dans le nouvel habitat colonisé (Sakai *et al.* 2001; Cox 2004). Des exemples spectaculaires illustrent ces adaptations rapides dans le milieu nouvellement envahi (e.g. Yeh 2004; Phillips *et al.* 2006). Cependant les changements adaptatifs lors des invasions sont susceptibles d'avoir lieu à n'importe quelle étape du processus d'invasion. L'étude des scénarios adaptatifs associés aux succès d'invasion des populations peut de ce fait, s'avérer complexe. Cette section a pour but de formaliser trois scénarios d'invasions principaux, en fonction de l'endroit et du moment où se déroulent les changements adaptatifs clefs lors du processus d'invasion. Ces scénarios ne

sont cependant pas mutuellement exclusifs. Différentes populations d'une même espèce peuvent à priori suivre des scénarios différents et/ou intermédiaires entre ces trois scénarios proposés. Ces scénarios sont schématisés dans la figure I.6.

III.1. Scénario 1 : Scénario d'invasion avec adaptations indépendantes post-introduction

Considérons dans un premier temps, le type de scénario le plus communément évoqué, qui implique des adaptations post-introduction dans les localités envahies. Dans ce cas, les changements adaptatifs ont lieu indépendamment dans les différentes zones envahies (voir Figure I.6-1). Un exemple illustrant ce scénario est l'invasion du millepertuis des Canaries (*Hypericum canariense*) dans trois sites d'invasion indépendamment, sur la côte Ouest des Etats-Unis et sur l'île Maui (Hawai) au cours des 50 dernières années (Dlugosch & Parker 2008a; Encadré I.1.). Lors des événements d'introduction, ces populations ont subi une perte d'environ 50 % de la diversité génétique par rapport aux populations natives. Cette importante réduction de diversité génétique, n'a pas empêché ces populations de s'adapter indépendamment aux conditions environnementales locales. En effet, les auteurs ont montré que la phénologie de floraison des populations sur les différents sites d'introduction est en accord avec le gradient latitudinal sur lequel ces populations se trouvent. Ce résultat indique clairement que les populations de *H. canariense* se sont adaptées aux variations saisonnières locales indépendamment les unes des autres. Un autre exemple illustrant très bien ce scénario concerne le copépode *Eurytemora affinis* (Lee *et al.* 1999; 2011). Cette espèce euryhaline, à l'origine établie dans les estuaires marins ou les marais salins, s'est établie à plusieurs reprises indépendamment dans des habitats d'eau douce en Europe, en Amérique du Nord et en Asie. A chaque introduction, les populations ont subi des adaptations parallèles à l'eau douce. Cette adaptation a d'ailleurs pu être observée en laboratoire sous sélection artificielle à partir de lignées océaniques en quelques générations (Lee *et al.* 2011).

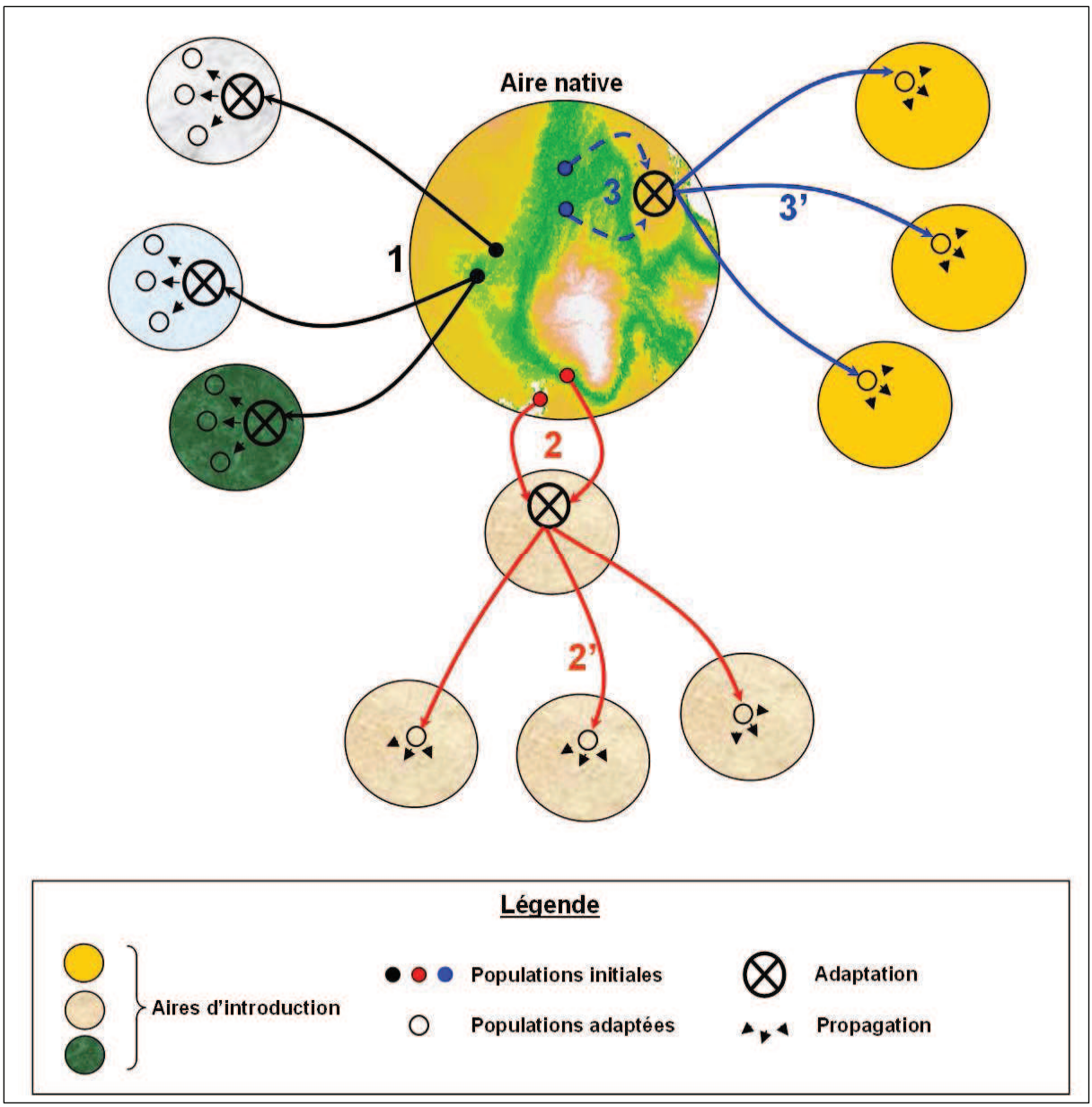
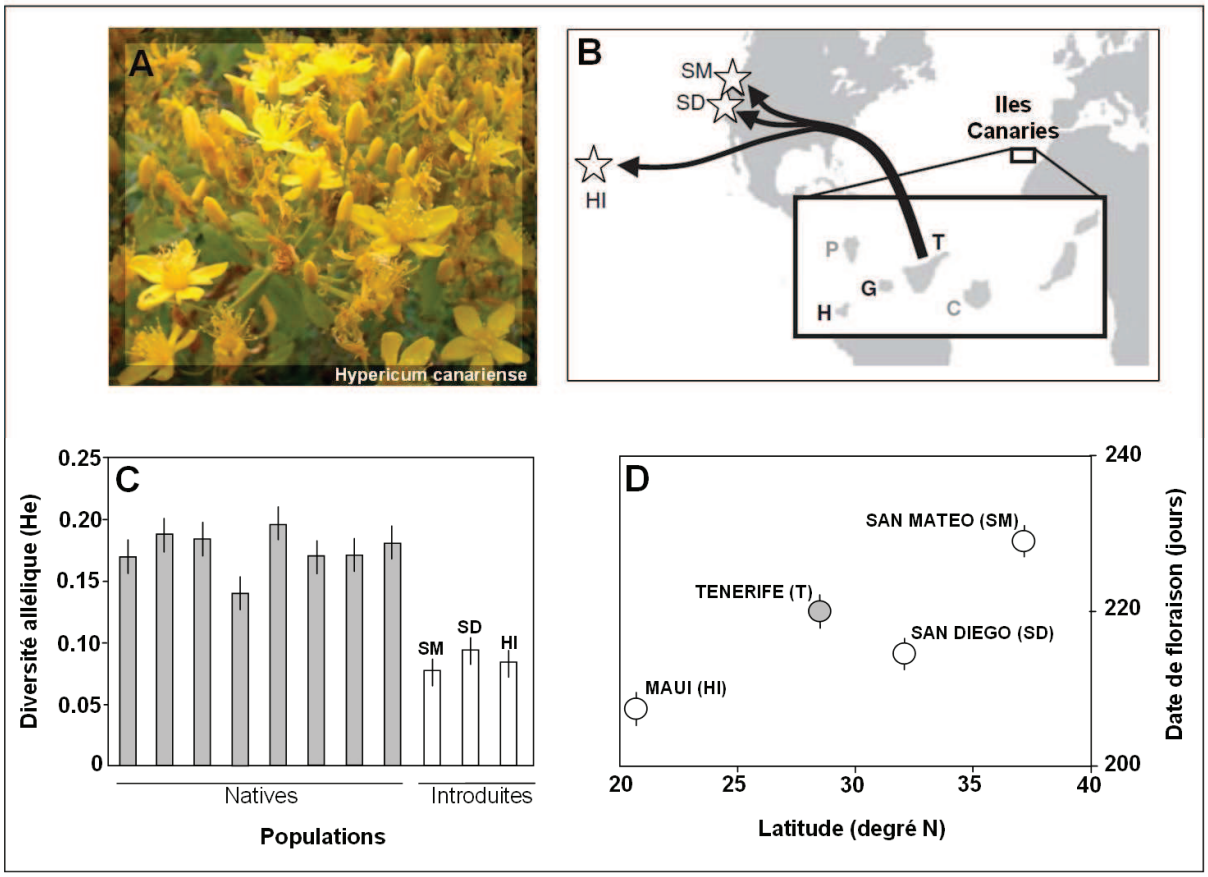


Figure I.6. Représentation schématique des trois scénarios éco-évolutifs des invasions biologiques. 1) Scénario d'invasion avec adaptations indépendantes post-introduction. Ce scénario implique que les éventuels changements adaptatifs associés au succès d'invasion des populations ont lieu dans chaque localité de manière indépendante. 2) Scénario d'invasion avec adaptation unique dans un population tête de pont lors duquel un seul événement adaptatif a lieu après introduction dans la population invasive «tête de pont». Ce changement adaptatif permet alors à des propagules «pré-adaptées» de disperser de la tête de pont vers d'autres localités présentant les mêmes pressions de sélection (2'). 3) Le scénario d'invasion avec adaptation pré-introduction est caractérisé par des changements adaptatifs ayant lieu au sein de l'aire native dans des habitats marginaux suivi d'événements de migration longues distances à partir de ces habitats marginaux (3').

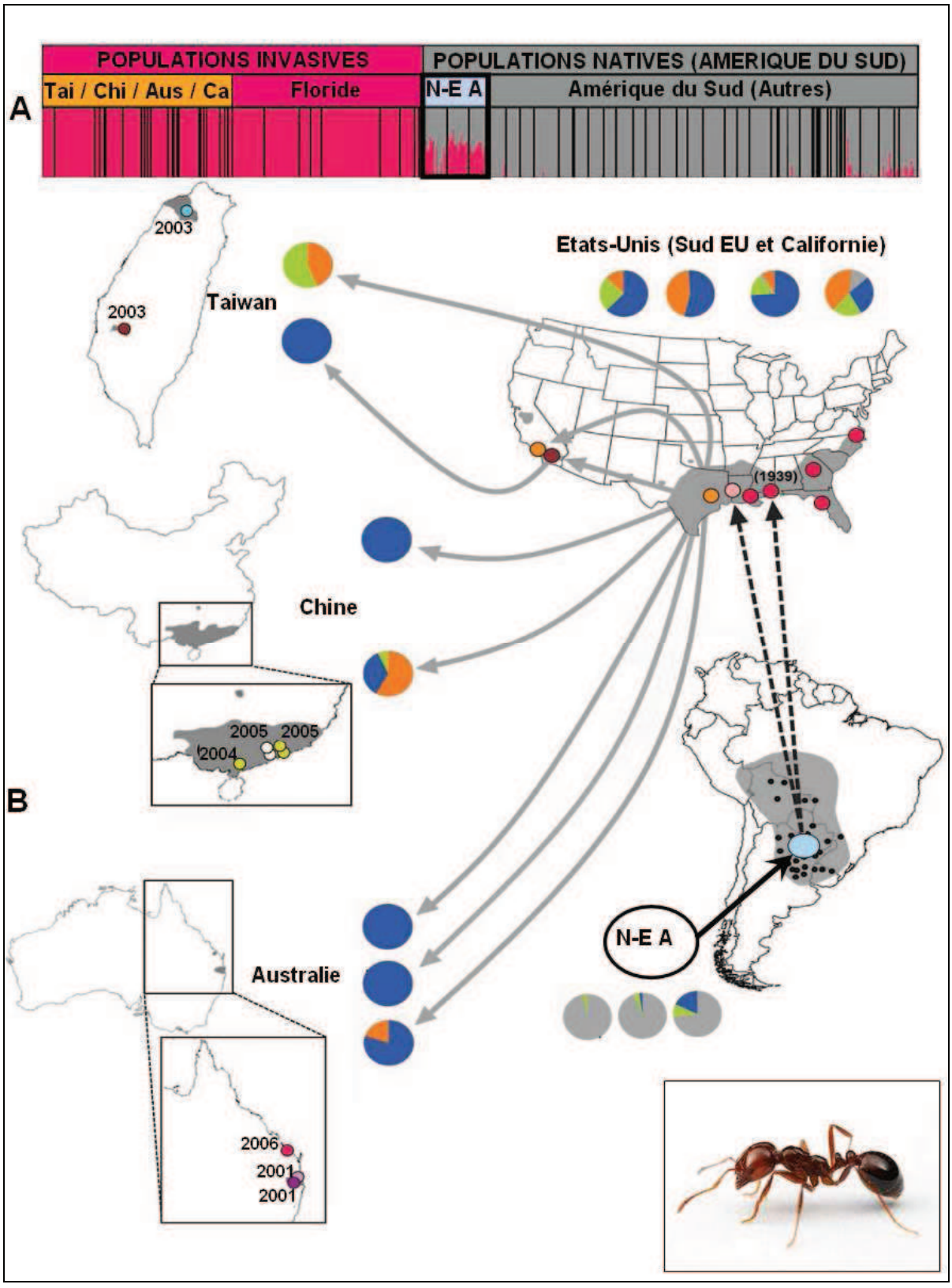


Encadré I.1. Scénario 1 : Le cas de l'invasion du millepertuis des Canaries (Figure tirée de Dlugosch *et al.* 2008a; 2008b). Le millepertuis des Canaries, *Hypericum canariense* (A), est endémique des îles Canaries. Cette plante est capable de disperser par bourgeonnement et de s'autoféconder. Cependant la majorité de la variation génétique au sein de l'aire native se distribue au sein des populations ce qui suggère que les croisements entre individus sont communs. Cette plante ornementale à été introduite indépendamment mais en petit nombre dans des plantations privées dans deux localités de Californie et sur l'archipel d'Hawai sur l'île Maui où elle s'est propagée très rapidement au cours des 50 dernières années (B). Les événements d'introduction ont été accompagnés de goulots d'étranglements intenses réduisant la diversité allélique de 50% dans les populations introduites par rapport aux populations natives (C). Malgré cette réduction de diversité génétique, les populations ont connu des changements adaptatifs en réponse aux conditions environnementales locales. En particulier, des expériences en environnement commun menées sur le Campus de l'Université de Californie à Santa Cruz (Etats-Unis) on montré que la date de floraison suit le gradient latitudinal des localités dans lesquelles les différentes populations ont été introduites (D). Ainsi, à partir d'une population source, des propagules se sont établies et se sont adaptées indépendamment dans chaque localité. Cet exemple illustre le scénario d'invasion avec adaptations indépendantes post-introduction (voir Fig I.6-1 et texte pour plus de détails).

III.2. Scénario 2 : Scénario d'invasion avec adaptation post-introduction unique dans une population «tête-de-pont»

L'intensification des échanges commerciaux entre populations humaines offre aux espèces des opportunités croissantes de sortir de leurs aires natives vers des régions géographiques potentiellement très éloignées. En particulier, les échanges commerciaux étant organisés en réseaux, des populations s'établissant dans une localité peuvent à leur tour agir comme des populations sources à partir desquelles des propagules sont susceptibles de disperser vers d'autres localités appartenant au même réseau. Ce scénario, basé sur un changement de régime de dispersion à partir d'une localité appartenant à un réseau particulièrement développé, a historiquement été mis en évidence de manière théorique et empirique sur des espèces aquatiques profitant des eaux de ballastes des bateaux marchands (Drake & Lodge 2004 ; Floerl *et al.* 2005; 2009). Mais un tel scénario a également été décrit sur des espèces terrestres (e.g. Miller 2005; Ascunce *et al.* 2011; encadré 1.2).

En plus d'un changement de régime de dispersion, ce scénario peut également impliquer des événements évolutifs dans les populations établies dans les sites primaires d'invasions (i.e. sites «tête de pont») avant d'éventuels événements de dispersion vers d'autres localités du réseau. L'exemple de l'anole brun illustre particulièrement bien ce dernier scénario (Kolbe *et al.* 2004). Ce lézard est originaire de la région Caraïbes et a été introduit à plusieurs reprises en Floride au cours du 19ème siècle à partir de populations provenant de différentes îles Caribéennes. Des propagules ont ensuite bénéficié des activités commerciales internationales accrues en Floride pour disperser vers des localités éloignées telles que, Hawaï, Taiwan ou d'autres régions des Etats-Unis (Kolbe *et al.* 2004). Au cours de ce scénario, les populations de Floride ont bénéficié d'un apport génétique continu leur permettant d'acquérir une diversité génétique supérieure à celle des populations natives insulaires, sur laquelle la sélection a pu agir efficacement et ainsi potentiellement permettre des changements adaptatifs clés dans le succès d'invasion. A cet égard, Kolbe *et al.* (2004) ont notamment observé une augmentation de la taille du corps des individus des populations de Floride par rapport aux populations natives des îles Caribéennes. Le lien entre ces modifications morphologiques et la valeur sélective des individus des populations envahissantes n'a cependant pas été mis en évidence et l'implication de ce trait dans le succès d'invasion des populations de Floride reste de ce fait hypothétique.



Encadré I.2. Scénario 2 : Le cas de la fourmi de feu *Solenopsis invicta* (Figure tirée de Ascunce *et al.* 2011).

Encadré I.2. Scénario 2 : Le cas de la fourmi de feu *Solenopsis invicta* (suite)

La fourmi de feu, *S. invicta* (en médaillon), est une fourmi originaire d'Amérique du Sud qui a profité des bateaux marchands pour envahir le Sud des Etats-Unis au début du 20ème siècle et depuis peu d'autres localités géographiques, à savoir, plusieurs sites sur l'île de Taiwan, la Chine et l'Australie. A partir de marqueurs génétiques (66 microsatellites), les routes d'invasion de ces populations ont été retracées (Ascunce *et al.* 2011). Des analyses de réassignation génétique, ont montré que la plupart des individus génotypés se réassignent à l'un des deux groupes obtenus, l'un constitué des populations envahissantes (Sud des Etats-Unis, Californie, Taiwan, Chine et Australie); l'autre des populations de l'aire native (A). Seuls les individus de populations natives du Nord de l'Argentine (N-E A) présentent des génotypes pouvant se réassigner aux deux groupes. De plus les populations envahissantes sont caractérisées par quatre haplotypes principaux, absents dans la majorité des populations de l'aire native mais présents dans les populations N-E A (B). Les auteurs en ont conclu que les populations envahissantes ont suivi un scénario «tête-de-pont», dont la première étape a consisté en l'établissement d'une première population envahissante dans le Sud des Etats-Unis à partir de propagules issues d'une région de l'aire native (N-E A). La seconde étape correspond à la dispersion récente de propagules vers Taiwan, la Chine et l'Australie à partir de cette population tête-de-pont du Sud des Etats-Unis. Il est cependant important de noter que cette étude est uniquement basée sur des données génétiques. Elle ne permet donc pas de proposer un scénario adaptatif associé au succès de la population tête-de-pont. En d'autres termes, on ne peut pas savoir si le succès d'invasion de la population de la tête-de-pont (Sud des Etats-Unis) s'explique principalement par un changement de régime de dispersion ou s'il y a eu au sein de cette population des changements adaptatifs favorisant l'établissement dans les localités lointaines.

Récemment, ce scénario éco-évolutif d'invasion a été formalisé et nommé «tête-de-pont» (Lombaert *et al.* 2010; Guillemaud *et al.* 2010), en référence à un dispositif militaire servant principalement à établir un périmètre à l'intérieur duquel l'armée peut manœuvrer dans le but d'augmenter ultérieurement le territoire conquis. Lombaert *et al.* (2010) ont montré que la coccinelle asiatique, originaire d'Asie a probablement suivi ce scénario en s'établissant dans une tête-de-pont localisée dans le Nord-Est de l'Amérique du Nord avant de disperser et de s'établir en Amérique du Sud, en Europe et en Afrique du Sud à partir de la tête-de-pont. Une étude montre par ailleurs que les populations introduites dans Nord-Est de l'Amérique du Nord mais aussi en Amérique du Sud, en Europe et en Afrique ont subi une purge d'allèles délétères leur permettant d'éviter les effets de dépression de consanguinité (Facon *et al.* 2011). Cette étude suggère que cette purge a probablement eu lieu une seule fois dans la population tête-de-pont l'aire primaire d'invasion et que ce changement évolutif ainsi qu'un changement de régime de dispersion ont pu faciliter l'établissement de certaines propagules vers des localités lointaines.

D'un point de vu évolutif, le scénario d'invasion avec adaptation post-introduction unique dans une population tête-de-pont est plus parcimonieux que le scénario d'adaptations indépendantes post-introduction car il minimise le nombre d'événements de changements évolutifs nécessaire aux populations envahissantes pour s'établir dans un nouvel environnement (FIGURE I.6-2). En effet, contrairement au scénario impliquant des changements évolutifs indépendants post-introductions dans chaque localité envahie, les changements évolutifs clés dans le succès d'invasion au cours du scénario tête-de-pont n'ont lieu qu'une seule fois dans la tête de pont avant dispersion vers d'autres localités présentant potentiellement des conditions environnementales semblables à celles du premier site d'invasion. Ce scénario n'exclut pas le fait que d'autres changements adaptatifs peuvent également avoir lieu localement dans les sites d'invasions secondaires.

III. 3. Scénario 3 : Scénario d'invasion avec adaptation pré-introduction

De plus en plus d'études montrent que les espèces envahissantes sont composées de populations fortement structurées et différenciées au sein de leur aire native et qu'une partie seulement de ces populations ont la capacité de devenir envahissantes (Lee & Gelembiuk 2008 et références citées). Des changements évolutifs semblent pouvoir avoir lieu dans des populations établies dans des habitats marginaux de l'aire native avant que des propagules issues de ces populations ne soient dispersées et ne s'établissent dans des localités géographiques éloignées présentant les mêmes pressions de sélection (FIGURE I.6-3). Les populations du séneçon du cap (*Scenecio inaequidens*) originaires d'Afrique du Sud, et établies en Europe centrale illustrent potentiellement ce scénario (Bossdorf *et al.* 2008). En effet, ces auteurs suggèrent que le changement adaptatif ayant permis l'augmentation du rapport racine/tige (permettant aux plantes de mieux capitaliser sur les ressources nutritives disponibles) dans les populations introduites a probablement eu lieu dans des régions montagneuses de l'aire native avant la dispersion de propagules «pré-adaptées» vers l'Europe. Cependant, la relation génétique entre les populations d'Europe et des régions montagneuses d'Afrique du Sud n'a pas été vérifiée. Ce scénario reste de ce fait hypothétique.

D'un point de vue évolutif, plusieurs arguments supportent l'occurrence d'invasions via un scénario avec adaptation pré-introduction. Les changements évolutifs associés aux populations des habitats marginaux des aires natives ont fait l'objet de nombreuses études (e.g. Kawecki 2000; 2008; Sexton *et al.* 2009). Les populations qui s'établissent dans ces

milieux sont souvent considérées comme des populations « puits » vouées à l'extinction et uniquement maintenue par un flux de migrants issus des habitats naturels avoisinants. Paradoxalement, ce flux de migrant permanent entraine un flux d'allèles bénéfiques dans l'habitat naturel mais « maladaptés » aux conditions marginales, effaçant ainsi l'effet potentiel d'allèles adaptés au nouveau milieu ayant émergés dans les populations marginales (i.e. «genetic swamping»; Kaweki 2004; Blondel 2006; Bridle & Vines 2007). Cependant, sous certaines conditions et notamment dans le cas d'un flux de gène limité des populations naturelles vers les populations marginales ou dans le cas de flux de gènes symétriques entre les populations marginales et naturelles, des événements adaptatifs peuvent avoir lieu permettant ainsi aux populations de se maintenir dans les milieux marginaux. Ces deux dernières situations aboutissent à des résultats différents. En effet dans le premier cas (i.e. flux de gène limité des populations naturelles vers les populations marginales), les populations marginales peuvent s'adapter localement et ainsi devenir « spécialistes » des nouvelles conditions environnementales (Kawecki 2000; Kawecki 2008). Les populations adaptées aux habitats marginaux ne sont alors plus adaptées aux conditions environnementales naturelles. Dans le deuxième cas (i.e. flux de gènes symétriques entre les populations marginales et naturelles), les allèles adaptés aux nouvelles conditions apparaissant dans les populations des habitats marginaux dispersent vers les populations naturelles ce qui amène à deux conséquences principales. D'une part, une partie des migrants issus des populations naturelles qui dispersent vers les habitats marginaux possèdent déjà ces allèles adaptés ce qui limite l'effet du «genetic swamping» (Kawecki & Holt 2002; Lenormand 2002). D'autre part, les populations des habitats marginaux, mais également des milieux naturels adjacents, s'adaptent à une gamme environnementale comprenant les conditions des habitats marginaux et celles des milieux naturels adjacents (à condition que les allèles apparus dans les habitats marginaux ne soient pas délétères dans le milieu naturel). On assiste donc à l'émergence de populations « généralistes » adaptées à une gamme environnementale supérieure à celle tolérée par les populations naturelles d'origine.

Les habitats marginaux semblent par ailleurs propices à l'émergence et à la propagation de lignées asexuées (Vandel 1928; Haag & Ebert 2004), ces dernières présentant, comme nous l'avons vu dans la section «II. Le rôle des systèmes de reproduction dans le succès d'invasion des populations», des capacités d'invasions supérieures à leurs congénères sexuées. En effet, dans la plupart des espèces de plantes et d'animaux qui présentent des lignées asexuées, celles-ci ont tendance à être localisées dans les habitats marginaux vers des

latitudes ou altitudes élevées, des habitats perturbés ou extrêmes, par rapport à leurs congénères sexuées (Vandel 1928). Ce patron spatial, appelé parthénogenèse géographique est expliqué par plusieurs facteurs (Backer 1965; Haag & Ebert 2004). i) Les lignées asexuées peuvent plus facilement coloniser ces habitats recevant peu de migrants du fait de leur assurance de reproduction. ii) Les lignées asexuées, isolées génétiquement de leurs congénères sexuées sont imperméables à d'éventuels flux de gènes «maladaptés» issus de l'habitat principal. iii) La réduction des interactions biotiques, observée au moins dans certains de ces habitats marginaux, est favorable au maintien de ces lignées. iv) Les populations dans ces habitats marginaux se comportent en métapopulations où les sites marginaux sont voués à des épisodes récurrents d'extinction et de colonisation. Or, lors des événements de colonisation, les lignées asexuées ne sont pas affectées par des effets fondateurs.

IV. Modèle d'étude : la petite fourmi de feu *Wasmannia auropunctata*

L'étude des processus évolutifs associés aux invasions biologiques requiert une quantité d'informations importante sur les espèces modèles. La petite fourmi de feu figure parmi les espèces envahissantes les plus prospères (Lowe *et al.* 2000) et de ce fait, a attiré l'attention de nombreux chercheurs. De nombreuses études ont notamment permis de mettre en évidence un polymorphisme original de son système de reproduction qui semble étroitement lié au succès d'invasion des populations. D'autre part, cette espèce a récemment fait l'objet d'études approfondies dans son aire d'introduction mais également au sein de son aire native. Cette section consiste à présenter les principales caractéristiques de cette espèce.

IV.1. Aire native de *Wasmannia auropunctata*

Le genre *Wasmannia* est endémique de la région néotropicale et ne comprend que dix espèces mais est largement surreprésenté écologiquement par la petite fourmi de feu, *Wasmannia auropunctata* (Longino & Fernandez 2007; Figure 1.7.). L'aire native de cette espèce s'étend du Mexique à l'Argentine. Bien que le plus souvent établies dans les plaines tropicales recouvertes par la forêt primaire, des populations ont été trouvées jusqu'à 3000m d'altitude en Equateur (M. Leponce, communication personnelle). La biologie de cette espèce au sein de son aire native est relativement bien documentée ce qui procure un avantage certain pour étudier les processus éco-évolutifs ayant lieu dans les populations envahissantes.

Figure I.7. Photo d'un nid de *W. auropunctata* en laboratoire. Les nids sont polygynes (i.e. plusieurs reines), chaque reine (5–6 mm) ayant été fécondée par un seul mâle (i.e. espèce monoandre). Les gynes (i.e. reines non fécondées) et les mâles sont ailés. Les ouvrières (1–2 mm) sont stériles. Des larves et plusieurs stades de nymphes (i.e. individus en développement) sont visibles sur la photo.

W. auropunctata fait partie des espèces sur lesquelles l'impact des activités humaines a considérablement modifié la biologie de certaines populations. Ainsi on peut distinguer deux types de populations en fonction du type d'habitat qu'elles occupent. Certaines populations sont confinées dans les zones de forêt primaire régulièrement perturbées par des facteurs naturels et en particulier dans les plaines forestières inondables (Figure I.8-A). Ces populations sont caractérisées par de faibles densités de nids dans lesquels la densité d'ouvrières est également faible (Orivel *et al.* 2009). Ces populations présentent un système de reproduction sexuée classique des hyménoptères haplo-diploïdes: les femelles, c'est-à-dire les reines et les ouvrières (stériles) sont diploïdes et sont produites par reproduction sexuée. Les mâles se développent à partir d'œufs non fécondés par parthénogenèse arrhénotoque et sont donc haploïdes (Figure I.9-A). Les populations caractérisées par un tel système de reproduction seront qualifiées de «sexuées» au cours de ce document.

Des populations ont régulièrement émergé de ces populations sexuées pour coloniser principalement des habitats profondément perturbés notamment par les activités humaines (e.g. carrières, forêts secondaires, bords de route, plantations; Foucaud *et al.* 2007a; 2009; Figure I.8-B). Ces habitats sont caractérisés par des conditions biotiques et abiotiques

différentes de celles des habitats naturels de forêt primaire (Orivel *et al.* 2009). En particulier, les amplitudes thermiques et hydriques sont plus importantes au cours de la journée. Dans ces milieux, les populations atteignent des densités beaucoup plus importantes et les nids concentrent une densité d'ouvrières et de couvains importante. La présence de ces populations est également associée à une réduction de la richesse spécifique de fourmis (Orivel *et al.* 2009). Il est cependant difficile de distinguer entre une dominance locale de *W. auropunctata* due au déplacement d'autres espèces ou due au fait que la plupart des autres espèces de fourmis n'occupent pas ces habitats. Du fait de cette forte densité de nids et d'individus et du fait que peu d'autres espèces cohabitent, ces populations sont considérées comme envahissantes dans ces milieux particuliers de l'aire native (Orivel *et al.* 2009). Dans ces populations, un système de reproduction particulièrement fascinant à été identifié (Figure I.9-B). En effet, alors que les ouvrières sont produites par reproduction sexuée classique entre les reines et les mâles, les reines (diploïdes) produisent les nouvelles gynes par parthénogenèse thélytoque et les mâles (haploïdes) transmettent également leur génome à leurs fils par clonalité. Ainsi, mâles et reines se comportent comme deux lignées évolutives indépendantes entre lesquelles il n'y a pas de transfert de génome nucléaire (Fournier *et al.* 2005; Foucaud *et al.* 2007a). Seules les ouvrières héritent des deux génomes parentaux. Les femelles et les mâles des couples de clones sont caractérisés par des génotypes très différents. En conséquence, les ouvrières de ces lignées ont en moyenne un niveau d'hétérozygotie plus élevé que les ouvrières des populations sexuées (Foucaud *et al.* 2007a). Ces populations seront appelées clonales dans le reste de ce document. Ce système de reproduction n'est cependant pas sans faille. En effet, Foucaud *et al.* (2006) ont montré dans certaines populations clonales en milieu naturel que de rares événements de sexualité avaient abouti à la production de gynes. De même ces auteurs ont identifié la présence de rares mâles issus de la parthénogenèse arrhénotoque de certaines reines clonales.

Ces deux types de populations qui se distinguent par le système de reproduction des reproducteurs et par les conditions environnementales des habitats qu'elles occupent, partagent cependant de nombreuses caractéristiques en commun. Cette espèce est généraliste tant au niveau des sites de nidifications qu'au niveau alimentaire. En effet, les individus s'agglutinent dans des refuges improvisés (e.g. écorce d'un arbre, branche creuse) directement au sol ou dans la végétation épiphyte. Ce comportement de nidification «vagabond» permet de garantir aux ouvrières de fournir aux reines et au couvain les conditions de température et d'humidité optimales et de se rapprocher des ressources disponibles. Leur nourriture est

principalement constituée d'arthropodes (Clark 1982), de nectars floraux ainsi que des exudats d'homoptères (cochenilles ; Delabie 1994). A cet égard, il semble que les populations clonales établies dans les milieux anthropisés (et notamment dans les plantations) profitent d'une abondance de ces hémiptères avec lesquels elles forment des associations mutualistes (Delabie *et al.* 1994; 2001).

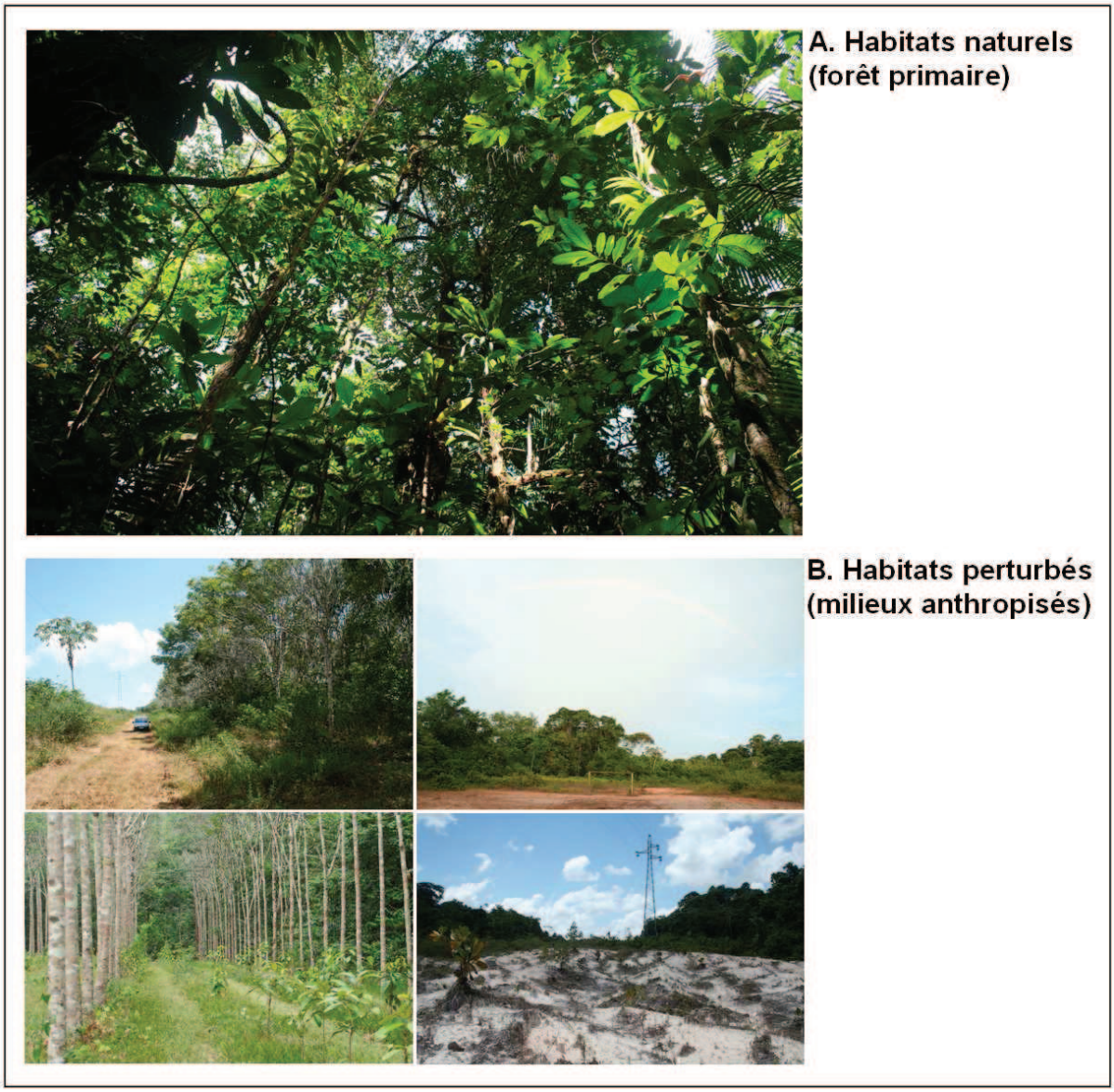


Figure I.8. Habitats classiques dans lesquels les populations de *W. auropunctata* sont observées dans l'aire native. Des populations caractérisées par de faibles densités de nids et d'ouvrières habitent les forêts primaires (A). Les reproducteurs des populations se reproduisent selon un schéma haplo-diploïde classique dans lequel les femelles (ouvrières et reines) sont produites sexuellement et les mâles sont issus de la parthénogenèse arrhénotoque. Des populations caractérisées par de fortes densités de nids et d'ouvrières habitent des habitats perturbés notamment par les activités humaines (B). Les reproducteurs sont caractérisés par un système de reproduction clonal particulier (détails dans le texte; voir également la Figure I.8.).

De plus, quelques soient les populations, les nids sont la plupart du temps polygynes (i.e. plusieurs reines dans un même nid) et organisés en supercolonies (Foucaud *et al.* 2009), lesquelles sont fortement structurées génétiquement. Cette structuration entre supercolonies tend à être plus prononcée dans les populations clonales ce qui suggère encore moins de flux de gènes entre les supercolonies clonales. Cette structuration s'explique en partie par le fait que la fondation de nouveaux nids au sein d'une supercolonie s'effectue par bourgeonnement. Une fois les reines fécondées à l'intérieur du nid, elles quittent le nid avec un cortège d'ouvrières par voie du sol. Les vols nuptiaux dans cette espèce sont souvent considérés comme inexistants ou inefficaces bien que certains individus reproducteurs volants aient été aperçus épisodiquement en milieu naturel (Torres *et al.* 2001; J.C.H Delabie communication personnelle). L'absence de vols nuptiaux efficace est supportée par le fait que les reines sont incapables de fonder une nouvelle population sans l'aide des ouvrières (Ulloa-Chacon 1990).

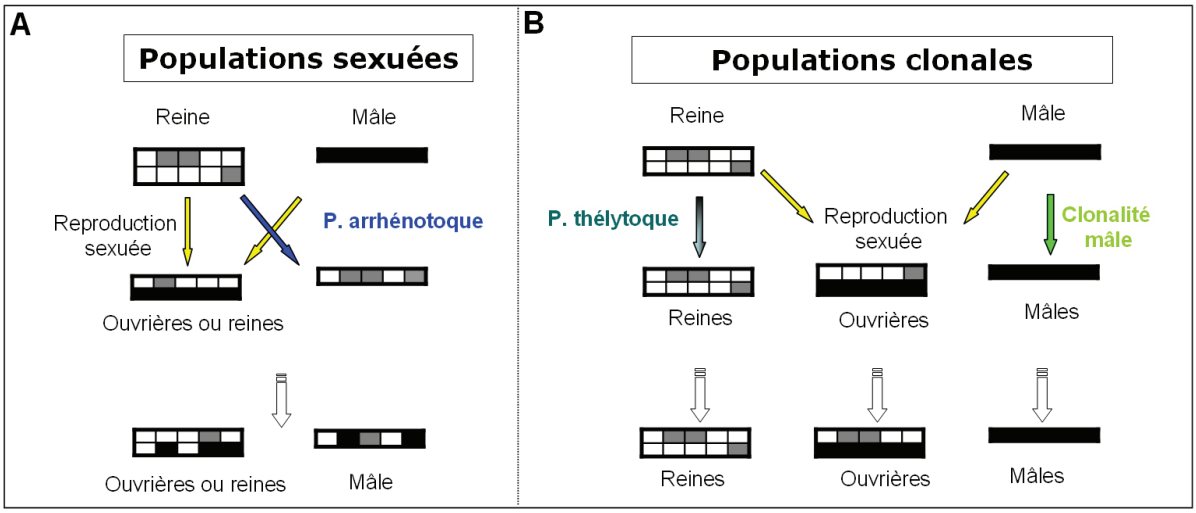


Figure I.9. Polymorphisme du système de reproduction de *W. auropunctata*. Certaines populations sont caractérisées par un système de reproduction classique des hyménoptères haplo-diploïdes (A). Dans ces populations, appelées ici «sexuées», les femelles, c'est-à-dire les gynes et les ouvrières (stériles) sont produites sexuellement et les mâles se développent à partir d'œufs non fécondés par parthénogenèse arrhénotoque. D'autres populations sont caractérisées par un système particulier de reproduction clonale (B). Dans ces populations, appelées ici « clonales», les gynes sont produites par parthénogenèse thélytoque et les mâles sont produits par androgenèse par l'intermédiaire des œufs produits par les reines. Les ouvrières (stériles) sont elles produites sexuellement.

IV. 2. Aire d'invasion de *Wasmannia auropunctata*

Au cours du XXème siècle, la petite fourmi de feu a été dispersée par l'homme et a envahi la quasi-totalité de la zone tropicale (Wetterer & Porter 2003; Foucaud *et al.* 2010; Figure I.10.). Cette espèce s'est également établie à l'extérieur de la zone tropicale, une première fois, en Floride en 1924 (région subtropicale) et plus récemment, dans le bassin Méditerranéen en Israël en 1998 (Figure I.10.). L'impact de *W. auropunctata* sur les écosystèmes envahis et sur les activités humaines lui a valu un classement parmi les « pires » espèces envahissantes (Lowe *et al.* 2000). En effet, les études effectuées sur les populations établies dans les différentes localités, en Afrique (Walker 2006; NDoutoume *et al.* 2007), sur les îles du Pacifique (Lazaro *et al.* 2000) ou en Israël (Vonshak *et al.* 2009), montrent toutes que cette espèce déplace les espèces de fourmis locales et peut même avoir des impacts sur d'autres espèces d'arthropodes ainsi que sur des vertébrés (Vonshak *et al.* 2009).

Quelque soit la zone envahie, les populations introduites sont associées aux activités humaines et sont globalement similaires aux populations dominantes de l'aire native du point de vue de leurs traits démographiques, de leur système de reproduction et de leur structure sociale (Foucaud *et al.* 2010). Les propagules introduites sont toujours constituées d'un unique couple de génotypes clonaux mâle et femelle, très différents l'un de l'autre, ce qui se traduit chez les ouvrières produites sexuellement par des individus au niveau d'hétérozygotie élevé. Les routes d'introduction ont en partie été retracées récemment à partir de marqueurs microsatellites (Foucaud *et al.* 2010; Figure I.10.). A la vue de leurs résultats, ces auteurs ont suggéré que les populations envahissantes suivent un scénario d'invasion avec adaptation pré-introduction, le changement de système de reproduction ayant lieu dans les milieux anthropisés de l'aire native avant que des propagules issues de ces populations ne soient dispersées vers d'autres habitats anthropisés dans des localités géographiques éloignées de l'aire native.

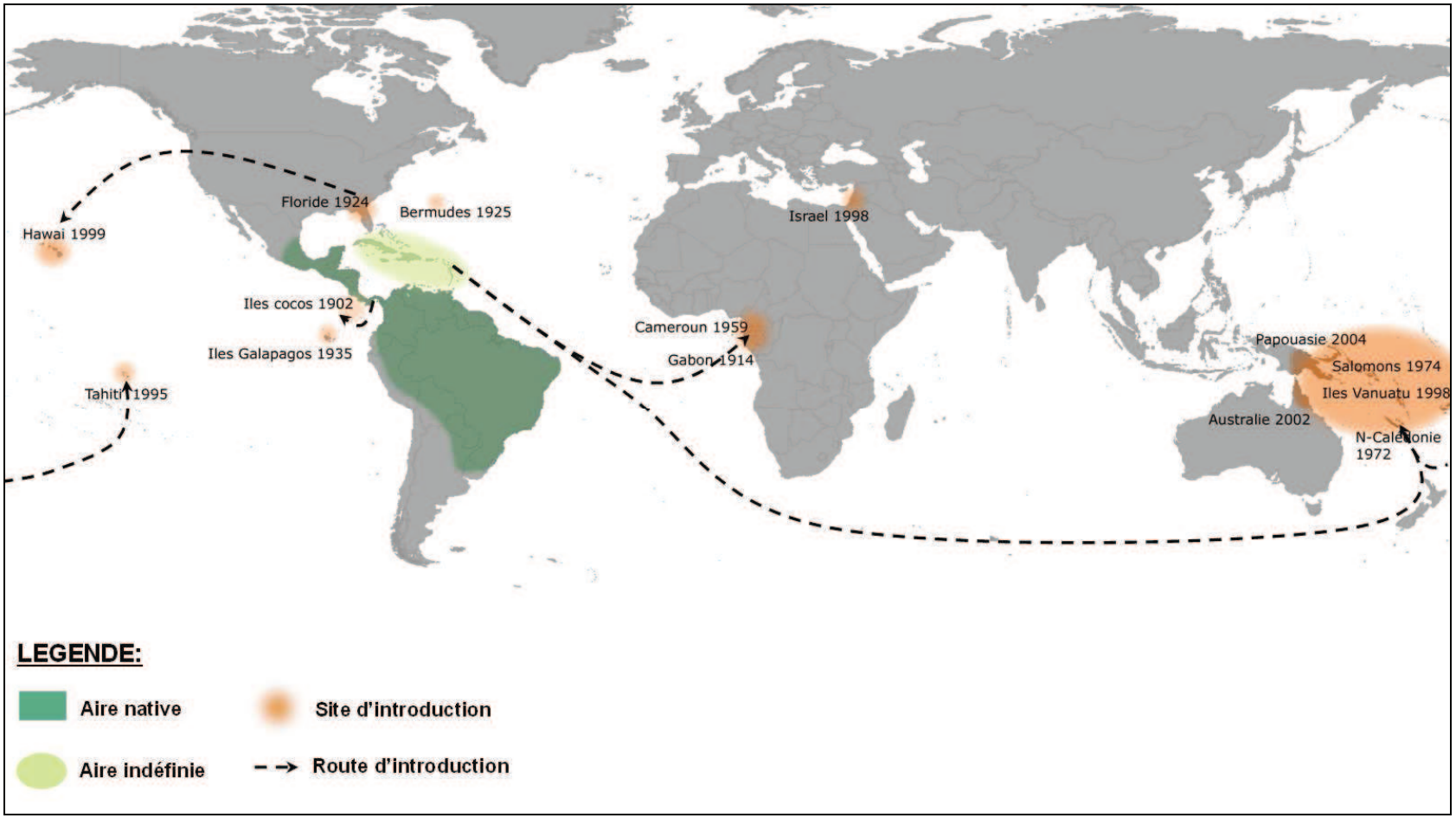


Figure I.10. Carte de distribution de la petite fourmi de feu *W. auropunctata*, routes d'invasions et dates d'introductions. Native de la région néotropicale (région verte foncée), *W. auropunctata* a dispersé au cours du XX^{ème} siècle et s'est établie dans plusieurs localités de la ceinture tropicale, en région subtropicale (Floride) et récemment en région Méditerranéenne (Israel). Les routes d'introduction ont partiellement été reconstruites à partir de marqueurs microsatellites (Foucaud *et al.* 2010). Le statut natif ou introduit de la zone Caraïbe (en vert clair) n'est pour l'instant pas clairement déterminé.

V. Principaux objectifs et articulation de la thèse

La petite fourmi de feu présente un polymorphisme de système de reproduction qui semble étroitement lié à des changements tant au niveau des habitats occupés qu'au niveau du statut écologique des populations (dominance et statut envahissant). En effet, les populations sexuées occupent majoritairement les fôrets tropicales primaires. A l'inverse, les populations clonales de l'aire native occupent principalement des habitats anthropisés. Enfin, toutes les populations envahissantes (clonales) sont établies dans des habitats dont les caractéristiques environnementales diffèrent, parfois de manière considérable dans le cas des populations établies en Israël, de l'habitat naturel ancestral de l'espèce (i.e. forêts tropicales primaires).

L'origine et les mécanismes du système de reproduction clonal de *W. auropunctata* sont encore mal connus et son rôle dans le succès d'invasion est encore flou. Ce système de reproduction ne confère aucun avantage démographique aux lignées clonales par rapport aux lignées sexuées. En effet la présence d'ouvrières est indispensable pour la fondation et le maintien des populations. Or celles-ci sont produites sexuellement ce qui implique que les mâles sont essentiels dans ces lignées asexuées. Il est donc vraisemblable que le système de reproduction clonal confère des avantages d'ordre évolutif. Comme nous l'avons vu précédemment, les conséquences évolutives de l'asexualité dépendent en grande partie des mécanismes sous-jacents. La compréhension du rôle du système de reproduction clonal dans le succès d'invasion des populations de *W. auropunctata* implique donc l'étude approfondie des mécanismes de ce système. D'autre part, l'invasion des populations de *W. auropunctata* étant étroitement liée à des changements de milieu importants, il est également important de tester si ce changement de système de reproduction de la sexualité vers la clonalité est accompagné de changements adaptatifs en réponse aux nouvelles conditions environnementales des milieux envahis. Enfin il est également intéressant de déterminer les conditions qui favorisent l'émergence de tels changements évolutifs et notamment de déterminer où ont lieu ces changements au cours du processus d'invasion.

Cette thèse s'articule donc autour de deux axes principaux constituant les deux chapitres suivants. Le premier est consacré à l'étude approfondie du système de reproduction caractérisant les populations clonales afin d'en déterminer son origine et les mécanismes sous-jacents et d'en apprécier les conséquences évolutives. Le second est consacré à l'étude des changements adaptatifs associés au succès d'invasion des populations clonales et aux

scénarios éco-évolutifs d'invasion suivis par les populations envahissantes tropicales d'une part, et par celles établies dans le bassin méditerranéen d'autre part. Enfin, l'ensemble des résultats obtenus dans cette thèse est discuté d'une manière globale dans un troisième chapitre.

CHAPITRE II

MÉCANISMES ET ENJEUX EVOLUTIFS DU SYSTÈME DE REPRODUCTION CLONAL DE *W. AUROPUNCTATA*

I. Introduction

Le but de ce chapitre est de mieux comprendre l'origine et les mécanismes des systèmes de reproduction des populations clonales de *W. auropunctata* afin d'en apprécier les enjeux évolutifs et leurs rôles dans le succès invasif de ces populations. Ce chapitre s'articule autour de trois articles scientifiques qui sont présentés brièvement dans une première section.

Nous avons vu dans le chapitre précédent que l'asexualité au sens large du terme, peut être issue d'événements d'hybridation entre des entités génétiques distinctes (Prentis *et al.* 2008; Judson & Normark 1996) ou d'avoir une origine infectieuse (Huigens et al. 2000) ou spontanée (Simons *et al.* 2003). Dans le cas de *W. auropunctata*, Foucaud *et al.* (2007a) ont trouvé des indications permettant d'écarter raisonnablement l'hypothèse d'une origine hybride. En effet, ces auteurs ont trouvé des niveaux d'hétérozygotie et des différences de tailles alléliques à des loci microsatellites similaires entre les reines des populations clonales et sexuées ce qui indique que les reines clonales ne sont pas issues d'événements d'hybridation entre des lignées génétiquement distantes. D'autre part, certains résultats de thèse de Julien Foucaud (2007b) suggèrent que *Wolbachia*, l'un des parasites de reproduction les plus repandus, et notamment connu pour induire la parthénogenèse chez les hyménoptères, ne joue pas de rôle primordial dans le déterminisme du système de reproduction des individus des populations clonales. Cependant ce dernier point n'a pas été formellement démontré et la présence et l'implication d'autres parasites de reproduction n'ont pas été vérifiées. Le premier objectif de ce chapitre est donc de vérifier la non-implication de cinq parasites de reproduction parmi les plus répandus, *Wolbachia*, *Cardinium*, *Rickettsia*, *Arsenophorus* et *Spiroplasma*, dans le polymorphisme de système de reproduction des populations de *W. auropunctata*. Cette étude fait l'objet d'un premier article scientifique en préparation (Article 1) présenté à la suite de cette section. Nos résultats montrent que seule la bactérie *Wolbachia* est présente dans des populations de *W. auropunctata*. *Wolbachia* est notamment présente dans les populations sexuées alors que les populations clonales sont majoritairement saines. Ainsi, aucun des parasites de reproduction ne semble induire le mode de reproduction asexuée chez *W. auropunctata*. Nous suggérons que le patron d'infection observé résulte d'une différence écologique entre les différents types de populations (sexuées et clonales).

Le deuxième objectif de ce chapitre est d'identifier les mécanismes sous-jacents aux modes de reproduction des femelles et des mâles des populations clonales. En ce qui concerne

la parthénogenèse femelle, les études menées jusqu'à présent n'ont pas permis de préciser les mécanismes cytologiques qui en sont responsables. Plus particulièrement, les patrons de transmission du génome observés dans les lignées femelles suggèrent un mécanisme qui, d'un point de vue fonctionnel, s'apparente, à première vue, à la parthénogenèse apomictique (i.e. sans méiose) permettant de conserver en grande partie l'hétérozygotie dans la descendance (Fournier *et al.* 2005; Foucaud *et al.* 2007a). En effet ces auteurs ont mis en évidence, à partir de 12 marqueurs microsatellites, que la majorité de l'hétérozygotie est conservée dans la lignée parthénogénétique femelle. Cependant ces mêmes auteurs ont également identifié des transitions de l'hétérozygotie à l'homozygotie ponctuelles et rares. Plusieurs mécanismes peuvent aboutir à ce résultat. Il est notamment difficile de distinguer la parthénogenèse apomictique associée à des événements de mutations et/ou de conversion génique, de la parthénogenèse automictique à fusion centrale associée à une forte réduction du taux de recombinaison génétique (voir Figure I.5. du chapitre précédent). Théoriquement, une manière élégante de distinguer ces deux mécanismes est de vérifier à l'aide de techniques cytogénétiques, la présence ou non d'événements de méiose lors de la production d'œufs parthénogénétiques. Ces méthodes se basent traditionnellement sur l'observation microscopique de gamètes dans des ovaires matures disséqués de femelles parthénogénétiques (Verma & Ruttner 1983). Cependant, dans le cas de *W. auropunctata*, de telles approches ne sont pas envisageables car la très grande majorité des gamètes produits par les reines parthénogénétiques sont haploïdes et utilisés pour la production d'ouvrières via la reproduction sexuée, et la production d'œufs parthénogénétiques pour la production de gynes est beaucoup moins fréquente et surtout imprévisible. Nous avons donc opté pour une solution indirecte basée sur l'étude des ouvrières produites sexuellement en testant les hypothèses suivantes. Sous un mécanisme d'automixie à fusion centrale, c'est-à-dire impliquant la méiose, associé à une réduction du taux de recombinaison, les oocytes haploïdes destinés à être fécondés pour la production d'ouvrières sont susceptibles d'être issus du même processus méiotique que les oocytes destinés à fusionner pour se développer en gynes parthénogénétiques. Dans ce cas, une réduction du taux de recombinaison est attendue au niveau du génome maternel hérité dans la descendance ouvrière des lignées clonales, relativement au taux de recombinaison mesuré dans une descendance ouvrière d'une lignée sexuée. Au contraire, sous l'hypothèse d'un mécanisme apomictique, deux processus indépendants sont alors impliqués, l'un impliquant la méiose pour la production d'oocytes destinés à être fécondés pour la production d'ouvrières et l'autre impliquant la mitose pour la production d'oocytes diploïdes pour la production de gynes apomictiques. Dans ce cas, on

s'attend à ce que le taux de recombinaison chez les reines clonales estimé à partir du génome hérité maternellement de leurs ouvrières ne soit pas différent de celui des reines sexuées estimé à partir de leurs ouvrières. D'un point de vue évolutif, ces deux systèmes de reproduction n'ont pas les mêmes conséquences. En effet, la parthénogenèse automictique, même associée à un mécanisme de réduction du taux de recombinaison méiotique entraine, tôt ou tard, une augmentation de l'homozygotie dans le génotype parthénogénétique. Cela peut conduire à l'expression d'allèles délétères récessifs jusqu'alors silencieux sous l'effet d'un allèle sain dominant et de ce fait induire une réduction de la valeur sélective au même titre que la dépression de consanguinité. D'autre part une baisse de recombinaison associée à un mécanisme d'automixie, qui affecte également la descendance ouvrière produite sexuellement, permet la transmission de complexes d'allèles co-adaptés au sein d'un même chromosome dans la descendance ouvrière. Il est intéressant de noter que la distinction entre les deux mécanismes peut également nous renseigner sur la fréquence d'émergence de la parthénogenèse. En effet, l'apomixie est un mécanisme cytoplasmique qui requiert des changements évolutifs *a priori* complexes ce qui impliquerait que l'émergence de la parthénogenèse chez *W. auropunctata* est un phénomène plutôt rare. Au contraire, l'automixie, du fait de la conservation des mécanismes méiotiques, est considérée comme étant la transition de la sexualité vers l'asexualité la plus simple à mettre en œuvre (Schwander & Crespi 2009). Dans ce cas, il est probable que la parthénogenèse puisse apparaître régulièrement dans les populations de *W. auropunctata*. Cette étude a fait l'objet d'un article scientifique paru en 2011 (Article 2). Nos résultats indiquent que les reines des populations clonales produisent une descendance de gynes (i.e. reines non fécondées) par parthénogenèse automictique à fusion centrale associée à une réduction du taux de recombinaison méiotique. Cette réduction du taux de recombinaison affecte également les ouvrières produites sexuellement.

Enfin ; l'étude de l'androgenèse des mâles est primordiale pour comprendre les enjeux évolutifs du système de reproduction clonal de *W. auropunctata* dans son ensemble. L'androgenèse est définie comme étant la production d'une descendance ne contenant que le génome nucléaire du parent mâle. Ce mode de reproduction permet donc aux mâles d'utiliser un œuf d'une femelle sans que celle-ci ne contribue au génome de la descendance. De ce fait, des auteurs considèrent l'androgenèse comme étant un phénomène de parasitisme des œufs de la part des mâles (Hedtke *et al.* 2008). Ce mécanisme est le principal mode de reproduction pour un faible nombre d'espèces appartenant à des groupes taxonomiques très différents. Ce

phénomène a été observé dans des populations naturelles de quatre espèces de praires d'eau douce hermaphrodites du genre *Corbicula* (Komaru *et al.* 1998; Byrne *et al.* 2000; Qiu *et al.* 2001), dans un complexe d'hybrides de phasme du genre *Bascillus* et dans le règne végétal, chez le cyprès hermaphrodite du sahara *Cupressus dupreziana* (Pichot *et al.* 2001). Récemment, ce phénomène a également été identifié dans des populations de deux espèces de fourmis, chez la petite fourmi de feu, *W. auropunctata* (Fournier *et al.* 2005) et plus récemment chez *Paratrechina longicornis* (Pearcy *et al.* 2011), et suspecté mais non confirmé chez la fourmi *Vollenhovia emeryi* (Ohkawara *et al.* 2006). Dans la mesure où ce mode de reproduction est apparu dans des groupes taxonomiques très divergeants, il est très probable que différents mécanismes en soient à l'origine et que ceux-ci n'aboutissent pas aux mêmes conséquences évolutives. Chez *C. dupreziana*, l'androgenèse semble résulter d'une apomixie mâle. Les embryons androgénétiques peuvent également se développer dans le tissu nourricier d'une graine d'une espèce sœur (Pichot et al. 2001). Dans le règne animal il semble que dans la plupart des cas, un mécanisme d'exclusion du génome maternel soit impliqué (McKone & Halpern 2003). Dans le cas des praires du genre *Corbicula*, les gamètes mâles possèdent un génome non-réduit semblable aux cellules somatiques des adultes (Komaru *et al.* 1997), résultant probablement de processus de divisions cellulaires améiotiques (i.e. apomixie). Il semble que lors de la fécondation, le génome nucléaire maternel soit exclu du zygote dans deux globules polaires (Komaru *et al.* 1998; 2000). Chez les phasmes du genre *Bascillus* les mâles diploïdes produisent des gamètes haploïdes ce qui implique un mécanisme de reploïdisation du zygote pour la production d'une descendance androgénétique diploïde. Le mécanisme le plus parcimonieux ayant été proposé pour expliquer la re-diploidisation du zygote implique la pénétration et la fusion de deux gamètes mâles dans un oocyte vidé de son bagage génétique nucléaire maternel (Tinti & Scali 1996; Mantonovi 1999). Mâles et femelles peuvent résulter d'un tel mécanisme bien que certains de ces hybrides ne semblent pouvoir produire que des descendants mâles (Tinti *et al.* 1995).

Les conséquences évolutives de l'androgenèse sont encore mal connues. En fait, jusqu'à présent, une seule étude théorique a tenté de décrire les enjeux évolutifs d'un tel système de reproduction (McKone & Halpern 2003). Cette étude ne concerne que les espèces diploïdes et tente de modéliser le devenir d'une mutation androgénétique dans une population en fonction i) du mode de reproduction des organismes dans lesquels cette mutation apparaît (i.e. hermaphrodite ou dioïque), ii) du mécanisme sous-jacent au mécanisme d'androgenèse (i.e. apomixie, fusion de plusieurs gamètes males, dédoublement d'un génome haploïde), iii)

de la dominance ou de la récessivité de la mutation, et iv) de quel sexe est hétérogamétique dans la population initiale. Dans cette étude, l'émergence de l'androgenèse aboutit souvent à la fixation de la mutation associée dans la population et à l'extinction des populations (McKone & Halpern 2003). Les populations qui conserveraient la capacité d'une reproduction femelle seraient logiquement plus épargnées de l'extinction.

Dans le cas des espèces qui présentent un système de reproduction qui s'éloigne d'un système diplo-diploïde, les mécanismes et les conséquences évolutives de l'androgenèse n'ont pas été traités par des modèles théoriques et demeurent de ce fait encore inconnus. Dans le cas des hyménoptères, et des insectes sociaux en particulier, les reproducteurs n'ont pas le même état de ploïdie; les femelles reproductrices diploïdes sont produites par reproduction sexuée, une partie de la descendance diploïde produite sexuellement ne se reproduit généralement pas (i.e. ouvrières) et les mâles, haploïdes, se développent par arrhénotoquie à partir d'un œuf non fécondé. La transmission du génome des mâles n'est assurée que par la production de femelles reproductives par reproduction sexuée, les ouvrières diploïdes étant stériles. Chez les espèces de fourmis pour lesquelles des événements d'androgenèse ont été identifiés, deux mécanismes hypothétiques qui s'opposent ont été proposés pour expliquer l'androgenèse à partir des études menées sur *W. auropunctata*. L'androgenèse dans cette espèce a été identifiée dans certaines populations initialement en 2005, à partir d'une étude de génétique des populations (Fournier *et al.* 2005). Dans cette espèce, l'androgenèse est étroitement liée à la parthénogenèse thélytoque des reines alors que les ouvrières qui sont stériles sont produites sexuellement (Fournier et al. 2005; Foucaud *et al*. 2007a; 1010 ; mais voir Foucaud *et al.* 2006). Cela semble également être le cas dans les deux autres espèces de fourmis pour lesquelles l'androgenèse a été identifiée ou suspectée. La parthénogenèse thélytoque aboutissant à la production d'une descendance femelle uniquement pourvue du génome maternel et les ouvrières étant stériles, Fournier *et al.* (2005) ont suggéré que l'androgenèse est une réponse adaptative des mâles pour contrecarrer l'effondrement de leur valeur sélective (réduite à zéro) du fait de l'émergence de la thélytoquie, s'engageant ainsi dans une «guerre des sexes». Ces auteurs ont suggéré un mécanisme d'élimination du génome maternel par le génome mâle lors de la fécondation. Cependant, Foucaud *et al.* (2007a) ont apporté plusieurs arguments à l'encontre de cette hypothèse. Ces auteurs considèrent notamment que sous ce scénario, les ouvrières qui sont produites sexuellement devraient également être affectées par le conflit génomique entre les sexes. Or, les ouvrières sont produites en large excès par rapport aux individus reproducteurs et notamment par rapport aux

mâles ce qui va à l'encontre d'un tel scénario. Ces auteurs ont donc suggéré que l'androgenèse pouvait être un trait femelle, notamment par la production d'œufs anucléés. Depuis aucune étude spécifique, impliquant notamment des croisements réciproques entre lignées clonales et sexuées, n'a été menée pour tenter de distinguer entre ces deux hypothèses principales (i.e. contrôle de l'androgenèse mâle ou femelle). C'est le sujet du troisième article qui compose ce chapitre (Article 3). Nos résultats suggèrent que l'androgenèse n'est pas un trait caractéristique des mâles mais au contraire, est contrôlé par les femelles parthénogénétiques. Dans le cas de *W. auropunctata*, nous considérons donc que l'androgenèse n'est pas un mécanisme de parasitisme d'œufs par les mâles mais plutôt une capacité des femelles à «kidnapper» un génome mâle dans la lignée clonale, pour la production d'ouvrières produites sexuellement.

II. Article 1: No evidence for endosymbiotic manipulation on the peculiar breeding system of the little fire ant *Wasmannia auropunctata*

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ABSTRACT

Some vertically transmitted endosymbiotic bacteria display the ability to manipulate the breeding system of their hosts to increase their own fitness. This can be achieved by a variety of mechanisms leading to as much as aberrant reproduction schemes in their hosts and often to reproductive isolation of populations. On the other hand, like other parasites, endosymbionts may be lost during habitat shift of some populations and especially during invasion. The maintenance of reproductive parasites in populations may therefore depend on the capacity of the endosymbionts to manipulate their hosts and the history of populations. The little fire ant *Wasmannia auropunctata* display a fascinating polymorphism in breeding system (including female parthenogenesis and male clonality) associated to its worldwide invasive success. To date no studies focused on the possible role of endosymbionts in the maintenance of the peculiar breeding system polymorphism among *W. auropunctata* populations. We here fill this gap by investigating for the presence of five major reproductive parasites (i.e. *Wolbachia*, *Arsenophonus*, *Cardinium*, *Rickettsia* and *Spiroplasma ixodetis*) in *W. auropunctata* populations. We screened a total of 86 individuals (queens and workers) from 43 nests covering most of the species's world distribution through the use of diagnostic PCR screenings specific to each endosymbiont. Further investigations were conducted on *Wolbachia* which was the unique endosymbiont detected in the present study by extending our screening on a larger set of samples and estimated its prevalence within and among populations in queens and workers and within nests in the worker caste. *Wolbachia* strains present in the infected populations of *W. auropunctata* were characterised using phylogeny based on the *wsp* gene and to a lower extant using a multilocus sequence typing approach. Our results revealed that most of clonal populations were *Wolbachia*-free while sexual populations were almost all infected. This clearly indicates that *Wolbachia* is not responsible for the clonal breeding system characterizing the invasive populations. We suggest that the strong association between the breeding system of individuals and the infection status within nests probably results from confounded environmental effects that differentiate clonal and sexual populations, in particular their habitat preferences.

INTRODUCTION

Endosymbiotic bacteria, as other cytoplasmic elements, are transmitted vertically and their fitness is hence tightly linked to their hosts' reproduction. Some of them behave as reproductive parasites by altering the breeding system of their host so that the number of infected daughters produced by an infected female exceeds the average production of daughters per uninfected female (Rousset 1992). Investigations in the field of reproductive parasitism have long been biased towards *Wolbachia*. This is logical, to some extent, given that this endosymbiont is the most widespread endosymbiotic bacteria within arthropods (Jeyaprakash & Hoy 2000; Werren & Windsor 2000). To date, this bacterium was found to induce four major classes of breeding system manipulation: cytoplasmic incompatibility (CI; Breeuwer 1997), feminisation of genetic males (F, Rousset *et al.* 1992), male killing (MK; Hurst *et al.* 1999) and induction of thelytokous parthenogenesis (P), the latter effect being specific to hymenopteran hosts (Stouthamer *et al*. 1999). In particular, the induction of parthenogenesis by *Wolbachia* occurs through a post-meiotic modification known as gamete duplication resulting in the production of a complete homozygous offspring (Stouthamer 1994). Finally, this reproductive parasite was also found to be obligate for oogenesis in the parasite wasp *Asobara tabida* (Dedeine *et al.* 2001). Since recently, other endosymbiotic bacteria are receiving increasing attention. *Cardinium* was found to induce the same four major phenotypic effects (i.e. CI, F, MK, P) in a variety of host species (reviewed in Duron *et al.* 2008). Hagimori *et al.* (2006) demonstrated that *Rickettsia* was also able to induce parthenogenesis in a hymenopteran species. This endosymbiont was already known to be associated with male killing in a buprestid beetle (Lawson *et al.* 2001). Finally, other bacteria from the genus *Arsenophonus* and *Spiroplasma* seem specialised in male killing within their hosts (reviewed in Duron *et al.* 2008). All of these reproductive parasites lead to biases in reproductive schemes beneficial for infected females and therefore may have drastic effects on the evolutionary dynamics of their hosts. It is therefore crucial to investigate the presence and the potential role of reproductive parasites in organisms that present peculiar breeding systems.

The little fire ant, *Wasmannia auropunctata*, has been both recognized to display a fascinating polymorphism in breeding system and a tremendous invasive success worldwide (Wetterer & porter 2003; Foucaud *et al.* 2007; 2010). In some populations (hereafter called "sexual populations"), queens and males are produced following a classical haplo-diploid scheme where diploid females (i.e. queens and sterile workers) are produced sexually and haploid males develop from haploid eggs through arrhenotoky. Some populations (hereafter called "clonal populations") emerged recurrently from these sexual populations, in which reproductives display a peculiar breeding system: queens use thelytokous parthenogenesis and sexual reproduction in a conditional manner to produce gynes (i.e. unfertilised queens) and (sterile) workers respectively (Fournier *et al.* 2005; Foucaud *et al.* 2010). A recent study revealed that gynes are produced via automictic parthenogenesis with central fusion (Rey *et al.* 2011). Moreover, contrary to other species in which queens reproduce by thelytoky, unmated queens were found to be unable to lay viable eggs (Foucaud *et al.* 2010). The production of parthenogenetic eggs, irrespective to their ploidy level (i.e. haploid or diploid), seems therefore strictly dependent on the fertilisation process. Finally, female parthenogenesis is tightly associated to male clonality (Fournier *et al.* 2005, Foucaud *et al.* 2009; 2010). The genome of males is also transmitted clonally via maternal eggs through a mechanism yet unresolved. To our knowledge no study has investigated the role of endosymbiotic organisms in this peculiar clonal breeding polymorphism. If endosymbiotic organisms are in fact involved in the breeding system of *W. auropunctata*, the expectation of infection pattern would be an excess of infection within clonal against sexual populations.

Interestingly, the breeding system polymorphism among *W. auropunctata* populations is strongly associated to the habitat used by populations and their invasive status. Indeed, while native non-dominant populations are mostly sexuals, native dominant and invasive introduced populations are all characterised by the clonal breeding system (Orivel *et al.* 2009; Foucaud *et al.* 2009; 2010). As a matter of fact, loss of parasites is frequent during invasion processes (Torchin *et al.* 2003). In this respect, the loss of manipulator endosymbiotic bacteria in invasive populations is common in ant species (e.g. Shoemacker *et al.* 2000; Tsuitsui *et al.* 2003; Reuter *et al.* 2005; Yang *et al.* 2010). From an invasion scenario perspective the expectation of infection pattern would therefore be at the opposite from the expectation under endosymbiontic manipulation hypothesis, that is clonal invasive populations being free and sexual native populations infected by endosymbionts. In this context, we investigated the infection status among and within sexual native and clonal invasive populations of *W. auropunctata* for five known reproductive parasites, *Wolbachia*, *Arsenophonus*, *Cardinium*, *Rickettsia* and *Spiroplasma ixodetis* in order to determine the driving force of infection pattern (i.e. reproductive system versus invasion process) in this species.

MATERIAL AND METHODS

Screening for endosymbiotic bacteria

We used specific PCR-amplification to investigate for the presence of *Wolbachia*, *Cardinium*, A*rsenophonus*, *Rickettsia* and *Spiroplasma ixodetis* on 86 individuals from a set of 43 nests covering most of the distribution area of *W. auropunctata* including its native and introduced range (Table 1). Twenty two of the 43 nests belonged to *W. auropunctata*'s native area. Based on previous studies, 11 were known to be clonal and 11 to be sexual (Foucaud *et al.* 2009). The other 21 nests belonged to the introduced range

	Native area				Introduced area		
	Location	Population	Individual screened		Location	Population	Individual screened
SEXUAL	French Guiana	FG1	WQ	CLONAL	Gabon	GA1	WQ
	French Guiana	FG2	WQ		Gabon	GA2	WQ
	French Guiana	FG3	WQ		Cameroon	CA1	WQ
	French Guiana	FG4	WQ		Cameroon	CA2	WQ
	French Guiana	FG5	WQ		Coco Isl.	CO1	WW
	French Guiana	FG6	WQ		Tahiti	TA1	WQ
	French Guiana	FG7	WQ		USA Florida	FL1	WQ
	Brazil	BR1	WW		USA Florida	FL2	WQ
	Brazil	BR2	WW		USA Florida	FL3	WW
	Costa Rica	CR1	WW		GUA	GU1	WQ
	Costa Rica	CR2	WW		GUA	GU2	WQ
CLONAL	French Guiana	FG9	WQ		Cuba	CU1	WQ
	French Guiana	FG10	WQ		Dominica	DO1	WW
	French Guiana	FG11	WQ		Dominican Rep.	DR1	WW
	French Guiana	FG12	WQ		New Caledonia	NC1	WQ
	French Guiana	FG13	WQ		New Caledonia	NC2	WQ
	French Guiana	FG14	WQ		Vanuatu Isl.	VA1	WQ
	French Guiana	FG15	WQ		Vanuatu Isl.	VA2	WQ
	French Guiana	FG16	WQ		Australia	AU1	WQ
	Brazil	BR3	WQ		Israel	IS1	WQ
	Brazil	BR4	WQ		Israel	IS2	WQ
	Brazil	BR5	WQ				

Table 1: *W. aruonpunctata* populations sampled from the native and introduced area for the broad screening of the five endosymbiotic bacteria.

and were all known to be clonal (Foucaud *et al.* 2010). When possible, a queen and a worker from each nest were screened. In nests in which no queens were sampled, two workers were screened (Table 1).

The total genomic DNA was extracted from all individuals following a standard CTAB protocol. We performed PCR-based screening using a protocol modified from Shoemaker *et al.'s* study (2000) for *Wolbachia* (see supplementary material), and following the protocols described in Duron *et al.* (2008) for the four other endosymbionts. We used an arthropod specific DNA fragment of the *EF1α* gene on *W. auropunctata* to serve as an internal control of DNA extractions and PCR quality using the universal primer set trs4F / trs9R (Ward *et al.* 2005). PCR products were then loaded and electrophoresed in 1.5 % aggarose gels and finally visualised under UV illumination after a staining in an ethidium bromide solution.

Wolbachia additional specific screening

Based on our first screening, we detected the presence of *Wolbachia* in several individuals (see results). We therefore extended our screening of *Wolbachia* on a larger dataset to better appreciate the distribution of this endosymbiont among and within populations (hereafter called the '*Wolbachia* additional specific screening'). Together with individuals screened previously, a total of 203 queens and 394 workers originating from 261 and 174 nests respectively from 60 populations were screened following the same protocol as detailed in the *screening for endosymbiotic bacteria* section.

We estimated the prevalence of *Wolbachia* within populations in the queen and worker castes based on this *Wolbachia* additional specific screening. The prevalence within a population was simply the number of infected individuals of a given caste on the total of individuals of the same caste screened in the population. To indirectly test whether *Wolbachia* is involved in the breeding system polymorphism of *W. auropunctata* we tested for statistical association between the breeding system within nests (i.e. sexual or clonal) and the infection status (i.e. presence or absence of *Wolbachia*) of individuals within the nests. Nests with at least one worker infected were considered as infected, and nests in which all individuals were *Wolbachia*-free were considered as non-infected. The association was

Area	Location	Population	Breeding system	N(nests)	N(Q)	Prevalence within population
Native	French Guiana	FG1	Sexual	1	1	1
	French Guiana	FG2	Sexual	4	4	1
	French Guiana	FG3	Sexual	5	5	1
	French Guiana	FG4	Sexual	2	6	1
	French Guiana	FG5	Sexual	10	13	1
	French Guiana	FG6	Sexual	3	3	1
	French Guiana	FG7	Sexual	1	1	1
	French Guiana	FG8	Sexual	2	2	1
	French Guiana	FG9	Sexual	1	1	1
	Brasil	BR2	Sexual	10	10	1
			Sexual	**39**	**46**	**1**
Native	French Guiana	FG14	Clonal	2	2	0
	French Guiana	FG9	Clonal	3	8	0
	French Guiana	FG10	Clonal	3	11	0
	French Guiana	FG11	Clonal	17	24	0
	French Guiana	FG12	Clonal	5	5	0
	French Guiana	FG15	Clonal	1	1	0
	French Guiana	FG16	Clonal	1	1	0
	Brasil	BR5	Clonal	5	5	0
	Brasil	BR3	Clonal	13	13	1
	Brasil	BR4	Clonal	10	10	1
Introduced	New Caledonia	NC1	Clonal	4	4	0
	New Caledonia	NC2	Clonal	7	8	0
	New Caledonia	NC3	Clonal	4	4	0
	Tahiti	TA1	Clonal	4	4	0
	Gabon	GA1	Clonal	7	7	0
	Gabon	GA2	Clonal	5	5	0
	Gabon	GA3	Clonal	2	2	0
	Gabon	GA4	Clonal	1	1	0
	Gabon	GA5	Clonal	2	2	0
	Cameroun	CA1	Clonal	4	4	0
	Cameroun	CA2	Clonal	5	5	0
	Cameroun	CA3	Clonal	5	5	0
	Cameroun	CA4	Clonal	4	4	0
	Israel	IS1	Clonal	1	1	0
	Israel	IS2	Clonal	1	1	0
	Israel	IS3	Clonal	1	1	0
	Israel	SI4	Clonal	1	1	0
	Guadeloupe	GU1	Clonal	1	1	0
	Guadeloupe	GU2	Clonal	1	1	0
	Cuba	CU1	Clonal	1	2	0
	Florida	FL1	Clonal	1	1	0
	Florida	FL2	Clonal	1	1	0
	Vanuatu Islands	VA1	Clonal	5	5	1
	Vanuatu Islands	VA2	Clonal	1	1	1
	Australia	AU1	Clonal	6	6	1
			Clonal	**135**	**157**	**0.143**

Table 2: Prevalence of *Wolbachia* infection in queens from nests sampled in the native and introduced range.

tested using a Fisher's exact test (Fisher 1922) and the measure of the strength of this association was assessed conducting a Cramer's V statistic (Cramer 1946).

In ants, the prevalence of *Wolbachia* within nests in the sterile workers caste (dead-end host) may vary from one nest to another (Russell 2011). We therefore specifically estimated the prevalence of *Wolbachia* within nests by screening 120 additional workers originating from eight distinct nests (i.e. 20 workers per nests). Four of these eight nests were known to be sexual and the other four were known to be clonal. The four sexual nests originated from two populations established in the primary forest, one in French Guiana (FG2) and one in Brazil (BR1) and were found to be infected by *Wolbachia* in the Wolbachia additional specific screening. Two of the four clonal nests originated from the native range, one from French Guiana (FG11) and the other from Brazil (BR4) and the two others originated from the introduced range, from Australia (AU1) and from New Caledonia (NC3). Based on the previous Wolbachia additional specific screening, two of these clonal nests were known to be infected by *Wolbachia* (AU1 and BR4) and the two others were estimated to be *Wolbachia*-free (i.e. FG11 and NC3).

Genetic characterisation of the Wolbachia strains

A phylogenetic analysis was performed to assess the position of the strains of *Wolbachia* detected in *W. auropunctata* individuals. To this aim, we sequenced a fragment of the *wsp* gene on 74 individuals from 17 populations (see the above "screening of endosymbiotic bacteria" section for PCR amplification details). PCR products were then purified and sequenced on an ABI 3730 DNA sequencer (Applied Biosystems). Individual electropherograms were checked for eventual errors using the Seqscape software (Applied Biosystems). Our phylogenetic analysis was performed on unique haplotypes (i.e. five haplotypes). We added to this sequence matrix, 21 *wsp* sequences of some *Wolbachia* strains infecting other ant species. Because ant species are generally known to be infected by strains from the phylogenetic supergroups A and B (Stouthamer *et al.* 1999; Lo *et al.* 2002), our set of reference sequences comprised nine and twelve haplotypes from these two supergroups respectively. A *wsp* sequence from the supergroup D was used to root the tree (Wsp ST-35). All sequences were aligned using clustalW (Thompson *et al.* 1997). A phylogenetic tree was constructed in MEGA v. 4 (Tamura *et al.* 2007) using the neighbour-joining method with Kimura two-parameter distance measure (Kimura 1980). Bootstrap analysis was performed with 1,000 replicates.

Some rare clonal populations were found to be infected by the same *Wolbachia* strain than most of sexual populations according to our phylogeny based on the *wsp* gene (see results below). Despite its extensive use in many studies, the *wsp* gene was found to be under strong diversifying selection and to undergo extensive recombination both within and between strains (Jiggins *et al.* 2001; Baldo *et al.* 2006; Baldo & Werren 2007). As a result, *Wolbachia* from different clusters might share similar *wsp* sequences (Baldo & Werren 2007). To verify whether these clonal populations actually share the same strain with sexual populations, we conducted an additional analysis on a small subset of 12 individuals from both sexual and clonal populations (See Figure 1) based on the multilocus sequence typing approach (MLST). This method is based on an unambiguous procedure for characterising strains of *Wolbachia* using the allelic profile at five conserved genes as molecular markers to genotype a strain (i.e. *GatB*, *FbpA*, *CoxA*, *FtsZ* and *HcpA*; Baldo *et al.* 2006). We amplified and sequenced fragments of these five genes following Baldo *et al.*'s protocols (2006). The allelic profile of each strain was then compared with identified strains in the MLST database (http://pubmlst.org/wolbachia/).

RESULTS

Screening of endosymbiotic bacteria

Among the five main endosymbiotic bacteria screened in the present study (i.e. *Wolbachia*, *Cardinium*, A*rsenophorus*, *Rickettsia* and *Spiroplasma ixodetis)*, *Wolbachia* was the only one detected in the sampled wild populations of *W. auropunctata*.

Our *Wolbachia* additional specific screening revealed a strong association between the reproductive system within *W.auropunctata's* nests and the infection status of individuals by *Wolbachia* (χ^2 = 143.24; p – value < 0.001; Cramer's V = 0.74). The mean prevalence within sexual populations was 1 and 0.99 within the queen and worker castes respectively (Table 2, 3). In the 39 sexual nests in which queens were sampled, all 46 sexually reproducing queens as well as all workers were infected by *Wolbachia*. However, we found four workers originating from two sexual nests that were *Wolbachia* – free. These workers belonged to two nests from populations of French Guiana (FG2, FG5; Table 1) in which no queens were sampled for these nests. Note that two others screened workers from the nest of the FG2 population were found to be infected while the other worker from FG5 was also W*olbachia*-free.

In clonal populations, the mean prevalence within populations was 0.14 and 0.13 within the queen and worker castes respectively (Table 2, 3). We found that 131 of the 157 clonal queens (i.e. 83.4 %) from the 135 sampled nests were *Wolbachia*-free irrespective to their origin (i.e. from the native or invasive range). All workers from these clonal nests were also non-infected. A total of 35 infected clonal queens were found within 23 nests of the native range in two Brazilian populations and twelve nests in the introduced range in Australia and in the Vanuatu Island. Workers from these populations were also all infected. Finally, five workers from a unique nest originating from Florida (U.S.A) were all infected by *Wolbachia*. No queen was sampled in this Floridian nest.

The prevalence of *Wolbachia* infection within nests matches a binary pattern. The 20 workers sampled from infected sexual and clonal nests were all infected. On the contrary, workers from the two *Wolbachia*-free clonal nests were all non-infected (data not shown).

Genetic characterisation of Wolbachia strains

Our *wsp-based* phylogeny indicated that five different *Wolbachia* strains infected the sampled populations of *W.auropunctata* (Figure 1). Most of individuals from sexual populations were infected by a single strain belonging to the supergroup B. Only the sexual populations originating from Brazil host *Wobachia* strains belonging to the supergroup A. Interestingly, we found that the few infected clonal populations host a single *Wolbachia* strain from the supegroup B, the one also found in most sexual populations.

In agreement with our *wsp*-based phylogeny, the MSLT approach indicated that the rare infected clonal populations share strictly the same *Wolbachia* strain than most of sexual populations. Interestingly enough, the allelic profile of the strain shared by clonal and sexual populations correspond to the allelic profile of a strain that was previously identified in *Solenopsis invicta* (ST-29; SinvA). This strain belongs to the supergroup A in the MLST classification but belong to the supegroup B when conducting phylogenies based on this unique *wsp* gene due to recombination events between *Wolbachia* strains (Baldo & Werren 2007).

Area	Location	Population	Breeding system	N(nests)	N(W)	Prevalence within population
Native	French Guiana	FG1	Sexual	1	1	1
	French Guiana	FG2	Sexual	8	14	0.93
	French Guiana	FG3	Sexual	10	18	1
	French Guiana	FG4	Sexual	6	14	1
	French Guiana	FG5	Sexual	15	22	0.91
	French Guiana	FG6	Sexual	10	16	1
	French Guiana	FG7	Sexual	6	10	1
	French Guiana	FG8	Sexual	4	8	1
	French Guiana	FG9	Sexual	1	2	1
	French Guiana	FG10	Sexual	2	4	1
	French Guiana	FG11	Sexual	3	6	1
	French Guiana	FG12	Sexual	4	8	1
	French Guiana	FG13	Sexual	3	6	1
	Brasil	BR1	Sexual	10	20	1
	Brasil	BR2	Sexual	10	15	1
	Costa Rica	CR2	Sexual	1	2	1
			Sexual	**94**	**166**	**0.99**
Native	French Guiana	FG9	Clonal	3	5	0
	French Guiana	FG10	Clonal	4	4	0
	French Guiana	FG11	Clonal	16	20	0
	French Guiana	FG12	Clonal	6	8	0
	French Guiana	FG17	Clonal	1	3	0
	French Guiana	FG13	Clonal	2	2	0
	French Guiana	FG14	Clonal	2	2	0
	French Guiana	FG15	Clonal	2	2	0
	French Guiana	FG16	Clonal	2	2	0
	Costa Rica	CR1	Clonal	1	2	0
	Brasil	BR5	Clonal	10	15	0
	Brasil	BR3	Clonal	13	27	0.78
	Brasil	BR4	Clonal	10	10	1

Table 3: Prevalence of *Wolbachia* infection in workers from nests sampled in the native and introduced range.

Area	Location	Population	Breeding system	N(nests)	N(W)	Prevalence within population
Introduced	New Caledonia	NC1	Clonal	4	4	0
	New Caledonia	NC2	Clonal	8	8	0
	New Caledonia	NC3	Clonal	4	4	0
	Tahiti	TA1	Clonal	4	9	0
	Gabon	GA1	Clonal	7	7	0
	Gabon	GA2	Clonal	6	6	0
	Gabon	GA3	Clonal	3	3	0
	Gabon	GA4	Clonal	1	1	0
	Gabon	GA5	Clonal	2	2	0
	Cameroun	CA1	Clonal	5	5	0
	Cameroun	CA2	Clonal	5	5	0
	Cameroun	CA3	Clonal	5	5	0
	Cameroun	CA4	Clonal	5	5	0
	Israel	IS1	Clonal	1	1	0
	Israel	IS2	Clonal	1	1	0
	Israel	IS3	Clonal	1	1	0
	Israel	SI4	Clonal	1	1	0
	Guadeloupe	GU1	Clonal	7	7	0
	Guadeloupe	GU2	Clonal	2	2	0
	Guadeloupe	GU3	Clonal	1	1	0
	Cuba	CU1	Clonal	2	7	0
	Cocos Isl.	CO1	Clonal	1	3	0
	Dominica	DO1	Clonal	2	6	0
	Dominican Rep.	DR1	Clonal	1	3	0
	Florida	FL1	Clonal	1	1	0
	Florida	FL2	Clonal	1	1	0
	Florida	FL4	Clonal	1	2	0
	Florida	FL3	Clonal	1	5	1
	Vanuatu Isl.	VA1	Clonal	5	10	1
	Vanuatu Isl.	VA2	Clonal	1	2	1
	Australia	AU1	Clonal	6	8	1
			Clonal	**167**	**228**	**0.131**

Table 3: Continued.

DISCUSSION

Among the five studied endosymbiotic bacteria in which some strains are known to manipulate the reproductive system of their host (i.e. *Wolbachia*, *Rickettsia*, *Cardinium*, *Arsenophorus* and *Spiroplasma ixodetis*), only *Wolbachia* was found in the sampled populations of *W. auropunctata*. This result is not surprising given that *Wolbachia* is estimated to infect more than one third of species from the Formicidae (Russel 2011) while the four other endosymbionts were found sporadically in few arthropods orders and seldom in ants (Duron *et al.* 2008; but see Sirvio & Pamilo 2010). Among the individuals screened, most of queens reproducing via thelytokous parthenogenetic were *Wolbachia – free*. These results clearly indicate that thelytokous parthenogenesis in *W. auropunctata* queens and the associated male clonality, is not induced by *Wolbachia* neither by any of the four other endosymbiotic bacteria screened in the present study. This finding echoes with those of Wenseleers & Billen's (2000) who showed that queens of six other ant species that reproduce via thelytokous parthenogenesis, were not infected by *Wolbachia*.

The second main result emerging from this study is the strong association between the reproductive system characterising *W.auropunctata*'s nests and the infection status by *Wolbachia* of individuals within nests. While individuals from clonal nests are mostly *Wolbachia*-free, all sampled queens and most of workers from sexual nests are infected by the bacteria. This pattern suggested that this bacterium is maintained if not favoured in sexual populations. However, none of the described mechanism by which *Wolbachia* alters the breeding system to enhance its own transmission (i.e. cytoplasmic incompatibility male killing and feminisation), can explain the maintenance of the bacteria in sexual nests better than in clonal nests.

First, cytoplasmic incompatibility is the most

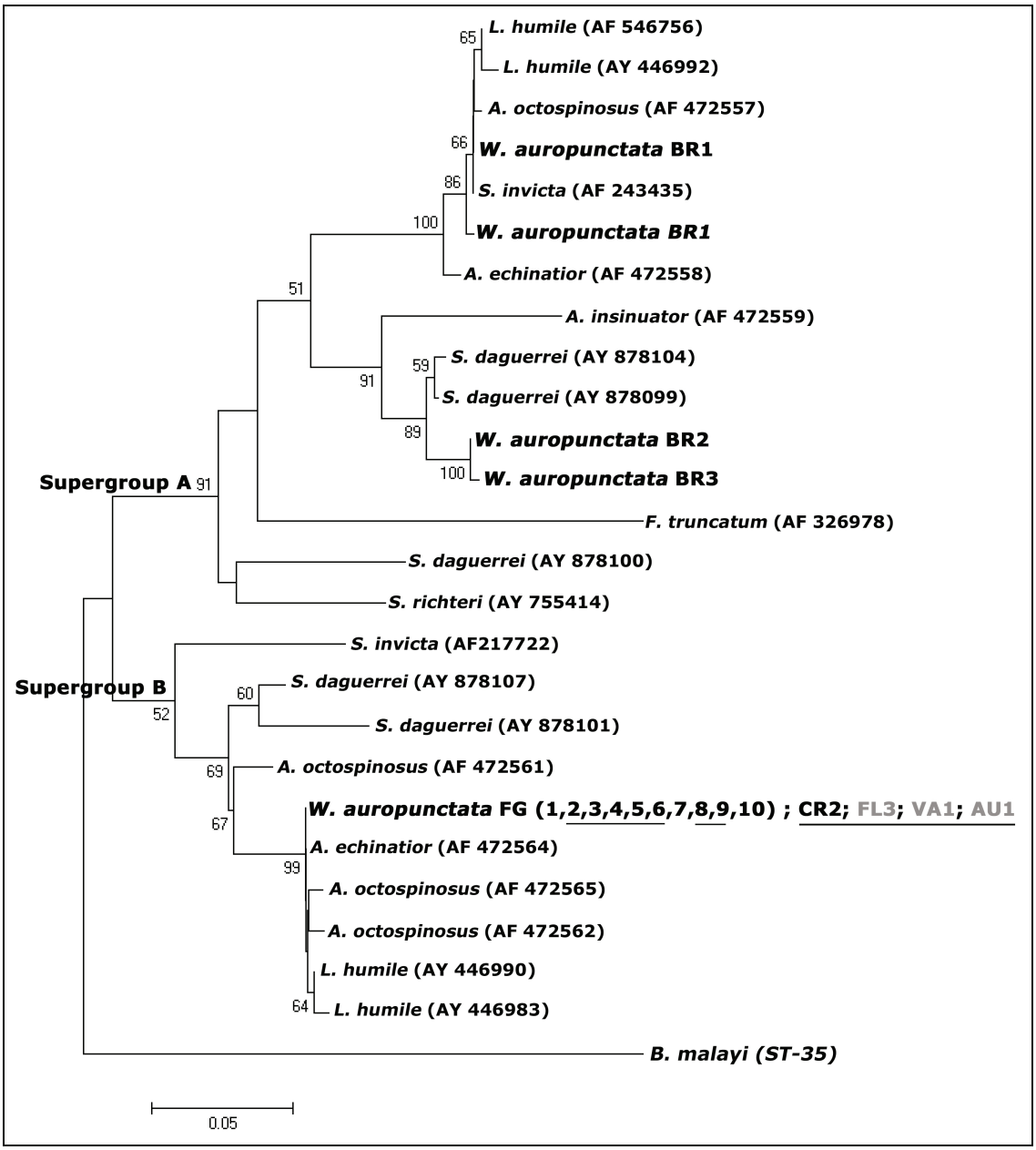

Figure 1: NJ tree based on the *wsp* nucleotide alignment of the different *Wolbachia* strains infecting native and introduced populations of *W. auropunctata*. Note: Each *Wolbachia* sequence is labelled with the name of its host species (GenBank Accession number is indicated in parenthesis). Only bootstrap values (computed from 1,000 replicates) of nodes are figured for values > 50%.*Wolbachia* strains found in individuals from clonal populations are in grey characters. Strains underlined were used for further analyses using the MLST approach (see text for more details). Note that the two different strains found in BR1 were found in different nests from the same population BR1.

prevalent phenotypic effect induced by *Wolbachia* from the super group A and B (Stouthamer *et al.* 1999). In the case of *W. auropunctata*, cytoplasmic incompatibility would lead to the same outcome on the transmission of the bacteria in both sexually and clonally reproducing queens (i.e. no improvement of transmission within the sexual nests compared to clonal ones) and therefore would not explain why *Wolbachia* is favoured in sexual populations. Second, male killing or feminisation induction seems unlikely to occur in *W. auropunctata*. Indeed, classical arrhenotokous males from sexual populations were found to be infected by the bacteria (data not shown) and there is no precedent for these two *Wolbachia*-induced phenotypes in any hymenopteran. Finally, *Wolbachia* was recently found to be obligatory for oogenesis in the hymenopteran genus *Asobara* (Dedeine *et al.* 2001). In the case of *W. auropunctata*, sex, and hence oogenesis also occur in *Wolbachia*-free clonal queens which use sexual reproduction for worker offspring production. Furthermore, some clonal queens producing their gyne offspring through automictic parthenogenesis (i.e. with meiosis) were found to be infected by the same *Wolbachia* strain than the one found in most of

sexually reproducing queens. The induction of oogenesis is therefore also unlikely to explain the infection pattern observed in *W. auropunctata* populations.

The stricking association between the breeding system and the infection status of individuals within nests might rather results from ecological / environmental confounding effects that differentiate sexual and clonal populations other than their breeding system itself. In particular, reproductive system is strongly associated to the invasive status of *W. auropunctata* (Foucaud *et al.* 2009). The loss of endosymbiotic bacteria in invasive populations is common in ant species (Shoemaker *et al.* 2000; Tsutsui *et al.* 2003; Reuter *et al.* 2005) and several hypotheses were proposed to explain this loss: i) *Wolbachia* can be eliminated through drift during introduction if all founders were un-infected. ii) *Wolbachia* can be lost in invasive populations after the introduction through drift or selection. In the case of *W. auropunctata*, we consider both of these explanations to be unlikely because invasive clonal populations have recurrently emerged from sexual populations (Foucaud *et al.* 2007) and none of all the sexually reproducing queen were found to be *Wolbachia-free* suggesting that the emergence of uninfected sexual populations may be extremely rare. Furthermore, the prevalence within populations and within nests was found to be fixed in all sexual populations suggesting that vertical transmission of *Wolbachia* is almost if not perfect. It is therefore unlikely that *Wolbachia* could have been lost in invasive population through the unique mean of drift. Interestingly, the invasive status of *W. auropunctata* population was found to be strongly associated with an important habitat change (Foucaud *et al.* 2009). We here propose two non-exclusive alternative hypotheses based on ecological features relative to this habitat change to explain the infection pattern in *W. auropunctata* populations.

Clonal populations might have lost *Wolbachia* by natural heat treatment. Indeed, while non-invasive sexual populations are established in primary forests, clonal populations settle in human perturbed areas. In this respect, Orivel *et al.* (2009) reported clear abiotic differences in habitats occupied by both types of populations. Human-modified habitats invaded by clonal populations are hotter and drier than the primary forests. Moreover, Foucaud *et al.* (submitted) have shown that clonal populations are adapted to these abiotic conditions allowing workers to tolerate temperatures as high as 36°C while the mortality rate in workers from sexual populations reach near 40% at this temperature. Furthermore, *Wolbachia* is known to be sensitive to hot temperature and heat treatments are commonly used to remove it from hosts for recently abandoned plantations where all human activity was stopped since at least 10 years. In those experimental purposes. Classically these treatments require rearing hosts' larvae at 33°C to 35 °C for few days to several generations (Wright & Wong 1980, Van Opijnen & Breeuwer 1999). While in primary forests these temperatures are seldom reached, they are commonly surpassed in human-modified habitats occupied by clonal populations (Orivel *et al.* 2009). Under this scenario, the loss of *Wolbachia* in most introduced populations of *W. auropunctata* is consistent with predictions of the enemy-release hypothesis (Torchin *et al.* 2003). Assuming that *Wolbachia* induces physiological costs to infected individuals in sexual populations (e.g. reduction of fecundity, adult survival and locomotor performance; Fleury *et al.* 2000; Wenseleers *et al.* 2002; but see Bouwma & Shoemaker 2011), the loss of *Wolbachia* this might in part explains the success of *Wolbachia*-free invasive clonal populations compared to their ancestral sexual relatives (Orivel *et al.* 2009).

A second hypothesis is that *Wolbachia* might have been lost in clonal populations through selection relaxation and/or counter-selection against infected individuals. Recent studies suggest that *Wolbachia* protect their *Drosophila* hosts against RNA viruses (Hedges *et al.* 2008, Teixeira *et al.* 2008), and might promote up regulation of immunity-gene expression in mosquitoes thus reducing their loads of *Plasmodium* and filarial nematodes (Kambris *et al.* 2009, 2010). Additionally, although no direct evidence exists, some correlations between infection status and prevalence of virulent RNA viruses within *Solenopsis invicta* wild nests suggested that *Wolbachia* might also play this defensive role in ant species (Valles & Hashimoto 2009; Yang *et al.* 2010). In the case of *W.auropunctata*, sexual populations are established in primary forests in which biotic pressures are likely to be more important than those undergone by clonal populations established in human perturbed areas (see Orivel *et al.* 2009). In such case, one might expect that while the defensive *Wolbachia* strains are selectively maintained in sexual populations, despite a probable physiological cost the decrease in biotic pressure in human-modified habitats would lead to the loss of *Wolbachia* through selection relaxation and/or through selection. Furthermore, if *Wolbachia* promote protection against pathogens, all individuals from infected nests including sterile workers considered as dead-end hosts are expected to be almost if not all infected. Interestingly, all workers within sexual nests were found to be infected; a pattern contrasting with the expected low prevalence of the bacteria in this dead-end sterile host offspring.

Despite this strong association between the breeding system and the infection status of individuals, some rare clonal nests were found to be infected by the *Wolbachia*. Interestingly, infected clonal nests from the native area (i.e. BR3 and BR4 in Brazil; Table 2, 3) were found in some particular habitats. Both Brazilian nests were sampled in peculiar sites, environmental conditions are different from those of the typical habitat where clonal

populations are usually found and rather would approximate to those found in native forests habitats (data not shown). This result strengthens the idea that environmental / ecological effects may drive the infection status of individuals of *W.auropunctata*'s nests.

Several infected individuals were also found in some introduced populations established in Florida, Australia and in the Vanuatu Islands, all of them being infected by the same strain (SinvA).

It was recently reported that Australia and Vanuatu Islands were characterised by the same single maternal clonal genotype and therefore most probably originated from the same unknown source population (Foucaud *et al.* 2010). This result is strengthens by the fact that these populations share the same mitochondrial haplotype (Rey *et al.* submitted). In the latter study, Florida was also found to share the same mitochondrial haplotype. Furthermore, the maternal clonal microsatellite genotypes in the Floridian populations are closely related to the genotype characterizing the oceanian populations (i.e. Australia, Vanuatu Islands, Foucaud *et al.* 2010; Rey *et al.* submitted). Although all together these results are not sufficient to assert that Oceania populations originate from Florida, it seems reasonable to hypothesize that populations from Florida and Oceanic Islands share at least a close ancestral maternal clonal lineage. The infection pattern of *Wolbachia* observed in the present study is in agreement with this hypothesis. Indeed, the populations from Florida, Vanuatu Islands and Australia are infected by the same *Wolbachia* strain. One hypothetical scenario to explain the infection pattern of these populations would then be that *Wolbachia* was already present in the original populations that have invaded Florida on one hand and Australia and Vanuatu on the other. Alternatively, Florida is the geographical source of invasive populations from Australia and Vanuatu and populations established in Florida were originally *Wolbachia-free* like all other invasive populations screened in the present study, and underwent a secondary infection locally before transportation to the pacific Islands. In agreement with this, although *Wolbachia* are more often vertically transmitted, some evidences indicated that *Wolbachia* may also benefit from horizontal transmissions between hosts via common food sources (Huigens *et al.* 2000) and between parasitoids and their hosts (Vavre *et al.* 1999). In this respect, horizontal transfers were suspected in some populations of *Solenopsis invicta* in North America and especially in Florida (Jeyaprakash & Hoy 2000).

In conclusion, this study revealed that none of the reproductive parasite screened in the present study out of *Wolbachia* infect wild populations of *W. auropunctata*. This result strengthens the previous findings suggesting that queen thelytokous parthenogenesis in this species is under a genetic determinism (Foucaud *et al*. 2006). The infection pattern of *Wolbachia* in wild populations of *W. auropunctata* echoes with previous studies revealing a loss of *Wolbachia* in invasive populations (e.g. Shoemacker *et al.* 2000; Tsuitsui *et al.* 2003; Reuter *et al.* 2005; Yang *et al.* 2010). The most likely explanation is that this loss resulted from a shift in habitat of invasive populations through natural heat treatment and / or selection relaxation. This latter hypothesis assuming both a defensive effect and/or a physiological cost induced by *Wolbachia* in sexual populations, it would be of interest to experimentally verify those hypothetical phenotypic effects in the most common *Wolbachia* strain found in sexual populations. Finally we found that invasive populations harbour the same strain than those infecting other invasive ant species. This suggests that these populations have undergone a secondary infection and hence reinforces the view that horizontal transmission of *Wobalchia* may be possible in ants.

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II. Article 2: Meiotic recombination dramatically decreased in thelytokous queens of the little fire ant and their sexually produced workers

Molecular Biology and Evolution

Research article

Meiotic Recombination Dramatically Decreased in Thelytokous Queens of the Little Fire Ant and Their Sexually Produced Workers

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Abstract

The little fire ant, *Wasmannia auropunctata*, displays a peculiar breeding system polymorphism. Classical haplo-diploid sexual reproduction between reproductive individuals occurs in some populations, whereas, in others, queens and males reproduce clonally. Workers are produced sexually and are sterile in both clonal and sexual populations. The evolutionary fate of the clonal lineages depends strongly on the underlying mechanisms allowing reproductive individuals to transmit their genomes to subsequent generations. We used several queen-offspring data sets to estimate the rate of transition from heterozygosity to homozygosity associated with recombination events at 33 microsatellite loci in thelytokous parthenogenetic queen lineages and compared these rates with theoretical expectations under various parthenogenesis mechanisms. We then used sexually produced worker families to define linkage groups for these 33 loci and to compare meiotic recombination rates in sexual and parthenogenetic queens. Our results demonstrate that queens from clonal populations reproduce by automictic parthenogenesis with central fusion. These same parthenogenetic queens produce normally segregating meiotic oocytes for workers, which display much lower rates of recombination (by a factor of 45) than workers produced by sexual queens. These low recombination rates also concern the parthenogenetic production of queen offspring, as indicated by the very low rates of transition from heterozygosity to homozygosity observed (from 0% to 2.8%). We suggest that the combination of automixis with central fusion and a major decrease in recombination rates allows clonal queens to benefit from thelytoky while avoiding the potential inbreeding depression resulting from the loss of heterozygosity during automixis. In sterile workers, the strong decrease of recombination rates may also facilitate the conservation over time of some coadapted allelic interactions within chromosomes that might confer an adaptive advantage in habitats disturbed by human activity, where clonal populations of *W. auropunctata* are mostly found.

Key words: parthenogenesis, thelytoky, recombination, inbreeding, biological invasion, *Wasmannia auropunctata*.

Introduction

The ubiquitous nature of sexual reproduction in the tree of life is a key question that is addressed by evolutionary biologists but not yet resolved. Sexually produced individuals need both a mother and a father, so sexual offspring are considered to suffer from the "2-fold cost of meiosis" (Maynard Smith 1971). Several theoretical models have been proposed to highlight the potential evolutionary benefits counteracting this cost of requiring two parents. These models have generated about 20 hypotheses accounting for the long-term advantage of sexual reproduction (reviewed in Kondrashov 1993 and Barton and Charlesworth 1998). However, the short-term advantages of sexual reproduction remain a matter of debate, and asexuality appears to emerge easily and independently from sexual lineages (Simon et al. 2003). There is both theoretical and empirical evidence to suggest that, in specific conditions, asexual populations displace related sexual populations in the short term (Burger 1999; Neiman and Linksvayer 2006; Hoffmann et al. 2008). The most direct line of argument in favor of asexual lineages is demographic, as such lineages are not subject to the 2-fold cost of meiosis. In other words, two parthenogens produce two offspring at the same time as a male and a female produce just one. However,

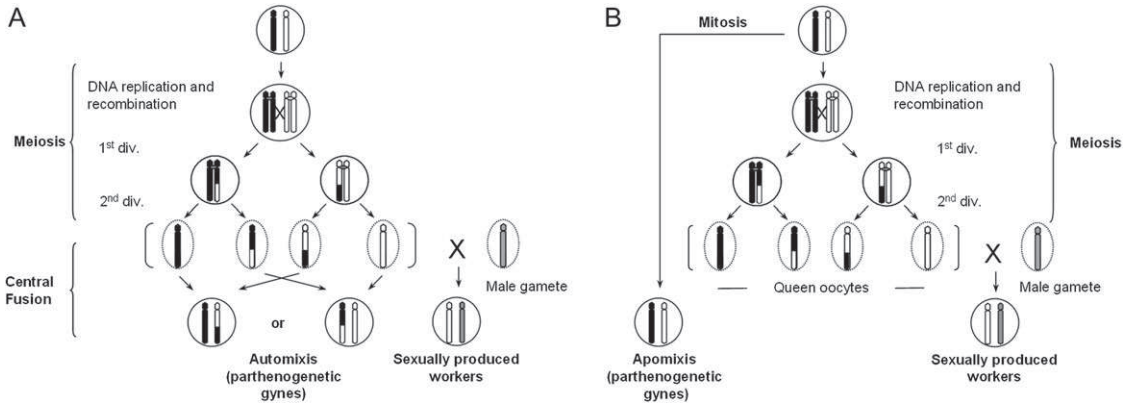

Fig. 1. Hypothetical cytological mechanism of parthenogenesis in queens displaying the ability to use parthenogenesis conditionally for the production of gynes and sexual reproduction for the production of workers. In (*A*), the queen lineage is automictic, and there is only one meiotic process for producing oocytes, which then either fuse together to generate parthenogenetic gynes or are fertilized by male gametes for the sexual production of workers. In (B), the queen lineage is apomictic, and the cytological mechanism of queen parthenogenesis is independent of the meiosis used to generate oocytes for fertilization by male gametes.

asexuality may also have other evolutionary advantages. In a relatively uniform environment with few biotic interactions, asexuality facilitates the conservation of favorable coadapted allelic interactions over time (Burger 1999; Neiman and Linksvayer 2006), whereas sexual reproduction breaks up both interchromosomal (i.e., via segregation) and intrachromosomal (i.e., via recombination) allelic associations. This advantage applies to strictly asexual lineages, but asexuality occurs in highly diverse forms.

Thelytokous parthenogenesis (i.e., thelytoky) is the development of a female individual from an unfertilized egg. Two main types of thelytoky, defined cytologically, are recognized (Suomalainen 1950 in Suomalainen 1962): ameiotic (i.e., apomictic) and meiotic (i.e., automictic). Apomictic thelytoky generates offspring that are strictly genetically identical to the parent (barring mutations and gene conversions), thus maintaining overall levels of heterozygosity. By contrast, meiosis, and hence recombination, occur in automictic thelytoky. A process for restoring diploidy in the resulting reduced oocytes is therefore required. Two such processes are known, each with different genetic consequences. First, diploid restoration through gamete duplication leads to the complete loss of heterozygosity, thus erasing genetic diversity in the resulting offspring. There are only a few known examples of this mechanism in nature, generally in insects, and in most cases, this situation has been shown to result from manipulation by endosymbiotic bacteria, such as *Wolbachia* (e.g., Stouthamer and Kazmer 1994; Gottlieb et al. 2002). Second, diploidy may also be restored by the fusion of two reduced oocytes. If both oocytes arise from the same nucleus (i.e., terminal fusion), global heterozygosity is lost throughout the genome. However, recombination events during the first meiotic division may conserve some heterozygosity, particularly for loci far from chromosomal centromeres. If oocytes from different nuclei fuse (i.e., central fusion), overall heterozygosity should be conserved, except at loci far from the centromeres, which are affected by recombination (fig. 1A). Regardless of the cytological mechanism underlying automixis described above, the offspring displays a partial, or total loss of heterozygosity, which may lead to inbreeding depression (Engelstädter 2008). There is a peculiar mode of automictic parthenogenesis, the premeiotic doubling mechanism, which preserves overall heterozygosity in the resulting offspring. In this case, a premeiotic doubling of chromosomes is achieved through endomitosis, and the number of chromosomes is subsequently reduced through meiosis. During the first division of meiosis, all chromosomes pair with their genetically identical counterpart eliminating the effect of recombination during pairing. As a result, the four resulting daughter cells are diploids and all identical to the mother cell (i.e., overall heterozygosity is preserved baring mutations and gene conversions). As for apomixy, the mechanism of premeiotic doubling only gives rise to diploid gametes and was described in species were females solely reproduce under parthenogenesis (e.g., Terhivuo and Saura 2006; Lutes et al. 2010). Because of its similarities with apomixis with respect to genomic outcomes (i.e., diploid resulting oocytes, preservation of overall heterozygosity), this peculiar mode of automictic parthenogenesis was classified as a specific case of apomixy in some studies (van Wilgenburg et al. 2006). We will hence hereafter use the term "apomixy" to refer to both standard apomictic parthenogenesis (i.e., mitotic parthenogenesis) and premeiotic doubling.

In some species, reproductive modes are a combination of both sexual and parthenogenetic reproduction. In social insects, males are classically haploid and develop from unfertilized reduced oocytes through arrhenotokous parthenogenesis, whereas diploid females are produced sexually (Crozier and Pamilo 1996). There is generally a reproductive cast (i.e., queens and males) responsible for reproduction, whereas the workers share the tasks necessary for the maintenance of populations. Automictic thelytoky with central fusion has recently been demonstrated in some individuals from three different social insect species. In the ant *Cataglyphis cursor*, queens take advantage of the caste organization to benefit from the

advantages of both sexual and asexual reproduction (Pearcy et al. 2004, 2006). Queens make use of automictic thelytoky with central fusion to produce female reproductive individuals (i.e., gynes), thus increasing the transmission of their own genes to production of the "germline" lineage, whereas they use sexual reproduction to produce workers, thereby increasing the genetic diversity of the "somatic" lineage. *Cataglyphis cursor* queen lineages would be expected to suffer a progressive loss of heterozygosity, due to the frequent recombination events associated with automictic thelytoky with central fusion. Pearcy et al. (2004, 2006) argued that the maintenance of populations over time implies the continual replacement of queens by sexually produced parthenogenetic queens or nonsterile workers (i.e., pseudoqueens). In the Cape honeybee, *Apis mellifera capensis*, pseudoqueens produce new queens by automictic thelytoky with central fusion (Verma and Ruttner 1983). Baudry et al. (2004) demonstrated a genetically controlled mechanism for reducing the frequency of recombination events during pseudoqueen meiosis. This process avoids the loss of heterozygosity resulting from thelytoky with central fusion automixis and is thought to provide pseudoqueens with a means of limiting inbreeding depression in the resulting queen offspring. A similar genetically controlled mechanism for reducing the recombination associated with thelytoky has been suggested in the ponerine ant, *Platythyrea punctata* (Kellner and Heinze 2011).

Thelytoky has also been demonstrated for queens of some native and all invasive populations of the little fire ant, *Wasmannia auropunctata* (Fournier, Estoup, et al. 2005; Foucaud et al. 2007, Foucaud, Estoup, et al. 2009). These populations (hereafter referred to as 'clonal' for the sake of simplicity; Foucaud et al. 2007) are found almost exclusively in ecological environments disturbed by human activity (e.g., plantations, open quarry). Molecular studies have indicated that these populations probably emerge recurrently from populations displaying classical sexual haplo-diploid reproduction (hereafter referred to as 'sexual'; Foucaud et al. 2007). Unlike clonal populations, sexual populations are found principally in primary forests. The specific ecological features of clonal populations, differentiating them from sexual populations, suggest that clonality may play a major role in the invasive potential of *W. auropunctata* populations (Foucaud, Orivel, et al. 2009, 2010; Orivel et al. 2009). As in *C. cursor*, the clonal queens of *W. auropunctata* use thelytoky and sexual reproduction in a conditional manner, to produce gynes and workers, respectively. *W. auropunctata* is unusual among thelytokous species, in that queen thelytoky is closely associated with androgenesis (i.e., the production of haploid sons strictly identical to their father baring mutations; Fournier, Estoup, et al. 2005; Foucaud, Estoup, et al. 2009). Moreover, contrary to other species in which queens reproduce by thelytoky, unmated queens of *W. auropunctata* (irrespectively to their reproductive system) were found to be unable to lay viable eggs (Foucaud, Estoup, et al. 2009). Hence, the production of parthenogenetic eggs by clonal queens seems strictly dependent on the fertilization process.

The underlying mechanism of thelytoky in *W.auropunctata* queens has yet to be demonstrated convincingly. In a recent study, Foucaud, Estoup, et al. (2009) observed a limited number of transitions from heterozygosity to homozygosity during female thelytoky. However, it was not possible, based on the results of this study, to discriminate between apomixis with few gene conversion events and automixis with central fusion associated with a loss of recombination, as described in Cape honeybee workers. The genomic consequences of these two mechanisms may differ considerably in the sexually produced worker offspring. Under apomixis (and premeiotic doubling), the production of diploid unfertilized eggs destined to develop into gynes would be independent of the production of haploid oocytes destined to be fertilized for the production of workers (fig. 1*B*). By contrast, if automixis occurs, oocytes destined to develop into queens (fusion of two maternal oocytes) or workers (fertilization of oocytes by sperm) may follow the same process of meiotic division. In particular, oocytes with different fates might originate from the same first meiotic division, during which potential meiotic recombination events occur (fig. 1A). In this case, sexually produced workers hatching from eggs laid by clonal queens would be expected to display a very low recombining maternal genome.

In this study, we first characterized the underlying mechanism of thelytoky in *W. auropunctata* clonal queens by 1) comparing global and locus-by-locus patterns of heterozygosity in both clonal and sexual queens and 2) by directly analyzing the rate of heterozygosity to homozygosity transition at 33 microsatellite loci in female reproductive offspring of clonal queens. We then investigated the potential decrease in recombination rate in sexually produced workers hatching from eggs laid by clonal queens. We did this by comparing recombination rates between these 33 microsatellite loci in workers produced by both clonally and sexually reproducing queens. We found several lines of evidence to suggest that clonal queens use automictic thelytokous parthenogenesis with central fusion combined with low recombination rates. Consistent with this finding, we demonstrated that clonal queens produced meiotic oocytes for the generation of sexually produced workers with dramatically low levels of recombination. These findings have evolutionary consequences for both queens and workers, in the particular ecological context of invasive clonal populations of the little fire ant.

Materials and Methods

Sampling, Experimental Set-Up, and Microsatellite Genotyping

In 2008 and 2009, we sampled 4 sexual and 10 clonal populations of *W. auropunctata* in the native range (i.e., French Guiana) and from various locations within the area of introduction (i.e., New Caledonia, Israel, Florida; table 1). Previous investigations had precisely determined the reproductive system (i.e., clonal or sexual) of all the sampled populations (Foucaud, Orivel, et al. 2009, 2010). Ten clonal

Table 1. Sampling Sites and Reproductive Systems of the Collected *Wasmannia auropunctata* Populations.

				Geographical coordinates		
Reproductive system	Range	Country	Site name	*x*	*y*	Number of lineages
Sexual	Native	French Guiana	M1	−52.980300	5.067717	2
Sexual	Native	French Guiana	M3	−52.950000	5.022817	5
Sexual	Native	French Guiana	M7	−52.974967	5.049300	1
Sexual	Native	French Guiana	Pi32	−52.959967	5.048367	1
					Total	9
Clonal	Native	French Guiana	Cay	−52.213234	4.485450	1
Clonal	Native	French Guiana	RN	−52.917417	5.270533	1
Clonal	Native	French Guiana	Ker	−53.045750	5.071333	1
Clonal	Native	French Guiana	M3 C	−52.949967	5.041300	1
Clonal	Native	French Guiana	M6 C	−52.973950	5.048650	1
Clonal	Native	French Guiana	P2	−52.917800	5.287783	1
Clonal	Native	French Guiana	P3	−52.914833	5.291750	1
Clonal	Introduced	USA (Florida)	Orl	−81.352333	28.590000	1
Clonal	Introduced	Israel	Hz	32.174577	34.834464	1
Clonal	Introduced	New Caledonia	Mp	−20.587833	164.821333	1
					Total	10

NOTE.—The number of analyzed monogyne families per site is indicated in the last column. Note that our experiment initially started from 33 sexual and 23 clonal lineages; many of queens died before laying enough worker offspring for genetic analysis.

and 9 sexual fertilized queens originating from the sampled clonal and sexual populations were isolated in the laboratory, together with 50 workers each from the same populations. These ants were placed in individual monogynous artificial nests consisting of 8 × 10.5 cm boxes (height × diameter). Microsatellite genotyping showed that each monogyne lineage was composed by different queen and male genotypes and were hence genetically different. These 19 monogyne lineages (i.e., families) were housed in a walk-in climatic chamber at a constant temperature of 25°C, with 70% relative humidity and a 12:12 h (light:dark) photoperiod. They were fed ad libitum with a honey–yeast–water solution and *Ephestia* eggs throughout the experiment. For each of the 19 families, we collected 48 newly produced workers at the last pupal stage, after at least 10 weeks, to ensure that these workers were produced by the focal queens. Workers were stored in 95% ethanol, and DNA was extracted for each of the 912 newly produced workers and their parents (i.e., queens and fathers), according to a Chelex-based protocol (Estoup et al. 1996). Individual genotypes for each worker were obtained for 33 microsatellite markers (Fournier, Foucaud, et al. 2005; Almany et al. 2009). Polymerase chain reaction products were separated on an ABI 3130 DNA sequencer (Applied Biosystems). Genetics profiles were analyzed with GeneMapper software version 4.0. (Applied Biosystems).

Indirect Evidence for the Underlying Mechanism of Thelytoky

Traditionally, cytological methods have been applied to dissected mature ovaries to demonstrate directly the mode of thelytoky (i.e., apomixis vs. automixis and mechanism of diploidy restoration; Verma and Ruttner 1983). Unfortunately, such methods cannot be used in *W. auropunctata* because unmated clonal queens of this species cannot lay viable eggs. Furthermore, once they are mated, queens predominantly lay fertilized eggs generating sexually produced workers. They lay parthenogenetic eggs for queen production unpredictably and much less frequently. We therefore looked for indirect evidence about the underlying mechanisms of thelytoky, by analyzing three different microsatellite data sets.

First, the most striking contrast between apomixis and automixis is that meiotic recombination events lead to a gradual loss of heterozygosity over generations in automictic lineages, whereas heterozygosity is maintained and may even increase through the accumulation of mutations in apomictic lineages (Suomalainen 1962). We therefore compared global and locus-by-locus heterozygosities at 33 microsatellite loci in the 10 clonal and 9 sexual queens of our monogynous nests reared in the laboratory. A nonparametric Mann–Whitney *U* test was carried out to assess the significances of differences in mean individual heterozygosity between clonal and sexual queens.

Second, as meiotic recombination does occur in automictic thelytoky, transitions from heterozygosity to homozygosity would be expected to occur frequently in the gyne offspring of a given queen. By contrast, heterozygosity in newly produced gynes would be expected to be retained following rare gene conversion events in apomictic thelytoky. We extended the individual multilocus genotypes obtained for 38 F1 gynes and their respective mothers from the various clonal lineages ($n = 8$) studied by Foucaud, Estoup, et al. (2009), by genotyping 21 new microsatellite markers (Almany et al. 2009), giving a total of 33 analyzed loci. We also produced two entirely new queen-gyne data sets, each for 96 gynes produced by a clonal queen sampled in Israel (both queens originated from the same clonal lineage). Genotypes from individuals displaying homozygosity to maternal heterozygote loci were replicated twice (i.e., two independent DNA extractions and two independent genotyping procedures) to ensure that homozygosity at the concerned loci were not due to genotyping error. Mean and single-locus rates of transition from heterozygosity to homozygosity were estimated directly from these queen-gyne data sets and compared with theoretical expectations for each mechanism of thelytoky.

Third, as the observed transition rate turned out to be very low in F1 gyne offspring (see Results), we analyzed an additional data set, in which we investigated the pattern of transition event accumulation on linkage groups over several generations in a populational sample of queens. This multigenerational pattern of transition events on linkage groups has the potential to inform on the parthenogenetic mode characterizing *W. auropunctata* clonal queens. Under apomixis, transitions to homozygosity result from gene conversion or mutational alteration. In this case, it is expected that, for a given maternal linkage group, a transition event to homozygosity will affect a unique marker of the group without altering heterozygosity at the neighboring markers. By contrast, under automixis, transition events to homozygosity are more likely to result from recombination events. When such a recombination event occurs between the centromere and a given linkage group, several if not all markers of the group are likely to transit from heterozygosity to homozygosity simultaneously. Under automixis, one might therefore expect an excess of nonindependent cotransitions to homozygosity over independent single locus transitions for loci located within the same linkage group. We therefore analyzed the microsatellite genotypes of 105 queens and gynes collected at different locations in the field for an invasive clonal population present in New Caledonia since about 1970 (Foucaud et al. 2006). All these queens were known to originate from a well-known single clonal lineage (i.e., the Q0 clonal lineage; Foucaud et al. 2006), making it easy to identify transition events. All individuals were genotyped at the same 33 microsatellite loci, and we focused our analysis of transition events on groups of microsatellite loci found to belong to the same linkage groups in *W. auropunctata* (see Mapping of Microsatellite Loci).

Analysis of Recombination Rates Based on Worker Families

Cytological Analysis

Analyses of mitochondrial DNA and microsatellite markers indicated that queens (or males) did not cluster according to their reproduction system (i.e., clonal or sexual) and hence that sexual and clonal individuals did not correspond to different species or subspecies (Foucaud et al. 2007). However, we nonetheless analyzed mitotic metaphases from workers from a subset of populations (i.e., three sexual and four clonal lineages), to identify potential differences in chromosome number between populations with different reproductive systems. Such differences might account, at least in part, for differences in recombination pattern between clonal and sexual populations. Chromosome preparations were obtained from the cerebral ganglia of worker nymphs, as described by Imai et al. (1988), but with the following modifications to the protocol. As the larvae of this species are very small, we placed the entire body in a hypotonic colchicine citrate solution (0.005%). We made a small hole in the cephalic capsule, allowing the colchicines solution to penetrate into the brain. Two hours later, the brain was extracted, dissociated directly on a slide, and fixed in a series of solutions of acetic acid, ethanol, and distilled water. Slides were stained with DAPI (i.e., 4′,6-diamidino-2-phenylindole) and observed under a Zeiss A1 microscope (Zeiss S.A.S., Le Pecq, France). Images were acquired, and the number of chromosomes was counted with Genus Software (Applied Imaging, Genetixn Queesway, United Kingdom).

Mapping of Microsatellite Loci

The 33 microsatellite loci studied were mapped based on the microsatellite genotypes obtained from worker families produced by queens from sexual populations. When maternal and paternal genotypes were not obtained directly, they were deduced indirectly from the genotypes of the workers. This inference is straightforward in *W. auropunctata*, as the queens are monoandrous (i.e., each queen mates only once; Foucaud, Estoup, et al. 2009). Once all detectable mistyping errors had been ruled out and the few mutations observed in worker genotypes had been discarded from the data set, we tested for distorted segregation at each locus, in each family, by performing χ^2 tests. In the absence of distorted segregation, the two maternal alleles should be even distributed among workers. The number of significant χ^2 (at the 0.05 threshold) observed for all sexual families was compared with the distribution of the number of significant χ^2 obtained by simulating the same number of families with the same number of genotyped workers (as in the observed data set) and assuming no segregation distortion. We simulated data for 10 million families with a custom-developed computer program for calculating the probability that the observed number of significant χ^2 could be obtained by chance, without segregation distortion. We tested for differences in segregation distortion between clonal and sexual lineages for 27 of the 33 loci studied (6 loci were homozygous in all clonal lineages), using a Wilcoxon signed-rank test.

We established linkage groups in our microsatellite loci at a two-point logarithm of odds (LOD) score threshold of 3.0, using the Carthagene software "group" option (de Givry et al. 2005). As the phase of the alleles was unknown, we expected there to be some bias when mapping loci in linkage groups including more than three loci. We therefore inferred the phase in each linkage group with more than two loci, as follows. We first identified a central locus (i.e., linked to all other loci of the linkage group) by a two-point analysis. We then arbitrarily assigned a code to each worker: A or H (corresponding to usual "F2 backcross" data types in genetic mapping software), depending on the particular maternal allele it displayed in each family. In parallel, we constructed a table in which, for each pair of linked loci, the number of recombinant and parental types was calculated (the parental type being the predominant association of two alleles at two loci). We then determined the phase of all linked loci, taking into account the identified parental and recombinant worker types at the central locus. Several genetic maps were then designed for each linkage group (from 3 to10, depending on the number of loci constituting the group) using the Build option of Carthagene software. Maps displaying the

maximum likelihood were eventually selected and genomic distances between loci were estimated.

Comparison of Recombination Rates in Sexual and Clonal Queen Lineages

The rates of recombination between each pair of loci (r) were calculated as the total number of recombinant worker genotypes divided by the total number of genotyped workers, based on a LOD score approach in multiple families originating from either clonal or sexual lineages.

We compared recombination rates between sexual and clonal lineages for pairs of loci within each linkage group. Three of the 28 microsatellite loci belonging to defined linkage groups were homozygous in all clonal lineages and were therefore discarded from the comparative analysis. We found that r estimates between some loci from different linkage groups mapped in sexual lineages were significantly lower than 0.5 in clonal lineages. We included these r estimates in our comparative analysis, which was thus based on a total of 29 pairwise r estimates calculated in both sexual and clonal lineages. We compared r values for each pair of loci, using Fisher's exact tests. We corrected the 5% significance threshold by the number of tests performed using the Benjamini and Hochberg false discovery rate procedure (Benjamini and Hochberg 1995). We then calculated mean recombination rates ($\bar{r}$) in clonal and sexual lineages. We assessed the significance of differences in $\bar{r}$ between clonal and sexual lineages, with a nonparametric Mann–Whitney U test.

Results

Mechanism Underlying Queen Thelytoky

The thelytokous queens of our experimental lineages displayed a significantly lower level of heterozygosity than sexual queens (mean $H_{obs} = 0.55$ and 0.80, respectively; $P = 9 \times 10^{-4}$, Supplementary table S1, Supplementary Material online). Locus-by-locus analysis revealed that the observed lower level of heterozygosity was accounted for mostly by six loci that were homozygous in all clonal queens but heterozygous in most, if not all sexual queens (Supplementary table S1, Supplementary Material online). By contrast, none of the 33 loci was found to be homozygous in all sexual populations.

There are several possible reasons for such a large decrease in heterozygosity at the same six loci in all clonal lineages. First, clonal lineages may originate from the same common ancestor, which was homozygous at these six loci. In this case, all clonal lineages would be expected to have the same allele at a given locus. The high degree of allelic diversity between clonal lineages observed for these six loci (from three to eight alleles per locus) does not support this hypothesis. Second, a lower level of heterozygosity may result from selection or bottleneck events occurring during the transition from sexuality to clonality or during the establishment of clonal populations. Transition to homozygosity would not be expected to occur for the same 6 microsatellite loci in all 10 clonal lineages under both evolutionary forces unless those 6 loci were genetically linked. However, our mapping results demonstrated that these six loci belonged to five different linkage groups (see below). Third, in conditions of apomixis, episodic transitions to homozygosity resulting from mutations and/or gene conversion might be expected. However, it is unlikely that such events would occur recurrently, at the same six loci, in different clonal lineages. By contrast, in conditions of automixis with central fusion, recombination events would be expected to lead to transitions to homozygosity for all loci located far from the centromeres, whatever the origin of the clonal lineage. The observed pattern would thus be easy to explain by a mechanism of automictic thelytoky if the six homozygous loci were actually located at some distance from the centromere.

We detected very few single-locus transitions to homozygosity in our various thelytokous queen-gyne offspring data sets. The mean transition rate per locus ranged from 0 to 2.8%. The rate of homogenization during automixis with central fusion is expected to range from 0 in the absence of recombination events to 1/3 in the presence of recombination (Pearcy et al. 2004; Oldroyd et al. 2008). The observed pattern of transition rates is hence consistent with what would be expected under conditions of automixis with central fusion only if the number of recombination events is globally very small all over the genome.

The small number of transition events observed in the queen-gyne offspring data sets concerned unlinked loci. We were therefore unable to check for the occurrence of cotransitions for loci from the same linkage group in these data sets. Such cotransitions would be expected to occur with automixis, but not with apomixis with episodic gene conversion events. Only when we analyzed our multigenerational population data set including data for 105 reproductive females from New Caledonia did we find a clear excess of nonindependent cotransitions to homozygosity ($n = 8/10$) over independent single-locus transitions ($n = 2/10$) for loci belonging to the same linkage group (Supplementary Table S2, Supplementary Material online).

Comparison of Recombination Rates in Sexual and Clonal Queen Lineages

We found that 28 of the 33 microsatellite loci genotyped in worker families produced in sexual queen lineages were dispersed over 11 different linkage groups (fig. 2). The five remaining loci displayed no significant linkage and could not, therefore, be mapped. We compared recombination rates between the 29 pairs of loci estimated from worker families produced in either clonal or sexual queen lineages (table 2; fig. 2). No recombination events were detected in worker families produced by clonal queens, for 19 of the 29 pairs of loci considered. The mean recombination rates were 0.555% in clonal queens (ranging from 0% to 2.50%) and 25.2% in sexual queens (ranging from 0.368% to 47.7%). The mean recombination rate was thus lower in clonal queens, by a factor of 45.3 (table 2). Consistent with these strikingly different patterns of recombination, several linkage groups that were considered independent in analyses of worker families from sexual queens were found to be

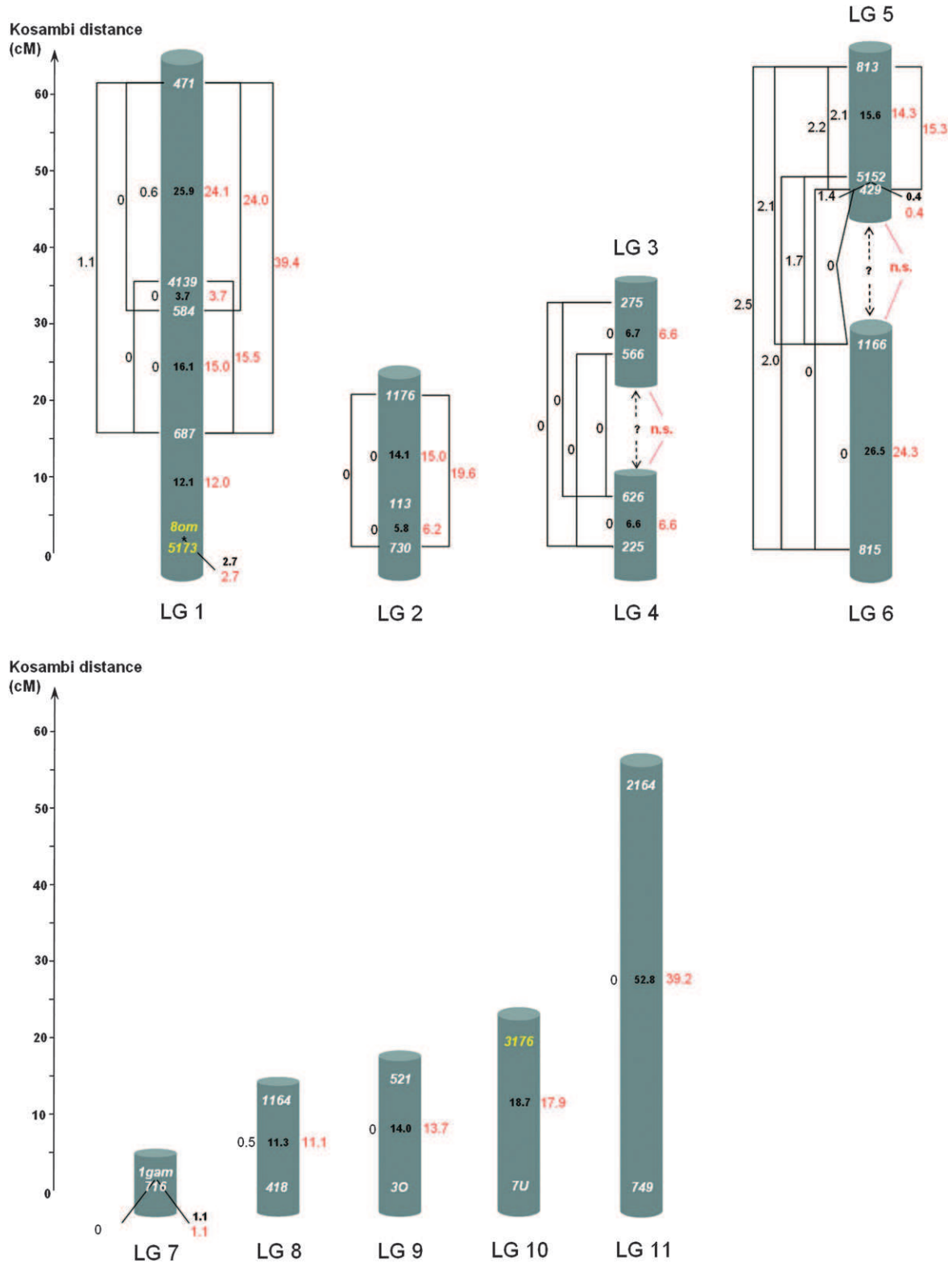

FIG. 2. Linkage groups in *Wasmannia auropunctata* deduced from families of workers from sexual lineages and estimates of recombination rates for both sexual and clonal queen lineages. Recombination rates estimated from sexual queen lineages are shown on the right of linkage group (red characters) whereas those estimated from clonal queen lineages are shown on the left (black characters). Kosambi distances between loci, inferred from sexual lineages, are shown in bold black characters within linkage groups. The names of microsatellite loci are shown in white. Microsatellite loci in yellow are homozygous in all clonal lineages analyzed, making it impossible to estimate the recombination rate for these loci in these lineages. Other microsatellite loci did not display any significant linkage.

Table 2. Mean Recombination Rates ($\bar{r}$) and Recombination Rates for Each Pair of Linked Markers (r) Estimated from Workers Produced Sexually in Clonal and Sexual Lineages.

		Clonal				Sexual					
Locus 1	Locus 2	*N* recombinant	*N* parental	*rc*	lod	*N* recombinant	*N* parental	*rs*	lod	*rc*/*rs*	*P* value
113	1176	0	172	0	51.777	45	255	15	35.235	0	7.92E-10
1166	429	0	340	0	102.350	116	158	42.336	1.404	0	2.20E-16
1166	5152	5	293	1.678	78.678	136	183	42.633	1.509	0.039	2.20E-16
1166	813	1	47	2.083	12.338	138	175	44.089	0.952	0.047	4.10E-10
1166	815	0	324	0	97.534	61	190	24.303	15.109	0	2.05E-01
1gam	716	0	360	0	108.371	1	92	1.075	25.595	0	0.21
2164	749	0	388	0	116.800	120	186	39.216	3.116	0	2.20E-16
225	566	0	85	0	25.588	66	73	47.711	0.077	0	0.02
225	626	0	82	0	24.684	12	171	6.557	35.852	0	2.20E-16
275	225	0	87	0	26.190	107	127	45.726	0.372	0	2.20E-16
275	566	0	130	0	39.134	15	211	6.637	44.069	0	0.001503
275	626	0	213	0	64.119	78	109	41.711	1.121	0	2.20E-16
418	1164	2	418	0.476	120.922	37	297	11.078	50.045	0.043	6.72E-12
429	5152	5	345	1.429	93.979	1	271	0.368	79.012	3.883	0.24
429	813	1	45	2.174	11.755	42	232	15.328	31.508	0.142	2.20E-16
429	815	0	288	0	86.697	85	127	40.094	1.819	0	2.20E-16
471	4139	1	180	0.552	51.796	74	233	24.104	18.782	0.023	1.14E-15
471	687	3	273	1.087	75.897	134	206	39.412	3.336	0.028	2.20E-16
5152	813	1	47	2.083	12.338	39	233	14.338	33.323	0.145	0.02
5152	815	5	241	2.033	63.444	106	146	42.063	1.385	0.048	2.20E-16
521	30	0	222	0	66.829	25	158	13.661	23.396	0	9.06E-10
566	626	0	81	0	24.383	59	84	41.259	0.954	0	1.23E-14
584	4139	0	136	0	40.940	10	258	3.731	62.134	0	0.02
584	471	0	118	0	35.522	63	200	23.954	16.287	0	7.84E-12
584	687	0	140	0	42.144	45	256	14.950	35.466	0	2.23E-08
687	4139	0	196	0	59.002	54	294	15.517	39.532	0	1.27E-11
730	113	0	171	0	51.476	22	332	6.215	70.769	0	2.34E-04
730	1176	0	185	0	55.691	70	287	19.608	30.734	0	1.81E-14
815	813	1	39	2.500	10.010	117	132	46.988	0.196	0.053	4.19E-09
Global			$\bar{r}_c$ = 0.555%				$\bar{r}_s$ = 25.161%			$\bar{r}_c/\bar{r}_s$ = 45.335%	

NOTE.—*r* values for each pair of loci were compared using Fisher's exact test.

linked in analyses of worker families from clonal queens (i.e., linkage groups 3 and 4, and linkage groups 5 and 6; fig. 2).

The observed much lower rates of recombination in clonal than in sexual queen meiosis could not be accounted for by differences in chromosome number between sexual and clonal lineages. Our cytological analysis indeed revealed that *W. auropunctata* workers had the same number of chromosomes ($2N = 32$) whether produced by clonally or sexually reproducing queens (Supplementary fig. S1, Supplementary Material online). There were therefore no major cytological differences between clonal and sexual *W. auropunctata* populations.

Finally, we tested for an excess of meiotic segregation distortion in low recombining clonal lineages due to more widespread hitchhiking effects in such lineages in comparison with sexual ones. We found only a small percentage of significant distortion segregation in the sexually produced worker offspring from either clonal or sexual queen lineages. The proportion of observed significant χ^2 values in all tests was, however, similar to the proportion expected in the absence of distortion segregation, and the observed distortion segregations were randomly distributed over loci and lineages. The proportion of significant distortion segregation at the 0.05 level did not differ significantly between workers from clonal and sexual lineages (Wilcoxon signed-rank test $P = 0.93$, $n = 27$). The segregation of our microsatellite loci during queen meiosis hence appeared similar in both clonal and sexual lineages, despite the large differences in recombination patterns.

Discussion

Thelytoky and Loss of Recombination in *Wasmannia auropunctata* Queens

Wasmannia auropunctata (Foucaud et al. 2007), like *Vollenhovia emeryi* (Ohkawara et al. 2006) and *C. cursor* (Pearcy et al. 2004, 2006), is one of the rare species of the Hymenoptera in which some queens are able to make conditional use of both sexuality and thelytoky in the production of workers and queens, respectively. In this study, we obtained indirect evidence that *W. auropunctata* queens from clonal populations give rise to gyne offspring via a mechanism of automictic thelytokous parthenogenesis with central fusion. This is not in agreement with van Wilgenburg et al. (2006) who classified *W. auropunctata* queen parthenogenesis as apomictic (premeiotic doubling) based on the first study showing parthenogenesis in some queens of this species (Fournier, Estoup et al. 2005). Automixis with central fusion, also found in *V. emeryi* and *C. cursor*, seems to be a common way for individuals to use both sexuality and thelytoky in the production of their offspring. This mechanism appears to be the simplest

mechanism for transition from sexuality to parthenogenesis, as it does not require major changes in the cytological mechanism of meiosis (Schwander and Crespi 2009). Clonal queens produce meiotic oocytes, which may either fuse together for gyne production or be fertilized by male gametes for the production of workers (fig. 1*A*).

Theoretically, automictic thelytoky with central fusion leads to partial genetic homogenization in the newly produced offspring, due to recombination events during meiosis. However, we found unexpectedly low rates of transition from heterozygosity to homozygosity in thelytokous queen lineages, confirming, for a much larger data set, the low rates previously observed by Foucaud, Estoup, et al. (2009). A genetically controlled mechanism for decreasing recombination, thereby limiting the transition to homozygosity, has been demonstrated in the Cape honeybee, whose orphaned workers (i.e., pseudoqueens) produce new queens via automictic thelytoky with central fusion (Moritz and Haberl 1994; Baudry et al. 2004). Kellner and Heinze (2011) provided indirect evidence of the existence of a similar mechanism in the ponerine ant *P. punctata*. In this study, we showed that workers produced sexually by thelytokous queens also displayed a much lower recombination rate than workers produced from sexual queens (by a factor of about 45). Our results hence demonstrate that *W. auropunctata* clonal queens produce meiotic oocytes with normal chromosome segregation but with very low rates of recombination during the first meiotic division. These cytological features apply to gyne production, as the diploid restoration system during automixis involves the fusion of reduced oocytes (i.e., central fusion) that probably originated from the same first meiotic division process (fig. 1*A*).

Baudry et al. (2004) argued that automictic thelytoky and low recombination rates were probably controlled by different genes in the Cape honeybee. We observed very low rates of recombination in all *W. auropunctata* clonal lineages (genetically different according to our microsatellite data), whereas such low rates of recombination were not observed in any of the nine sexual queens from four genetically different populations studied. This suggests that, in *W. auropunctata*, thelytoky and the reduction of recombination are two coadapted traits that have emerged together, several times, in different lineages, during the evolutionary shift from sexuality to clonality. We therefore suggest that, in *W. auropunctata*, both traits are likely to be genetically controlled by either the same gene(s) or by gene(s) close enough together to be fixed jointly in various clonal lineages. It is also possible that selection pressure against transitions to homozygosity is particularly strong in thelytokous queen lineages, resulting in rapid counter selection against clonal lineages not bearing the gene(s) for low recombination rates.

Evolutionary Consequences of Reducing Recombination Rates During Automixis

Given the high genomic recombination rates reported in others social insects (Gadau et al. 2000; Wilfert et al. 2007), the number of linkage groups found in *W. auropunctata* worker families produced in sexual queen lineages (i.e., 11 groups of 2 or more markers) might seem at first sight particularly low considering that only 33 microsatellite markers was used in this study. This might indicate that genomic recombination rates are already low in sexual queens of this species. Unfortunately, the present data set has a too limited resolution (i.e., include a too small number of markers) to allow a robust comparison of the number and configuration of linkage groups with previous mapping studies conducted in other social insects.

The decrease in recombination rates in *W. auropunctata* thelytokous queen meiosis compared with their sexually reproducing relatives seems to be much greater than that reported for Cape honeybees (factor of >45 vs. >10, respectively; Baudry et al. 2004). This extreme decrease in recombination rates is probably a response to particularly strong selective pressure against recombination in queens during the establishment and maintenance of clonal lineages.

Decrease in meiotic recombination is expected to limit the negative effects of the loss of heterozygosity associated with automictic thelytoky with central fusion (particularly for genes far from the centromere). Theoretically, this loss of heterozygosity may lead to inbreeding depression, which has been shown to have various negative effects on life history traits in many species (Keller and Waller 2002; Charlesworth and Willis 2009). In some ant species, brood quality, queen life span, male quality, and, more generally, colony survival are negatively affected by inbreeding depression (Schrempf et al. 2006; Haag-Liautard et al. 2009; but see Thurin and Aron 2009). Moreover, additional costs of inbreeding are likely in Hymenoptera due to the system of single-locus complementary sex determination in this order (van Wilgenburg et al. 2006), resulting in individuals homozygous at the sex locus developing into nonviable or sterile males (Petters and Mettus 1980; Cook 1993; but see Cowan and Stahlhut 2004).

The maintenance of heterozygosity through a large decrease in recombination rate in *W. auropunctata* thelytokous queens would therefore be expected to increase the life time of clonal lineages. In *C. cursor*, no decrease in recombination has been observed in queens reproducing by automictic thelytokous parthenogenesis with central fusion, and populations are thought to be maintained partly by a renewal of parthenogenetic lineages suffering from inbreeding depression (Pearcy et al. 2004). This renewal is achieved through both sexual events for gyne production and the production of new queens from sexually produced workers, by thelytokous parthenogenesis (Pearcy et al. 2004). By contrast, *W. auropunctata* workers are sterile in both sexual and clonal populations, and although sexual reproduction events to produce new queens do occur in clonal populations, they are much rarer than parthenogenesis events (Foucaud et al. 2006; Foucaud, Estoup, et al. 2009). Moreover, the presence within each clonal population of a single maternal genome as well as a single paternal genome reproducing through androgenesis would lead to the rapid increase of homozygosity for both gyne and

worker offspring within such sexually produced lineages (Foucaud et al. 2006, 2010). The large decrease in recombination observed in clonal populations of *W. auropunctata* thus constitutes the only means by which this species can preserve clonal lineages over time. Consistent with this hypothesis, Mikheyev et al. (2009) suggested that a particular *W. auropunctata* clonal lineage has been maintained in Gabon (Africa) since the beginning of the last century.

In a lineage reproducing by automictic thelytoky with central fusion associated with a large decrease in recombination, the most if not the entire genome is transmitted to the next generation and selection acts on the genome as a whole. In a given stable environment, an emerging thelytokous parthenogenetic lineage adapted to the environment will conserve interactions of coadapted alleles over time (Maynard Smith 1978; Lynch 1984; Burger 1999). Consistent with this hypothesis, several authors have noted that thelytoky often occurs in habitats in which biotic interactions are limited, the diversity of biotic interactions being a significant element in environmental uncertainty (Lynch 1984). *Wasmannia auropunctata* clonal populations are found mostly in habitats disturbed by human activity, which often display fewer biotic interactions than natural habitats (Wetterer and Porter 2003; Orivel et al. 2009). Well-adapted thelytokous queens may therefore have an advantage over their sexual relatives in such areas. Furthermore, in the sterile worker offspring with limited recombination, lower rates of recombination during queen meiosis may also preserve maternal allelic interactions, at least on individual chromosomes. Although sterile, workers make a major contribution to the inclusive fitness of reproductive individuals, including queens in particular (Crozier and Pamilo 1996). Furthermore, whereas queens are generally confined to favorable conditions within the nest, selection is more likely to act on workers, which are faced with biotic and abiotic environmental factors during foraging. In changing environments, restricted meiotic recombination may make it difficult for clonal lineages to adapt sufficiently rapidly (Lynch 1984; Otto and Michalakis 1998). Consistent with this, sexual *W. auropunctata* populations are maintained in native primary forests, which are characterized by major biotic interactions (Orivel et al. 2009) and in which very few clonal populations have ever been found (Foucaud, Orivel, et al. 2009; Rey O and Orivel J, personal observation).

In the present study, we provided new insights on the extraordinary reproductive system of *W. auropunctata* clonal lineages. In particular, we determined how clonal queens produce their female offspring (i.e., worker and gyne). Our findings indicate that parthenogenetic queens produce a unique kind of meiotic oocytes with a drastic reduction of recombination that may either fuse together for gyne production (automictic parthenogenesis with central fusion) or be fertilized by male gametes for the production of workers. Unfortunately, our results do not inform on how and to which extent, queens control the conditional production of workers or gynes. The mechanism underlying the capacity of parthenogenetic queens to lay eggs that develop into haploid males strictly identical to their father (i.e., the so-called male clonality reproductive system) is also still unresolved. We are currently leading some laboratory cross experiments between reproductives (i.e., queens and males) originating from lineages characterized by different reproductive systems to clarify the respective role of the male and queen genetic contribution in this uncommon reproductive system.

Supplementary Material

Supplementary tables and figure are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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SUPPLEMENTARY MATERIAL:

Reproduction sytem	Queen lineage	Microsatellite locus																
		418	**730**	**3176**	**1gam**	**275**	**716**	**225**	**2164**	**1166**	**680**	**521**	**566**	**1164**	**429**	**113**	**584**	**1176**
Sexual	M1-1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	0	1	1
	M1-2	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1
	M3-4	1	1	1	1	1	1	1	1	0	0	1	1	1	0	1	1	1
	M3-5	1	1	0	1	1	0	1	1	1	1	0	0	1	1	1	1	1
	M3-11	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
	M3-12	1	1	0	1	1	-	1	1	1	1	1	0	1	1	1	1	0
	M3-14	1	1	1	1	0	0	1	1	1	1	1	0	1	0	1	1	1
	M7-10	0	1	1	1	1	0	0	1	1	0	0	1	0	0	1	0	1
	Pi32	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1
Mean over sexual lineages		**0.89**	**1.00**	**0.78**	**1.00**	**0.89**	**0.25**	**0.78**	**0.89**	**0.89**	**0.78**	**0.56**	**0.67**	**0.78**	**0.67**	**0.89**	**0.78**	**0.89**
Clonal	Cay	1	1	0	1	1	1	0	1	1	0	1	0	1	1	1	1	1
	Flo	1	0	0	0	1	1	0	1	1	0	0	0	1	1	0	0	0
	Isr4	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	0	0
	Ker	1	1	0	1	1	1	0	1	1	0	1	0	1	1	1	0	1
	M3C	1	1	0	1	0	1	0	1	1	0	0	0	1	0	0	0	0
	M6	1	0	0	1	0	1	0	1	0	0	0	0	0	1	0	1	0
	P2	1	1	0	1	1	1	0	1	1	0	1	0	1	1	0	0	0
	QO	1	1	0	1	1	1	0	1	1	0	1	0	1	1	1	0	1
	RN	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	0	1
	P3	1	0	0	0	1	1	0	1	1	0	-	1	1	1	0	1	1
Mean over clonal lineages		**1.00**	**0.70**	**0.00**	**0.80**	**0.80**	**1.00**	**0.20**	**1.00**	**0.90**	**0.00**	**0.56**	**0.30**	**0.90**	**0.90**	**0.40**	**0.30**	**0.50**

Table S1: Single-locus and mean heterozygosity values for sexual and clonal queens. Note: The 6 loci that were found to be homozygous in all clonal queens are highlighted in gray.

Reproduction sytem	Queen lineage	Microsatellite locus																
		5173	**749**	**30**	**626**	**471**	**7U**	**5152**	**8179**	**8om**	**687**	**385**	**297**	**815**	**813**	**4139**	**872**	**Mean overall loci**
Sexual	M1-1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0.85
	M1-2	1	1	1	1	1	1	1	1	0	1	0	1	0	1	1	1	0.82
	M3-4	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	0.82
	M3-5	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	0.79
	M3-11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0.94
	M3-12	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	0.84
	M3-14	1	1	1	0	1	1	1	0	1	1	0	0	1	0	1	1	0.73
	M7-10	1	0	1	0	1	1	0	0	1	1	1	1	0	1	1	1	0.61
	Pi32	1	1	1	0	1	0	1	1	1	1	1	1	1	1	0	1	0.85
Mean over sexual lineages		**1.00**	**0.89**	**0.89**	**0.56**	**0.89**	**0.78**	**0.78**	**0.78**	**0.89**	**1.00**	**0.56**	**0.67**	**0.78**	**0.89**	**0.89**	**0.89**	**0.80**
Clonal	Cay	0	1	1	1	1	1	1	1	0	1	0	0	1	0	1	0	0.70
	Flo	0	1	1	1	1	1	1	1	0	0	0	0	-	0	0	0	0.44
	Isr4	0	0	1	1	1	0	1	0	0	1	0	0	1	0	1	1	0.61
	Ker	0	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	0.64
	M3C	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0.30
	M6	0	1	1	1	1	0	1	1	0	1	0	0	0	0	1	0	0.42
	P2	0	1	1	0	1	1	1	0	0	1	0	0	1	0	0	0	0.52
	QO	0	1	1	1	1	0	0	0	0	1	0	0	1	0	1	1	0.61
	RN	0	1	1	1	1	0	1	1	0	0	0	0	1	0	0	1	0.64
	P3	0	1	1	0	1	1	1	0	0	1	0	0	1	0	1	1	0.59
Mean over clonal lineages		**0.00**	**0.90**	**0.90**	**0.60**	**1.00**	**0.50**	**0.80**	**0.40**	**0.00**	**0.70**	**0.00**	**0.00**	**0.89**	**0.10**	**0.50**	**0.50**	**0.55**

Table S1: Continued

Linkage group	Linkage group 2						Linkage group 8				Linkage group 11			
Kosambi distance	5.8 cM			14.1 cM			11.3 cM				52.8 cM			
Microsatellite loci	730		113		1176		418		1164		749		2164	
Reference Q0 gynotype	*153*	*177*	*355*	*364*	*238*	*246*	*97*	*112*	*162*	*164*	*415*	*417*	*288*	*290*
BSQ_12-8	153	153	364	364	238	238	97	112	162	164	415	417	288	290
MPG_5-18	153	153	364	364	238	238	97	112	162	164	415	417	288	290
MPQ_5-5	153	177	355	364	238	246	97	112	162	164	415	417	288	288
BSQA_13-3	153	177	364	364	238	246	112	112	164	164	415	417	288	290

Table S2: Single-locus transitions and multiple locus cotransitions from heterozygosity to homozygosity observed at linked loci in clonal queens. Note: Queens were collected in the field, from different sites, from an invasive clonal population in New Caledonia. Linkage groups were defined on the basis of data for workers produced sexually by queens originating from sexual lineages (see Figure 2). The transitions observed are highlighted in grey. The reference Q0 genotype is in italic characters.

		Clonal				Sexual					
Locus1	**Locus2**	**$N_{recombinant}$**	**$N_{parental}$**	**r_c**	**lod**	**$N_{recombinant}$**	**$N_{parental}$**	**r_s**	**lod**	**r_c / r_s**	**P-value**
113	1176	0	172	0	51.777	45	255	15	35.235	0	7.92E-10
1166	429	0	340	0	102.350	116	158	42.336	1.404	0	2.20E-16
1166	5152	5	293	1.678	78.678	136	183	42.633	1.509	0.039	2.20E-16
1166	813	1	47	2.083	12.338	138	175	44.089	0.952	0.047	4.10E-10
1166	815	0	324	0	97.534	61	190	24.303	15.109	0	2.05E-01
1GAM	716	0	360	0	108.371	1	92	1.075	25.595	0	0.21
2164	749	0	388	0	116.800	120	186	39.216	3.116	0	2.20E-16
225	566	0	85	0	25.588	66	73	47.711	0.077	0	0.02
225	626	0	82	0	24.684	12	171	6.557	35.852	0	2.20E-16
275	225	0	87	0	26.190	107	127	45.726	0.372	0	2.20E-16
275	566	0	130	0	39.134	15	211	6.637	44.069	0	0.001503
275	626	0	213	0	64.119	78	109	41.711	1.121	0	2.20E-16
418	1164	2	418	0.476	120.922	37	297	11.078	50.045	0.043	6.72E-12
429	5152	5	345	1.429	93.979	1	271	0.368	79.012	3.883	0.24
429	813	1	45	2.174	11.755	42	232	15.328	31.508	0.142	2.20E-16
429	815	0	288	0	86.697	85	127	40.094	1.819	0	2.20E-16
471	4139	1	180	0.552	51.796	74	233	24.104	18.782	0.023	1.14E-15
471	687	3	273	1.087	75.897	134	206	39.412	3.336	0.028	2.20E-16
5152	813	1	47	2.083	12.338	39	233	14.338	33.323	0.145	0.02
5152	815	5	241	2.033	63.444	106	146	42.063	1.385	0.048	2.20E-16
521	30	0	222	0	66.829	25	158	13.661	23.396	0	9.06E-10
566	626	0	81	0	24.383	59	84	41.259	0.954	0	1.23E-14
584	4139	0	136	0	40.940	10	258	3.731	62.134	0	0.02
584	471	0	118	0	35.522	63	200	23.954	16.287	0	7.84E-12
584	687	0	140	0	42.144	45	256	14.950	35.466	0	2.23E-08
687	4139	0	196	0	59.002	54	294	15.517	39.532	0	1.27E-11
730	113	0	171	0	51.476	22	332	6.215	70.769	0	2.34E-04
730	1176	0	185	0	55.691	70	287	19.608	30.734	0	1.81E-14
815	813	1	39	2.500	10.010	117	132	46.988	0.196	0.053	4.19E-09
Global				**$\overline{r_c}$ = 0.555%**				**$\overline{r_s}$ = 25.161%**		**$\overline{r_s}$ / $\overline{r_c}$ = 45.335**	

Table S3: Mean recombination rates ($\bar{r}$) and recombination rates for each pair of linked markers (r) estimated from workers produced sexually in clonal and sexual lineages. Note: r values for each pair of loci were compared using Fisher's exact test.

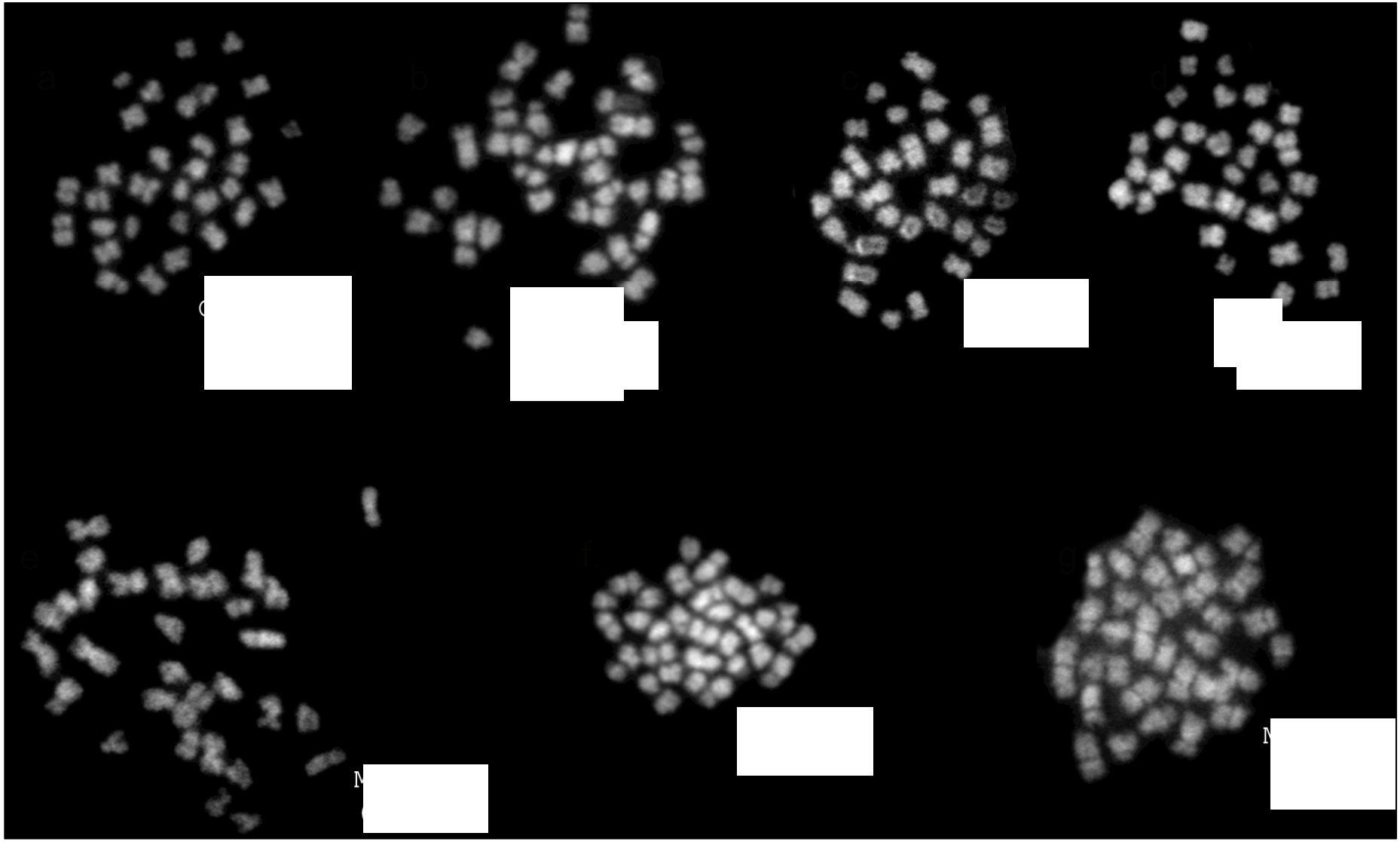

Figure S1: Metaphase chromosomes obtained from worker larvae originating from clonal and sexual populations of *W. auropunctata*. Clonal populations are located within the native range (a, Cayenne and b, Ker are from French Guiana) and the introduced range (c, Israel and d, New Caledonia). Sexual populations are located within the native range (e, M11, f, M3 and g, M7), with all sites located in French Guiana. All metaphasic plates display 2N=32 chromosomes

III. Article 3: Androgenesis in the little fire ant corresponds to a male genome kidnapping by females rather than a male egg parasitism.

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Running title: Androgenesis in *Wasmannia auropunctata*

ABSTRACT

Among the variety of modes of asexual reproduction, androgenesis is probably the least studied and the most puzzling. Androgenesis is the production of an offspring containing only the nuclear genome of the fathering male via the maternal eggs and their nutrient reserves. This hijacking of eggs often leads evolutionary biologists to consider androgenesis as an "eggs parasitism" strategy by males. While populations of the little fire ant *Wasmannia auropunctata* are generally characterized by a classical sexual haplo-diploid reproduction mode, in some populations androgenesis occurs in tight association with queen parthenogenesis and workers are produced via sexual reproduction. We conducted laboratory reciprocal-crosses experiments involving reproductives from both types of populations and analysed their reproductive offspring at 12 microsatellite markers and one mitochondrial gene to decipher the role of males and females in androgenesis. Our results demonstrate that androgenesis in *W. auropunctata* does not correspond to an egg-parasitism strategy by males but is rather under the control of parthenogenetic queens, hence corresponding to a so called "male genome kidnapping" strategy by females. We argue that this male genome kidnapping mechanism allows parthenogenetic females lineages to increase their adaptive potential indirectly through sexually produced workers, hence allowing them to persist over a long time in changing environments.

INTRODUCTION

Parthenogenesis is a mechanism in which the offspring develop from unfertilized eggs, laid by a virgin female, into females (thelytoky) or males (arrhenotoky; Suomalainen 1950). In strict thelytokous parthenogenetic species, males are absent (e.g. Himler *et al.* 2009). Gynogenesis, and androgenesis are two other mechanisms that lead to the production of an offspring that contains the genetic nuclear from only one parent, but differ from parthenogenesis in that two sexual partners are required for the development of the offspring. As a result, one of the two sexual partners invests in the mating without transmitting its nuclear genome. Due to its use of sperm from heterospecific males for egg activation, gynogenesis is considered as "sperm parasitism" (Hughes 1989). Similarly, the hijacking of eggs by males' chromosomes during androgenesis often leads to consider this breeding system as "egg parasitism" (Hedtke *et al*. 2008). Androgenesis has been identified as the main form of reproduction in very few species from four distinct taxa; in the hermaphroditic Saharan cypress tree, *Cupressus dupreziana* (Pichot *et al* 2001), in four hermaphroditic species of freshwater clams in the genus *Corbicula* (Komaru et al. 1998; Ishibashi et al. 2003; Byrne et al. 2000; Korniushin 2004), in a hybrid complex of some stick insects in the genus *Bacillus* (Mantovani & Scali 1992; Tinti & Scali 1996) and recently in two ant species, *W. auropunctata* (Fournier *et al.* 2005a; Foucaud *et al.* 2007) and *Paratrechina longicornis* (Pearcy *et al.* 2011).

Androgenesis is traditionally regarded as a trait under the control of males, giving them a substantial fitness benefit compared to their sexually reproducing relatives (McKone & Halpern 2003). As a consequence, evolutionary models predict that an androgenesis mutation will often spread rapidly to fixation but consequently lead populations to extinction (McKone & Halpern 2003). However, extinction may be avoid in populations that retain female reproductive capacity (McKone & Halpern 2003), for instance, through hermaphrodism as it is the case for *C. dupreziana* and the clams of the genus *Corbicula*, or when androgenesis result from hybridization between sexual relatives as in the stick insects *Bacillus* hybrid complex (Tinti & Scali 1995; 1996).

We are not aware of theoretical or empirical studies that have investigated the evolutionary outcomes of androgenesis in haplo-diploid organisms such as ants. In *W. auropunctata*, androgenesis occurs in some populations in which female reproductives (i.e. queens) reproduce via parthenogenesis (i.e. thelytoky) and workers (i.e. sterile individuals) are produced via sexual reproduction (Fournier *et al.* 2005a; Foucaud *et al.* 2007; 2010). In the single population of *P. longicornis* studied so far, a similar mating system was found (Pearcy *et al.* 2011). It remains however unknown whether the whole species is only made up of such populations. By contrast, Foucaud *et al.* (2007) demonstrated that populations displaying an androgenesis-parthenogenesis system (hereafter referred to as 'clonal populations' for the sake of simplicity), have recurrently emerged from classical haplo-diploid sexual populations (hereafter referred to as 'sexual') whereby females (i.e. queens and sterile workers) develop from diploid fertilized eggs and males develop from haploid unfertilized eggs (arrhenotoky).

The emergence of a thelytoky mutation in a sexual population theoretically reduces the male reproductive success to zero and potentially lead to the extinction of males (e.g. Himler et al. 2009). Fournier *et al.* (2005a) therefore argued that, in *W. auropunctata*, androgenesis emerged in males as a response face to female parthenogenesis, to preserve their reproductive success. The same authors also suggested that androgenesis might involve a maternal genome elimination of diploid eggs by males (MGE). However, as stated by Foucaud *et al.* (2007), because workers are diploid females produced sexually, they should also suffer from the above conflict between sexes. As a result, a drastic decrease of worker production at the expense of males would be expected. Yet, males in these populations are scarce and clonal nests in the field display great worker densities compared to their sexual relatives (Orivel et al. 2009). These observations weakened the MGE hypothesis. Foucaud *et al.* (2007) suggested two alternative mechanistic hypotheses to explain androgenesis. First, they proposed a variant of the MGE hypothesis, the ''permissive MGE'' hypothesis under which the sperm is adapted for replacing the egg's nucleus whenever the egg lacks counteradaptations to prevent it. This modified hypothesis stems from the expected arms race between males and females for access to the egg. In this case, the production of androgenetic males may result from a process controlled by both sexes. Alternatively, androgenesis may result from the production by parthenogenetic queens of anucleate oocytes later fertilized by males. In the last hypothesis, androgenesis is a trait characteristic of females.

Despite the magnitude of the evolutionary outcomes behind the three above hypotheses, no specific studies so far attempted to decipher the role of males and females in androgenesis. To fill this gap, we conducted a laboratory controlled reciprocal-crosses experiment involving reproductives (i.e. females and males) from sexual and clonal populations and used genetic markers to determine how their sons were produced to infer the role of males and females in androgenesis. This was achieved by comparing the nuclear microsatellite genotypes and the mitochondrial DNA haplotypes of the produced sons to those of their parents. Because, androgenesis is tightly associated to female

parthenogenesis in natural clonal populations, we also genetically determine how female reproductive progeny resulting from these crosses were produced to better understand the association between these two genetic systems. We found that in *W. auropunctata*, androgenesis is not induced by males but rather is a parthenogenetic female trait characteristic. We argue that the capacity to kidnap the nuclear genome of males from other populations may allows parthenogenetic queen lineage to indirectly increase their adaptive potential through sexually produced workers and hence allowing them to persist over long-time in changing environments.

MATERIALS AND METHODS

Sampling and cross experiments

We sampled a total of nine populations, eight in French Guiana and one in Israel (see supplementary materials), consisting in approximately 20 – 30 fertilized queens and 5000 – 10 000 workers. Based on previous genetic studies of population samples collected in the field at the same locations (Foucaud *et al.* 2007;Vonshak et al. 2009; Rey *et al*. 2011), we could predict that four of the collected populations were sexual and five were clonal. The mode of reproduction of each population sampled was confirmed *a posteriori* through prior genetic analyses on a subset of reproductives.

Ants were maintained at constant temperature and humidity (25°C; 70% RH; L:D 12:12) and fed *ad libitum* with *Ephestia* eggs and a honey-yeast-water solution. These populations were bred as source populations for the production of males and alated (unfertilized) queens that were in turn used for the reciprocal cross-experiments. The production of reproductive individuals in laboratory source populations of *W. auropunctata* is laborious and stochastic. Crosses were thus strongly constrained by the synchronous production of males and females in sources populations characterized by different mating systems. Crosses consisted in the fertilization of queens from sexual populations by males from clonal populations (hereafter denoted "F_S x M_C") and in the fertilization of queens from clonal populations by males from either sexual populations (hereafter denoted "F_C x M_S") or from unrelated clonal populations (hereafter denoted "F_C x M_C"). We set up 90 crosses between reproductives from the different clonal and sexual populations as follow. One to six virgin (i.e. alated) queen(s) from a given population were placed in an artificial nest isolated in sealed and meshed boxes with one to ten males, and without workers until at least some of the queens lose their wings (cf. they lose their wings only after mating or with age; Ulloa-Chacon 1990). All the potential fathering males involved in the crosses were collected and sacrificed for subsequent genetic analyses.

Each freshly fertilized (i.e. dealated) queen was then individualized in a new box with 50 workers from their source population and food was supplied *ad libitum*. These monogynous lineages were then reared under the same laboratory conditions than sources populations. We checked twice a week and collected the reproductive progeny (i.e. daughters and sons) produced in ethanol vials until the queen died or after a maximum time of 621 days. At the end of the experiments all mothering queens and their workers were collected.

Genetic analyses

Crosses effectiveness and identification of the fathering males

Among the 90 initial crosses, only 34 monogynous dealated queen lineages were obtained among which 27 produced female and male reproductive progeny and in some cases gynandromorphic sexuals (i.e. individuals displaying female and male phenotypic characters; see result and discussion section). To check the effectiveness of the 27 successful crosses and identify the fathering males, we genotyped at 12 microsatellite markers all males that have participated to the cross, the queen and eight of the adult workers collected at the end of the experiment. Individual DNA extraction and microsatellite genotypes were obtained following Fournier *et al.* (2005a; 2005b). For a given monogynous cross, multilocus genotype of each worker was compared to the genotype of the queen and all males involved in the cross. A male was identified as the effective fathering male if its allele at each locus was compatible with the paternal allele identifiable from the genotypes of the eight workers.

Determination of the mode of offspring production

A total of 398 reproductive offspring, 178 males and 220 females, from 22 of the 27 successful crosses were genetically analysed at the same 12 microsatellite markers following the same protocol. The numbers of male and female offspring genotyped for each cross are given in Table 1. We inferred the mode of production of the offspring as follows: A male offspring was considered as being produced by androgenesis when his haploid multilocus genotype was identical to the one of the fathering male and by arrhenotoky when his haploid multilocus genotype was solely composed of maternal alleles. A male offspring was considered as polyploid when his genotype included more than one allele at one locus at least. A female progeny was considered as being produced by parthenogenesis when her diploid multilocus genotype was identical to the one of the mothering queen, and by sexual reproduction when her diploid multilocus genotype at each 12 loci was included an allele inherited from each parent. A female progeny was considered as polyploid when her genotype included more than two alleles at one locus at least.

Type of cross	Cross id.	Crosses			Reproductive produced				Male analysed	Female analysed
		Date of fertilization	Date of end	Life-time	Male	Female	Sex ratio	Gynandromorph		
Female (sexual) x Male (clonal)	S1xC1-I	22/01/2010	13/05/2011	476	203	9	4.25	0	35	9
	S1xC1-II	22/01/2010	24/06/2011	518	18	152	89.41	0	16	16
	S1xC2-I	07/12/2009	13/05/2011	522	170	3	1.73	0	16	2
	S1xC2-II	07/12/2009	13/05/2011	522	159	20	11.17	0	32	4
	S2xC2-II	03/12/2009	24/06/2011	568	14	47	77.05	0	8	6
	S2xC2-III	03/12/2009	24/06/2011	568	1	8	88.89	0	0	0
	S3xC2-I	16/01/2010	24/06/2011	524	0	45	100.00	0	0	16
	S4xC2-IV	16/11/2009	11/06/2011	572	0	60	100.00	0	0	7
	TOTAL				565	344	37.84	0	**107**	**60**
Female (clonal) x Male (sexual)	C2xS1-I	12/01/2010	13/05/2011	486	16	236	93.65	2	16	16
	C2xS1-III	12/01/2010	24/06/2011	528	2	63	96.92	5	2	16
	C2xS1-IV	12/01/2010	16/11/2010	155	15	104	87.39	7	15	15
	C3xS1-I	18/01/2010	13/05/2011	480	0	50	100.00	0	0	8
	C3xS4-I	25/11/2009	11/02/2011	443	4	35	89.74	0	4	15
	C3xS4-II	25/11/2009	08/07/2011	590	0	27	100.00	0	0	0
	C3xS4-III	25/11/2009	08/07/2011	590	0	7	100.00	0	0	6
	C3xS4-V	25/11/2009	08/07/2011	590	8	66	89.19	1	0	8
	C3xS4-VI	25/11/2009	08/07/2011	590	3	33	91.67	0	3	0
	C4xS1-I	19/01/2010	23/08/2011	581	0	75	100.00	0	0	16
	TOTAL				48	696	93.55	15	**40**	**100**
Female (clonal) x Male (clonall)	C3xC2-I	10/12/2009	11/06/2011	548	0	35	100.00	0	0	12
	C3xC2-III	10/12/2009	11/06/2011	548	0	2	100.00	0	0	0
	C3xC2-V	10/12/2009	23/08/2011	621	0	3	100.00	0	0	0
	C4xC1-II	09/06/2010	23/08/2011	440	1	19	95.00	7	0	12
	C4xC2-I	25/01/2010	13/05/2011	473	18	199	91.71	14	0	12
	C4xC2-II	25/01/2010	13/05/2011	473	11	80	87.91	10	6	0
	C4xC2-III	25/01/2010	13/05/2011	473	10	168	94.38	14	10	12
	C4xC2-V	25/01/2010	23/08/2011	575	3	0	0.00	7	0	0
	C4xC5-I	05/05/2010	23/08/2011	475	9	106	92.17	6	5	12
	TOTAL				52	617	92.22	58	**31**	**60**
	GLOBAL				665	1657		73	**178**	**220**

Table 1: Details on the number of reproductives (i.e. reproductive females and males) produced and analysed in each of the 27 successful crosses studied. Note: Sex ratio correspond to female over male offspring production.

A subset of 49 individuals from seven monogynous lineages was used to determine how the mitochondrial genome was transmitted to the next generation in each type of cross and each female and male offspring (For details on the numbers and types of genotyped specimens see supplementary materials). We amplified a 700 – 710 bp fragment of the mitochondria *COI* (*Cytochrome oxidase I*) gene from each of these 49 individuals following Foucaud *et al.* (2007). Individual electropherograms were checked for potential errors with Seqscape software (Applied Biosystems). Aligned sequences were analysed visually by comparing the sequence of each reproductive offspring to the sequences of their parents.

RESULTS AND DISCUSSION

Genotypic data show that all male offspring from the F_S *x* M_C crosses developed from unfertilised eggs through arrhenotoky (Table 2). The so called "androgenetic males" from clonal populations are hence, incapable of producing a male offspring via androgenesis when fertilizing a female from sexual populations. Additionally, female offspring from these crosses were exclusively produced via sexual reproduction (Table 2). Hence, males from clonal populations do not influence the breeding system of queens from sexual populations.

We found that queens from clonal populations produce haploid male offspring through androgenesis almost exclusively (i.e. all but one; Table 2), and this irrespectively of having been fertilized by males from others unrelated clonal populations (i.e. F_C x M_C) or from sexual populations (i.e. F_C x M_s). This result echoes with previous studies indicating that queens from clonal populations seldom produce arrhenotokous male progeny both *in natura* (Foucaud *et al.* 2006) and in laboratory conditions (Foucaud *et al.* 2010). The single arrhenotokous male produced by the parthenogenetic queen in this study is thus probably not a consequence of her crossing with a male from a sexual population. In agreement with this, the parthenogenetic queen that produced the arrhenotokous male also produced eight androgenetic males (Table 2). We also demonstrated that the mitochondrial genome is always transmitted through the cytoplasm of the mothering queen whatever both the nature of the offspring (i.e. arrhenotokous or androgenetic male and sexually produced or parthenogenetic female progeny) and the nature of the cross.

Altogether, our results indicate that in *W. auropunctata* androgenesis is not under the control of males but rather of the parthenogenetic females. This leads us to unambiguously refute the hypothesis of Fournier *et al.* (2005a) stating that androgenesis emerged as an evolutionary response of males face to female parthenogenesis to preserve their reproductive success. Two mutually exclusive hypotheses might account for the origin of androgenesis in *W. auropunctata.* First, the

Type of cross	Cross id.	Offspring							
		Males				Females			
		Arrhenotoky	Androgenesis	Polyploidy	TOTAL	Sexual	Parthenogenesis	Polyploidy	TOTAL
Queen (sexual) x Male (clonal)	S1xC1-I	35	0	0	35	9	0	0	9
	S1xC1-II	16	0	0	16	16	0	0	16
	S1xC2-I	16	0	0	16	2	0	0	2
	S1xC2-II	32	0	0	32	4	0	0	4
	S2xC2-II	8	0	0	8	6	0	0	6
	S3xC2-I	0	0	0	0	16	0	0	16
	S4xC2-IV	0	0	0	0	7	0	0	7
	TOTAL	**107**	**0**	**0**	**107**	**60**	**0**	**0**	**60**
Queen (clonal) x Male (sexual)	C2xS1-I	0	7	9	16	10	6	0	16
	C2xS1-III	0	1	1	2	2	14	0	16
	C2xS1-IV	1	8	6	15	13	0	2	15
	C3xS1-I	0	0	0	0	3	5	0	8
	C3xS4-I	0	3	1	4	0	15	0	15
	C3xS4-III	0	0	0	0	0	6	0	6
	C3xS4-V	0	0	0	0	0	6	2	8
	C3xS4-VI	0	1	2	3	0	0	0	0
	C4xS1-I	0	0	0	0	1	13	2	16
	TOTAL	**1**	**20**	**19**	**40**	**29**	**65**	**6**	**100**
Queen (clonal) x Male (clonall)	C3xC2-I	0	0	0	0	0	12	0	12
	C4xC1-II	0	0	0	0	0	12	0	12
	C4xC2-I	0	9	1	10	1	11	0	12
	C4xC2-II	0	6	0	6	0	0	0	0
	C4xC2-III	0	8	2	10	0	12	0	12
	C4xC5-I	0	3	2	5	0	12	0	12
	TOTAL	**0**	**26**	**5**	**31**	**1**	**59**	**0**	**60**
	GLOBAL	108	46	24	**178**	90	124	6	**220**

Table 2: Number and type of males and females offspring produced by the 22 crosses genetically analysed at 12 microsatellite markers.

androgenesis mutation may appear in the queen lineage independently from the emergence of the female parthenogenesis mutation. This hypothesis seems however unreasonable for at least three reasons. i) So far not any parthenogenetic queen has been found producing exclusively arrhenotokous males; ii) occurrence of androgenesis was never observed in sexual populations; and iii) this scenario is not parcimonious as it requires two independent evolutionary changes in the same parthenogenetic queen lineage.

In the second hypothesis, androgenesis may emerge simultaneously in queens as a by-product of female parthenogenesis (see Foucaud *et al.* 2007). This second hypothesis is more parcimonious than the first one as only one evolutionary change is required for the emergence of both parthenogenesis and androgenesis and may better explain the tight association between the two genetic systems in natural populations. Furthermore, Rey *et al.* (2011) recently demonstrated that queen parthenogenesis involved meiosis (automixy) with central fusion of oocytes with drastic reduction of genetic recombination during meiosis. Mechanistically, recombination is primordial for proper chromosome segregation during meiosis (Bascom-Slack 1997) and a reduction of recombination potentially lead to the missegregation of chromosomes and consequently to the production of aneuploid eggs (Baker *et al.* 1976, Bascom-Slack 1997). This would render a mechanism of maternal genome exclusion easier or even directly lead to the production of empty eggs fertilized by males to produce androgenetic drones. Yet, this mechanism remains purely hypothetic and further studies are required to better understand the cytological process that lead to androgenesis. A detailed study of the gynandromorphs produced such as those observed in some of the crosses studied here, might potentially give new insights on the fertilization process as suggested by Dobata *et al.* (2011). We will come back to this point later in this results and discussion section.

The evolutionary consequences of androgenesis controlled by parthenogenetic females are far-reaching. First, androgenesis may allow the reproduction between females and males from the same cohort without inbreeding depression effects in their sexually produced workers. Second, this mechanism may maintain over time high level of heterozyosity and/or beneficial genetic combinations in the sexually produced worker offspring, as previously suggested by Foucaud *et al.* (2007). Finally, the kidnapping of new males' nuclear genome may indirectly confer to parthenogenetic queen lineages the ability to adapt to changing environment and hence increase their life-time through time. Theory predicts that diploid asexual lineages are devoted to dead-end in particular because of their limited ability to create novel genomes through syngamy of two meiotically recombined gametes (Fisher 1930; Muller 1932). This cost of asexuality may be reduced in *W. auropunctata* parthenogenetic female lineages by creating genetic diversity among parthenogenetic-androgenetic lineages

at the level of the sexually produced worker offspring. Selection may act on the combination of female's and male's genomes indirectly on workers but will always preserve the same parthenogenetic female lineage. In a changing environment, the kidnapping of a new adapted male genome by a parthenogenetic queen for the production of the worker offspring may allow selection to fix a better adapted combination between a new male genome and the same conserved female genome in the clonal population. In eusocial species, whereas queens are generally confined to favorable conditions within the nest, selection is more likely to act on workers which are faced with biotic and abiotic environmental factors during foraging. We therefore argue that this gene kidnapping mechanism might be an unprecedented manner for parthenogenetic queens to increase their adaptive potential and hence their life-time in changing environments.

The benefit of the genome kidnapping process (i.e. androgenesis) for parthenogenetic queens would be accentuated if such queens would allocate their resource preferentially in the production of female compared to male reproductives, hence increasing their genome transmission in the female offspring while producing a minimum of male reproductives that do not share their genome, for the production of the worker offspring. In agreement with this, we found that parthenogenetic queens produced 93.55% and 92.23% of reproductive females in the F_C x M_S and the F_C x M_C crosses respectively, while sexually reproducing queens produced 37.84% of reproductive females when fertilised by males from clonal populations (Table 1).

Interestingly, we found that 47.5 % of the male progeny from the F_C *x* M_S crosses corresponds to polyploid individuals according to their microsatellite genotypes (Table 2). Although morphologically identified as males, we suggest that these polyploid individuals are actually gynandromorphs (i.e. individuals displaying both female and male phenotypic characters) with female internal tissues. As a matter of fact, gynandromorphs were also morphologically easily identified in the offspring of these crosses and were found to be polyploid too. Such polyploid male progenies were found at a significantly lower frequency in the F_C *x* M_C crosses involving queens and males from distinct clonal populations (16.13%; χ^2 = 3.97, p-value = 0.04). The production of polyploid males hence appears to be, at least partly, a consequence of the fertilization of a parthenogenesis queen by a male from a sexual population. Although the fitness of polyploid individuals remains unknown it seems reasonable to hypothesize that their ability to reproduce is reduced, and hence represent a cost for the parthenogenetic mothering queens. It is worth stressing that about one third (i.e. 29 %) of the female offspring resulting of the F_C x M_S was produced sexually. This proportion is substantially higher than that of observed in crosses involving reproductive from unrelated clonal populations (i.e. 1.67% in this case; χ^2 = 13.53; p-value = 2.35×10^{-4}; Table 2) or reproductives from the same clonal populations (see Foucaud *et al.* 2010 for comparison).

We here hypothesize that the production of polyploid males, gynandromorphs and sexually produced reproductive females in the F_C *x* M_S crosses may result from similar negative interactions between cytoplasmic male factors inherited from the sperm and those in the fertilized oocytes. In most of sexually reproducing organisms, crucial cytoplasmic elements controlling the organisation of the cytoplasm and the progress of cell divisions (i.e. centrioles) are generally inherited by the fathering males (Kellogg *et al.* 1994). Moreover, the emergence of arrhenotokous parthenogenesis in hymenopteran involved modifications of some maternal cytoplasmic elements allowing them to organize unfertilized egg-cells without the implication of the male material (Riparbelli *et al.* 1998; Tram & Sullivan 2000; Riparbelli *et al.* 2010). Finally, although not yet demonstrated in hymenopterans, female parthenogenesis may also result from modifications of the cytoplasmic material in females (Riparbelli & Callaini 2008). We therefore argue that our results are compatible with the view that cytoplasmic elements transferred by sperm from males originating from sexual populations may interfere with the probably modified cytoplasmic material of eggs produced by parthenogenetic queens, leading to the production of a fraction of aberrant zygotes developing into irregular reproductive individuals. Under this hypothesis we would expect that normally developed androgenetic haploid males resulting from the F_C x M_S crosses have inherited from maternal cytoplasmic material. This would nullify the negative effect of cytoplasmic conflict in the following generation when they fertilize a female progeny from the same cohort (cf. with the same cytoplasmic material). This transition step may be required for the emergence of parthenogenetic - androgenetic stable lineages when the genome of a male is introgressed into a clonal lineage. Further F2 cross-experiments would be useful to confirm this hypothesis. It still remain unclear however, why the worker progeny produced by parthenogenetic queens fecundated by males from sexual populations do not appear to suffer from the above cytoplasmic conflicts (cf. not any polyploidy or gynandromorph workers were observed).

In conclusion, this study demonstrates that, instead of being an "egg-parasitism" male trait characteristic, androgenesis is controlled by parthenogenetic female in *W. auropunctata* and therefore rather corresponds to a male genome kidnapping mechanism. The tight association between the two genetic systems observed *in natura* most likely results from the fact that androgenesis is a by-product of the mechanism of parthenogenesis. The ability of kidnapping

the nuclear genome of males from other populations may allows parthenogenetic queens to indirectly increase their adaptive potential through the sexually produced workers, hence allowing such parthenogenetic lineages to persist over a long time in changing environments. This finding leads to the paradox that parthenogenetic queens use sex to preserve their own asexuality through time.

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Supplementary material:

Supplementary table 1: Sampling design of sexual and clonal populations.

Reproduction system	Country	Site name	Date of sampling	Geographical coordinates	
				X	Y
Sexual	French Guiana	S1	September 2009	-52.974967	5.049300
Sexual	French Guiana	S2	September 2009	-52.937499	5.030233
Sexual	French Guiana	S3	September 2009	-52.980300	5.067717
Sexual	French Guiana	S4	September 2009	-52.950000	5.022817
Clonal	French Guiana	C1	September 2009	-52.917800	5.287783
Clonal	French Guiana	C2	September 2009	-53.045750	5.071333
Clonal	French Guiana	C3	September 2009	-52.914833	5.291750
Clonal	Israel	C4	March 2009	32.174577	34.834464
Clonal	French Guiana	C5	September 2009	-52.213234	4.485450

CHAPITRE III

CHANGEMENTS ADAPTATIFS ET SCÉNARIOS D'INVASION

Le but de ce chapitre est i) de tester si des changements adaptatifs ont accompagné l'invasion des populations envahissantes de *W. auropunctata* en réponse aux conditions environnementales des localités envahies de la ceinture tropicale et de la région Méditerranéenne; et ii) d'identifier où ont eu lieu ces changements adaptatifs. Nous nous sommes intéressés à la capacité des ouvrières à tolérer des stress thermiques, un trait particulièrement important chez les insectes qui sont totalement ou partiellement dépourvus de système de régulation thermique interne.

I. Les outils utilisés en biologie de l'invasion

L'étude des invasions biologiques en général fait appel à différentes approches méthodologiques, chacune permettant de répondre à des questions bien précises: Quel est le potentiel invasif d'une espèce? D'où viennent les populations envahissantes ? Comment les populations deviennent envahissantes ? Dans cette section, ces différentes approches sont brièvement présentées.

I.1. Modèles de distribution d'espèces

Ces approches dites SDM, pour *species distribution modelling*, ont été utilisées pour prédire l'expansion de l'aire de répartition d'espèces envahissantes de groupes taxonomiques très variés (e.g. Muñoz & Real 2006; Kulhanec *et al*. 2010; Ghirardi 2011). Elles se basent sur le fait que les populations envahissantes conservent leur niche écologique (i.e. tendance d'une espèce à maintenir les requis écologiques ancestraux; Wiens & Graham 2005), et supposent donc que les populations envahissantes ne s'adaptent pas au cours du processus invasif. Des prédictions réciproques permettent cependant de mettre en évidence de tels changements de niche associés aux invasions (Broennimann *et al.* 2007; Roedder & Lotters 2009). Cela consiste à calibrer un modèle spatial de niche à partir de données d'occurrence de populations dans l'aire native puis à prédire l'occurrence de l'espèce à partir de ce modèle dans l'aire d'introduction et réciproquement c'est-à-dire, calibrer un autre modèle à partir des occurrences connues dans l'aire d'introduction et prédire l'occurrence de l'espèce dans l'aire natif à partir de cet autre modèle (Figure III.1.). L'adéquation ou non des distributions obtenues à partir des modèles prédictifs, peut mettre en évidence un conservatisme ou un changement de niche lors du processus d'invasion (Broennimann *et al.* 2007, Figure III.1.). Dans certains cas, cette méthode peut également informer grossièrement sur l'aire (i.e. aire native ou aire d'introduction) dans laquelle le changement de niche a eu lieu.

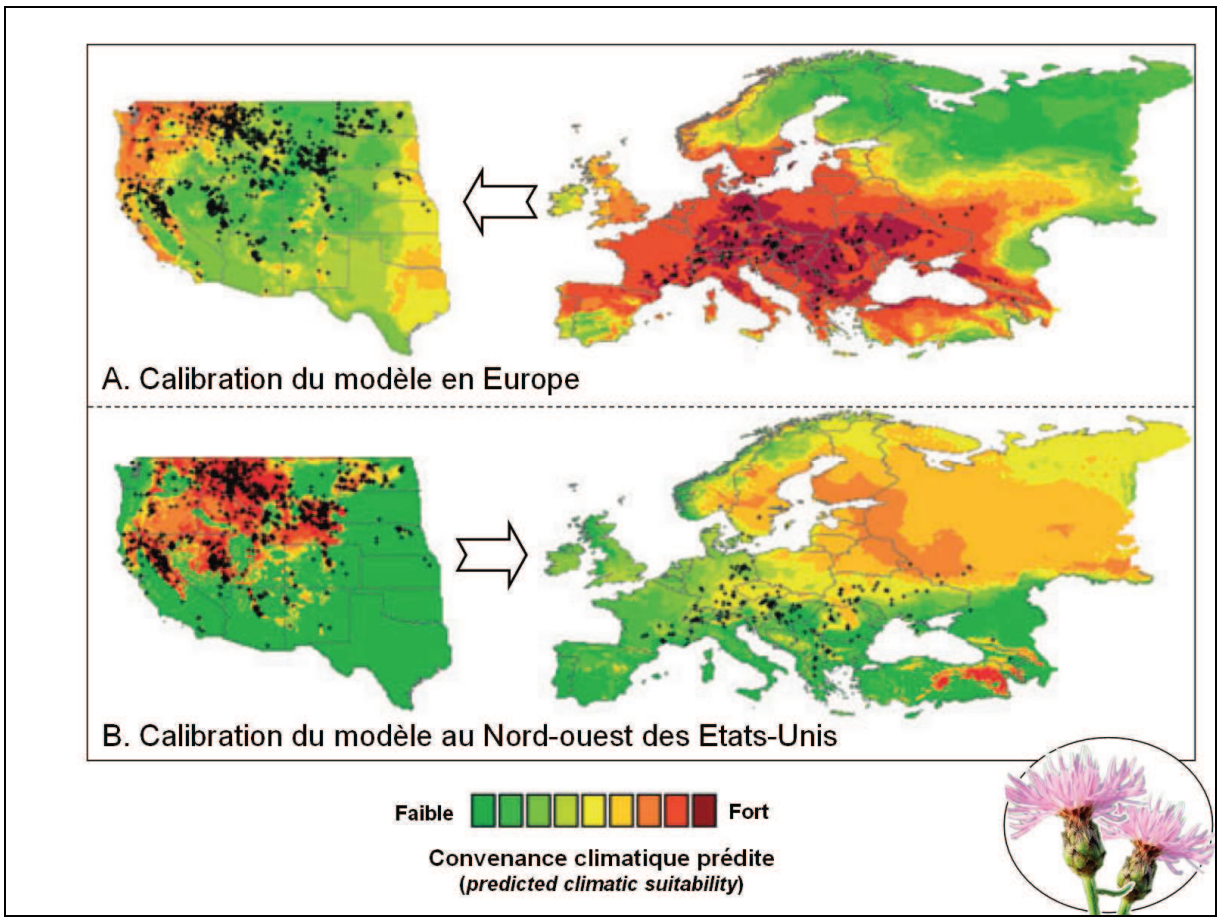

Figure III.1. Exemple d'approche de modèles de distribution d'espèces (approche SDM) réciproques, appliquée à une espèce de plante envahissante la centaurée maculée, *Centaurea maculosa* (en médaillon). Cette figure représente les cartes de prédiction des occurrences de *C. maculosa*, à partir des modèles calibrés en Europe (A) et au Nord-ouest des Etats-Unis (B) et de chacun des modèles projetés dans l'autre aire (Figure tirée de Broennimann *et al.* 2007). Les ronds noirs sont les occurrences réelles de *C. maculosa* au sein de l'aire native et dans l'aire d'introduction. Les modèles incluent 19 variables bioclimatiques relatives à la température, à l'humidité et à l'évapotranspiration. *C. maculosa* est originaire d'Europe et a été introduite aux Etats-Unis à la fin du 19ème siècle. Les modèles calibrés en Europe et projetés aux Etats-Unis ne permettent pas de prédire avec certitude l'expansion des populations de *C. maculosa* aux Etats-Unis ce qui reflète un changement de niche de ces populations. De plus, comme aucune population de l'aire native ne se trouve dans la zone climatique «principale» définie sur les populations de l'aire d'introduction, les auteurs suggèrent que le changement de niche a très probablement eu lieu dans l'aire d'introduction.

I.2. Approches génétiques pour retracer les routes d'invasions

Les approches génétiques permettent d'identifier les routes d'invasions et donc les populations sources desquelles sont issues les populations envahissantes. Historiquement, les analyses les plus souvent utilisées pour retracer les routes d'invasions impliquent la construction de dendrogrammes basés sur des données génétiques obtenues à partir de différents types de marqueurs neutres (e.g. microsatellites, gènes mitochondriaux). Bien que relativement efficaces, ces méthodes se heurtent à certaines limitations. En effet, les phénomènes d'invasions biologiques étudiés sont la plupart du temps contemporains et les temps de divergence entre populations natives et introduites sont relativement cours. Les signatures génétiques de ces temps de divergence (i.e. différentiation génétique inter-populations) sont généralement faibles ce qui rend difficile et/ou incertaine l'inférence les routes d'introduction. Des événements de goulots d'étranglement génétique pendant de longue période suivant l'introduction peuvent faciliter ces inférences, la dérive génétique pouvant augmenter la divergence génétique entre populations.

Des méthodes plus récentes ont été développées afin de permettre non seulement de retracer les routes d'invasion mais également d'identifier des événements démographiques associés aux invasions (i.e. présence et durée de goulots d'étranglement; Estoup & Guillemaud 2010). Ces approches sont basées sur des inférences statistiques Bayesiennes (*Approximate Bayesian Computation* ABC; Beaumont 2002) permettant d'approximer des vraisemblances postérieures de différents modèles (i.e. scénarios d'invasion) par le biais de simulations de jeux de données et en comparant les données observées aux données simulées. Les approches ABC se sont révélées efficaces pour retracer des routes d'introduction parfois complexes (Miller *et al.* 2005; Lombaert *et al.* 2010; voir également encadré I.2. dans le Chapitre I).

I.3. Analyses de traits d'histoire de vie

L'analyse comparative de traits d'histoire de vie entre les populations envahissantes et les populations natives est une des méthodes les plus directes qui permet de mettre en évidence des changements adaptatifs associés aux invasions. Ces analyses peuvent être effectuées en laboratoire ce qui permet de contrôler précisément les conditions environnementales. Les études en laboratoire ne prennent cependant pas en compte la complexité des interactions biotiques du milieu naturel. D'autre part, ces études portent généralement sur des mesures de traits phénotypiques mesurés sur des organismes élevés dans des conditions d'élevages optimales et rarement sur les conditions environnementales qui caractérisent les milieux dans lesquelles les populations étudiées ont été

récoltées. Ces mesures peuvent également être effectuées en conditions semi-contrôlées dans des «jardins communs» ce qui permet de tester des conditions climatiques plus «naturelles». Il est intéressant de noter que la plupart des études utilisant ces méthodes dans un contexte d'invasion, tentent de mettre en évidence des différences de traits phénotypiques entre populations au sein de l'aire d'introduction (Hierro *et al.* 2005). Cela traduit une vision biaisée à travers laquelle les éventuels changements adaptatifs sont généralement suspectés d'avoir lieu dans l'aire d'introduction. D'autre part, les rares études qui montrent des différences entre populations de l'aire native et de l'aire d'introduction négligent souvent de mesurer les relations de filiations entre populations (i.e. qui est la source de qui ?), par exemple en estimant les relations de proximité génétique entre les populations comparées. Ce dernier point est pourtant essentiel pour affirmer avec certitude que des éventuels changements adaptatifs ont bien eu lieu dans les populations envahissantes étudiées par rapport à leur(s) population(s) source(s). Notons que ces dernières peuvent être établies dans l'aire native et/ou dans une autre localité de l'aire envahie.

Dans le cadre de l'étude des scénarios éco-évolutifs des populations envahissantes, l'idéal serait certainement d'avoir recours simultanément aux trois approches présentées ci-dessus. Cependant, une telle démarche nécessite une quantité d'information et de matériel qui n'est pas forcément accessible pour toutes les espèces. Ceci explique, au moins en partie, pourquoi jusqu'à présent peu d'études ont permis d'identifier avec certitude un scénario éco-évolutif d'invasion. Dans le cadre de cette thèse, nous avons étudié les scénarios d'invasions de populations de *W. auropunctata* établies dans la ceinture tropicale et dans la région Méditerranéenne. Ces deux cas d'études n'ont malheureusement pas pu faire l'objet d'approches multidisciplinaires. En effet dans le cas des populations envahissantes établies dans la ceinture tropicale nous avons été confrontés à des limitations techniques que nous discuterons plus en détail dans le chapitre IV de discussion générale. Dans le cas des populations établies dans la région Méditerranéenne, nous avons pu développer une approche pluridisciplinaire couplant des analyses de SDM, des analyses de génétique des populations et des mesures de traits d'histoire de vie afin de retracer le scénario éco-évolutifs d'invasion de ces populations.

II. Evolution de la thermotolérance et scénario évolutifs associés aux invasions de *W. auropunctata*

II.1. Populations envahissantes établies dans la ceinture tropicale.

Foucaud *et al.* (2010) ont montré que toutes les populations envahissantes de *W. auropunctata* dans l'aire d'introduction sont caractérisées par un système de reproduction clonal. Ces auteurs ont également suggéré que les populations envahissantes suivent un scénario d'invasion avec adaptation pré-introduction impliquant des populations clonales de l'aire native à partir desquelles des propagules dispersent vers des localités éloignées géographiquement hors de l'aire native. D'autre part, ces populations clonales de l'aire native s'établissent majoritairement dans des habitats marginaux anthropisés (e.g. plantations, bord de route; Foucaud *et al.* 2009). Orivel *et al.* (2009) ont montré que ces milieux sont caractérisés par des conditions environnementales particulières, notamment des températures élevées et de faibles taux d'humidités, par rapport à celles qui caractérisent les milieux naturels ancestraux (forêts primaires; Figure III.2.) principalement occupés par des populations sexuées. Nous avons testé au cours de cette thèse si le succès d'invasion des populations clonales etait également associé à des changements adaptatifs permettant aux ouvrières de tolérer des températures élevées. Cette étude fait l'objet d'un article soumis dans la revue *Evolution* (cf. Article 4: *Thermotolerance adaptation to human-modified habitats occurs in the native range of the invasive ant Wasmannia auropunctata before long-distance dispersal*.) présenté dans ce chapitre. Nos résultats montrent que les ouvrières des populations clonales, indépendamment du fait qu'elles soient établies dans l'aire native ou dans l'aire d'introduction, tolèrent mieux les températures élevées que les ouvrières issues des populations sexuées établies dans l'habitat ancestral (i.e. forêts primaires). Ce résultat suggère fortement que les populations envahissantes sont issues de populations clonales établies dans les milieux anthropisés de l'aire native et donc adaptées à des températures élevées. Ces résultats supportent l'hypothèse d'un scénario d'invasion avec adaptation pré-introduction dans des milieux perturbés par les activités humaines.

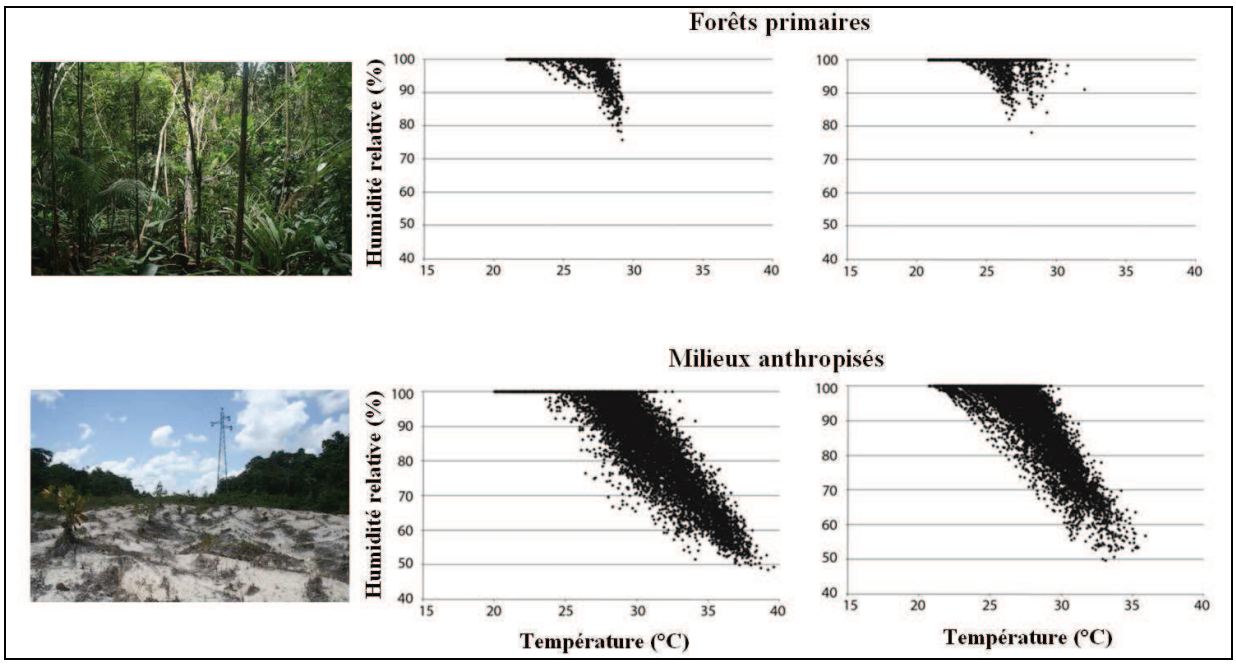

Figure III.2. Distribution de données de température et d'humidité enregistrées toutes les 20 minutes entre Avril 2006 et Janvier 2008, dans (A) deux habitats naturels (i.e. forêt primaire) et (B) deux habitats anthropisés (i.e. forêt secondaire ; bas-côté d'une piste). Graphiques tirés de Orivel *et al.* 2009.

II.2. Populations envahissantes de la région Méditerranéenne

Dans un deuxième temps nous avons voulu comprendre comment des populations d'une espèce de fourmi native des forêts tropicales de l'Amérique du Sud ont pu envahir une localité de la région Méditerranéenne caractérisée par des saisons et notamment par des hivers particulièrement froids. *W. auropunctata* a été identifié en Israël pour la première fois en 2005 mais est probablement présente dans cette région depuis 1998 (Vonshak *et al.* 2010). Malgré les températures basses de cette région, les populations de *W. auropunctata* qui y sont établies présentent une écologie semblable à celle des populations tropicales (Vonshak *et al.* 2010). En effet, les nids se trouvent dans des débris végétaux ou sous des pierres et les ouvrières sont actives toute la journée même en hiver. Il semble de ce fait que ces populations ont subi des changements adaptatifs permettant aux ouvrières de ces populations de tolérer les températures froides caractéristiques des hivers Israéliens.

Vonshak *et al.* (2010) ont suggéré que ces populations ont suivi un scénario d'invasion avec adaptation indépendante post-introduction. D'après ces auteurs, les populations de *W. auropunctata* établies en Israël auraient été accidentellement introduites depuis le Brésil via l'importation de bois dans une usine de contreplaqué située dans la vallée du Jourdan, cette région étant caractérisée par des températures relativement clémentes par rapport au reste du pays. Dans cette vallée, certaines

populations se seraient adaptées aux conditions plus rudes que dans d'autres régions d'Israël et auraient alors envahi l'ensemble du territoire (i.e. adaptation post introduction). Cette hypothèse se confronte cependant au fait que ces populations envahissantes sont clonales et ne sont constituées que d'un seul couple parental clonal. (i.e. un seul génotype reine et un seul génotype mâle) sur l'ensemble du territoire (Vonshak *et al.* 2009; 2010).Ces populations présentent donc un potentiel adaptatif relativement limité bien que les reines de ces populations peuvent kidnapper des génomes mâles d'autres populations (cf. Article 3). Cette caractéristique permet en effet, au moins temporairement, d'augmenter la diversité génétique au sein des ouvrières sur laquelle la sélection peut agir. Il semble cependant peu probable que ce seul moyen d'augmenter la diversité génétique puisse permettre à une population tropicale de s'adapter à des températures aussi basses, à moins qu'un génome mâle kidnappé ne provienne d'une population pré-adaptée au froid. Cependant aucun autre génotype mâle n'a été identifié en Israël y compris dans la vallée du Jourdan qui a été définie comme le site primaire d'introduction de *W. auropunctata* en Israël, ceci étant également valable pour des échantillons récoltés peu après le signalement de l'invasion (Vonshak *et al.* 2010).

Nous avons étudié les changements adaptatifs de ces populations et développé une approche multidisciplinaire pour identifier l'origine des changements adaptatifs ayant permis aux ouvrières de tolérer des températures froides. Cette approche a consisté à coupler des analyses SDM, des analyses de génétiques de populations et des mesures de traits phénotypiques en conditions contrôlées, ces trois outils étant généralement utilisés de manière indépendante en biologie des invasions (voir section précédente). Cette étude fait l'objet d'un article actuellement en préparation (Article 5: *Where do adaptive shifts occur during invasion? A multidisciplinary approach to unravel cold adaptation in a tropical ant species invading the Mediterranean zone.*). Nos résultats indiquent que les ouvrières des populations établies en Israël sont mieux adaptées aux températures froides de cette région comparativement aux ouvrières des populations tropicales de l'aire native ou de l'aire d'introduction. Nous avons également mis en évidence que les populations envahissantes établies en Israël, sont très probablement originaires du Nord-est de l'Argentine, une région qui présente des températures similaires à celles d'Israël, et où des populations de *W. auropunctata* très proches génétiquement de celles d'Israël se sont avérées également adaptées aux températures froides. Ces résultats suggèrent fortement que les populations envahissantes d'Israël ont suivi un scénario d'invasion avec adaptation pré-introduction.

III. Article 4: Thermotolerance adaptation to human-modified habitats occurs in the native range of the invasive ant *Wasmannia auropunctata* before long-distance dispersal

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Running title: Thermotolerance adaptation in the native range.

ABSTRACT

Key evolutionary events associated with invasion success are traditionally thought to occur in the introduced, rather than the native range of species. In the invasive ant *Wasmannia auropunctata*, however, a shift in reproductive system has been demonstrated within the native range, from the sexual non-dominant populations of natural habitats to the clonal dominant populations of human-modified habitats. Because abiotic conditions of human-modified habitats are hotter and dryer, we performed lab experiments on workers from a set of native and introduced populations, to investigate whether these ecological and genetic transitions were accompanied by a change in thermotolerance and whether such changes occurred before establishment in the introduced range. Thermotolerance levels were higher in native populations from human-modified habitats than in native populations from natural habitats, but were similar in native and introduced populations from human-modified habitats. Differences in thermotolerance could not be accounted for by differences in body size. Our findings highlight the importance of human land use in explaining major contemporary evolutionary changes within the native range of species. Transitions from natural to human-modified habitats of native populations can occur in a few decades, and ultimately pave the way for new bioinvaders.

INTRODUCTION

Most recent studies trying to decipher the reasons for the success of invasive species have focused explicitly on the introduced range of invasive species (Richardson et al. 2000; Sakai et al. 2001; Colautti and MacIsaac 2004). This is logical, to some extent, given that post-introduction ecological and evolutionary challenges are likely to be more important than those occurring before long-distance dispersal. After their introduction into a new area, migrant individuals generally have to cope with new environmental conditions when compared to their native habitats. Such new demographic (in case of a founder effect), biotic and abiotic conditions can constitute severe barriers to successful invasion (Blackburn et al. 2011). In this respect, comparisons between native and introduced populations of invasive species implicitly seek to unravel the evolutionary changes that may have occurred in the introduced range (Keller and Taylor 2008; van Kleunen et al. 2010).

However, critical biological switches can also occur within the native range of invasive species (Bossdorf et al. 2008; Lee and Gelembiuk 2008). The role of preadaptation (or prior adaptations, see Hufbauer et al. 2011) of certain native populations to explain invasive success has not received adequate attention (van Kleunen et al. 2011), despite early studies underlying this possibility (Elton 1958). In particular, native populations adapted to human-modified habitats may be particularly prone to become invasive elsewhere (Ehrlich 1989). Human activities are now widely recognized as a major force promoting biological invasions (e.g. King and Tschinkel 2008; Leprieur et al. 2008), both through large-scale transport (Floerl and Inglis 2005; Suarez et al. 2005; Tatem et al. 2006) and biotic homogenization (e.g. McKinney and Lockwood 1999; Olden et al. 2004; McKinney 2006). Human activities provide native species with an opportunity to cross habitat boundaries locally, and to adapt to human-driven biotic and abiotic conditions within their native range (i.e., pre-adapt). In addition to triggering a general decrease in species abundance, functional diversity and other biotic effects (Olden et al. 2004; Ekroos et al. 2010), human activities tend to alter the abiotic properties of habitats, such as soil chemical and physical features, water resources and properties, air filtering, light incidence and climatic conditions (Pickett et al. 2001; Kozlov and Zvereva 2007; Hrodey et al. 2009). Native populations adapting locally to such profound changes to their environment may subsidiarily benefit from the opportunity for long-distance dispersal provided by human activities. For instance, the worldwide airline transportation network provides numerous high capacity routes between geographically distant but climatically similar locations (Tatem and Hay 2007). Native species that adapted to human-modified habitats are thus likely to access new areas characterized by similar habitat alterations, potentially multiple times, following human-driven introduction events (e.g., McKinney 2006; Foucaud et al. 2010; Hufbauer et al. 2011).

While the idea of human activities promoting invasion success through prior adaptations in the native range is not new (Elton 1958; Ehrlich 1989), a renewed attention has been turned to the study of the native range and evolutionary changes occurring before introduction (Dlugosch and Parker 2007; Lee and Gelembiuk 2008; Valéry et al. 2009; Hufbauer et al. 2011). However, this interest has been mostly theoretical, and the importance of prior adaptations to human-modified habitats in generating successful invaders has seldom been studied using natural populations (van Kleunen et al. 2010). Notable exceptions are studies comparing life-history traits between native populations of invasive and non-invasive species (Schlaepfer et al. 2010; Jenkins and Keller 2011; van Kleunen et al. 2011), or population history of native populations differing in their physiological ability to invade novel habitats (Winkler et al. 2008). To date, these studies all underline the importance of prior adaptations to human-modified or marginal habitats within the native range in explaining successful invasions events. However, the relative contribution of prior adaptation and other processes (multiple introductions, post-introduction adaptation) to invasion success remains speculative, given the scarcity of experimental studies of native populations of invasive species.

Wasmannia auropunctata, an invasive ant species ranked among the most destructive invaders worldwide (Lowe et al. 2000), presents the opportunity to test for the hypothesis of important adaptations occurring within the native range before long-distance introductions. Previous studies have shown that this ant underwent major biological shifts within its native range before the long-distance dispersal events leading to worldwide invasion. In natural areas of its native range (primary forests of South America), *W. auropunctata* forms low-density populations (Orivel et al. 2009). However, this ant has also successfully colonized human-modified habitats within its native range, and this habitat change was paralleled by a striking switch in ecological dominance (Foucaud et al. 2009; Orivel et al. 2009). A switch in reproductive system also took place at the time of habitat transition: primary forest populations generally display a classical haplo-diploid reproductive system (hereafter referred to as "sexual"), whereas populations from human-modified areas are almost entirely clonal (Foucaud et al. 2007). All introduced populations studied to date appear to be drawn from dominant populations of human-modified habitats of the native range of the species (Foucaud et al. 2010).

We have previously shown that abiotic conditions differ considerably between the natural and human-modified environments of *W. auropunctata* in its native range (Orivel et al. 2009). Over the course of the year, temperature remains stable at values below 30°C and humidity never reach less than 80% in natural habitats, whereas, in human-modified habitats, temperatures may reach 40°C and humidity may drop to 50%. The worker caste, in charge of the foraging outside the colony, may be particularly affected by these conditions. These abiotic differences clearly constitute a major ecological challenge, which has apparently been met by some native *W. auropunctata* populations. However, it remains unknown whether populations from natural and human-modified habitats differ in their ability to tolerate hot and dry abiotic conditions. If

Range	Region of origin	Population	Habitat	Reproductive system
Native	French Guiana	M3-F	Natural	Sexual
Native	French Guiana	M7	Natural	Sexual
Native	French Guiana	M11	Natural	Sexual
Native	French Guiana	Ker	Human modified	Clonal
Native	French Guiana	P2-1	Human modified	Clonal
Native	French Guiana	P2-2	Human modified	Clonal
Native	French Guiana	Cay	Human modified	Clonal
Native	French Guiana	Pi41	Human modified	Clonal
Introduced	Florida	Fl	Human modified	Clonal
Introduced	Cameroon	Cam	Human modified	Clonal
Introduced	New Caledonia	NCQ0	Human modified	Clonal

TABLE 1: Sampling design for native and introduced *W. auropunctata* populations. Note: The locations of the sampling sites and their names correspond to previously studied sites (see Fournier et al. 2005a; Foucaud et al. 2007; Foucaud et al. 2009). Note that the habitat and reproductive system factors are confounded (see Materials & Methods section for details).

both native and introduced populations from human-modified habitats can tolerate such conditions, and native populations from natural habitats cannot, this would indicate the occurrence of a major evolutionary shift before long-distance dispersal into the introduced range.

A possible way for certain populations to tolerate difficult abiotic conditions could be to display variation in worker body size. Worker body size has been shown to play an important role in thermotolerance in several ant species (Kaspari 1993). In particular, *Cataglyphis velox* workers, desert ants that have to deal with extreme heat, have been demonstrated to make use of their large body size to forage at higher temperatures (Cerda and Retana 1997, 2000). In *W. auropunctata*, previous studies have shown that workers from populations established in the introduced range are smaller than those found in the native range (McGlynn 1999; Mikheyev & Mueller 2009). However, these studies did not compare the body sizes of native and introduced workers originating from populations established in different habitats (i.e. natural versus human-modified habitats, as in this study).

The main goal of this study was thus to investigate a putative evolutionary shift within the native range before long-distance dispersal (i.e. prior adaptation of certain native populations to become invasive), by testing in laboratory the putative differences in thermotolerance between native populations from natural and human-modified habitats as well as introduced populations from human-modified habitats. We further investigated whether observed thermotolerance differences were due to body size variations. The importance of prior adaptation to human-modified habitats within the native range in explaining invasive success is discussed.

MATERIALS AND METHODS

Sampling

We collected ca. 140 queens and several thousand workers from 11 populations from natural and human-modified habitats within the native range (i.e., French Guiana) and the introduced range (i.e., Cameroon, Florida and New Caledonia) of *W. auropunctata* (Table 1). Sampling was conducted between December 2007 and March 2008. From a phylogenetic point of view, the sampled introduced populations are derived from various introduced populations from the Caribbean area (Foucaud et al. 2010). Those intermediate Caribbean populations show a large amount of genetic similarity with French Guianese populations, and may originate from the northern part of the native range of *W. auropunctata*. Therefore, sampled native and introduced populations used in this study are not phylogenetically distant, considering the vast amount of genetic structure present in the native range of the species, despite some divergence in the mitochondrial genome (Mikheyev and Mueller 2007). Based on our previous genetic studies of the sampled populations, we expected three of the sampled populations to be sexual and eight to be clonal (Table 1; Foucaud et al. 2006; Foucaud et al. 2007). This was confirmed by genotyping a minimum of three queens and 30 workers from each population at 33 microsatellite loci, as described by Fournier et al. (2005b) and Almany et al. (2009). The reproductive system was determined by visually inspecting genotypes and using a program to identify identical multilocus genotypes that we had developed in Pascal object programming language (available upon request

to the authors; see Fournier et al. 2005a; Foucaud et al. 2007).

All the populations sampled from natural habitats were sexual, whereas all the populations sampled in human-modified habitats were clonal (Table 1): the factors "habitat" and "reproductive system" are thus confounded. In previous studies (e.g., Foucaud et al. 2007), we encountered, on very rare occasions, clonal populations in natural habitats (only twice in French Guiana) and sexual populations in human-modified habitats (only once in French Guiana). As these types of populations are extremely rare and difficult to sample, we were unable to include them in this study. Furthermore, clonal populations from natural habitats have so far been sampled only from the canopy, whereas sexual populations are found only at ground level. It therefore seems likely that clonal and sexual populations from natural habitats do not share the same ecological niche. We were thus unable to assess the interaction between habitat and reproductive system in this study, essentially because it was seldom present in the field.

We set up 11 populations (Table 1), consisting of more than ten queens and at least 500 workers originating from three to 10 nests sampled in locations known to harbor a single population (Foucaud et al. 2009). These laboratory populations were kept in a climate chamber at a constant temperature of 25°C, with a humidity of 60% to 70%. Food (sugar water and worms) and water were provided *ad libitum* once per week.

Thermotolerance assays

We assessed the tolerance of the workers to abiotic conditions in a resistance test in which mortality rate was determined in groups of workers. We placed ten individual workers in a Petri dish sealed with a grid and maintained for three hours at constant temperature and humidity conditions within a climate chamber. The number of dead workers at the end of this three-hour period was used as a measurement of the worker abiotic tolerance (hereafter referred to as 'thermotolerance', *sensu largo*). Workers from experimental populations were tested more than 40 days after sampling, to ensure that all the workers tested had been produced in the laboratory and not sampled from the field (the life expectancy of *W. auropunctata* workers is about 30 days, Ulloa-Chacon 1990). This time lag to the start of the experiments minimized the impact of the acclimation factor in the interpretation of our results. In addition, evaluation of the impact of acclimation on the measure of upper thermal limit in *Linepithema humile* shows that in our case of 25°C acclimation temperature and a high rate of temperature change, no acclimation effect should be expected (Chown et al. 2009). Similar experiments carried out on workers from populations housed in the laboratory for more than a year provided qualitatively similar results, confirming that acclimation is unlikely to account for our findings (data not shown).

Preliminary experiments were conducted in the laboratory on one sexual population from a natural habitat (M7) and one clonal population from a human-modified habitat (Ker), to determine the range of temperature and humidity conditions yielding moderate to high worker mortality rates. We studied a total of 14,250 workers in 39 combinations of temperature (seven values from 25°C to 42°C) and humidity conditions (seven values from 100% to 25%; see Supplementary Figure S1 for details). The proportion of dead workers per groups of ten workers (i.e., the mortality rate) at the end of the incubation period was used to assess the tolerance of the workers to the abiotic conditions to which they were exposed. Based on the findings of these preliminary experiments, we chose four temperature and humidity combinations giving mild to high worker mortality rates for the main experiment: 36°C-55%, 38°C-65%, 39°C-70% and 40°C-75%. We chose to co-vary temperature and humidity in our main experiment, because mild levels in mortality in *W. auropunctata* workers also co-vary with these two factors (see Figure S1).

TABLE 2: Significance of fixed effects in the statistical analysis of thermotolerance. Note: Degrees of freedom for the numerator and denominator are provided for each effect (columns Num DF and Den DF, respectively). Effect 'Type' corresponds to the three different types of habitat), 'cond' to the temperature-humidity conditions tested and 'rack' to the vertical position of the Petri dish in the climate chamber. See Materials & Methods section for details.

Effect	Num DF	Den DF	F Value	*p* value
Type	2	8.632	10.46	0.0049
Cond	3	4.541	8.62	0.0248
Rack	4	39.35	36.68	< 0.0001
Cond*Rack	12	842.5	6.38	< 0.0001

During the main experiment following our preliminary tests, we tested a total of approximately 8,800 workers as follows. For each of the four chosen sets of abiotic conditions, we tested all 11 populations twice, using ten Petri dishes of ten individuals (i.e., 4 conditions * 11 populations * ten Petri dishes * two runs = 880 Petri dishes, minus one missing). All eight assays were conducted in the same climate chamber, in which two Petri dishes per population were placed at random positions on five vertical racks. As before, we used the proportion of dead workers per Petri dish (i.e., the mortality rate) as a measurement of the tolerance of the workers to the abiotic conditions to which they were exposed.

Statistical analysis of thermotolerance

Our main goal was to investigate putative differences in abiotic tolerance between populations of different origins (native natural, native human-modified and introduced human-modified), through the use of a statistical model. Explanatory variables consisted only in categorical factors. We used five categorical factors: 'type' (corresponding to the three different types of habitat), 'pop' (the sampled population), 'cond' (the tested temperature-humidity conditions), 'rep' (the number of the replicate, from one to two), and 'rack' (the vertical position of the Petri dish in the climate chamber, from rack 1 to rack 5). Note that 'pop' was nested within 'type' and that the variable 'rep' was nested within 'cond' (hereafter denoted 'pop(type)' and 'rep(cond)'). Both the 'pop' and 'rep' factors were treated as random effects, whereas the remaining factors ('cond', 'rack' and 'type') were treated as fixed effects. For the purposes of interpretation, only the following interactions were included in the full model: cond*type, cond*pop(type), rack*pop(type), rack*rep(cond) and rep(cond)*pop(type). Given that the response variable was a proportion, we assumed a binomial distribution.

With fixed and random effects and a binomially distributed independent variable, we ran a generalized linear mixed model with a logit link function (Zuur et al. 2009). We were principally interested in estimating the magnitude of the difference in mortality rates between the three types of population, while correcting for several clustering factors (e.g., population). We therefore used Penalized Quasi-Likelihood methods (PQL; Breslow and Clayton 1993), as implemented in SAS 9.1, to fit the model (PROC GLIMMIX, SAS Institute 2002). PQL methods may yield biased estimates of variance components in some situations (Lin and Breslow 1996). However, based on previous work (Breslow and Clayton 1993; Breslow and Lin 1995; Bolker et al. 2009), we conclude that our estimates may only be subject to a negligible bias, given that (i) the data were only slightly unbalanced, (ii) all fixed effects reached high levels of significance (all p-values of interactions or main effects < 0.005, see Results), (iii) all variance component estimates were small (< 0.36, see Results) and the overdispersion coefficient was moderate (1.53, see below). The significance of fixed effects was assessed through approximate F-tests and the number of denominator degrees-of-freedom for each test was computed using the Kenward-Roger approximation, as recommended by Bolker et al. 2009).

It is worth stressing that our full model yielded an estimate of 0 for the variance component of the interaction between replicate and vertical position within the climate chamber (i.e., 'rack*rep(cond)') and the interaction between the type of habitat and the conditions tested (i.e., 'type*cond') was clearly non significant ($F_{[6,\ 25.8]} = 1.15$ $P \sim 0.36$). Therefore, as suggested by Zuur et al. (2009), we fitted a second model from which these two terms were removed. With this model, the coefficient of overdispersion was moderate (1.53 $SE = 0.08$), estimates of variance components were not large (i.e., << 1, Lin and Breslow 1996) and all fixed effects other than the conditions tested ('cond') gave small p-values (see Table 2). This second model was therefore selected for subsequent analyses.

Effect of body size on thermotolerance

We tested for a possible effect of body size on our thermotolerance results, by investigating worker body size in five native populations — two from natural habitats (M3 and M11) and three from human-modified habitats (Ker, P3 and Cay) — and two introduced populations (New Caledonia and Australia). The introduced Australian population, which was not tested in our main thermotolerance assay, has previously been shown to be clonal (Foucaud et al. 2010), and subsequently to exhibit thermotolerance levels similar to those of other introduced populations (results not shown). We collected and point-mounted 20 foraging workers from each population. Multifocus images (x40) of worker heads were acquired with a GT Entovision System and Archimed™ software (Microvision Instruments). Four morphological parameters of the head were measured on each worker (maximum head width, maximum head length, and head width in front of and behind the eyes) with Cartograph software (Microvision Instruments).

Preliminary analysis showed that all morphometric measurements were highly correlated (all Pearson's product-moment correlations > 0.66). Thus, as maximum head width is often considered a standard proxy for body size (Hölldobler and Wilson 1990), we used only this morphological variable for subsequent analyses. After testing for normality and variance homogeneity, we investigated putative differences in body size between populations originating from different habitats in both ranges (native natural, native human-modified and introduced human-modified), by fitting a linear mixed model implemented in R (R Development Core Team 2005) and including the following factors: 'type' (fixed factor corresponding to our three types of population) and 'pop' (random factor corresponding to the sampled population, nested within 'type').

RESULTS

Thermotolerance assays

Mortality rate was significantly higher for workers of populations from natural habitats of the native range (NaN) than for workers of populations from human-

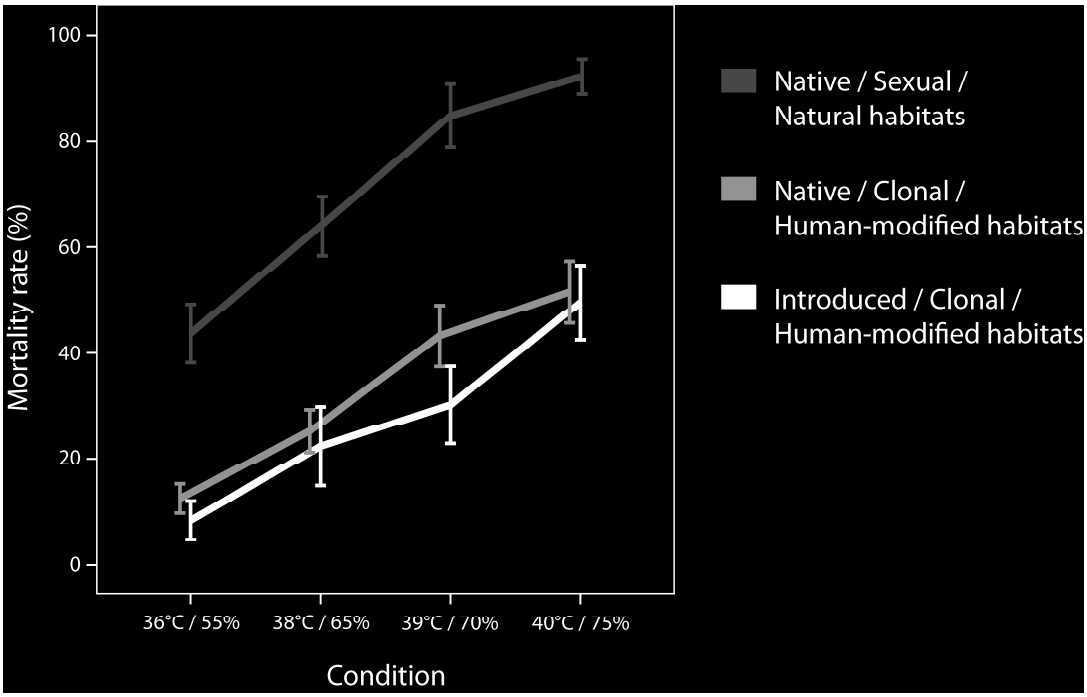

FIGURE 1: Mortality rates of workers from three *W. auropunctata* population types for each experimental condition of the thermotolerance assay. Note: Populations types are sexual populations from natural habitats in the native range, clonal populations from human-modified habitats in the native range and clonal populations from human-modified habitats in the introduced range. Mortality rates are mean proportions of dead workers per group of ten workers placed in the same Petri dish. Error bars indicate 95% confidence intervals.

modified habitats of the native (NaH) or introduced ranges (IntH) (mortality $_{NaN}$ = 0.79, $IC_{95\%}$ = [0.60; 0.91]; mortality $_{NaH}$ = 0.29, $IC_{95\%}$ = [0.16; 0.47]; mortality $_{IntH}$ = 0.21, $IC_{95\%}$ = [0.09; 0.40]; $t_{NaN\text{-}NaH}$= -4.97, df = 7.94, p = 0.0011 and $t_{NaN\text{-}IntH}$= -4.63, df = 7.85, p = 0.0018). By contrast, no significant difference was found between the mortality rates of the workers of populations from human-modified habitats of the native and introduced ranges ($t_{NaH\text{-}IntH}$= -0.95, df = 7.85, p = 0.37).

We also found a significant condition effect (Figure 1; Table 2), meaning that our four sets of conditions resulted in moderate to high mortality rates, as expected on the basis of our preliminary results (summarized in Supplementary Figures S1 to S3). The lack of interaction between the factors "type of population" and "condition" indicates a constant relationship between population type and mortality from mild to harsh abiotic conditions (Figure 1). However, without a priori power computations, we cannot discard definitively that these interactions existed and were not significant because of a lack of power. The vertical position of the Petri dish within the climate chamber had a significant effect on worker mortality rate (Table 2). This effect was expected, given the pitfalls of current climate chamber technology (i.e., the occurrence of vertical gradients), and was controlled through the equilibration of our experimental design (i.e., equal mixing of all populations at each vertical position within the climate chamber).

Body size and thermotolerance

Maximum individual head width was normally distributed (Shapiro-Wilk test: W = 0.989, p = 0.41) and individual variances were homogeneous for workers from different population types — that is, for workers from native natural, native human-modified and introduced human-modified habitats (Brown-Forsythe Levene-type test: p > 0.09). The linear mixed model analysis revealed that the type of population factor was not significant (χ^2 = 0.6287, df = 2, p = 0.73; Figure 2). Workers thus had similar body sizes regardless of their habitat of origin (i.e. native natural, native human-modified and introduced human-modified).

DISCUSSION

Adaptive shift between natural and human-modified habitats

Our study of the thermal and hygrometric tolerance levels of native and introduced populations of *W. auropunctata* from different habitats indicated that a major phenotypic transition likely occurred within the native range of this worldwide invasive species. Workers from native sexual populations sampled in natural habitats were less tolerant to hot and dry conditions than both native and introduced clonal populations sampled in human-modified habitats. By contrast, populations established in human-modified habitats in the native and introduced ranges displayed similar, higher levels of thermotolerance. A previous study has shown that climatic conditions differ considerably between adjacent natural and human-modified

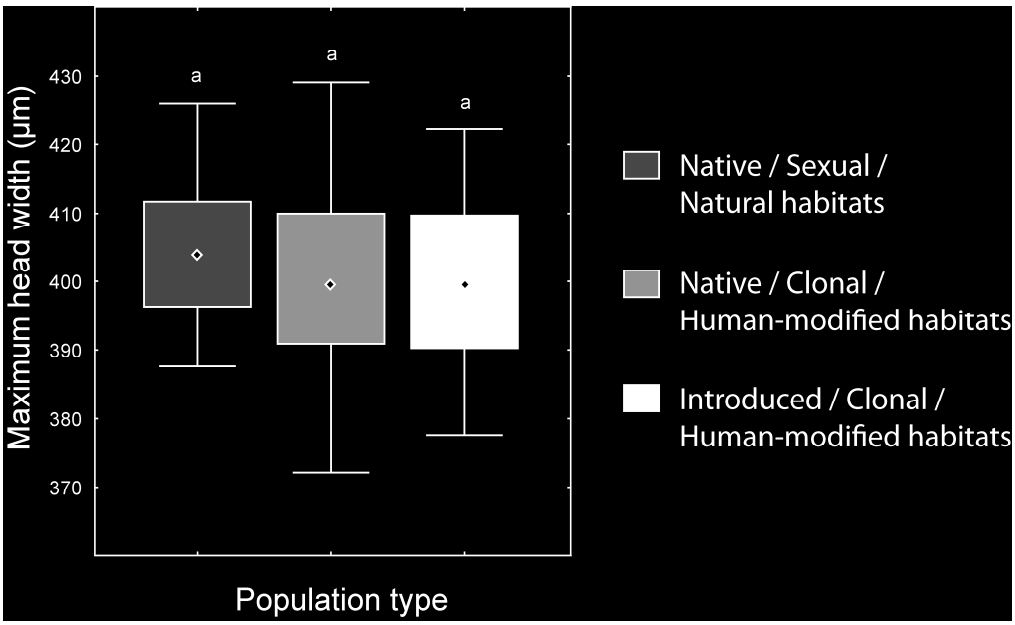

FIGURE 2: Maximum head width of workers from the three population types. Note: Diamonds indicate means, blocks and horizontal bars indicate 50% and 95% percentiles, respectively.

in the native range of *W. auropunctata*, with the latter reaching higher temperatures and lower humidity (Orivel et al. 2009). Some of the abiotic conditions tested in our study correspond to the worst possible conditions found in the field during an 18-month period, rather than to conditions likely to be experienced on a regular basis by workers in the field. However, the higher mortality rates for workers from native sexual populations in the conditions tested likely translates in a lower foraging efficiency and lower fitness in the actual conditions measured in native human-modified habitats when compared to heat-tolerant clonal populations. Consistent with this hypothesis, Cerdá et al. (1998) have shown that, in a Mediterranean ant community, heat-tolerant species have a higher foraging efficiency at high temperatures than heat-intolerant species.

These findings highlight the importance of selective processes due to human activities in the native range. Indeed, the switch from heat-intolerant sexual populations confined to natural moist and cool habitats to heat-tolerant clonal populations establishing in hot and dry human-modified habitats is probably a key first step towards invasive success, as it allows the species to gain access to new resources and to new transport opportunities, specific to human areas. These results are consistent with the "two-step" scenario of invasion process put forward by Foucaud et al. (2010), which is largely based on the potential of native populations to evolve under direct human pressure on landscape properties (see also Hufbauer et al. 2011).

The hypothesis of human selective pressures modifying species within their native range, driving them towards invasive characteristics, is supported by a growing number of studies on various species. For instance, the ant *Tapinoma sessile*, native to America, is showing a striking transition in social system between populations from natural habitats and populations from highly urbanized habitats within its own native range (Buczkowski 2010). The urban populations also evolved invasive characteristics within the native range, such as improved demography and ecological dominance. In this species, the role of human disturbance in the form of urbanization has been clearly demonstrated (Buczkowski 2010; Menke et al. 2010). So far this species is not considered invasive because it has not established itself outside its native range. Given the dispersal opportunities provided by human-dominated landscapes, it is nevertheless probable that *T. sessile* will invade distant urban areas in the near future. Agricultural activities also cause major changes in the biology of native species (e.g., distribution, migration, dominance, demography), especially with novel perturbation regimes or the introduction of new hosts (Via 1990). In the butterfly *Euphydryas editha*, a host plant shift occurred within 10 years following the introduction by humans of two plant species, *Plantago lanceolata* and *Collinsia torreyi*, during logging and cattle ranching in previous natural habitats (Singer et al. 1993). The reproductive output of *E. editha* has been shown to be much higher on its new hosts (Singer et al. 1993; Singer and Thomas 1996) and, if its potential for migration were not so low (Brussard and Ehrlich 1970), it might have colonized vast areas in which its new hosts were introduced through agriculture (e.g., in California and Nevada). In some cases, host-switching species evolve invasive features, thrive in their human-modified native range and eventually invade remote areas. This was the case for the Colorado potato beetle, *Leptinotarsa decemlineata*, which switched from its historical *Solanum* hosts to *Solanum tuberosum* following its introduction for farming. It subsequently invaded North America, Europe and Asia to become the most threatening pest of potato crops (Alyokhin et al. 2008).

Effect of body size on thermotolerance

In some ant species, differences in thermotolerance can be accounted for by differences in worker body size (Kaspari 1993; Clemencet et al. 2010). For instance, large *Cataglyphis velox* workers withstand temperatures 6 to 8°C higher than those tolerated by smaller workers (Cerda and Retana 1997). However, morphological measurements indicated that this was not the case in *W. auropunctata,* as workers of both heat-tolerant populations (i.e. established in human-modified habitats in both the native and the introduced range) and heat-intolerant populations (i.e. established in natural habitats) had bodies of similar size. McGlynn (1999) and Mikheyev and Mueller (2007) found that workers from introduced populations were smaller than those of native populations, based on a wide geographic sampling range. Additional Student's *t* tests on pairs of population types revealed only a non-significant trend of this type in our own data set (results not shown). Most importantly, the goal of our morphological analysis was to assess the possible relationship between body size and levels of thermotolerance in population samples for which we had thermotolerance data. In that respect, we found no significant association between these two variables. Thermotolerance in *W. auropunctata* is therefore probably achieved through physiological, biochemical or behavioral processes, or through a combination of such processes, rather than through an increase in body size. For example, in the small workers of thermophilic *C. rosenhaueri*, thermotolerance results partly from an increase in metabolic rate and a decrease in cuticular water loss, together with differences in body posture (Cerda and Retana 2000).

Physiological or other processes explaining the polymorphism in thermotolerance remain to be investigated in *W. auropunctata*. In addition to other experimental investigations, direct approaches using functional loci could be envisaged to study the genetic determinism of thermotolerance in *W. auropunctata*. Our thermotolerance assays could thus be complemented with transcriptomic data, through the use of dedicated microarrays or investigation of the expression patterns of candidate genes, such as genes from the heat shock protein family (e.g., Dahlgaard et al. 1998; Fangue et al. 2006).

CONCLUSION

This study highlights the importance of human land use in bringing about major contemporary evolutionary changes within the native range of species. Those native adaptations to human habitats can constitute prior adaptations when introduced in remote human habitats, often showing little variability. This human-induced process is ultimately paving the way for the emergence of new bioinvaders. Human modifications of ecosystems are usually substantial, so most species may fail to cross such large valleys in the adaptive landscape (see Ronce and Kirkpatrick 2001; Lenormand 2002; Ravigné et al. 2009 for theoretical studies on this subject). However, *W. auropunctata* provides a striking example of a species that eventually crossed such a valley, thereby gaining access to new resources and new areas. This may not be an uncommon process, considering that most of the worst cases of biological invasion are due to human commensals (Ehrlich 1989; Lowe et al. 2000). More empirical studies in the native and introduced ranges of invaders are needed to draw general conclusion on the relative contribution of prior adaptations (with a special emphasis on human habitats) and post-introduction evolutionary events (e.g., multiple introductions, purging of deleterious mutations through moderate bottlenecks, "bridgehead" effect, hybridization; Ellstrand and Schierenbeck 2000; Abbott et al. 2003; Bossdorf et al. 2005; Durka et al. 2005; Facon et al. 2008; Lombaert et al. 2010; Facon et al. 2011) to the success of invasive species (Lee and Gelembiuk 2008; van Kleunen et al. 2010; Hufbauer et al. 2011). This later point is crucial to help building biologically coherent managing policies to prevent invasions worldwide.

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SUPPLEMENTARY MATERIAL

FIGURE S1: Preliminary experimental results for rates of worker mortality induced by thermal and hygrometric stress in two native populations of *W. auropunctata*

Note: (A) Mortality rates of one native clonal population from a human-modified habitat (KER) and one native sexual population from a natural habitat (M7) to various abiotic conditions in a preliminary experiment. (B) Sampling size in number of Petri dishes of 10 workers for each temperature and humidity combination. The color scale represents different ranges of worker mortality rate from low (green) to high (red). Untested conditions are indicated by empty, gray cells.

(A) Proportion of dead workers

		Temperature													
		25℃		30℃		34℃		36℃		38℃		40℃		42℃	
		Ker	M7	Ker	M7	Ker	M7	Ker	M7	Ker	M7	Ker	M7	Ker	M7
Relative humidity	100%	0.1	0	0	0			0	0	0.03	0	0.37	0.43	1	1
	80-85%			0	0	0.01	0.02	0.09	0.08	0.07	0.12	0.56	0.73	1	1
	70-75%	0	0	0	0	0.01	0.03	0.05	0.08	0.12	0.24	0.69	0.83	1	1
	60-65%	0	0.01	0	0.01	0.03	0.03	0.03	0.1	0.29	0.48	0.93	0.98	1	1
	50-55%	0	0.01	0	0.03	0	0.03	0.15	0.33	0.52	0.72	0.95	0.99	1	1
	35%	0	0	0.03	0.055	0.23	0.39	0.42	0.57	0.98	1				
	25%					0.19	0.48								

0 - 0.1
0.1 - 0.4
0.4 - 0.6
0.6 - 0.9
0.9 - 1

(B) Sampling size (in Petri dish of ten individuals)

		Temperature														
		25℃		30℃		34℃		36℃		38℃		40℃		42℃		Total
		Ker	M7	Ker	M7	Ker	M7	Ker	M7	Ker	M7	Ker	M7	Ker	M7	
Relative humidity	100%	4	3	3	5			10	10	9	8	32	36	12	12	144
	80-85%			6	5	10	10	26	30	35	40	30	30	10	10	242
	70-75%	10	10	10	9	10	10	39	39	39	40	49	50	10	10	335
	60-65%	10	10	10	8	10	10	29	30	69	70	30	30	10	10	336
	50-55%	10	10	9	10	10	10	29	29	26	25	11	9	10	10	208
	35%	10	10	10	10	10	10	30	30	10	10					140
	25%					10	10									20
	Total	44	43	48	47	60	60	163	168	188	193	152	155	52	52	1425

FIGURE S2: Mortality rates of workers from each of the sampled populations over all tested thermal and hygrometric conditions.
Note: Squares indicate means, blocks and horizontal bars indicate 50% and 95% percentiles, respectively.

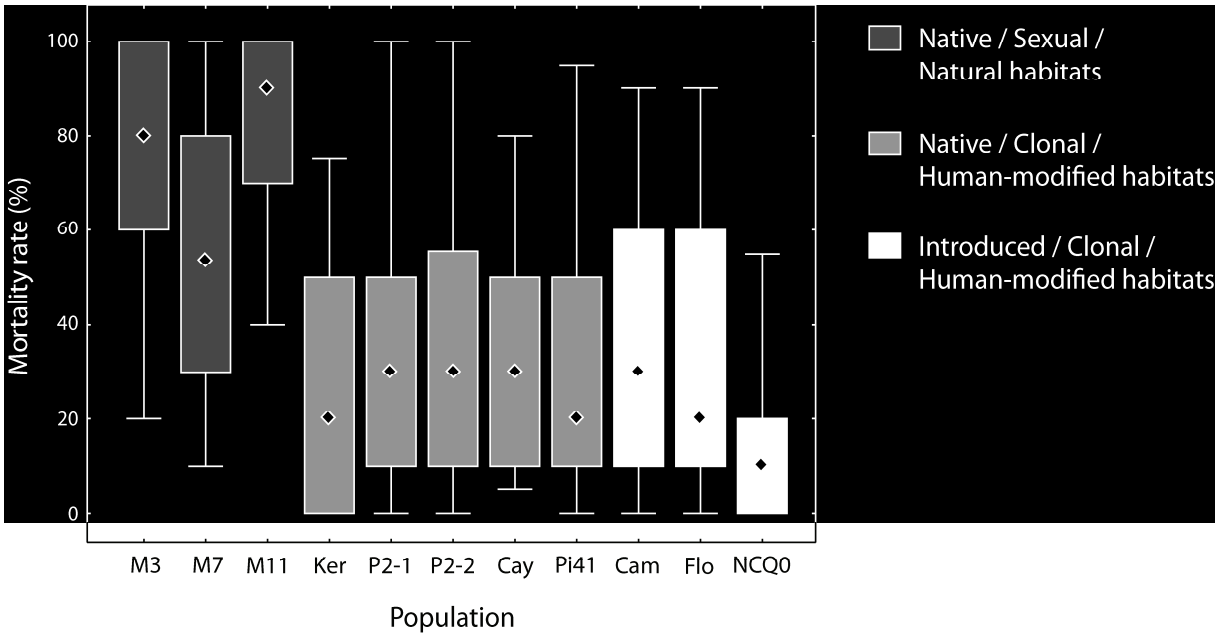
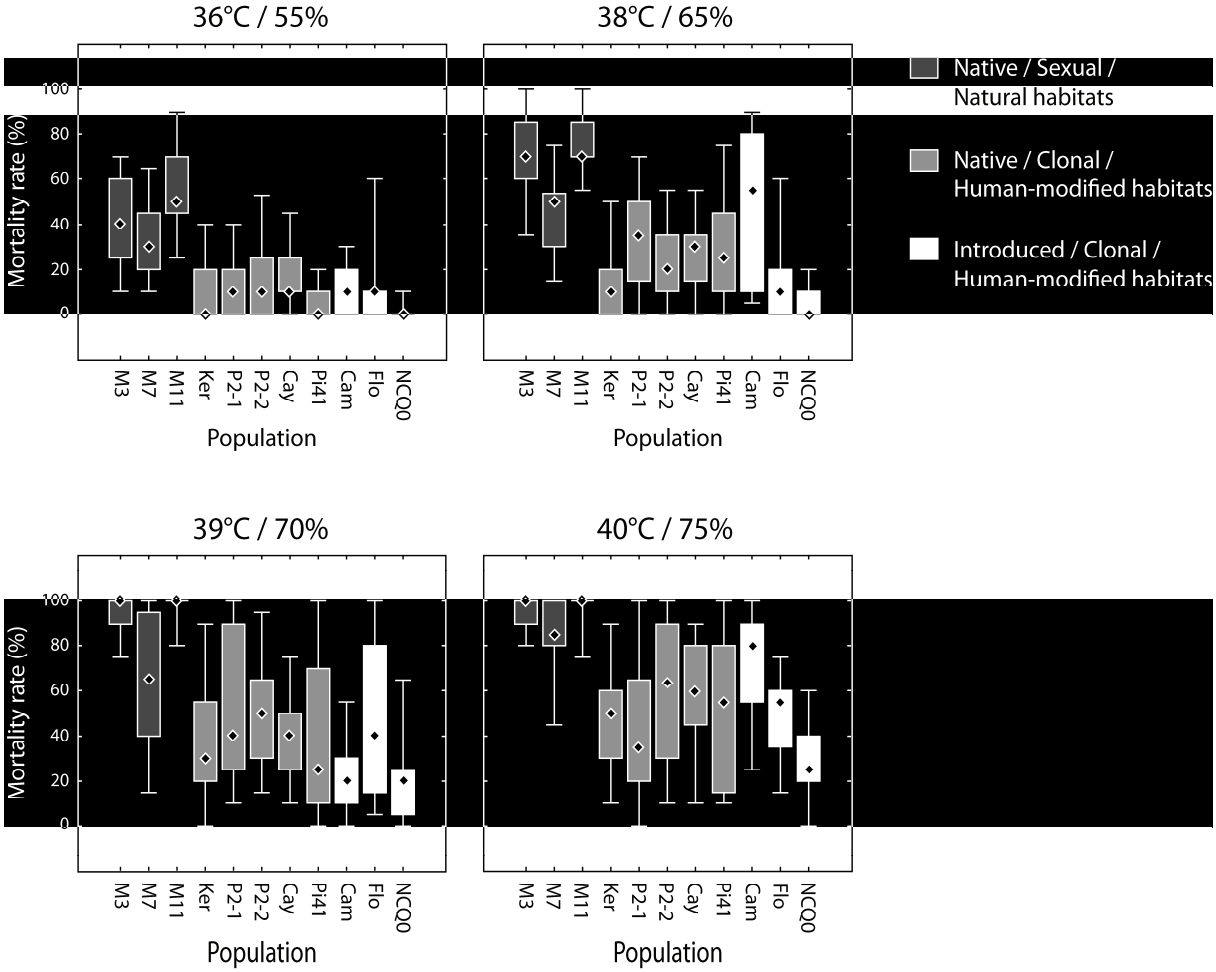


FIGURE S3: Thermotolerance of workers from each sampled populations for each set of thermal and hygrometric conditions tested.

Note: Squares indicate means, blocks and horizontal bars indicate 50% and 95% percentiles



IV. Article 5: Where do adaptive shifts occur during invasion? A multidisciplinary approach to unravel cold adaptation in a tropical ant species invading the Mediterranean zone.

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Keywords: *Wasmannia auropunctata,* adaptation, biological invasion, cold temperature, climatic niche shift, Mediterranean zone

Running head: Cold adaptation of an invasive ant

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ABSTRACT

Evolution is now recognised to improve the invasive success of populations but it often remains unclear where and when key adaptation events occur. We used a multidisciplinary approach to disentangle the eco-evolutionary scenario of the invasion of a Mediterranean region (Israel) by the tropical ant *Wasmannia auropunctata*. Laboratory experiments showed that Israeli populations were better adapted to cold temperatures than tropical native and invasive populations. Species distribution modelling showed that adaptation to the colder-temperature conditions typical of Israel may have occurred at a primary site of invasion (Florida) and/or at the southern edge of the native range (north-eastern Argentina). Phylogeographic analyses indicated that the Israeli populations were loosely related to populations from Florida but remarkably similar to populations from Argentina. Our results strongly suggest that the Israeli populations were established following a two-step invasion scenario, with prior adaptation to cold in north-eastern Argentina before long distance dispersal to Israel.

Contribution by authors
OR, AE, JF and BF conceived the study; OR, AE and BF wrote the manuscript; OR, AL, AE, BF performed the main laboratory experiment and LCA performed the additional laboratory experiment on Argentinean populations; OR, JF and AL produced the genetic data; OR, AE and BF performed data treatments with the contribution of SB, JPR and GJK for statistical analyses, development of species distribution models and phylogenetic analyses respectively; OR, AE, MV, AL, JF, JO, LC, LC, ML and TS collected samples. All authors contributed substantially to the correction of the final draft of the manuscript.

INTRODUCTION

Evolution is now recognised to play an important role in increasing the invasive success of some populations (e.g. Lee 2002; Cox 2004, Suarez & Tsutsui 2008). Most studies addressing this concern has a temporal and geographical focus on evolutionary changes that occur following introduction into a usually remote new location, as an evolutionary response to novel biotic and/or abiotic selection regimes (e.g. Yeh 2004; Phillips 2006). This scenario implies that evolutionary changes occur independently in each distinct invaded locations. A notable illustration of this pattern is the marine copepod, Eurytemora affinis, that has invaded and has adapted to freshwater habitats multiple times independently with the advent of ballast water shipping and discharge into freshwater lakes on several continents (Lee 1999).

Two alternative (although not exclusive) eco-evolutionary scenarios of invasion have been recently formalized to explain the success of some invasive populations. First, adaptation results from evolutionary processes occurring in a primary invaded site followed by subsequent introductions into other territories from this site, a scenario recently described as the "bridgehead" effect (Lombaert *et al.* 2010). Under this scenario, adaptive change(s) may occur only once in the bridgehead area. The adaptive changes in the initial site of introduction may break biotic and/or abiotic barriers to invasion and therefore rendering easier subsequent establishment into sometimes remote biogeographical areas, displaying selective pressures similar to those in the bridgehead area. In the second invasion scenario, key evolutionary changes necessary for the invasion of new biogeographic regions may also occur within the native range, before long-distance dispersal events into the introduced range (Bossdorf *et al.* 2008; Lee & Gelembiuk 2008; Jenkins & Keller 2011; Hufbauer *et al.* in press). This scenario was recently called "two-step" (Foucaud *et al.* 2009) and more specifically AIAI for "anthropogenically-induced adaptation to invade", when prior-adaptation is related to human-altered habitats (Hufbauer *et al.* in press).

Unravelling of the eco-evolutionary scenario underlying invasive populations has important implications for both invasion theory, to better understand circumstances that favour adaptive changes during invasion processes, as well as for management policies, as it may guide actions to prevent or control undesirable invasions. Yet, the distinction between the three main eco-evolutionary scenarios of invasion requires a substantial amount of information that is so far difficult to pile up for most species (Hufbauer *et al.* in press). Required evidences include phenotypic analyses involving both native and introduced populations to test whether adaptation occurred during invasion; some genetic data for documenting the genetic relationship between native and invasive populations and retrace the routes of invasions; and finally environmental data (climatic and/or biotic) to assess selection pressure on key life history traits in both the native and introduced populations. Common-garden experiments and phenotypic analyses have been successfully used to illustrate post-establishment adaptation of invasive populations (e.g. Yeh 2004; Phillips 2006). Genetic tools were successfully used to decipher complex invasion histories (e.g. Lombaert *et al.* 2010 and Ascunce *et al.* 2011) and niche-based models were successfully used to identify ecological niche shift associated to invasion processes (e.g. Broennimann *et al.* 2007). However, to our knowledge, experimental, genetic and niche modelling approaches have not been combined simultaneously on a single species to test the assumptions proposed by Hufbauer *et al.* (in press).

We here propose an integrative multidisciplinary approach combining phenotypic, genetic and niche-modelling analyses, to tease apart the three main above mentioned eco-evolutionary scenarios of invasion, using the successful invasive ant *Wasmannia auropunctata*, as biological model. Some populations of this tropical ant recently successfully invaded the Mediterranean zone in Israel (Vonshak *et al.* 2010). First we tested whether the establishment of *W. auropunctata* populations in the Mediterranean zone was accompanied by an adaptation of workers to local cold temperature. Then we assessed whether this adaptation occurred within the introduced range in Israel, or before transportation to Israel, either at a primary site of invasion (i.e. bridgehead scenario) or within the native range in some peculiar habitats (i.e. two step scenario). We addressed the first question by carrying out laboratory experiments to compare the response to cold stress of workers from Israeli populations and workers from a large set of populations established in either the native or the introduced range of the species. We addressed the second question by first conducting species distribution modelling (SDM) analysis, to identify geographic regions where native or invasive populations of *W. auropunctata* may be subject to cold temperature selection pressures similar to those in Israel. We then conducted a worldwide phylogeographic analysis based on mitochondrial and microsatellite genetic DNA, to determine the putative genetic origin of the Israeli lineage, focusing more specifically on the candidate geographic areas identified in our SDM analysis.

MATERIALS AND METHODS

Study species

W. auropunctata originates from the neotropic ecozone, from Mexico to Northern Argentina (Wetterer & Porter 2003) and displays two different types of populations within its native range. Ancestral native populations are confined to primary forests, occupying naturally disturbed areas (e.g. floodplains), and are characterised by low nest and worker densities (Wetterer & Porter 2003; Orivel *et al.* 2009). Some populations have repeatedly successfully invaded human-modified habitats within the native range (e.g. road sides, plantations; Wetterer & Porter 2003; Orivel *et al.* 2009). This change of habitat within the native range is associated with a major ecological shift with high worker and nest densities (Orivel *et al.* 2009) and a genetic shift in the reproductive system, from a classical haplo-diploid sexual model to clonally reproducing queens and males (Foucaud *et al.* 2009).

Site	Area	Climatic zone	Country	Habitat
M11	Native	Tropical	France (FG)	Natural
M3	Native	Tropical	France (FG)	Natural
M7	Native	Tropical	France (FG)	Natural
Cay	Native	Tropical	France (FG)	Human-modified
P2	Native	Tropical	France (FG)	Human-modified
Ker	Native	Tropical	France (FG)	Human-modified
Mp	Introduced	Tropical	France (NC)	Human-modified
Cam	Introduced	Tropical	Cameroon	Human-modified
Orl*	Introduced	Subtropical	USA (Florida)	Human-modified
Zarate 1*	Margin native	Subtropical	Argentina	Human-modified
Zarate 2*	Margin native	Subtropical	Argentina	Human-modified
MaAb	Introduced	Mediterranean	Israel	Human-modified
NeveUR	Introduced	Mediterranean	Israel	Human-modified
NY	Introduced	Mediterranean	Israel	Human-modified
Hz	Introduced	Mediterranean	Israel	Human-modified

Table 1: *W. auropunctata* populations sampled for laboratory experiments assessing cold tolerance. Note: About 20 fertilised queens and 2000 – 5000 workers per population were sampled. FG= French Guiana; NC = New Caledonia. Refer to the "material and methods" section for further details on different types of areas. * Populations more specifically used to verify where adaptation to cold occurred given the results obtained from the SDM and genetic analyses (see Figure 2, 3 and 4).

Since the beginning of the 20th century, *W. auropunctata* has successfully invaded many countries in tropical and subtropical zones (Wetterer & Porter 2003; Foucaud *et al.* 2010). In its introduced range, the invasive populations settle in human-altered habitats with several traits similar to those of the clonal populations established in the native area (i.e. ecological dominance and clonality; Foucaud *et al.* 2010). In 2005, established populations of *W. auropunctata* were found in Israel, in the Mediterranean zone, which is characterised by climatic features, especially colder winter temperatures, very different from those in the tropical core habitat (Vonshak *et al.* 2010). Despite the harshness of the abiotic conditions of the Mediterranean zone with respect to the tropics, the Israeli populations display nesting and foraging behaviour similar to that observed in the tropical and subtropical areas (Vonshak *et al.* 2010). The ability of foraging workers to cope with cold winter temperatures suggests that the Israeli population may have undergone adaptation to cold temperatures to successfully invade this new biogeographic area.

Laboratory Experiments

Sampled populations

We sampled a total of 15 *W. auropunctata* populations (Table 1). Twelve were used for verifying whether adaptation to cold occurred in Israeli populations during invasion and three were used to more specifically verify where adaptation occurred given the results obtained from the SDM and genetic analyses (see results section). The first set of twelve populations includes: (i) six populations from the native tropical area representing the core habitat of the species, with three populations from natural habitats (i.e. primary forest) and three populations from human-modified habitats (i.e. plantation or roadside) and (ii) six populations from the invasive range, two populations from the tropical zone and four populations from the Mediterranean zone (Israel). The three other populations, more specifically used to verify where adaptation occurred given the results obtained from the SDM and genetic analyses, were sampled as following: two populations from Zarate (Buenos Aires province, Argentina), a zone located at the southern margin of the native range and one population from Florida which corresponds to a subtropical zone of the introduced range. All but the two Argentinean populations were reared and studied at the CBGP laboratory (Montpellier, France). The two populations from Argentina were reared and studied at the SABCL laboratory (Buenos Aires, Argentina) due to the strong legislation constraints associated to the importation of live *W. auropunctata* from Argentina in Europe. In both laboratories, ants were reared in a walk-in climatic chamber maintained at constant temperature and humidity (25°C; 70% RH; L:D 12:12) for at least two months, to ensure that all the workers tested in the experiments had been produced in the laboratory. This time lag before the experiments minimised the impact of the acclimation factor in the interpretation of our results. During this period, ants were fed *ad libitum* with *Ephestia* eggs and a honey-yeast-water solution.

Cold tolerance

Time to recovery from chill coma (a reversible state of narcosis induced by cold) is an appropriate phenotypic surrogate for the assessment of cold adaptation in insects (Gibert *et al.* 2001; Hoffman *et al.* 2003). We therefore measured the time to recovery and the survival rate of workers after a cold stress. All populations were tested following the same protocol. We placed 50 workers from each population in five sealed and meshed boxes (10 workers per box) and fed them with 0.5 ml of honey-water for one hour. Workers were then placed in a climatic chamber at 2°C for 16 hours. This temperature is close to the lowest absolute minimum air temperature recorded in the winter months between 1981 and 2000 in Israel (January: 2.5°C and February: 2.6°C, Israeli meteorological service). At the end of the cold stress period, boxes were placed in a walk-in climatic chamber with standard climatic conditions (i.e. 25°C, 70 % RH). The proportion of workers recovering from cold stress in each box was determined at 11 time points: just after the cold shock, after 27, 40 and 60 minutes and then every 30 minutes until 5 hours. This experiment was then repeated except for the two tested Argentinean populations hence reaching a total of 1400 tested workers (50 workers x 13 populations x 2 runs at the CBGP and 50 workers x 2 populations at the SABCL).

We investigated putative differences in the response of workers to cold stress as a function of biogeographic origin (native area in natural habitats, native area in human-modified habitats, introduced area in tropical zones, subtropical populations in Florida, subtropical populations in Argentina, and introduced area in the Mediterranean zone). For each of those six origins, we investigated the relationship between the proportion of workers that had recovered and the time since cold stress by fitting a non-linear mixed model (nlme) according to a reparameterised Gompertz relationship implemented in R statistical software (R Development Core Team, 2010), with a mean function defined as follows:

$$f(x) = plateau * \exp(-\exp(c * (d - x)))$$

Two parameters, the "*plateau*" and the parameter "*d*", which represent the maximum proportion of surviving workers and the abscissa at the inflection point (i.e. a good proxy of the time for workers to recover), respectively, were estimated and compared for each six biogeographic origin. The parameter "*c*", for which the biological interpretation is difficult in this case study, was not used in subsequent statistical analysis. Parameter estimates with non-overlapping confidence intervals were considered to be significantly different. The data from Argentinean populations were included in the main statistical analyses but the estimates should be interpreted carefully as the experiment was conducted in a different laboratory by different observers.

We also conducted complementary analyses based on general linear mixed models (GLMM), using both the maximal proportion of surviving workers and the time taken to reach half the maximal proportion of recovered workers as response variables (see Appendix S1 in supporting information for details).

Species distribution modelling analyses

We used the "domain" algorithm (Carpenter *et al.* 1993) to model the distribution of *W. auropunctata* on the basis of 88 geo-referenced occurrences of the species in Israel. Half of these occurrences were used for model fitting, the other half being used for model evaluation. Climate data included four variables describing the cold-temperature conditions typical of the habitats occupied by *W. auropunctata* in Israel: the minimum temperature of the coldest month, the mean temperature of coldest quarter, the annual mean temperature and the annual range of temperature (available from the WORLDCLIM database; Hijmans *et al.* 2005). Data were managed with the GIS software GRASS (GRASS Development Team 2008), interfaced with R via the R packages spgrass6 (Bivand 2011) and raster (Hijmans & van Etten 2010). Models were fitted and evaluated with the R package dismo (Hijmans *et al*. 2010).

The performance of the models was evaluated for the Israeli region, by comparing model predictions with observations, through calculations of the area under the curve (AUC) of a receiver operating characteristics (ROC) plot (Fielding & Bell 1997). As only data for occurrences were available, pseudo-absences were randomly generated in the geographical region over which the model was defined, except for populated and urban areas (the necessary land-use information was obtained from the Natural Earth website: http://www.naturalearthdata.com/). This approach was adopted because *W. auropunctata* is known to be intimately associated with human-modified habitats in its area of introduction, particularly in Israel (Vonshak *et al.* 2009). The domain algorithm applied to our climatic data set yielded a model that was ultimately used to spot worldwide geographical regions displaying similar climatic conditions and in which the presence of *W. auropunctata* populations had previously been reported.

Phylogenetic analyses

We conducted phylogeographic genetic analyses to investigate the branching position of Israeli individuals within a large set of samples from tropical/subtropical native and invasive populations (see Figure SF1 in supporting information). This analysis was based on mitochondrial and microsatellite data. DNA was extracted and purified from all individuals analysed, by a standard CTAB-based protocol.

Mitochondrial data

We amplified a 700 – 710 bp fragment of the mitochondrial *COI* (*Cytochrome oxidase I*) gene from 136 individuals (83 workers and 53 queens), with two sets of primers (See Appendix S2). In total, 123 of these individuals originated from 31 native and 20 introduced populations in the tropical/subtropical zone, whereas the other 13 individuals analysed (five queens and eight workers) originated from seven nests established in Israel (see Figure SF1). Two sequences from the closely related species

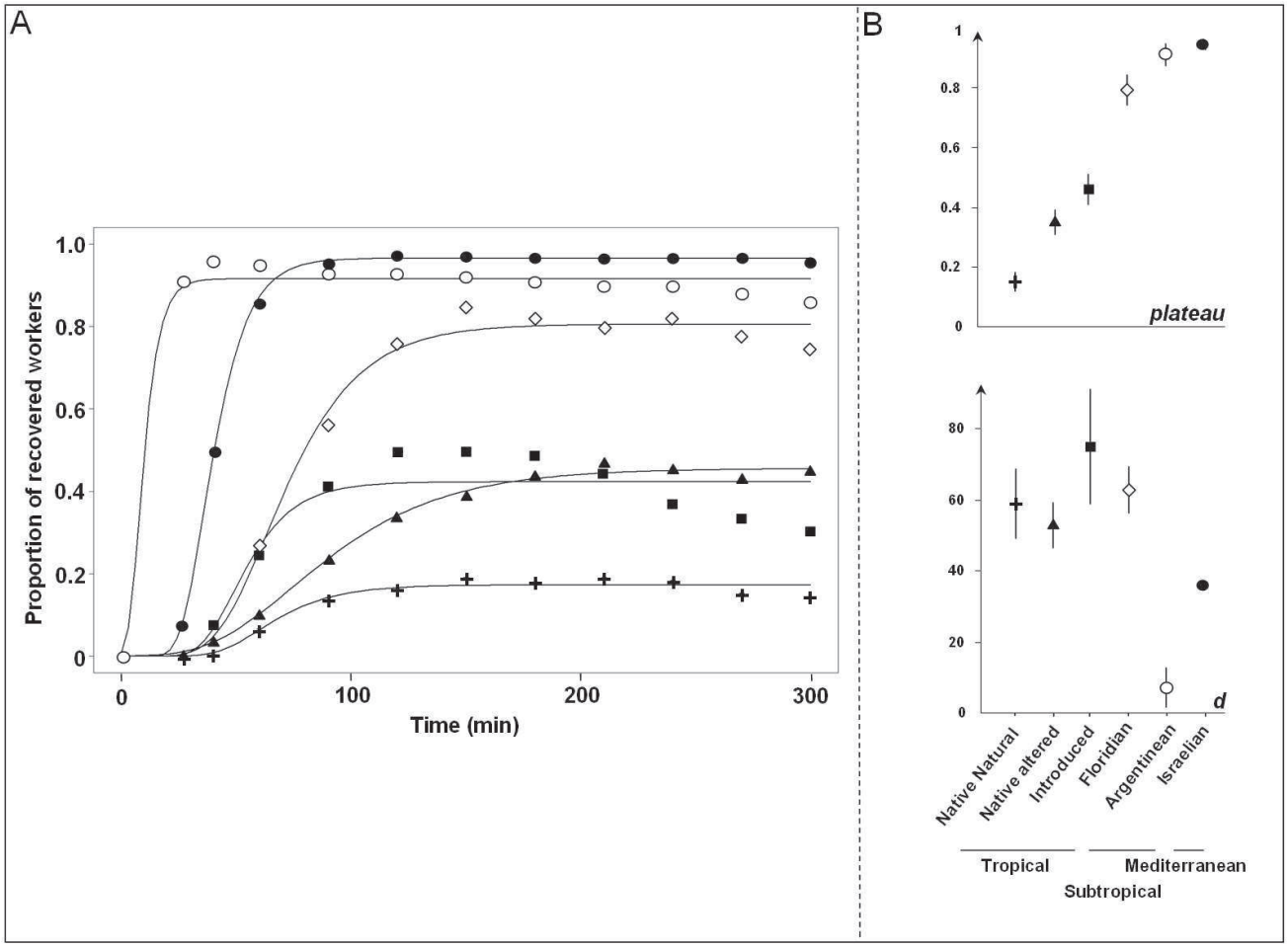

Figure 1: Cold tolerance in laboratory experiments using Gompertz relationships. Note: Box (A) shows the fits of non-linear mixed models using Gompertz functions to the proportion of recovered workers at each observation time. The Symbols correspond to the mean proportion of recovered workers at each observation time in all populations of each of the six biogeographic origins considered: ✚ = native area in natural habitats; ■ = native area human-modified habitats; ▲ = introduced area in the tropical zone; ● = introduced area in the Mediterranean zone (i.e. Israel); ◊ = introduced area in the subtropical zone (Florida); ○ = Zarate (Buenos Aires province, Argentina). Plain symbols correspond to populations used to test whether adaptation occurred in the Mediterranean populations compare to populations established in the core habitat of the native range. Open symbols correspond to the populations used to more specifically verify where adaptation to cold occurred given the results from both the SDM and genetic analyses (see Figure 2, 3 and 4). The curves are the fitted Gompertz functions. Box (B) shows the comparison of Gompertz function parameters estimated for each biogeographic origin of populations, for the parameter *plateau* at the top, and parameter *d* at the bottom (see text for details). Cold tolerance of workers for all but the two Argentinean populations was assessed at the CBGP (Montpellier France). Cold tolerance of workers for the two Argentinean populations was assessed at the SABCL laboratory (Argentina).

Wasmannia rochai (GenBank accession numbers EF459732 – EF759824) were used as an outgroup.

Our phylogenetic analyses were performed on unique haplotypes (35 in total). Sequences were partitioned into codon positions (cp) and models of evolution were independently selected for five sets of partitions (1^{st} cp; 2^{nd} cp; 3^{rd} cp; $1^{st}/2^{nd}$ cp; $1^{st}/2^{nd}/3^{rd}$ cp). This was achieved by comparing Akaike's information criteria corrected for samples of small size (i.e. AICc, Posada & Buckley 2004 and references therein) in JMODELTEST (Posada 2008). Relationships between *W. auropunctata* haplotypes were then constructed by Bayesian inference, with MRBAYES v3.1.2 (Ronquist & Huelsenbeck 2003). Three distinct partitioning strategies (PS) were used (unpartitioned analysis, one partition per cp, one partition for $1^{st}/2^{nd}$ cp + one partition for 3^{rd} cp). For each PS, two independent runs were carried out, for 20 million generations each, with eight Markov chains. The best PS was then identified by comparing Bayes' factors (Kass & Raftery 1995) and the corresponding tree was constructed with a conservative burn-in of 25%. Node support was estimated from posterior probabilities (i.e. the proportion of post burn-in trees recovering a particular clade; Ronquist & Huelsenbeck 2003).

More specifically, we evaluated the robustness of the affiliation of the Israeli haplotype to clade B (see results). We constrained the position of the Israeli haplotype by forcing it into the other clade (i.e. clade A), using the settings for the best PS. We then used

Bayes' factors to determine whether there was significantly less statistical support for the constrained analysis than for the unconstrained analysis.

Microsatellite data

In *W. auropunctata* clonal populations, the nuclear genomes of queens and males behave as two independent lineages (Fournier *et al.* 2005a; Foucaud *et al.* 2007). We therefore carried out microsatellite analyses on the two sexes separately. We added to the data set published by Foucaud *et al*. (2010) a new data set including the genotypes of 12 males and 33 queens from seven populations sampled from Argentina. A global data set of 453 male and 1093 queen genotypes was therefore analysed in this study. For sites from which no males were sampled, we analysed the spermatheca content of the queens, when possible (for details, see Fournier *et al.* 2005a). If the spermatheca was empty, we deduced the paternal genotype from workers in clonal populations. All individuals were genotyped at 12 microsatellite loci (Fournier *et al.* 2005b). Dendrograms were constructed from individual genotypes with the neighbour-joining algorithm (Saitou & Nei 1987) and using a variant of Chakraborty & Jin's allele shared distance (Chakraborty & Jin 1993; see Fournier *et al.* 2005a).

RESULTS

Cold tolerance

All estimates of the Gompertz function parameters fitted for the six biogeographic origins were significantly different from zero, indicating a good fit of this function to the pattern of worker recovery following cold stress over the period of observation (Figure 1A).

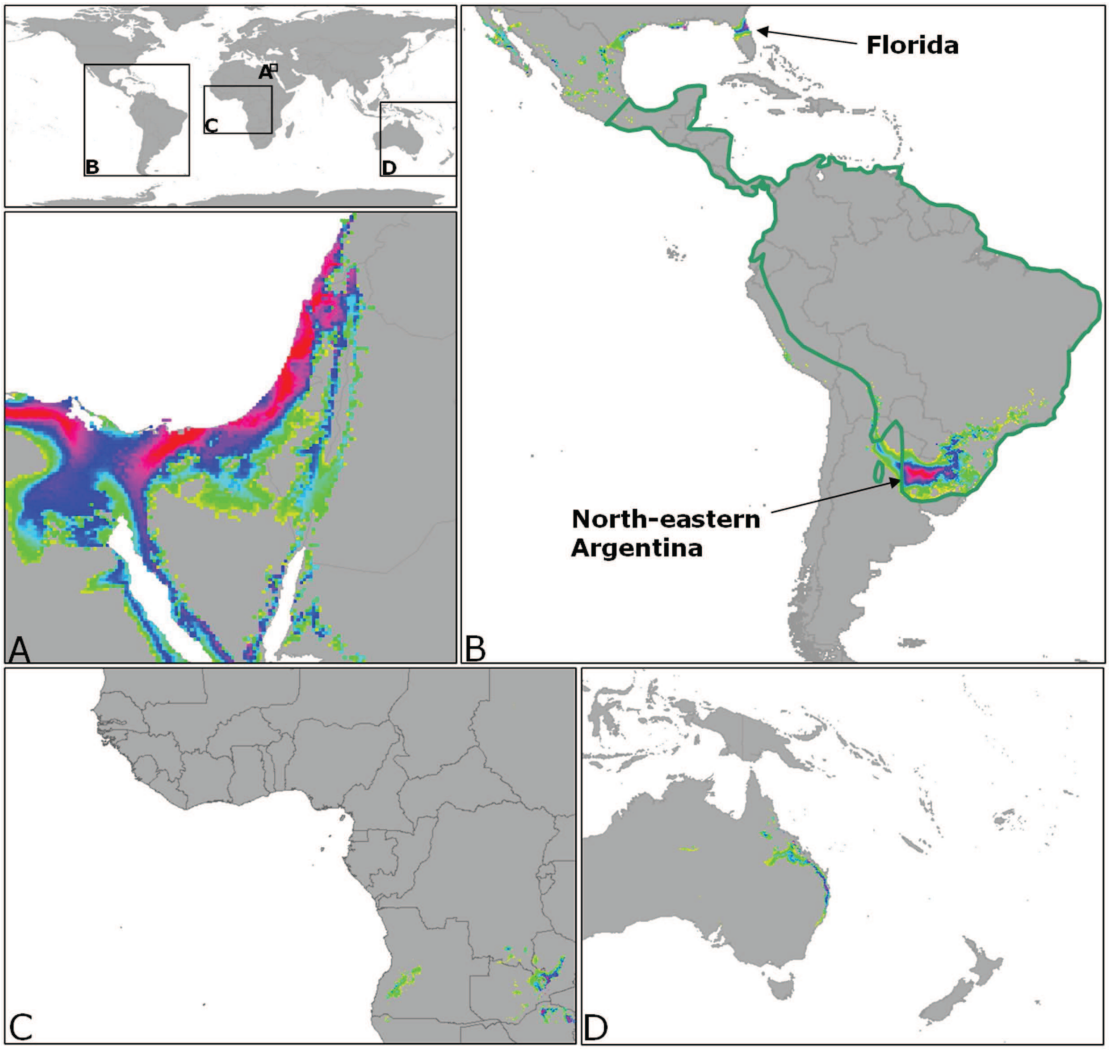

Figure 2: Species distribution modelling (SDM) results. Note: Box (A) show the SDM result in the region for which the model was fitted, Box (B), SDM results within the native range and surrounding invaded areas and Boxes (C-D), SDM results in regions remote from the native range in which the presence of invasive *W. auropunctata* populations has been reported. Yellow to red patches indicate the goodness of fit, with red patches corresponded to the highest level of agreement between the model and the observed data. The native range of *W. auropunctata*, according to Wetterer & Porter (2003), is surrounded by a green strip in (B). Results of model fitting for the tropical islands of Hawaii and Tahiti, where *W. auropunctata* is established, are not shown as these islands displayed no climatic similarities to Israel.

Workers from Israeli populations survived significantly better (*plateau* = 0.95,) and recovered faster (*d* = 35.67,) than workers from natural habitats (*plateau* = 0.15, ; *d* = 59.01,) and human-modified habitats (*plateau* = 0.35, ; *d* = 52.86,) in the native area, and than populations established in the tropical introduced area (*plateau* = 0.46, ; *d* = 74.95; Figure 1B). Workers from subtropical populations, Florida (*plateau* = 0.79, ; *d* = 62.83,) and Argentina (*plateau* = 0.91, ; *d* = 7.24,) also recovered better and faster than tropical populations. Workers from Israeli populations survived better and recovered faster than populations originating from Florida but did not do better than Argentinean populations.

Additional analyses based on GLMMs revealed similar trends and led to the same main conclusion (See Appendix S1).***Species distribution modelling***

The climatic envelope model defined on the basis of the occurrences of *W. auropunctata* nests in Israel accurately predicted the presence of *W. auropunctata* nests throughout this geographic region (Figure 2A; AUC=0.95). Our predictive analyses across the global distribution range of *W. auropunctata* highlighted a small number of regions with climatic conditions similar, in terms of coldness, to those of the locations at which *W. auropunctata* occurs in Israel. We identified the north-eastern region of Argentina as the largest geographical regions with similarities to the climatic envelope defined in Israel within the native range of this species and the surrounding regions (Figure 2B). In the introduced range, some regions of North America, Australia and Africa were also found to display locally similar cold temperatures (Figure 2 B-D). However, Florida was the only such region in which the presence of populations of *W. auropunctata* was reported.

Phylogeographic analyses

Our tree based, on the COI region of mtDNA, encompasses two main clades: clades A and B (Figure 3; see also Mikheyev & Mueller 2007). All the populations established in Israel were characterised by a unique haplotype branching off within clade B. Our data provide positive evidence that the alternative topology (i.e. Israeli haplotype constrained into clade A) was less supported than the best topology obtained (Bayes' factor = 5.1). The Israeli haplotype was rigorously identical to that found in clonal populations established in Zárate, Buenos Aires province (Argentina C5, Figure 3 and see map in Appendix SF1) and differed from the haplotype of the clonal lineage established in the city of Buenos Aires by only one nucleotide (Argentina C4, Appendix SF1). Clade B also contains all the Argentinian haplotypes sampled in this study, together with haplotypes from Paraguay and southern Brazil, all of which were obtained from populations located in the southern margin of the native range. The Florida haplotype was assigned to clade A, indicating a loose genetic relationship between the populations of Florida and Israel.

The individual nuclear microsatellite data obtained from both queens and males confirmed that Israeli populations were established from a single pair of queen and male genotypes reproducing clonally. These clonal genotypes were strictly identical to those found by Vonshak *et al.* (2009) in older samples collected in 2005. Both the queen and male genotypes from Israel were assigned to genetic clusters including only genotypes of individuals from Argentina (Figure 4). For queens, the closest Argentinian genotypes were those of individuals originating from populations established near the city of Buenos Aires and in the Ocloyas mountain region (C4, C5 and C3; Figure 4; see also Supplementary figure SF1). The Israeli clonal male lineage had a multilocus haploid genotype remarkably similar to that of clonal males from Argentinian populations established near Buenos Aires (C5 and C6; Figure 4). Consistent with phylogeographic results based on mtDNA analyses, the Israeli queen and male microsatellite genotypes were only distantly related to those of populations from Florida (Figure 4).

DISCUSSION

Adaptation of invasive populations to a new environment

Adaptation to local temperature conditions is particularly crucial for ectotherms, which have little or no capacity for regulating their body temperature. The invasion of *W. auropunctata,* from the tropical zone to the Mediterranean zone, is an excellent case study to investigate whether and where physiological adaptation occurs during invasion within a biogeographical region that strongly differs in climate from the core habitats of the native range.

Our results clearly indicated that workers from populations established in the Mediterranean zone are adapted to local cold temperatures compared to populations from the introduced or native range within the tropical zone. This adaptation may have made it possible for workers to continue foraging throughout the year, even in the harsher winter conditions of the Mediterranean climate of Israel than of the tropical zone of origin. This result echoes with other studies showing that evolutionary changes may facilitate invasion (e.g. Yeh 2004; Phillips 2006). Interestingly enough, most of the studies that investigated on adaptive changes during invasion of animals focused on life history traits such as growth, reproduction or dispersal and few focused on physiological traits (but see Lee & Gelembiuk 2008). Our results provide one of the few illustrations that adaptation on physiological traits, here tolerance to cold, may also improve the success of invasion of some populations.

When and where adaptation occur during invasion processes?

The use of phenotypic experiments is crucial to demonstrate that adaptation occurred but does not provide information about where evolutionary changes responsible for adaptation took place. Our phenotypic experiment conducted on tropical and Mediterranean populations could lead us to consider that adaptation occurred locally following introduction. This scenario is however unlikely in the case of *W. auropunctata* Israeli populations. This sce-

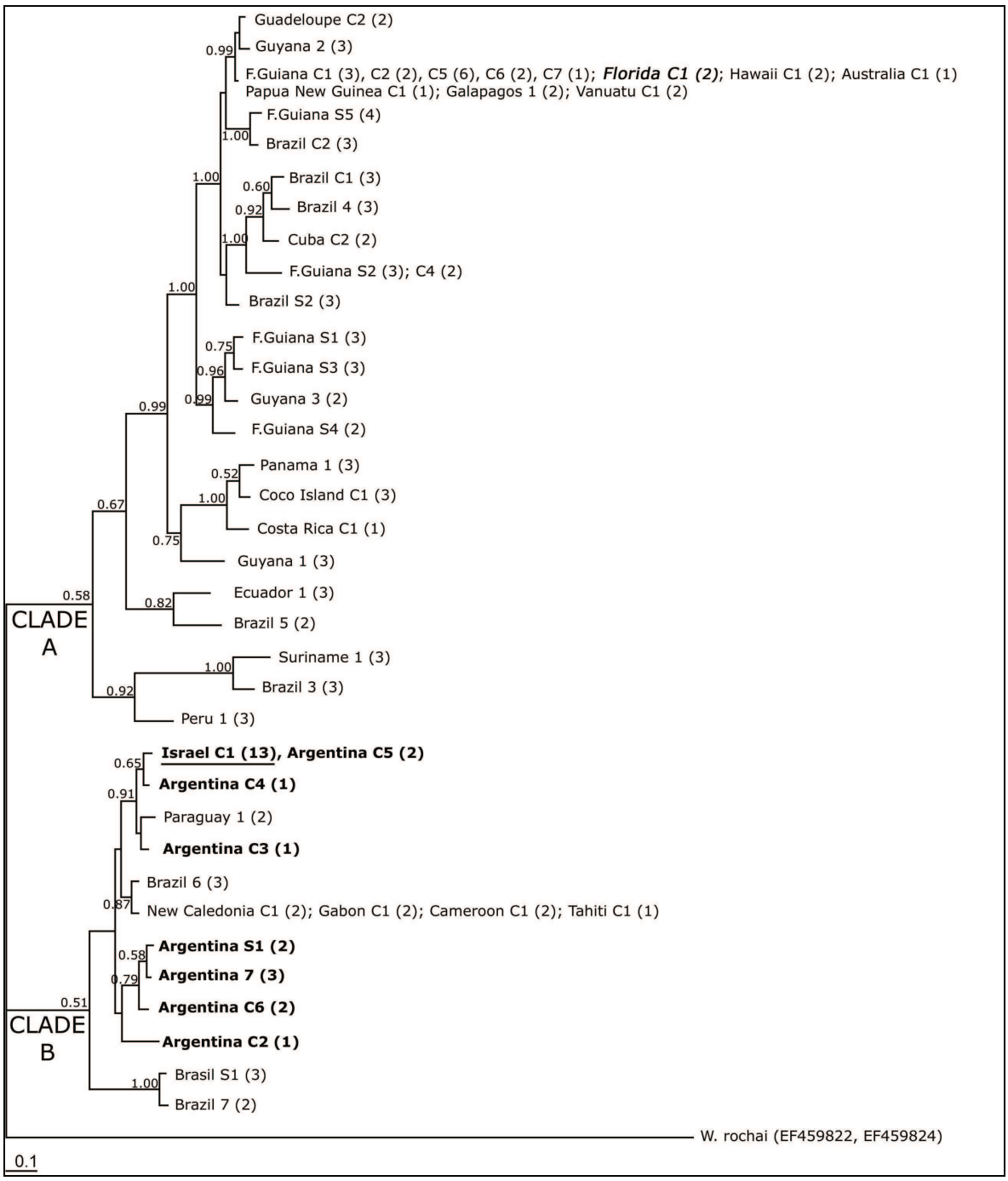

Figure 3: Consensus tree for the phylogenetic relationship between native and introduced populations.
Note: Posterior probabilities of nodes are shown for values > 50%. For each shared haplotype, the name of the sampled population is provided and the number of individuals sequenced per population is indicated in brackets. The Argentinian haplotypes are shown in bold characters, the Florida haplotype is shown in bold italic characters and the Israeli haplotype is shown in underlined bold characters. The closely related species *Wasmannia rochai* was used as an outgroup.

-nario requires the genetic diversity in the original propagule introduced to be sufficiently high for local adaptation to occur. Adaptive genetic variation is favoured by large size of the original propagule and/or by admixture events between genetically differentiated introduced populations (Facon *et al.* 2008). We found that the Israeli populations were established from an initial propagule including only a single pair of queen and male genotypes reproducing clonally and probably originating from the same or two genetically similar populations (see discussion below). Moreover, multilocus microsatellite genotypes of reproductive individuals from Israeli populations sampled in this study were strictly identical to those reported for populations sampled in this area five years previously by Vonshak *et al.* (2009). It seems therefore unlikely that cold adaptation occurred after transportation to Israel. The sole use of phenotypic experiments is thus not sufficient to pinpoint where cold adaptation most likely occurred.

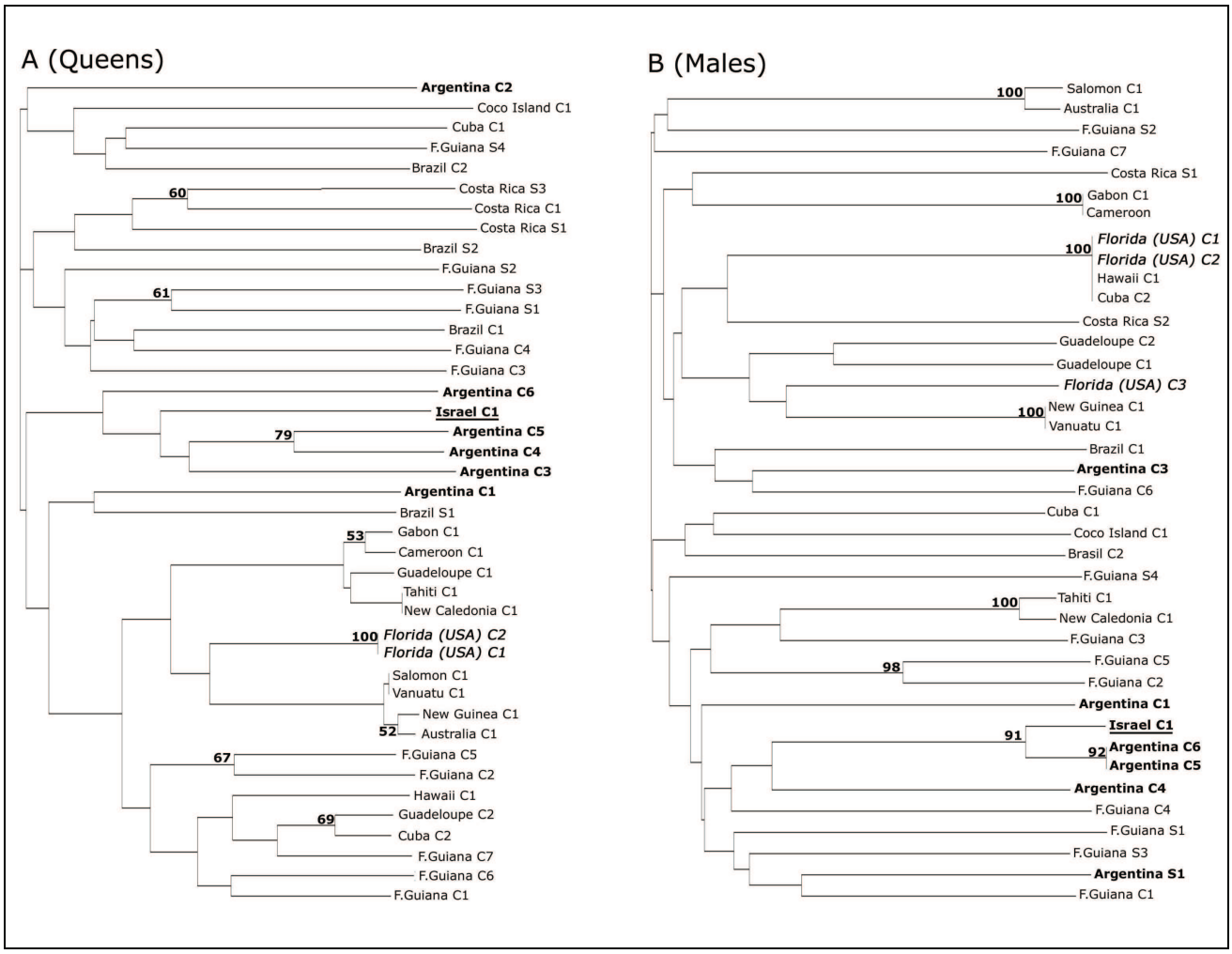

Figure 4: NJ dendrograms of the microsatellite allele shared distances between individual queens (A) and males (B) from populations sampled in the native and introduced ranges. Note: The Argentinian genotypes are shown in bold characters, the Florida genotypes are shown in bold italic characters and the Israeli genotype is shown in underlined bold characters. Dendrograms are not rooted, due to the lack of PCR amplification for many *W. auropunctata* microsatellite loci in the closely related species *W. rochai* used as an outgroup in figure 3. Bootstrap values (computed from 1,000 replicates) for nodes are shown only when > 50%. Note that low bootstrap values are usually obtained when individual trees are bootstrapped over microsatellite loci.

SDM approaches are useful to investigate geographical regions among the world where populations undergo similar selective pressure (i.e. similar climatic characteristic) than within the target introduced range (here Israel) and may hence provide an overview of candidate regions where adaptive changes may have occurred before the dispersal of populations in the invaded area. We found, using SDM, that population established in north-eastern Argentina, at the colder limit of the native distribution of the species, and in Florida (USA), in the introduced range, are likely to undergo similar cold selection pressure than populations established in Israel. At this stage, two main invasion scenarios are possible relative to the two geographical zones identified by the SDM approach.

First, adaptation may have occurred in populations established in north-eastern Argentina, before long-distance dispersal events directly to Israel, according to a two-step invasion process (Bossdorff *et al.* 2008; Lee & Gelembiuk 2008; Foucaud *et al.* 2010; Jenkins & Keller 2011). According to the *W. auropunctata* distribution map produced by Wetterer & Porter (2003), north-eastern Argentina is located at the southern and, hence, coldest edge of the native range of the species. The limits of the range of an organism are often viewed as zones of heterogeneous habitats, in which numerous evolutionary processes may occur (Kirkpatrick & Barton 1997; Thomas *et al.* 2001). Furthermore, the potential to evolve in response to new conditions is generally considered to be greater within or at the edge the native range than in the introduced range, due to the larger effective population sizes, genetic variation and propagule pressure (Hufbauer *et al.* in press). Finally, consistent with this hypothesis (i.e. adaptation to cold in Argentina), we found that workers from the Argentinean populations survived as well as workers from Israeli populations, to the same cold stress experiment.

In the second scenario, the Israeli lineage may stem from a successful invasive population already adapted to cold temperatures in Florida. Under this so called

bridgehead scenario (Kolbe *et al*. 2004; Floerl *et al.* 2009; Lombaert *et al.* 2010), adaptation could have occurred after the introduction of some tropical native populations within Florida. In agreement with this, *W. auropunctata* was first reported in Florida in 1924 (Wheeler 1929), long before the Israeli population has been introduced. Many evolutionary processes may have occurred over this period, enabling the Florida populations to adapt to cold (e.g. admixture, Facon *et al.* 2008). Furthermore, consistent with this hypothesis, we found that workers from populations sampled in Florida tolerated cold stress better than workers from other tropical populations (native or introduced). Alternatively, populations established in Florida may originate from an Argentinean population pre-adapted to cold temperatures and could have then been moved and successfully established in Israel in a second time. In this case, adaptation to cold might have occurred only once in Argentina. A similar invasion scenario was recently proposed for the fire ant *Solenopsis invicta,* which originated from Argentina, and that have invaded Florida before invading more distant territories (e.g. New Zealand, China; Ascunce *et al.* 2011). Yet, Ascunce *et al.* (2011) did not assess whether and where adaptive changes occurred during the invasion process.

Our phylogeographic survey allowed us to distinguish between the above invasion scenarios. We found that the Israeli lineage was strongly related to populations established in the southern part of the native range of the species and in particular to populations located near Buenos Aires (Argentina). By contrast, the Israeli mtDNA haplotype as well as the microsatellite multilocus genotypes were found to be only loosely related to those of the Florida population samples. These results indicate that, the two bridgehead scenarios involving populations from Florida in the invasive success of Israeli populations should be ruled out and that the Israeli lineage directly originates from Argentina, where prior adaptation to cold most likely occurred.

Altogether, our multidisciplinary results support the view that the adaption to cold of Israeli populations occurred at the southern limit of the native range of the species before long-distance dispersal to Israel. However, there is a slight spatial incongruence between the phylogeographic identification of the origin of the Israeli lineage (i.e. the city of Buenos Aires) and the region at the edge of the native range found to display cold temperatures similar to those found in Israel using SDM (i.e. north-eastern Argentina). This slight discrepancy might come from the fact that the actual source population of the Israeli lineage may be established further north than Buenos Aires, in the climatic regions identified using SDM, but not sampled in this study. Alternatively, the Israeli lineage may actually originate from populations located in the region of Buenos Aires characterised by temperatures colder than those found in Israel (a climatic feature confirmed by various standard bioclimatic databases; results not shown). In this latter case workers from populations established close to Buenos Aires and, hence, also those from Israeli populations would be expected to tolerate colder temperatures than those used in our experimental design. Consistent with this hypothesis, we found that although the cold temperature conditions tested in our laboratory experiment reflected the extremes of temperature found in Tel-Aviv (Israel), many, if not all workers from the Israeli populations recovered rapidly from cold stress. Moreover, preliminary results indicate that workers from Argentinean populations collected around Buenos Aires were able to survive to negative temperatures (L.C.A and L.C unpublished data). The notion that the Israeli populations may have originated from Buenos Aires is also supported by the important commercial ports in this city, which might have facilitated the long distance dispersal of *W. auropunctata*.

In conclusion, our integrative multidisciplinary study is, to our knowledge, the first to unambiguously identifies the biogeographic origin of evolutionary changes undergone by an invasive population established in an ecological niche that on average greatly differs from the core habitat within the native range. The eco-evolutionary scenario of invasion of Israeli population illustrates a "two-step" invasion scenario. Our study highlights the need for studies focusing on populations established in marginal habitats and areas surrounding the native range of the species, with a view to identifying populations capable of invading remote areas characterised by similar environmental conditions.

We believe that our integrative multidisciplinary approach could be successfully apply to other invasive species to identify the origin of adaptive changes during invasion. Yet some limitations may weaken such approach when applied to at least some species. In particular, biotic interactions may also play an important role in the establishment and spread of invasive populations (Kennedy *et al.* 2002). The approach used in this study does not account for this type of factors although integrating georeferenced biotic data could be considered. On a more practical view, a large number of live populations are required for phenotypic studies in the laboratory. Because the transfer of live invasive species is under increasingly stronger legislation constraints, it becomes more and more difficult to conduct phenotypic studies analyses on such populations simultaneously.

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SUPPORTING INFORMATION:
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Appendix S1: Details and results of statistical analyses based on general linear mixed models using both the proportion of surviving workers and time to reach half the maximal proportion of recovered workers after cold stress as response variables.

Analytical methods:

We assessed the effect of the biogeographic origin of each population on the maximal proportion of surviving workers observed throughout the experiment and the time to reach half the maximal proportion of surviving workers. The maximal proportion of surviving workers in populations was determined regardless of the time at which this maximum was reached. The biogeographic origin of each population was included as a categorical fixed factor (six levels: native area in natural habitats, native area in human-modified habitats, area of introduction in tropical zone, Florida in subtropical zone, Argentina in subtropical zone and area of introduction in the Mediterranean zone), whereas run number and population locality (nested in the "biogeographic origin" factor) were considered as random factors. For the maximal proportion of surviving workers, we used a general linear mixed model to consider binomial error terms and Laplace approximation to estimate parameters. The significance of the fixed factor was assessed in a likelihood ratio test. We used generalized linear mixed models (using GLMER in R) to assess the effect of the origin of each population on the time to reach half the maximal proportion of surviving workers. Gaussian error terms were considered and we used restricted maximum likelihood approximation (REML) to estimate parameters. The significance of the fixed factor was assessed in a likelihood ratio test. We then compared the time taken to reach half the maximal proportion of surviving workers for each origin of populations in Student's *t* tests.

Results

Maximal proportion of surviving workers after cold shock

The table below indicates the mean proportions of surviving workers from populations of different biogeographic origins after cold shock at 2°C for 16 hours and the results of the general linear mixed model for populations from the introduced area in the Mediterranean region (Israel) as the baseline level.

Biogeographic origin	**Mean**	**Estimate**	**Std. Error**	**Z Value**	**P-Value**
Introduced area in the Mediterranean zone	0.98	-0.01799	0.10226	-0.176	0.86034
Native area, in natural habitats	0.33	-1.13936	0.18707	-6.091	1.13×10^{-9}
Native area, in human-modified habitats	0.57	-0.56064	0.16441	-3.41	6.50×10^{-4}
Introduced area in the tropical zone	0.56	-0.57968	0.18799	-3.084	2.05×10^{-3}
Florida in subtropical zone	0.96	-0.02297	0.20388	-0.113	0.91
Argentina in subtropical zone	0.88	-0.11157	0.23117	-0.483	0.63

Time to reach half the maximal proportion of surviving workers after cold stress

Our comparison of models revealed that the biogeographic origin of populations had a significant effect on worker survival (p-value = 5.15×10^{-4}). Time to reach half the maximal proportion of surviving workers was significantly shorter for Israeli populations (mean = 42.60) than for tropical introduced populations (mean = 111.73, p-value = 1.57×10^{-6}) or populations originating from the native area, whatever the type of habitat (human-modified habitat: mean = 86.87, p-value = 1.82×10^{-4} or natural habitat: mean = 117.5, p-value = 3.80×10^{-5}). Workers from populations established in Florida (mean = 81.0, p-value = 7.54×10^{-5}) recovered also slower than workers from Israeli populations. It seems that workers from Argentina recovered faster (mean = 14.49) than all populations tested in this study. However, this result must be interpreted carefully as these Argentinean populations were tested independently at the SABCL laboratory (Buenos Aires, Argentina) and this measure (i.e. **t**ime to reach half the maximal proportion of surviving workers after cold stress) is sensible to how workers were considered by observers as recovered.

Appendix S2: Details on the amplification and sequencing protocols of the mitochondrial DNA of cytochrome oxydase (*COI*) sequences.

LCO and HCO universal primers (Folmer *et al.* 1994) were used for 97 individual DNA to PCR amplify a 710 bp fragment. However, we did not obtain any PCR product with this set of primer for 39 individuals from several localities (i.e. French Guiana, Guiana, Peru, Brazil, Panama, Surinam, Ecuador, Paraguay, Dominican Republic, Costa Rica and Coco Island). For these individuals, we combined the LCO primer with a new designed internal Reverse primer called newCOI-R (TGY-TGG-TAT-AAA-ATA-GGG-TCT-C) to amplify a 700 pb DNA fragment. For both sets of primers we used the same PCR mixtures as in Foucaud *et al.* (2007). Thermal cycling conditions were as follows: denaturation at 95 °C for 3 min, then 37 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C (48°C for LCO/newCOI-R primer set) for 1 min, and extension at 74 °C for 1 min, followed by a final extension at 74 °C for 10 min. PCR products were purified and sequenced on an ABI 3730 DNA sequencer (Applied Biosystems). Individual electropherograms were checked for eventual errors using Seqscape software (Applied Biosystems).

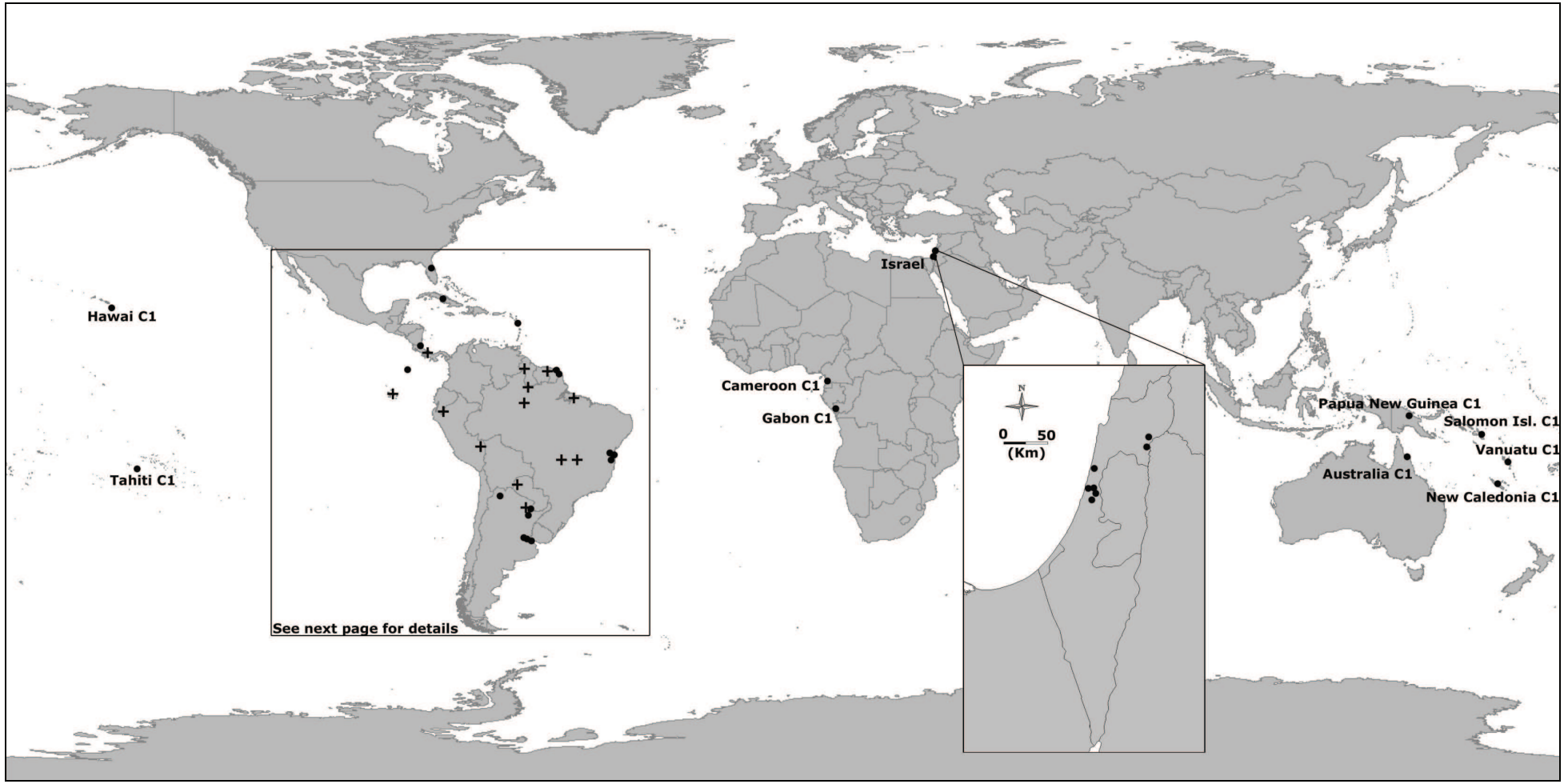

Figure SF1: Map of sites sampled for *W. auropunctata* in the phylogeographic analyses. Black dots are sampled populations for which both mtDNA and microsatellites were analysed. Black crosses correspond to population samples used for mtDNA analyses only.

Figure SF1: Continued

CHAPITRE IV

DISCUSSION GÉNÉRALE

Les résultats obtenus au cours de cette thèse nous ont permis d'acquérir une meilleure connaissance des changements évolutifs associés au succès d'invasion des populations. Bien que nos résultats concernent principalement notre modèle d'étude *Wasmannia auropunctata*, ceux-ci ont cependant une portée plus générale en biologie des invasions et en évolution des systèmes de reproduction.

I. Les facteurs évolutifs associés au succès d'invasion de *W. auropunctata*

I.1. Le rôle du système de reproduction

Foucaud *et al.* (2009) ont suggéré que le succès d'invasion des populations de *W. auropunctata* est indirectement associé au système de reproduction asexué original qui les caractérise. Les résultats obtenus au cours de cette thèse ont permis d'identifier les mécanismes de ce système de reproduction et permettent de formuler des hypothèses robustes sur son rôle dans le succès d'invasion de ces populations.

En général, on considère que les organismes asexués ont un potentiel d'invasion supérieur à leurs congénères sexués du fait de leur croissance démographique plus rapide (i.e. pas de coût de deux) et de leur capacité à éviter les effets liés aux populations de petites tailles (i.e. assurance de reproduction et évitement de la dépression de la consanguinité) lors de l'établissement et de la propagation dans le milieu colonisé. Du fait de l'absence de recombinaison, l'asexualité permet également de conserver des combinaisons d'allèles coadaptés dans le temps. Ces populations souffrent cependant d'un potentiel adaptatif limité et accumulent des mutations délétères. Elles sont de ce fait vouées à une extinction plus ou moins rapide, en fonction de leur fond génétique, même dans des environnements stables.

L'asexualité pratiquée par les reines des populations clonales de *W. auropunctata* ne suit pas les mêmes règles que l'asexualité classique. Ces reines utilisent de manière conditionnelle la parthénogenèse (i.e. asexualité) pour la production de la descendance reproductive femelle (i.e. reines), l'androgenèse pour la production des mâles et la sexualité pour la production d'ouvrières stériles. Les avantages que procurent ce système de reproduction ne sont pas d'ordre démographique. En effet, ce type d'asexualité implique un coût associé à la production de mâles indispensables au maintien des populations. Les reproducteurs ne bénéficient *a priori* pas non plus de l'assurance de reproduction, bien que

dans ces populations clonales, l'accouplement a certainement lieu au sein du nid entre individus issus de la même cohorte, ce qui réduit considérablement le coût associé à la recherche de partenaires sexuels (cf. absence de vols nuptiaux; Ulloa-Chacon 1990). D'autre part, l'émergence de la parthénogenèse femelle a suivi le scénario de transition «le plus simple» entre la sexualité et l'asexualité dans la mesure où celui-ci conserve les mécanismes de méiose (i.e. automixie; Schwander & Crespi 2009). Cela explique certainement en partie pourquoi les reines parthénogénétiques conservent la capacité à utiliser la reproduction sexuée (i.e. production d'oocytes haploïdes) pour la production d'ouvrières. L'automixie implique cependant une perte, au moins partielle, de l'hétérozygotie dans la descendance parthénogénétique du fait de la recombinaison méiotique, ce qui entraine potentiellement l'expression d'allèles délétères à l'état homozygote (mécanisme semblable à la dépression de consanguinité). Les reines parthénogénétiques de *W. auropunctata* évitent cependant en partie ce coût grâce à un mécanisme de réduction du taux de recombinaison méiotique qui réduit considérablement le taux de transition à l'état homozygote (Article 2). Ce mécanisme permet également de conserver à plus long terme des combinaisons d'allèles coadaptés au sein du génome reine.

L'avantage que procure ce système de reproduction par rapport à l'asexualité classique, réside dans la capacité des reines parthénogénétiques à conserver la reproduction sexuée pour la production d'ouvrières et à produire des mâles par androgenèse. L'androgenèse permet à une même lignée parthénogénétique femelle (i.e. génotype reine) de s'associer à plusieurs génotypes mâles différents (Article 3). Dans le cadre des invasions biologiques, et contrairement aux lignées asexuées «classiques», un même génotype femelle peut ainsi bénéficier d'apports de gènes issus d'autres propagules ce qui peut engendrer, au moins temporairement, une augmentation de diversité génétique dans la descendance ouvrière produite sexuellement. Cette caractéristique est donc susceptible d'augmenter le potentiel adaptatif d'une même lignée parthénogénétique femelle. Cet apport de gènes issus d'autres populations peut également conduire à d'éventuels effets de vigueur hybride dans la descendance ouvrière. Certains résultats empiriques *in natura* semblent d'ailleurs indiquer que des événements de kidnapping «multiples» de génomes mâles ont eu lieu au cours du processus d'invasion dans cette région. En effet, des études phylogénétiques basées sur des marqueurs microsatellites montrent que certaines populations introduites dans des localités différentes en Océanie (i.e. Australie, Iles Salomon, Vanuatu et Nouvelle Guinée) sont caractérisées par des génotypes reines identiques mais par des génotypes paternels différents

(Foucaud *et al.* 2010). Le fait qu'il n'y ait qu'un couple de génotypes parentaux dans chaque localité peut résulter de la perte de l'un des génotypes paternels par dérive. Cela peut également résulter de la sélection ayant fixé un couple de génotypes parentaux particuliers indirectement via la performance des ouvrières. Il semble cependant que les événements d'introgression de gènes issus de nouveaux migrants mâles dans les populations de l'aire d'introduction soient rares, ces populations étant majoritairement caractérisées par un seul génotype paternel.

Le système de reproduction clonal de *W. auropunctata* permet également de conserver dans le temps une combinaison de deux génotypes parentaux. Cette caractéristique confère deux avantages principaux. Premièrement, il permet de conserver des combinaisons de gènes coadaptés portés par des mêmes chromosomes dans la descendance ouvrière produite sexuellement dans le temps. Deuxièmement, les génotypes parentaux étant fixés d'une génération à l'autre, les individus reproducteurs d'une même cohorte peuvent se reproduire entre eux sans que la descendance ouvrière produite sexuellement ne souffre d'effets liés à la dépression de consanguinité. Ainsi, une propagule constituée d'une seule reine fécondée suffit théoriquement à engendrer une nouvelle population clonale dans un milieu nouvellement colonisé. Il serait intéressant de quantifier l'intensité des effets de la dépression de consanguinité dans cette espèce, afin d'apprécier le bénéfice d'un tel système de reproduction. Cela pourrait être testé par des expériences contrôlées en laboratoire sur des populations sexuées, en forçant des individus apparentés à se reproduire. Cependant, la manipulation en laboratoire de cette espèce est délicate et il est notamment particulièrement difficile de maintenir des lignées sexuées plus d'un an en laboratoire. Il est intéressant de noter que cette difficulté d'élevage ne concerne pas les populations clonales. Il est possible que cela reflète un effet d'évitement de la reproduction entre individus trop apparentés dans les populations sexuées, ce qui se traduit par l'extinction de la population.

I.2. Adaptation aux conditions environnementales des localités envahies

Le rôle de l'évolution dans le succès d'invasion est aujourd'hui reconnu et illustré par de nombreuses études empiriques (e.g. Phillips *et al.* 2006; Yeh 2004). La plupart des études effectuées sur des organismes du règne animal ont cependant porté sur des traits d'histoire de vie relatifs aux propriétés intrinsèques des populations (e.g. dispersion, croissance, fécondité), mesurés dans des conditions de laboratoire optimales, sans tenir compte des spécificités des

environnements natifs et de l'aire d'introduction. Dans notre étude, nous avons étudié des changements de traits d'histoire de vie en réponse à des conditions environnementales caractéristiques des nouveaux milieux dans lesquels les populations envahissantes s'établissent.

Nos résultats indiquent que le succès d'invasion des populations clonales établies dans des localités de la ceinture tropicale (Article 3) ou dans la région Méditerranéenne (Article III-2), est associé à des changements adaptatifs permettant aux ouvrières de tolérer des températures extrêmes par rapport à celles caractérisant l'habitat ancestral de l'espèce (i.e. forêts tropicales primaires). Les changements adaptatifs sur des traits physiologiques relatifs aux températures locales sont cruciaux chez les insectes qui ne sont pas (ou peu) capables de réguler leur température interne. Chez les insectes sociaux en particulier, et notamment chez les fourmis, le maintien d'une colonie est directement associé à la capacité des ouvrières à récupérer efficacement des ressources dans le milieu environnant (Crozier & Pamilo 1996). La mortalité est élevée lors de la recherche de ressources (e.g. Schmid-Hempel & Schmid-Hempel 1984) et une adaptation aux conditions environnementales peut permettre de limiter la perte d'ouvrières. Dans le cas de *W. auropunctata*, ces changements adaptatifs permettent aux ouvrières d'être actives à l'extérieur du nid à n'importe quel moment de la journée et de l'année. A cet égard, les ouvrières des populations établies en Israël semblent très actives même pendant l'hiver (Vonshak *et al.* 2009). Cette capacité des ouvrières à rester actives indépendamment des conditions environnementales confère un avantage certain pour s'approprier et exploiter une ressource face aux espèces dont les ouvrières ne sortent du nid que pendant certaines heures de la journée, comme c'est le cas pour de nombreuses espèces de fourmis Méditerranéennes (Cros *et al.* 1997)

Chez les fourmis, plusieurs stratégies d'adaptation face aux conditions environnementales ont été décrites. Celles-ci impliquent des changements de comportement (Cros *et al.* 1997), des changements physiologiques permettant la réduction de la transpiration cuticulaire, des changements de métabolisme (Cerda & Retana 2000) ou encore des changements morphologiques (Clémencet *et al.* 2010). Dans le cas des populations de *W. auropunctata* établies dans des localités de la ceinture tropicale, nous n'avons trouvé aucune différence significative entre la taille des ouvrières des populations de forêts et de celles des milieux anthropisés de l'aire native ou de l'aire d'introduction (Article 4). Des études complémentaires sont nécessaires pour identifier les mécanismes associés à la tolérance des

ouvrières des populations envahissantes tropicales de *W. auropunctata*, à des températures élevées. Dans le cas des populations établies en Israël, des mesures morphologiques ont également été effectuées sur les ouvrières des populations d'Israël, et indiquent que ces dernières sont en moyenne plus grandes que des ouvrières des populations tropicales natives ou introduites[1] (Figure IV.1.). La plus grande taille des ouvrières de ces populations pourrait être associée à leur capacité à tolérer des températures basses. Cependant, cette association ne peut pas être vérifiée statistiquement sur des données populationnelles. Cela pourrait être vérifié en comparant des mesures morphologiques au sein d'une même population entre des ouvrières tolérantes et intolérantes au choc thermique.

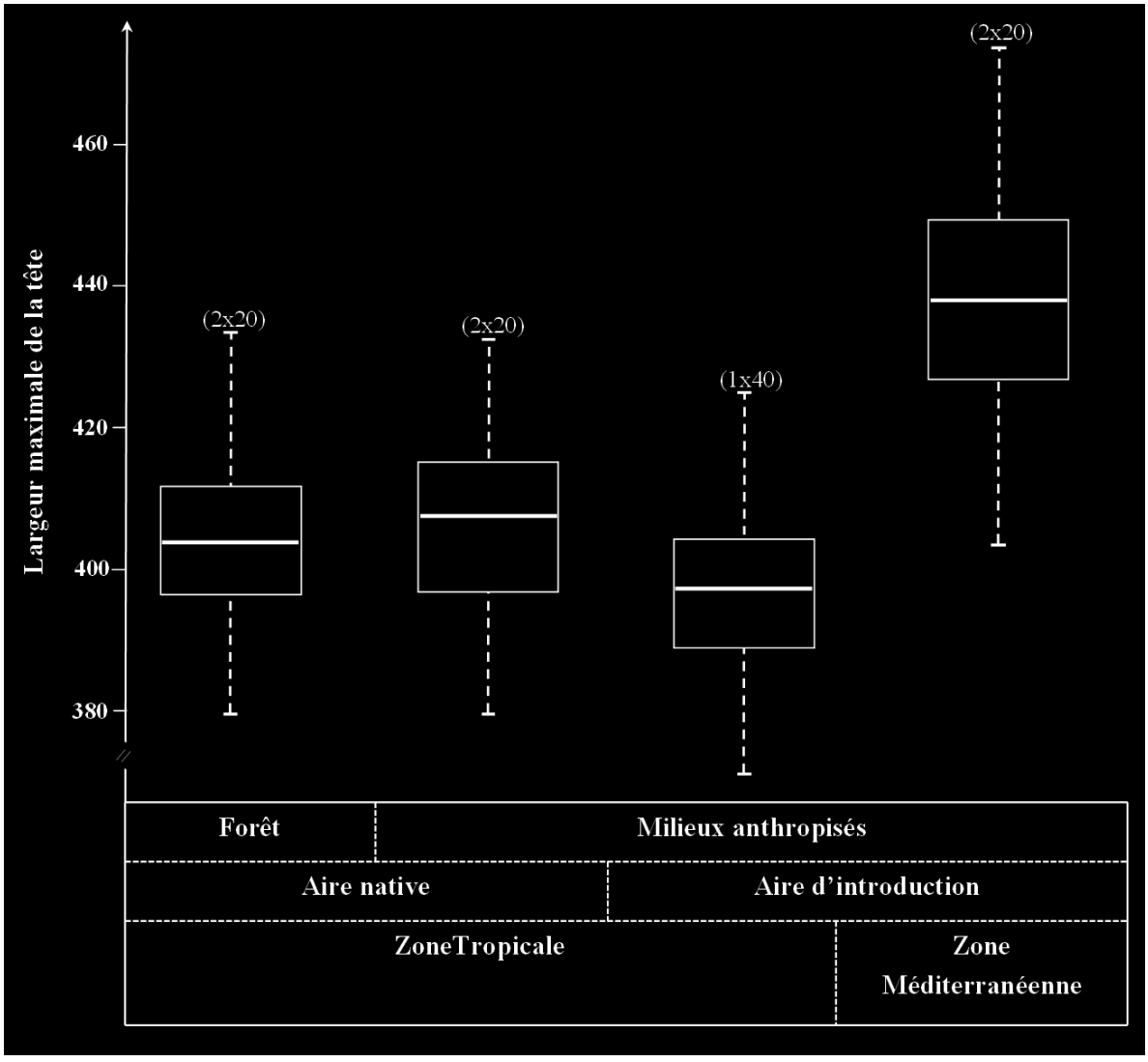

Figure IV.1. Largeur maximale moyenne de la tête d'ouvrières issues de populations établies dans quatre types d'habitats différents. Cette mesure est un bon proxy de la taille totale des individus. Le nombre de populations par habitat et le nombre d'ouvrières mesurées par population est indiqué entre parenthèses.

[1] Cette étude a fait l'objet d'un stage de master 1 visant à étudier des différences entre les populations sexuées et les populations clonales sur d'autres traits d'histoire de vie, afin d'identifier d'éventuels compromis adaptatifs dans les populations clonales. Ce stage à été réalisé par Paul Courtoisier sous la direction de Benoit Facon et de moi-même en 2010.

I.3. Les phénotypes invasifs sont-ils généralistes ?

Les changements adaptatifs permettant aux ouvrières de tolérer des températures particulières ont été étudiés indépendamment et pour des conditions de température fixées: au chaud pour les populations tropicales (Article 4), et au froid pour les populations Méditerranéennes (Article 5). Cependant, les ouvrières des populations envahissantes, qu'elles soient établies en zone tropicale ou Méditerranéenne, sont confrontées à des amplitudes thermiques plus importantes que celles subies par les ouvrières des populations établies en forêt primaire. Des études théoriques suggèrent qu'en réponse à des conditions environnementales fluctuantes «rapides» (i.e. à l'échelle de la durée de vie d'un organisme), l'évolution tend à favoriser des génotypes généralistes permettant aux individus de tolérer des larges gammes environnementales par rapport aux individus des populations ancestrales (Slatkin & Land 1976; Fierst 2011). Ce changement évolutif se traduit par une valeur sélective des génotypes généralistes plus élevée que celle des génotypes ancestraux pour des conditions environnementales qui s'éloignent des conditions optimales (i.e. conditions stressantes). On considère généralement que cette augmentation de tolérance de gamme environnementale est associée à un compromis évolutif qui résulte en une réduction de la valeur sélective des génotypes généralistes dans les conditions optimales. En ce qui concerne *W. auropunctata*, les populations clonales envahissantes établies dans les milieux anthropisés de la zone tropicale sont confrontées à des régimes de fluctuation de températures quotidiennes plus marquées que les populations établies en forêts (Orivel *et al.* 2009). Il est donc attendu que la sélection fixe des combinaisons de génotypes parentaux clonaux de type généralistes, qui produisent des ouvrières capables de mieux tolérer des conditions environnementales stressantes. Par ailleurs, les populations d'Israël sont susceptibles de faire face à des fluctuations quotidiennes plus importantes encore que les populations tropicales et également à des fluctuations saisonnières qui s'étendent sur un laps de temps supérieur à la durée de vie des ouvrières. Ainsi, alors que les ouvrières subissent les amplitudes thermiques diurnes, les reines vivent le temps de plusieurs saisons. La sélection agit cependant sur les génotypes parentaux, en grande partie indirectement sur la descendance (stérile) ouvrière produite sexuellement (Crozier & Pamilo 1996). Il est de ce fait attendu que les changements adaptatifs dans ces populations Méditerranéennes soient également en accord avec une stratégie généraliste, et que les ouvrières soient plus tolérantes aux conditions environnementales stressantes, que les ouvrières des populations tropicales.

Nous avons compilé l'ensemble des données de thermo-tolérance récoltées au cours de cette thèse (i.e. données des pré-manips, des expériences en laboratoire et des témoins utilisés au cours des expériences) et reporté graphiquement la survie des ouvrières issues de populations établies dans quatre types d'habitats (i.e. Natif tropical en forêt, Natif tropical en milieu anthropisé, introduit tropical en milieu anthropisé et introduit méditerranéen en milieu anthropisé) pour trois températures : Une température optimale (i.e. condition de maintien des populations en laboratoire : 25°C) et deux températures stressantes, une basse (i.e. 2°C) et une haute (i.e. 40°C). Ce graphique indique que les populations envahissantes ont évolué selon une stratégie généraliste leur permettant d'être plus tolérantes face à des températures stressantes que les populations natives occupant les forêts primaires (Figure IV.2.). Comme attendu, les populations établies en Israël semblent mieux tolérer l'ensemble de la gamme de températures que les populations introduites dans la région tropicale. Il est intéressant de noter que nos données n'ont pas mis en évidence de compromis évolutif des génotypes clonaux généralistes et sont de fait en accord avec le «*general purpose genotype* » proposé par Backer (1965), lequel présente une valeur sélective supérieure au génotype ancestral quelques soient les conditions environnementales.

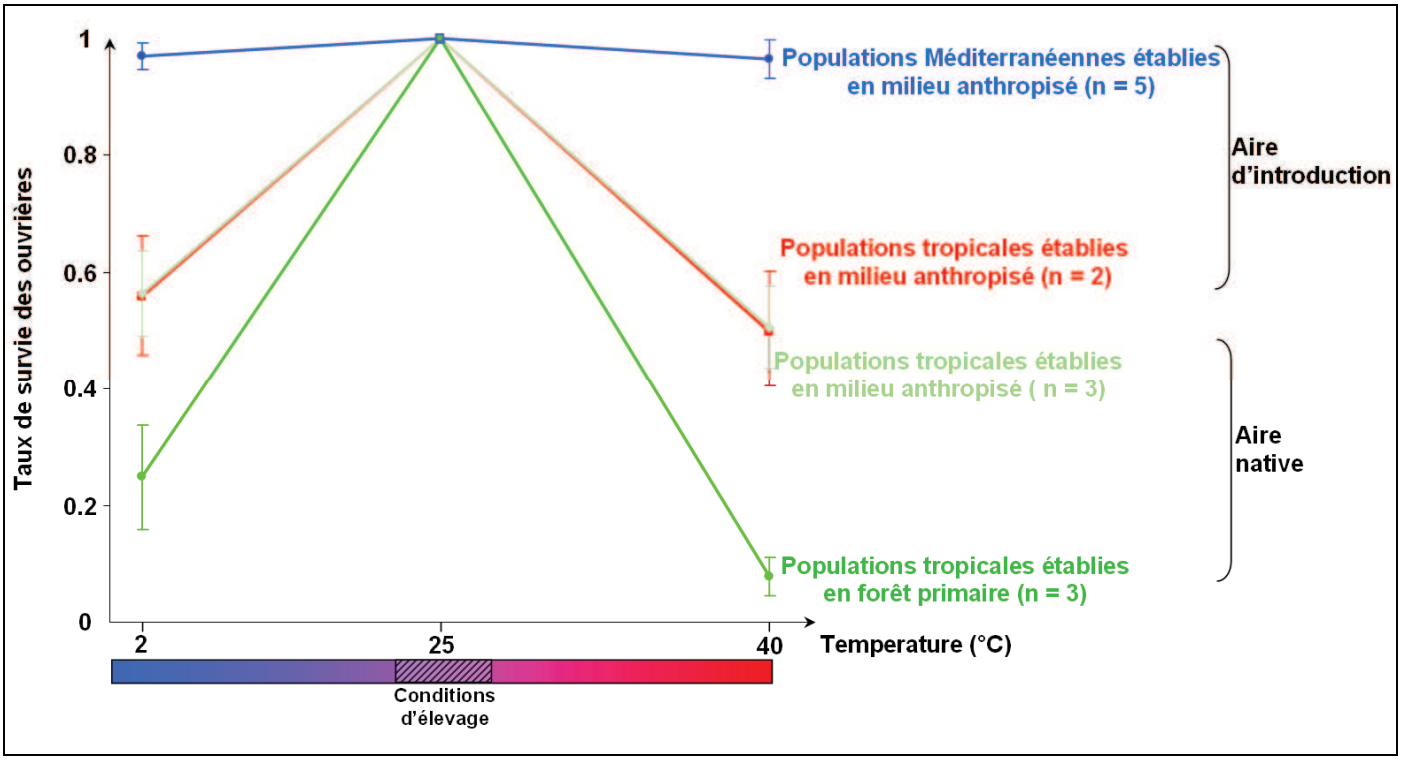

Figure IV.2. Taux de survie des ouvrières issues de populations établies dans quatre types d'habitats différents, mesurés pour trois conditions de températures 2°C, 25°C et 40°C. Le nombre de populations testées dans chaque type d'habitat et indiqué entre parenthèses. Les symboles correspondent aux moyennes du taux de survie des ouvrières pour chaque habitat et les barres verticales représentent les intervalles de confiance à 95%.

I.4. Perte d'un pathogène et succès d'invasion : quid de *Wolbachia* ?

Notre étude sur l'occurrence et le rôle éventuel de bactéries endosymbiontiques dans le polymorphisme du système de reproduction des populations de *W. auropunctata* indique que seule *Wolbachia* est présente. Nous avons mis en évidence que les populations sexuées sont très majoritairement infectées alors que les populations clonales sont très majoritairement saines, ceci indépendamment du fait qu'elles soient établies dans l'aire native ou dans l'aire d'introduction (Article 1). Cette bactérie est surtout connue en tant que parasite de reproduction. Etant absente dans la plupart des populations clonales, *Wolbachia* n'est certainement pas impliquée dans le système de reproduction asexuée de *W. auropunctata*. L'une des hypothèses permettant d'expliquer le patron d'infection observé, est liée au changement d'habitat que connaissent les populations clonales. En effet, les populations clonales sont établies dans des habitats marginaux caractérisés par des températures élevées (Orivel *et al.* 2009). Or ces bactéries peuvent être perdues par leurs hôtes en milieu contrôlé lorsque ces hôtes sont élevés à des hautes températures (Wright & Wong 1980; Van Opijnen & Breeuwer 1999). Il est donc possible que les populations clonales de *W. auropunctata* puissent bénéficier d'un traitement thermique naturel qui pourrait éliminer cette bactérie. Dans le cas où *Wolbachia* induirait un coût aux populations infectées, comme c'est le cas chez la fourmi *F. trucatum* (Wenseelers *et al.* 2002), la perte de cette bactérie dans les populations clonales pourrait également contribuer au succès d'invasion de ces populations sous un effet de relâchement de la pression biotique (i.e. enemy release; Torchin *et al.* 2003). Cette hypothèse est extrêmement difficile à tester. La façon la plus directe pour tester l'effet de *Wolbachia* sur ses hôtes, est de comparer au sein d'une même lignée des individus infectés et des individus dans lesquels les bactéries ont été éliminées, soit par traitement antibiotique soit par traitement thermique. Cependant ces études demandent généralement de tels traitements sur plusieurs générations. Du fait de la difficulté à maintenir des populations de *W. auropunctata* en laboratoire et du temps de génération élevé chez cette espèce, il est difficile d'envisager de telles expériences.

I.5. Conclusion

Le succès d'invasion des populations de *W. auropunctata* semble s'expliquer, au moins en partie, par des changements évolutifs qui impliquent un changement du mode de reproduction de l'asexualité vers un système asexué particulier ainsi que par des changements

adaptatifs qui permettent aux ouvrières des populations envahissantes d'être plus tolérantes à des températures stressantes que les ouvrières des populations ancestrales sexuées. L'association entre le système de reproduction clonal et la tolérance des ouvrières est encore mal définie. Il est cependant possible que la clonalité femelle et/ou mâle participe au maintien d'une architecture génétique particulière dans la descendance ouvrière produite sexuellement responsable de la tolérance des ouvrières. Il est intéressant de noter que bien que le système de reproduction clonal de *W. auropunctata* suit un schéma très différent de l'asexualité classique (i.e. conservation de la reproduction sexuée pour la production des ouvrières stériles), la combinaison des génotypes clonaux parentaux permettant la production d'une descendance ouvrière tolérante à des conditions environnementales stressantes, peut être considérée comme une combinaison du type «*general purpose genotype*» (Backer 1965). Cette caractéristique, commune aux systèmes de reproduction asexuée quels que soient les mécanismes sous-jacents, semble donc être un trait particulièrement important pour le maintien des lignées asexuées. D'autre part, ce type d'asexualité permet également de profiter d'avantages normalement attribués uniquement à la sexualité en évitant les inconvénients qui lui sont associés. En effet, les reines des populations clonales de *W. auropunctata* peuvent ponctuellement bénéficier de l'apport de nouveaux génomes mâles migrants issus d'une autre population pour la production des ouvrières et ainsi augmenter leur potentiel adaptatif. Du fait de la fixation des génotypes parentaux d'une génération à l'autre, des reproducteurs issus d'une même cohorte peuvent se reproduire entre eux en évitant les effets liés à la dépression de consanguinité dans la descendance ouvrière produite sexuellement.

II. Scénarios éco-évolutifs des invasions

Bien que l'évolution puisse manifestement jouer un rôle majeur dans le processus d'invasion comme nous l'avons clairement illustré dans le cas de *W. auropunctata*, l'origine biogéographique des changements adaptatifs est souvent mal connue et on suppose généralement que ces changements ont lieu après l'établissement des populations dans la localité nouvellement envahie (Sakai *et al.* 2001; Cox 2004). Comme nous l'avons vu dans l'introduction, les événements évolutifs sont pourtant susceptibles d'émerger n'importe quand au cours du processus d'invasion. Nos études ont permis de retracer l'origine biogéographique des changements adaptatifs observés dans les populations envahissantes de *W. auropunctata*.

II.1. Populations introduites dans des localités de la ceinture tropicale

Nous avons mis en évidence que les ouvrières des populations envahissantes de la ceinture tropicale tolèrent mieux les températures élevées et les déficits hydriques caractéristiques des milieux anthropisés, que les populations de forêts primaires de l'aire native (Article 4), une caractéristique qui doit largement participer au succès d'invasion de ces populations (voir section précédente). Récemment, Foucaud *et al.* (2010) ont suggéré que les populations ont suivi un scénario d'invasion avec adaptation pré-introduction. Ce scénario implique des populations établies dans les habitats anthropisés de l'aire native ayant subi un changement de système de reproduction à partir desquelles des propagules ont dispersé à l'extérieur de l'aire native dans d'autres habitats anthropisés. Nos résultats montrent que ce scénario, initialement formalisé à partir du système de reproduction clonal, est également valable pour des changements adaptatifs qui permettent aux ouvrières de tolérer les températures particulièrement stressantes des milieux anthropisés. En effet, nous avons montré que les ouvrières des populations établies dans les milieux anthropisés de l'aire native tolèrent aussi bien les températures élevées que les ouvrières des populations introduites dans les localités de la ceinture tropicale et mieux que les ouvrières des populations du milieu naturel (i.e. forêts tropicales primaires; Article 4; Figure IV.2.).

Il est important de souligner que les activités de l'homme pourraient jouer un rôle central dans ce scénario d'invasion avec adaptation pré-introduction des populations envahissantes de *W. auropunctata*. En effet, la transformation locale de l'habitat naturel de cette espèce (i.e. forêts primaires) en habitat marginal (i.e. milieux anthropisés tel que des plantations ou des bords de route) favorise l'émergence de lignées clonales (Foucaud *et al.* 2010). Ce patron est en accord avec le fait que dans les organismes dont certaines lignées sont asexuées, ces dernières sont généralement établies dans des milieux marginaux de l'aire native (Vandel 1928; Haag & Ebert 2004; voir aussi chapitre 1 de cette thèse). Nous avons également montré que ces milieux anthropisés caractérisés par des fluctuations environnementales plus importantes que dans les milieux naturels natifs (i.e. forêts primaires) favorisent l'émergence de changements adaptatifs permettant aux ouvrières de mieux tolérer les températures stressantes que ne le font les ouvrières des populations sexuées. D'autre part, les connexions établies par l'homme entre localités géographiquement éloignées permettent à certaines de ces populations de disperser sur de longues distances à l'extérieur de l'aire native. Enfin, du fait de l'homogénéisation des habitats par les activités humaines, ces populations

pré-adaptées aux milieux anthropisés tropicaux peuvent facilement s'établir et envahir ces nouvelles localités à l'extérieur de l'aire native dans la ceinture tropicale. La contribution de l'Homme à l'émergence de populations prédestinées à devenir envahissantes, au sein même de l'aire native des organismes, a été formalisée dans un article auquel j'ai participé et qui est actuellement sous presse («*Anthropogenically-Induced Adaptation to Invade (AIAI): Contemporary adaptation to human-altered habitats within the native range can promote invasions*», voir annexe 3 : Article 8).

Bien que ce scénario d'invasion avec adaptation pré-introduction soit très fortement soutenu par nos résultats, plusieurs informations seraient nécessaires pour le valider de manière définitive. En effet, nous n'avons pas réussi à mettre en évidence des relations de très forte proximité génétique entre les populations clonales de l'aire d'introduction et les populations clonales établies dans les milieux anthropisés de l'aire native. L'origine de ces populations n'a donc pas été précisément identifiée. Cela s'explique notamment par la forte structuration géographique des populations au sein de l'aire native. Il est de ce fait difficile d'échantillonner (par chance) les populations sources des populations envahissantes de l'aire d'introduction. D'autre part, bien qu'il soit raisonnable d'assumer que les milieux anthropisés de la ceinture tropicale présentent des conditions environnementales très semblables, nous n'avons pas vérifié que les pressions de sélection sur les traits étudiés (i.e. thermotolérance) sont véritablement similaires dans les milieux anthropisés de l'aire native et ceux de l'aire d'introduction. Une manière directe de vérifier cette similarité serait de mesurer directement les températures et l'humidité des localités occupées par les populations clonales envahissantes de *W. auropunctata* dans l'aire native et dans l'aire d'introduction ce qui, d'un point de vue logistique est relativement lourd. Une autre solution serait d'utiliser des approches indirectes par des techniques de système d'information géographique (SIG). Cependant, il n'existe pas de données suffisamment précises pour extraire des données climatiques à l'échelle à laquelle les populations de *W. auropunctata* s'organisent dans l'aire native. En effet, la plupart des populations clonales de l'aire native s'établissent dans des milieux anthropisés de faible surface entourés de forêts tropicales primaires (e.g. piste, plantations). Or les couvertures de données SIG ont une résolution encore trop faible (250 mètres) pour pouvoir les utiliser convenablement dans le cas de *W. auropunctata*.

II.2. Populations introduites dans la région Méditerranéenne

Afin de retracer le scénario éco-évolutif d'invasion des populations établies en zone Méditerranéenne (i.e. Israël), nous avons développé une approche intégrative en couplant des analyses de modélisation spatiale de niche, des analyses génétiques et des mesures de trait d'histoire de vie. Cette approche permet d'identifier, au moins dans les grandes lignes, l'origine biogéographique probable des changements adaptatifs associés au succès d'invasion des populations. La démarche suivie consiste à déterminer, dans un premier temps, les zones géographiques pour lesquelles les pressions de sélection sont similaires à celles exercées dans la localité envahie, en utilisant des approches de modélisation spatiale de niche. De telles approches permettent d'identifier des zones de l'aire native (i.e. sites d'adaptation pré-introduction potentiels) et/ou de l'aire d'introduction (i.e. sites d'adaptation post-introduction en tête-de-pont potentiels), pour lesquelles des populations sont susceptibles d'avoir subi des changements adaptatifs similaires. Cette première approche permet d'identifier des zones qui sont importantes à échantillonner pour des analyses génétiques subséquentes dans le but de retracer les routes d'invasion. Ces deux approches combinées (modèles de niche et analyses génétiques) peuvent permettre d'identifier des populations potentiellement sources ou au moins une région géographique dans laquelle la population source de la population envahissante est très probablement présente. Des analyses comparatives de traits d'histoire de vie en milieu contrôlé, comparant les populations envahissantes, les populations identifiées comme étant potentiellement à l'origine des populations envahissantes, ainsi que les populations établies dans l'habitat principal de l'aire native de l'espèce, permettent finalement d'identifier l'origine biogéographique des changements adaptatifs.

Dans le cas de *W. auropunctata*, cette approche nous a permis de montrer que les populations envahissantes d'Israël proviennent très probablement de populations clonales établies dans la région Nord-est de l'Argentine, confrontées à des températures basses et à des amplitudes thermiques annuelles similaires à celles observées en Israël. De plus, nos analyses de traits d'histoire de vie ont montré que les ouvrières issues de populations Argentines présentent une adaptation au froid similaire à celle des ouvrières des populations établies en Israël. L'origine de la lignée clonale établie en Israël n'a pas été précisément identifiée au niveau populationnel, bien que le génotype mâle caractérisant cette lignée ait été observé dans certaines lignées clonales d'Argentine. Cependant, notre étude nous a permis de pointer une région géographique relativement restreinte, au sein de laquelle d'autres études plus fines

seraient utiles pour préciser où et comment ont eu lieu ces changements adaptatifs. Il est intéressant de noter qu'à l'instar des populations envahissantes de la ceinture tropicale, les populations établies en région Méditerranéenne semblent également avoir suivi un scénario d'invasion avec adaptation pré-introduction.

Cette étude nous a également permis de mettre en évidence que les populations établies en Floride, confrontées à des températures froides semblables à celles ressenties en Israël, se sont également adaptées au froid mais dans une moindre mesure, et de manière indépendante (Article 5). En effet, les relations génétiques entre ces populations et celles établies en Argentine ou en Israël sont très relâchées. Cela suggère que les populations envahissantes établies en Floride ont suivi une route d'invasion différente et encore méconnue actuellement.

Le rôle de l'homme dans l'organisation spatiale et écologique des populations en Argentine est encore flou et des études approfondies sont nécessaires, notamment dans la région géographique identifiée par notre étude. Il est intéressant de noter que les populations clonales en Argentine ont toutes été observées dans des milieux plus ou moins perturbés par l'homme (Luis Calcaterra, communication personnelle). Cependant, de part la situation biogéographique de l'Argentine, il est probable que certaines populations clonales de cette zone se soient adaptées à des conditions froides dans des milieux marginaux qui n'ont pas été façonnés par l'homme. En effet, l'Argentine se répartit selon un gradient climatique latitudinal qui comprend une région tropicale à l'extrême Nord et des conditions polaires à l'extrême Sud. La limite de distribution de *W. auropunctata* se situe dans ce gradient dans la partie nord de l'Argentine. Cette situation de limite de distribution est propice à l'émergence de populations adaptées à des milieux marginaux (Kawecki *et al.* 2000; 2008; voir chapitre I). Des études approfondies sur les populations Argentines semblent, de ce fait, indispensables si l'on veut mieux cerner le rôle de l'homme dans le succès d'invasion des populations d'origine Argentine de *W. auropunctata* établies en Israël.

II.3. Conclusion

Nos résultats s'accordent sur le fait que les populations envahissantes de *W. auropunctata* établies dans les zones tropicales et Méditerranéennes semblent avoir suivi un même scénario d'invasion, avec adaptation pré-introduction, impliquant au début du

processus d'invasion, des populations «clefs» établies dans des milieux marginaux de l'aire native. Ce scénario illustre le fait que certains milieux marginaux de l'aire native peuvent imposer des régimes particuliers de sélection, qui amènent des populations à développer des traits leur conférant un succès d'invasion dans certaines régions géographiques à l'extérieur de l'aire native. Nos études montrent notamment que les milieux perturbés par l'homme, ainsi que les limites de distribution de l'aire native sont propices à l'émergence de telles populations. Ces changements évolutifs sont possibles en partie du fait i) de l'hétérogénéité des habitats et de l'étendue de l'aire native, ii) de la structuration génétique des populations et iii) des caractéristiques intrinsèques des populations (e.g. système de reproduction). Il est de ce fait important de considérer les aires natives comme étant une mosaïque d'habitats, composée d'un habitat principal parsemé d'habitats marginaux dans lequel peuvent émerger des populations susceptibles de devenir envahissantes. Une connaissance de l'impact de ces milieux sur les populations natives est essentielle dans l'étude des invasions.

III. Emergence des lignées clonales envahissantes dans l'aire native

Les invasions de *W. auropunctata* étudiées au cours de cette thèse semblent toutes avoir suivi un même scénario avec adaptation pré-introduction impliquant, au départ, des populations établies dans des milieux marginaux de l'aire native. Ces milieux correspondent à des habitats perturbés par l'Homme et/ou aux limites Sud de l'aire de distribution de l'espèce. Les populations établies dans ces milieux marginaux ont subi un changement de système de reproduction, ainsi que des changements adaptatifs permettant aux ouvrières de tolérer une gamme de conditions environnementales supérieure à celle tolérée par les ouvrières des populations ancestrales établies en forêts (Articles 4 et 5). L'ensemble de nos résultats permet de formuler une hypothèse pour expliquer comment ces populations clonales, adaptées à des conditions abiotiques particulières et à l'origine des populations envahissantes, apparaissent dans les milieux marginaux de l'aire native. L'émergence de la parthénogenèse femelle suit le mode de transition de la sexualité vers l'asexualité le plus simple (i.e. parthénogenèse automictique, Article 2). Il est de ce fait probable que la mutation de la clonalité puisse apparaître régulièrement. Ce processus est d'ailleurs supporté par une étude de génétique des populations montrant l'émergence de plusieurs lignées clonales indépendantes en Guyane et au Brésil (Foucaud *et al.* 2007a). Comme nous l'avons vu, le système de reproduction asexuée de *W. auropunctata* contourne certaines règles traditionnelles de l'asexualité en permettant notamment des flux de gènes entre les lignées sexuées et les lignées clonales

(Article 3; mais voir Simon et al. 2002). Lors de l'émergence d'un génotype parthénogénétique femelle, celui-ci peut donc s'associer à différents génotypes mâles et ainsi donner différents types de descendance ouvrière sur laquelle la sélection peut agir. Les populations de l'aire native étant génétiquement très structurées (Foucaud *et al.* 2007b), les lignées parthénogénétiques émergentes peuvent bénéficier d'un apport de mâles génétiquement très différents, ce qui augmente d'autant plus la diversité génétique potentielle dans la descendance ouvrière issue d'un seul génotype maternel parthénogénétique. La sélection peut alors agir et fixer une combinaison génétique parentale adaptée aux nouvelles conditions environnementales, notamment une combinaison génétique permettant aux ouvrières de tolérer de larges gammes de températures (Figure IV.3-A). Une fois cette combinaison génétique créée, elle est maintenue dans le temps du fait de l'association de la parthénogenèse avec l'androgenèse. Ce maintien des génotypes parentaux permet également d'éviter les effets de dépression de consanguinité dans la descendance ouvrière produite sexuellement.

Ce scénario d'émergence de lignées clonales envahissantes dans l'aire native reste hypothétique. Une première conséquence notable de ce scénario est que, au sein de l'aire native, des lignées parthénogénétiques femelles peuvent kidnapper des génomes de mâles issus de populations sexuées avoisinantes. A cet égard, Foucaud (2007b) a identifié deux lignées clonales distinctes, établies sur une piste forestière, caractérisées par des génomes mâles issus de populations sexuées avoisinantes. Dans ces lignées, les reines sont génétiquement différentes des reines des populations sexuées d'où sont originaires les deux mâles. Ce résultat illustre potentiellement la notion de kidnapping de génomes de mâles migrants, probablement issus de populations sexuées, qui a été mis en évidence au cours de cette thèse via des expériences de croisements en conditions contrôlées (Article 3).

Une deuxième conséquence notable de ce scénario est la possibilité d'une coexistence, au moins transitoire, de plusieurs génotypes mâles associés à un génotype parthénogénétique femelle, lors de l'émergence mais aussi au cours de la vie de ces populations clonales envahissantes. En accord avec cette hypothèse, deux lignées parthénogénétiques femelles ont été trouvées chacune associée à deux génotypes mâles distincts au sein de deux populations clonales, l'une établie dans une carrière abandonnée, l'autre dans une plantation (Foucaud et al. 2007b). Ces situations sont cependant relativement peu fréquentes. La rareté de ces lignées femelles associées à différents génotypes mâles *in natura* peut s'expliquer d'au moins trois

façons. i) Les événements d'introgression de génomes mâles issus de populations sexuées dans les lignées clonales sont rares. ii) Les populations clonales étudiées jusqu'à présent sont anciennes et la sélection a déjà agi, ne conservant le plus souvent qu'une seule combinaison génétique. iii) Les événements d'introgression sont nombreux mais le coût de transition associé à ces événements (i.e. production de reproducteurs aberrants; Article 3; Figure IV.3-B.) est élevé. Dans ce cas, seules les combinaisons génétiques parentales ayant une valeur sélective très élevée, indirectement via la performance des ouvrières, se maintiennent. Il est important de noter cependant que deux lignées clonales ayant passé le premier filtre sélectif associé au coût de la transition, peuvent s'échanger des mâles migrants sans souffrir de ce coût de transition. En effet, ce coût associé à la production d'individus reproducteurs génétiquement aberrants (i.e. gynandromorphes, polyploïdes imparfaits) ne concerne pas les croisements entre reproducteurs de populations clonales différentes (Article 3). A cet égard, dans le cadre d'un scénario d'invasion avec adaptation pré-introduction, les populations clonales établies dans l'aire d'introduction sont susceptibles de recevoir des migrants, majoritairement issus de populations clonales des milieux marginaux de l'aire native. Cet apport de nouveaux génotypes mâles migrants dans les populations envahissantes n'est donc pas associé à ces coûts de transitions.

Enfin, troisièmement, ce scénario implique qu'une même lignée parthénogénétique femelle associée à différents génotypes mâles produise des descendances ouvrières dont la tolérance aux conditions environnementales varie en fonction du génome paternel. En ce qui concerne le trait étudié dans notre étude, cela impliquerait que la thermotolérance des ouvrières soit dépendante du génome paternel fixé dans la lignée clonale. Des expériences de thermotolérance sur des ouvrières issues de croisements impliquant des reines d'une même lignée parthénogénétique (i.e. même génotype) fécondées par des mâles issus de populations clonales différentes et de populations sexuées permettraient de vérifier cette hypothèse. Il serait particulièrement intéressant de conduire une expérience similaire sur des lignées maternelles ayant kidnappé différents génotypes paternels *in natura*.

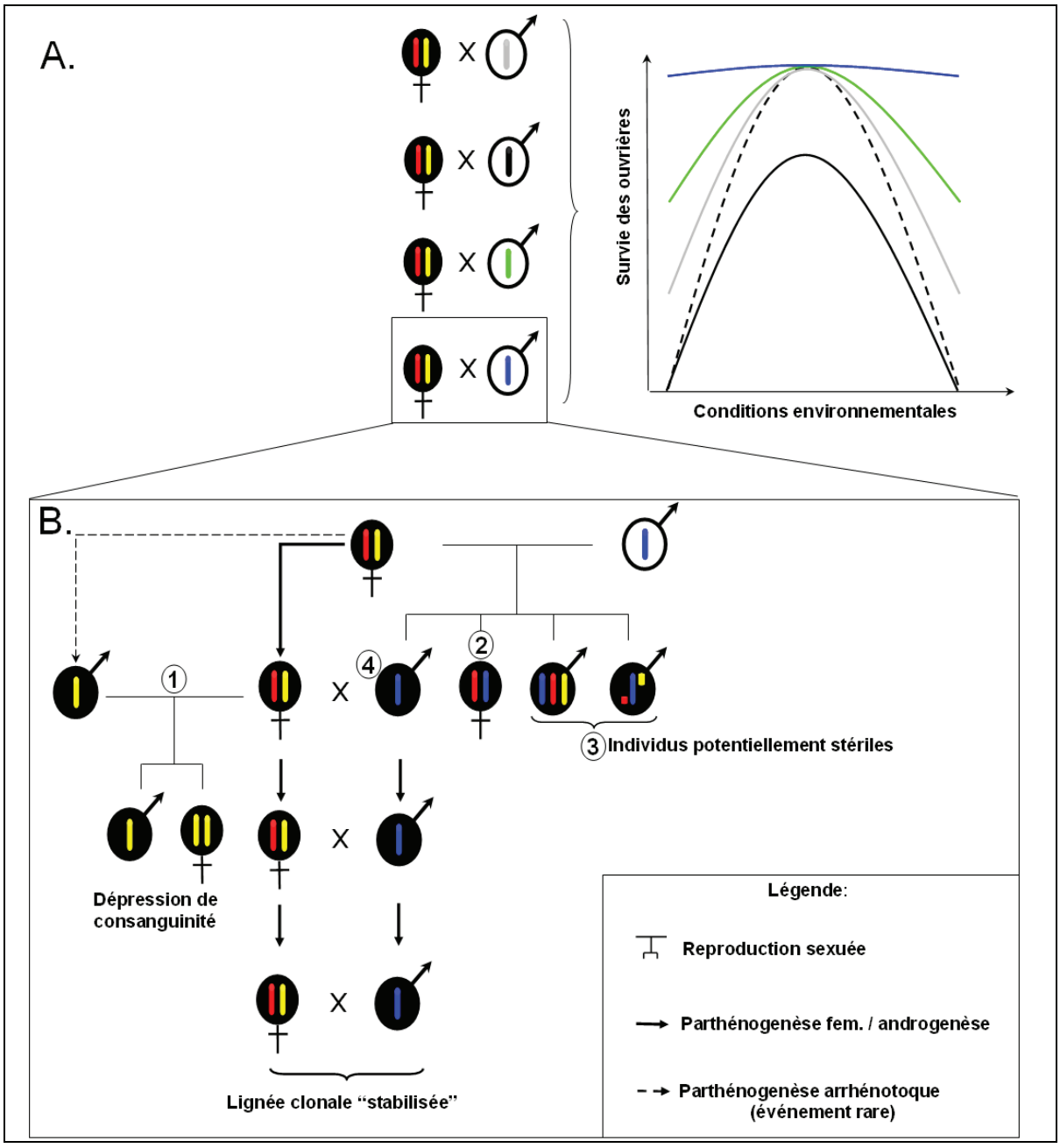

Figure IV.3. Emergence d'une lignée clonale potentiellement à l'origine de populations envahissantes. A. Une reine kidnappe différents génotypes mâles et produit des descendances ouvrières dont les performances sont différentes. La sélection fixe le(s) couple(s) de génotypes parentaux pour lesquels la descendance ouvrière est la plus performante. B. Le kidnapping d'un génome mâle issu d'une population sexuée engendre un coût de transition probablement dû à des interactions cytoplasmiques négatives. Une reine fécondée par un mâle issu d'une population sexuée produit en effet une partie de ces fils par arrhénotoquie (1), des reines par sexualité (2) et des individus reproducteurs génétiquement aberrants (3). Ce coût est très probablement transitoire et disparaît une fois que la lignée a fixé un matériel cytoplasmique unique (4).

IV. Conclusion générale

Trois résultats principaux découlent de cette thèse. i) Le succès d'invasion des populations de *W. auropunctata* est associé à un changement de système de reproduction, de la sexualité vers un système de reproduction clonal particulier que nous avons pu caractériser finement. ii) Le succès d'invasion repose également en partie sur des changements adaptatifs permettant aux ouvrières de mieux tolérer des températures caractéristiques des localités envahies comparativement aux ouvrières des populations non-envahissantes de l'aire native. iii) Ces changements évolutifs (i.e. système de reproduction et thermotolérance) ont émergé dans des habitats marginaux de l'aire native qui présentent des conditions environnementales similaires à celles des localités envahies.

Dans cette section nous discuterons de deux retombées de cette étude. D'une part, nous reviendrons sur la méthodologie suivie dans notre cas d'étude pour retracer les scénarios éco-évolutifs des invasions biologiques et discuterons de la pertinence de cette méthodologie pour l'étude d'autres organismes. D'autre part, nous verrons comment les résultats «fondamentaux» de nos travaux peuvent être transférer dans le domaine de l'appliqué et en particulier dans la gestion des populations envahissantes de *W. auropunctata*.

IV.1. Approches méthodologiques pour retracer l'histoire des invasions

Au cours de cette thèse nous avons eu recours à de nombreuses approches méthodologiques pour étudier le rôle de l'évolution dans les processus d'invasion. Cette multidisciplinarité nous a permis de retracer, à partir des populations envahissantes établies dans des localités distinctes, l'histoire évolutive de ces populations jusqu'à leurs émergences.

Notre étude permet de proposer un protocole couplant une approche de modélisation de distribution d'espèces (SDM), des analyses de génétique des populations et des mesures de traits d'histoire de vie permettant de retracer les routes d'invasions des populations pour identifier l'origine de certains changements adaptatifs pouvant émerger au cours des processus d'invasions. Les méthodes de SDM permettent d'identifier des zones géographiques au sein de l'aire native mais aussi dans l'aire d'introduction caractérisées par des conditions environnementales en adéquation avec celles qui caractérisent le site envahi étudié. En terme évolutif, cela permet d'identifier des localités dans lesquelles les pressions

de sélection sont similaires et dans lesquelles des changements évolutifs importants pour le succès d'invasion ont pu avoir lieu. Pour notre étude, nous nous sommes focalisés sur des changements adaptatifs en réponse à des conditions de température. Mais cette approche peut également être envisagée pour d'autres caractéristiques abiotiques (e.g. humidité, luminosité,…) ou même biotiques, par exemple, dans le cas d'un changement d'hôtes au cours d'une invasion de parasites. La limitation de ces approches est principalement liée à la disponibilité des données géoréférencées et de leurs résolutions. Cependant, ce domaine de recherche est en pleine expansion en biologie. Ces données seront donc de mieux en mieux adaptées aux problématiques de la biologie des invasions. Dans un deuxième temps, des analyses de génétique des populations sont importantes pour estimer les relations de filiations entre populations, c'est-à-dire pour identifier la source et retracer l'histoire démographique des populations envahissantes. Cela demande un effort d'échantillonnage considérable dans l'aire native mais également dans l'aire d'introduction. Les méthodes SDM permettent notamment de cibler des régions géographiques pour lesquelles il est crucial d'avoir des échantillons. Dans notre cas, nous n'avons pas eu recours aux méthodes ABC (voir Chapitre 3) du fait de la difficulté de les utiliser dans un cas comme celui de *W. auropunctata* (polymorphisme de système de reproduction, organisme haplo-diploïde, asexualité particulière). Il est cependant évident que ces méthodes sont à privilégier lorsqu'elles sont applicables. Enfin, des mesures de traits d'histoire de vie pour comparer les populations envahissantes, les populations de l'habitat principal et les populations identifiées comme sources potentielles au travers des deux approches précédentes permettent de mettre en évidence où le changement adaptatif a eu lieu.

IV.2. Retombées en termes de gestion des populations envahissantes de *W. auropunctata*

On distingue deux grandes stratégies pour lutter contre les espèces envahissantes. D'une part, les stratégies de prévention qui sont destinées à empêcher les espèces de s'implanter dans une nouvelle région géographique. Celles-ci se basent notamment sur le contrôle de quarantaine. D'autre part, les stratégies «de guérison», qui consistent à freiner l'expansion de l'espèce une fois établie dans le nouveau milieu. Cette stratégie requiert souvent l'utilisation de produits chimiques mais peut également faire appel à la lutte biologique (Mack *et al.* 2000). En termes de coûts, Mack *et al.* (2000) ont souligné l'intérêt de centrer les efforts sur les stratégies de prévention en accentuant les contrôles de quarantaine. En effet, la plupart des invasions impliquent initialement l'établissement de propagules de

petites tailles. Le coût associé à la destruction de ces propagules est, de ce fait, plus faible que le coût qu'engendrent les efforts de lutte contre une espèce bien établie dans le milieu (Mack *et al.* 2000; Leung *et al.* 2002). Idéalement, l'identification des zones géographiques susceptibles d'être sources de populations envahissantes et des zones géographiques qui risquent d'être envahies permettrait de cibler les efforts à fournir (i.e. quelles zones à surveiller). Mais ces localités sont la plupart du temps mal connues. Des études comme celles menées pour *W. auropunctata* permettent de mettre en évidence des zones particulièrement propices à l'émergence de populations à caractère envahissant. C'est le cas notamment de la région Nord-est de l'Argentine à partir de laquelle des populations ont été dispersées et ont réussi à s'implanter avec succès en Israël en s'adaptant à des températures particulièrement froides. Des contrôles de quarantaines de marchandises (pour l'export) dans cette région seraient donc particulièrement utiles. D'autre part des approches de modèles de distribution d'espèces pourraient permettre de prédire où cette espèce est susceptible d'envahir en prenant en considération l'adaptation au froid des populations mise en évidence dans notre étude. Cela permettrait de définir des zones à risques dans lesquelles il serait nécessaire de renforcer les contrôles de quarantaine surtout sur des produits provenant d'Argentine.

Les contrôles de quarantaine en zone d'introduction (i.e. à l'import) sont en effet également importants notamment pour éviter toute nouvelle introduction d'espèces potentiellement envahissantes mais aussi pour éviter les introductions multiples. En effet, comme nous l'avons vu, des introductions multiples peuvent engendrer une augmentation de diversité génétique et augmenter le potentiel adaptatif des populations envahissantes (e.g. Kolbe *et al.* 2004; Lavergne & Molovsky 2007). Ces introductions multiples peuvent également conduire à des effets de vigueur hybride à la suite d'événements de reproduction entre des individus de populations génétiquement très divergentes (e.g. Facon *et al.* 2005; 2008). Dans le cas de *W. auropunctata*, les résultats de cette thèse indiquent clairement que des flux de gènes entre populations clonales peuvent avoir lieu via les mâles (i.e. kidnapping de gènes; Article 3). Il est ainsi primordial de poursuivre les contrôles de quarantaine pour cette espèce même dans les zones déjà envahies.

Parmi les stratégies de «guérison», c'est-à-dire destinées à freiner l'expansion de l'espèce établie dans le milieu envahi, il faut distinguer la lutte chimique de la lutte biologique (Mack *et al.* 2000). La lutte chimique est la plus utilisée pour lutter contre les organismes envahissants. Plusieurs produits tels que l'Amdro®, l'Esteem® ou le Conserve® permettent

de réduire fortement les densités de *W. auropunctata* (Wetterer & Porter 2003; Causton *et al.* 2005; Souza *et al*. 2008) mais il est rare que les populations soient définitivement éradiquées des localités traitées. A notre connaissance, un seul cas d'éradication complète de *W. auropunctata* à l'aide de molécules chimiques a été répertorié et ce, sur l'île de Maui (Hawai ; Vanderwoude *et al.* 2010). Cependant, les auteurs précisent que la densité de *W. auropunctata* sur cette île était faible. Ces résultats soulignent qu'un traitement précoce peut être efficace tant que les populations n'atteignent pas des densités trop importantes. Néanmoins, une fois bien implantée, *W. auropunctata* est extrêmement difficile à contrôler, du moins par voie chimique. Il faut noter que ces méthodes présentent en plus de nombreux désavantages. Elles peuvent se révéler néfastes pour l'environnement du fait d'un effet généraliste sur des organismes de différents groupes taxonomiques, peuvent également constituer un danger pour la santé publique et sont souvent onéreuses.

Une solution possible pour lutter contre les espèces envahissantes, est la lutte biologique (Hoddle 2002). Cette possibilité a déjà été proposée par Foucaud (2007b). Cet auteur a soumis l'idée d'utiliser *Wolbachia* si celle-ci s'avérait être impliquée dans la manipulation de la reproduction, notamment en imposant une reproduction sexuée. En effet, les populations envahissantes étant très majoritairement constituées d'un seul couple de génotypes parentaux clonaux, il suffit d'un seul événement de reproduction sexuée pour que le degré d'apparentement entre reproducteurs (i.e. reines et mâles) qui en résulte soit élevé (i.e. plein frères). La reproduction de ces individus produits sexuellement est de ce fait susceptible d'entrainer des effets de dépression de consanguinité et éventuellement une réduction de la densité des populations. Cependant, nos résultats montrent que *Wolbachia* ne joue pas de rôle dans le déterminisme du système de reproduction des populations de *W. auropunctata*. Par contre, l'effet de cette bactérie endosymbiotique sur le métabolisme des individus n'est pas encore connu, et il n'est pas exclu que cette bactérie puisse représenter un coût important pour les populations. Dans ce cas *Wolbachia* pourrait quand même s'avérer être un moyen de lutter contre *W. auropunctata*. Cette solution a d'ailleurs été proposée pour lutter contre d'autres espèces de fourmis envahissantes du genre *Solenopsis* et pour *Linepithema humile* (Tsutsui *et al.* 2003).

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ANNEXES

ANNEXE 1:

ARTICLE 6: "Characterisation of 21 novel microsatellite markers for the little fire ant *Wasmannia auropunctata*.(in Almany *et al.* 2009)"

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Characterisation of 21 novel microsatellite markers for the little fire ant Wasmannia auropunctata

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Characterisation of 21 novel microsatellite markers for the little fire ant *Wasmannia auropunctata*

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Running title: Twenty-one novel microsatellite loci for *W. auropunctata*

Abstract

Polymorphic genetic markers are useful tools for evolutionary studies in social insects. We have characterised 21 novel microsatellites in the invasive ant, *Wasmannia auropunctata*. All loci genotyped on individuals originating from two populations were polymorphic. Within population, the number of alleles ranged from 1 to sixteen and observed heterozygosity from 0.000 to 1.000. Cross-species amplification tests were achieved on six other ant species. Several primer pairs yielded reproducible results in two other major invasive ants, eight in the Argentine ant *Linepithema humile* and 11 in the red fire ant *Solenopsis saevissima*.

The little fire ant *Wasmannia auropunctata* is one of the most threatening invasive ant species. Native from South America, it has been expending its range all around the tropical zones where its impact is worrying (e.g. Wetterer *et al*. 2003). Twelve microsatellite markers were developed previously in order to investigate the genetic structure and mating system in populations from both the native and the introduced ranges of the species (Fournier *et al*. 2005a, Fournier *et al.* 2005b, Foucaud *et al.* 2006, Foucaud *et al.* 2007). Theoretical and empirical studies both indicate that invasive success might have a strong genetic component in several species, including *W.auropunctata* (Sax *et al*. 2005, Foucaud *et al.* 2006). In order to study the association between genetic markers and phenotypic traits related to invasive success (e.g. through linkage disequilibrium or correlation with levels of multilocus heterozygosity), it is crucial, however, to increase the amount of pre-existent genetic markers. We report here the isolation and characterization of 21 novel microsatellite loci for *W.auropunctata*.

A partial DNA library of 1768 recombinant clones was obtained without enrichment from *W. auropunctata* individuals originating from New Caledonia, and was screened using a nonradioactive method (detailed protocols available at http://www.inapg.inra.fr/dsa/microsat/microsat.htm; Estoup *et al*. 1998). One hundred and forty of the 1768 recombinant clones showed a strong hybridization signal with $(GT)_{10}$ and $(CT)_{10}$ probes. Sixty-seven of the 78 sequenced inserts contained microsatellites and 26 were tested for amplification using fluorescent polymerase chain reaction (PCR). DNA extraction was performed following a Chelex-based protocol (Estoup *et al.* 1996). PCR amplifications were performed with a Mastercycler ep gradient (Eppendorf) under the following conditions: an initial denaturing step of

15 min at 94°C followed by 35 cycles of 30 s at 94°C, annealing at 57°C for 90 s, and extension at 72°C for 60 s; then a final extension at 60°C for 30 min. Twenty-one loci were selected on the following criteria: good quality of allelic patterns (i.e. low number of stutter bands) and ranges of allelic size compatible with the design of a total of four PCR multiplex sets (see below). Primer sequences and various features are given for each selected locus in Table 1. The flanking regions of each microsatellites loci have been blasted with the arthropoda genomes database provided by GenBank before being deposited (Accession Nos FJ970003 to FJ970023). We found some significant alignments with sequences all belonging to the *Apis mellifera* genome, for four of our 21 loci (Wa2-97, Wa3-85, Wa4-29 and Wa4-71; Table 1). Based on allele size ranges, the 21 markers were co-amplified in four independent PCR sets using Multilocus Amplification Kit (QIAGEN) in a 10-µL volume containing 1× QIAGEN Multiplex Master Mix, 2µM of each primer and 10 - 50 ng of genomic DNA. The forward primer of each pair of microsatellite DNA primers was labelled with a fluorescent dye (FAM, VIC, PET or NED) in order to detect alleles at all 21 loci using only two electrophoresis runs (see Table 1).

For each locus we estimated the number of alleles, the observed and the expected heterozygosities by typing 30 workers from a population from New Caledonia (introduced area) and 30 workers from a population from French Guiana (native area). Within population, the number of alleles per locus, the observed and the expected heterozygosities ranged between 1 and 16, 0.000 and 1.000, and 0.240 and 0.871, respectively (Table 1). Differences between values of observed and expected heterozygosity are a result of both relatedness of individuals within nests and of the reproduction systems of each populations (i.e. clonal for males and

females in the Caledonia and sexual in French Guyana; Foucaud *et al*. 2006 and Foucaud *et al.* 2007).

We could not apply any of the methods and programs traditionally used in population genetics to test for linkage equilibrium or for the presence of null alleles. This is because such methods and programs are based on an assumption (random mating within population and hence Hardy-Weinberg equilibrium), that does not apply to a social insect such as *W. auropunctata* for which the genotyped workers are more or less related. The genotyping of several monogynous laboratory families (i.e. full sister workers) have shown, however, that the large majority of the cloned microsatellites were statistically independent and there were only few evidence of null alleles in the analyzed families.

We examined the conservation of the primer sequences and the level of polymorphism of the 21 selected loci in six other ant species. For each species, extraction and PCR amplification were performed on five individuals as described above. Results are reported in Table 2. All loci were successfully cross-amplified in at least one species. Six of them were cross-amplified in at least five species suggesting a strong conservation of the flanking regions of those loci among the tested species. While 17 and 16 loci were amplified in the two tested species of the genus *Wasmannia* (*W. rochai* and *W. Sigmoidae* respectively), a minimum of three and a maximum of 19 loci were amplified in species of the *Blepharidatta* and *Allomerus* genus, respectively. Several primer pairs yielded reproducible results in two other major invasive ants: eight in the Argentine ant (*Linepithema humile*) and 11 in the red fire ant (*Solenopsis saevissima*). This novel sets of 21 microsatellite loci will be useful to investigate the association between particular genomic combinations

99 and invasive traits in *W.auropunctata* and will increase the number of genetic
100 markers available in other invasive ant species.

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Table legends

Table1. Microsatellite loci developed for the ant *Wasmannia auropunctata*. (1), (2), (3) and (4) refer to co-amplification PCR set. Cloned allele size is based on New Caledonian individuals. The observed allele size range, number of allele (N_a) and observed (Ho) and expected (He) heterozygosities were estimated from 30 workers collected in a population from French Guyana (FG) and 30 workers collected in a population from New Caledonia (NC). Blast result corresponds to the accession number of the alignment sequences, the identities (proportion of identical sites) and expected values of observing the same identity by chance (E). n.s: non significant alignment.

Table 2. Cross-species PCR tests for 21 *W.auropunctata* microsatellite loci in six ant species. Tested species included two species of the Genus Wasmannia (*Wasmannia rochai* and *Wasmannia sigmoidea*), three species of the subfamily Myrmicinae and belonging to the three Genus Allomerus, Solenopsis and Blepharidatta genus (*Allomerus decemarticulatus*, *Solenopsis invicta* and *Blepharidatta spp*) and one species belonging to the subfamily Dolichoderinae (*Linepithema humile*). The numbers of alleles and the size range of alleles (between parentheses) are based on 5 individuals genotyped for each species. Amplification failure is indicated by a dash.

Table 1

	Locus	Cloned allele: Core repeat (cloned allele)	Cloned allele: Size (bp)	Size range (bp)	Na: FG	Na: NC	Ho/He: FG	Ho/He: NC	Dye	Primers sequences 5'- 3'	Genbank Accession No.	Blast result
Electrophoresis run 1												
(1)	Waur-1164	$(CA)_{15}$	180	162-201	7	3	0.867 / 0.726	1.000 / 0.624	NED	F:CCATTGTTCACAAGCGGA R:CCTAAGTATTGTCGTCGGAG	FJ970003	n.s
(1)	Waur-429	$(GA)_{14}$	278	267-287	10	3	0.867 / 0.871	1.000 / 0.616	NED	F:CGACGAGTCGAGGACATAGG R:GCGTCTAATCAGGCTGCTC	FJ970004	NW_001253420 100 / 117 (E=2e-24)
(1)	Waur-113	$(CA)_{12}(TA)_{5}$	366	351-364	5	3	0.700 / 0.617	1.000 / 0.624	NED	F:TCAGTTCTGAGTCACGTCTGG R:GGACTTGCGAGAATTCGAG	FJ970005	n.s
(1)	Waur-584	$(CT)_{9}CG(CT)_{7}$	144	139-143	3	2	0.200 / 0.240	0.033 / 0.328	FAM	F:CTCTATCTCGCACGCGTTC R:CGACGATGAGCCTTGATG	FJ970006	n.s
(2)	Waur-1176	$(CA)_{25}$	233	215-265	12	5	0.867 / 0.832	1.000 / 0.644	FAM	F:GGATTCAACCTGGACCGAC R:GTAGAAGTTACACCAACGGCAG	FJ970007	n.s
(1)	Waur-5173	$(GA)_{11}$	317	312-404	11	2	0.567 / 0.776	1.000 / 0.500	FAM	F:CATAAGCTCATGCCTCTCAC R:GTTCTAACGAGTCGCATCC	FJ970008	n.s
(1)	Waur-749	$(CT)_{13}$	416	400-428	10	3	0.733 / 0.837	1.000 / 0.623	FAM	F:GCGGTTAATCGAACGTCAC R:CTTATCTCTGGCAGCCGTATC	FJ970009	n.s
(1)	Waur-3O	$(CT)_{6}N_{14}(CT)_{10}$	192	183-209	8	3	0.767 / 0.785	1.000 / 0.624	VIC	F:GTCGGTGGTATCCACGAGGTAT R:CCGAGCAGACTCATTGTAACG	FJ970010	n.s
(1)	Waur-626	$(CT)_{11}$	298	288-315	7	3	0.733 / 0.648	1.000 / 0.605	VIC	F:GTCATCGGCGAAGTTGG R:GGCCGCATATTACACTACG	FJ970011	n.s
(2)	Waur-471	$(AT)_{16}(GT)_{15}$	406	400-447	12	3	0.556 / 0.803	0.967 / 0.616	VIC	F:GAAGCGCACCTCACCGTACT R:GTGTATCGGCGGCAACAGAG	FJ970012	NW_001253017 154 / 163 (E=2e-64)
(1)	Waur-7U	$(GA)_{24}$	188	160-202	7	5	0.633 / 0.713	1.000 / 0.677	PET	F:GTACTTTCGTGCCCATCTGC R:GCCATCGACCTAAATACGGA	FJ970013	n.s
(2)	Waur-5152	$(CT)_{25}$	327	314-346	10	2	0.733 / 0.776	1.000 / 0.500	PET	F:GCCGCAATCATCAACAGACG R:TGTCAACAGCACCAATCGCA	FJ970014	n.s

Table 1 Continued

Locus	Cloned allele: Core repeat (cloned allele)	Cloned allele: Size (bp)	Size range (bp)	Na: FG	Na: NC	Ho / He: FG	Ho / He: NC	Dye	Primers sequences 5'- 3'	Genbank Accession No.	Blast
Eletrophoresis run 2											
(3) Waur-8179	$(CT)_8$	158	155-167	5	1	0.633 / 0.605	0.000 / 0.000	NED	F:GCTGATTCGTTGCACCTGAC R:GACCGTGAGACGGCACATT	FJ970015	n.s
(3) Waur-8Ω	$(GA)_{15}$	255	246-268	10	2	0.867 / 0.811	1.000 / 0.500	NED	F:GTAGTTGGCGAGACCGGATG R:CTCCAGCTGTGGTTCCGATG	FJ970016	n.s
(4) Waur-687	$(GA)_{28}$	360	326-360	12	4	0.833 / 0.792	1.000 / 0.632	NED	F:GCGAGACCTGTCAGAACTGTGG R:CGATCACCACGCGATGCTTC	FJ970017	n.s
(3) Waur-385	$(GA)_{10}$	141	133-144	4	2	0.633 / 0.583	1.000 / 0.500	VIC	F:GATTGAAGCTCTTCGTGCAG R:AAGCCAGGAATGTACGGTG	FJ970018	NW_001253028 290 / 390 (E=3e^{-18})
(4) Waur-297	$(CA)_{14}$	225	210-236	8	2	0.667 / 0.760	1.000 / 0.500	VIC	F:GTCAGCCGAGTGTCACTCAGTC R:CAATGAGAATGCGCGTGC	FJ970019	NW_001253350 118 / 128 (E=3e^{-43})
(4) Waur-815	$(CA)_{11}N_{16}(TA)_{20}$	343	326-386	9	4	0.750 / 0.824	1.000 / 0.626	VIC	F:GGCTCAATTGCGAACATGC R:CTGTCTTCAACGTCCGCCA	FJ970020	n.s
(3) Waur-813	$(GA)_{17}$	117	97-149	13	2	0.900 / 0.866	1.000 / 0.500	FAM	F:GTCCAAGGATGAATGTATTCAC R:GCCGCTAGAAGAAGAATGAC	FJ970021	n.s
(3) Waur-4139	$(GT)_5N_{64}(GT)_{11}$	296	296-351	12	3	0.800 / 0.830	1.000 / 0.625	FAM	F:GGTCTCAAGTTCAGCGACATAC R:GACAAGTTACGCCGCAAG	FJ970022	n.s
(3) Waur-872	$(GA)_{12}AA(GA)_{23}$	404	359-418	16	3	0.967 / 0.888	1.000 / 0.613	FAM	F:CGTGCTATCCTCGACGAAGT R:GCCGACAGTACAACAAACAGC	FJ970023	n.s

Table 2

Locus	*W. rochai*	*W. sigmoidea*	*A. decemarticulatus*	*B. spp*	*L. humile*	*S. saevissima*
Waur-113	-	-	1 (347)	-	-	-
Waur-1164	-	1 (155)	-	-	-	1 (147)
Waur-1176	6 (196 - 251)	-	3 (234 - 242)	-	2 (188 - 218)	1 (296)
Waur-297	5 (166 - 188)	2 (222 - 228)	3 (242 - 246)	-	1 (184)	1 (196)
Waur-3O	2 (201 - 205)	-	1 (228)	-	-	-
Waur-385	2 (159 - 161)	1 (139)	2 (133 - 137)	2 (133 - 137)	3 (133 - 137)	1 (126)
Waur-429	3 (265 - 271)	2 (285 - 294)	1 (250)	-	2 (327 - 237)	3 (278 - 288)
Waur-471	3 (386 - 390)	-	1 (379)	-	2 (382 - 387)	-
Waur-5152	-	2 (292 - 294)	-	-	-	-
Waur-5173	5 (333 - 353)	2 (312 - 314)	2 (354 - 414)	-	-	1 (378)
Waur-584	-	1 (128)	1 (122)	-	1 (178)	-
Waur-626	1 (294)	1 (293)	2 (290 - 354)	-	-	-
Waur-749	4 (406 - 421)	2 (394 - 398)	2 (434 - 436)	-	1 (386)	2 (418 - 421)
Waur-7U	2 (175 - 177)	1 (218)	2 (219 - 223)	1 (170)	-	2 (173 - 196)
Waur-297	5 (166 - 188)	2 (222 - 228)	3 (242 - 246)	-	1 (184)	1 (196)
Waur-4139	3 (338 - 342)	2 (335 - 339)	2 (309 - 318)	-	-	-
Waur-687	1 (330)	1 (302)	2 (352 - 360)	-	-	-
Waur-813	1 (94)	1 (107)	3 (132 - 138)	-	-	1 (97)
Waur-815	4 (258 - 276)	-	-	-	-	-
Waur-8179	2 (141 - 144)	1 (161)	1 (144)	-	-	1 (194)
Waur-872	-	-	3 (359 - 404)	-	-	-
Waur-8Ω	4 (258 - 271)	2 (263 - 265)	3 (275 - 281)	4 (260 - 271)	-	-

ANNEXE 2:

ARTICLE 7: "Thelytokous parthenogenesis, male clonality and genetic caste determination in the little fire ant: new evidence and insights from the lab"

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ORIGINAL ARTICLE

Thelytokous parthenogenesis, male clonality and genetic caste determination in the little fire ant: new evidence and insights from the lab

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Previous studies indicate that some populations of the little fire ant, *Wasmannia auropunctata*, display an unusual reproduction system polymorphism. Although some populations have a classical haplodiploid reproduction system, in other populations queens are produced by thelytokous parthenogenesis, males are produced by a male clonality system and workers are produced sexually. An atypical genetic caste determination system was also suggested. However, these conclusions were indirectly inferred from genetic studies on field population samples. Here we set up experimental laboratory nests that allow the control of the parental relationships between individuals. The queens heading those nests originated from either putatively clonal or sexual populations. We characterized the male, queen and worker offspring they produced at 12 microsatellite loci. Our results unambiguously confirm the unique reproduction system polymorphism mentioned above and that male clonality is strictly associated with thelytokous parthenogenesis. We also observed direct evidence of the rare production of sexual gynes and arrhenotokous males in clonal populations. Finally, we obtained evidence of a genetic basis for caste determination. The evolutionary significance of the reproduction system polymorphism and genetic caste determination as well as future research opportunities are discussed.
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Keywords: reproduction system; thelytokous parthenogenesis; male clonality; genetic caste determination; *Wasmannia auropunctata*

Introduction

Understanding the evolution of the wide diversity in reproduction systems is a major goal in evolutionary biology (Maynard Smith, 1978; Bell, 1982; Normark, 2003). A promising pathway to fulfil this ambition is the uncovering and careful study of extant organisms showing both sexual and asexual reproduction (for example, see Vrijenhoek, 1993; Kearney and Shine, 2004).

Recent genetic studies on the little fire ant, *Wasmannia auropunctata*, highlight the coexistence of two distinct reproduction systems, both indirectly inferred from the genotyping of individuals sampled in the field (Figure 1; Fournier *et al*., 2005a; Foucaud *et al*., 2007). The first reproduction system indirectly inferred is haplodiploidy (*sensu* Normark, 2003; that is, fertilized, diploid eggs produce female queens and workers and unfertilized, haploid eggs produce arrhenotokous males), which is classically the rule for Formicidae (Hölldobler and Wilson, 1990). This reproduction system will be hereafter referred to as 'sexual reproduction'. The second reproduction system indirectly inferred from field populations is more unusual, but not unique (Ohkawara *et al*., 2006), as it includes three distinct types of offspring production, one for each caste and sex (Fournier *et al*., 2005a). Female queens are produced by thelytokous parthenogenesis (that is, diploid females produced uniquely by female genetic material), males are produced through a male clonality system (that is, males are genetically identical to their haploid father, although laid by queens) and female workers are produced sexually (Figure 1). This reproduction system will be hereafter referred to as 'clonal reproduction'. Previous genetic data from field population samples also indicated that sexual queens and arrhenotokous males might be still rarely produced within populations displaying clonal reproduction (Foucaud *et al*., 2006).

Some *W. auropunctata* populations may also possess another unusual feature: a genetic caste determination system (that is, a genetic influence over the determination of the queen versus worker phenotype), at least in clonal populations (Foucaud *et al*., 2006). Previous field genetic studies on *W. auropunctata* indicate that, in clonal populations, thelytokous individuals were most probably targeted to the reproductive caste (that is, queens) whereas sexual individuals were almost invariably directed to the worker caste, with some rare exceptions (Foucaud *et al*., 2006). This pattern suggests that the caste fate of diploid eggs may have a genetic basis in *W. auropunctata*. However, in ants, the caste fate of a

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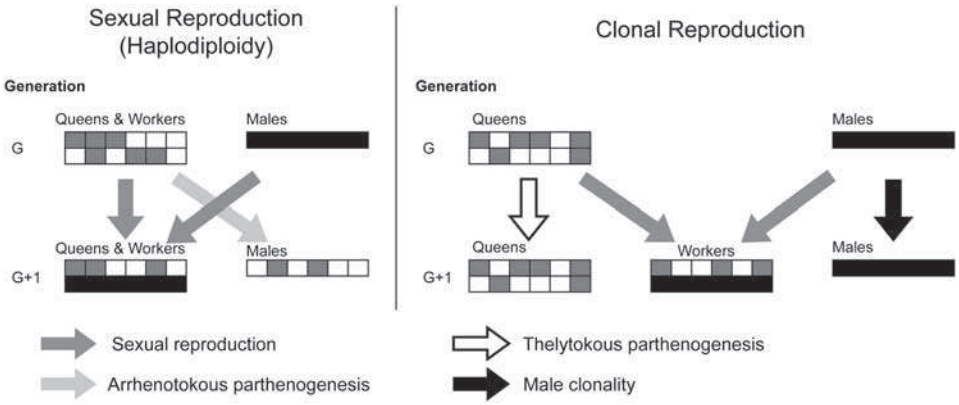

Figure 1 Schematic representation of the gene transmission between two consecutive generations in the two types of reproduction system found in *W. auropunctata*. Note: individual genotypes are represented by rectangles (diploid females: two lines; haploid males: one line), constituted of several loci represented by squares (white and gray squares: 'female' alleles; black squares: 'male' alleles). Sexuality, arrhenotokous parthenogenesis, thelytokous parthenogenesis and male clonality events are represented by dark gray, light gray, white and black arrows, respectively. It should be noted that clonal males are produced from eggs laid by female queens.

particular diploid egg (that is, queen or worker) is generally thought to be environmentally driven and controlled by the workers (Hölldobler and Wilson, 1990; Crozier and Pamilo, 1996; Queller and Strassmann, 1998). The genetic content of diploid eggs has been shown to influence its caste fate in only a few ant species (Fraser *et al*., 2000; Helms Cahan *et al*., 2004; Anderson *et al*., 2008a). As a consequence, genetic effects on caste determination are thought to be relatively rare in eusocial species (Crozier and Pamilo, 1996, but see Anderson *et al*., 2008a; Lo *et al*., 2009).

In this study, we seek to replace the indirect inferences accumulated so far in *W. auropunctata* with direct and unambiguous evidence of (i) a reproduction system polymorphism (that is, sexual and clonal populations), and (ii) a genetic caste determination system (that is, a genotype influence on caste). To this end, we set up experimental nests in the laboratory, each with a single mothering genotype, and characterized at 12 microsatellite loci the reproductive and worker offspring they produced. Unlike previous population genetic studies, our laboratory–nest design allowed the control of both the number of egg-laying queens and parental relationships between these queens and their male, queen and worker offspring.

Materials and methods

Sampling and experimental conditions

We collected 132 queens and several thousand workers from nine populations from French Guiana and New Caledonia (Table 1). On the basis of previous genetic studies of population samples collected in the field at same locations (Foucaud *et al*., 2006, 2007), we could predict that three of the collected populations were probably sexual and six were probably clonal (Table 1).

We set up 117 experimental nests, the large majority of them being mothered by a single dealate queen ($n = 112$), and a few of them including four dealate queens (only for one putatively clonal population with a single queen genotype, $n = 5$). These queens will be hereafter referred to as F0 queens. All nests were initially founded with at least 200 workers from the same population as the queen. These nests were held in climate chambers at a constant temperature of 25 °C and at a 65% humidity rate. Food (sugar water and mealworms) was provided *ad libitum*. For each nest, we collected at the pupal stage all reproductive individuals (queens and males) as well as a dozen workers; collecting offspring at the pupal stage allowed to distinguish castes by morphology and ensured that all have been produced by the mothering F0 queen after the setting up of the experimental nest. This offspring will be hereafter referred to as F1 queens, males or workers. The F1 reproductive individuals were collected after a couple of months and until 1.5 years after the beginning of our experimental setup. We also collected around 20 F1 adult workers in each experimental nests older than 90 days (cf. life expectancy of *W. auropunctata* workers was measured to be around 30 days; Ulloa-Chacon, 1990). Finally, F0 mother queens were collected when they died or at the end of the nest-rearing experiment (that is, after 1.5 years for the oldest nests).

We also tested whether virgin queens (that is, F1 alate gynes produced by our F0 queens) from both clonal and sexual populations could produce viable offspring, with a particular interest in the possibility of virgin parthenogenetic queens to produce queen daughters. To this end, we set up two types of nests: 15 parthenogenetic gynes were installed in individual nests and 15 parthenogenetic gynes and 14 sexual gynes were installed in seven nests containing one to six gynes according to their population of origin. About 0–100 workers of the corresponding population were added into every nest. Nutritional resources were added equally and simultaneously to all other experimental nests. Some experiments that we carried out independently indicated that newly mated queens produced their first adult offspring after a single month (unpublished data). We therefore conducted this experiment on virgin queens for over four months (or less when a gyne died) to control for an effect of the gyne age on its production of adult offspring. It is important to note that there is no obligatory mating flight for *W. auropunctata* queens, as observed both in the field and in our experimental stock colonies. We recorded whether eggs, larvae and adults were produced every 2–3 days.

Table 1 Numbers and origin of laboratory-settled nests and number of produced gynes and males

Sampled site	*Putative reproduction system*	*Number of laboratory-settled nests*	*Number of nests with produced reproductives (gynes/males)*	*Number of produced gynes*	*Number of produced males*
Ker	Clonal	20	10 (9/5)	32	8
RN	Clonal	15	2 (2/1)	2	1
M6-C	Clonal	13	1 (1/0)	15	0
M3-P	Clonal	2	0	0	0
P2	Clonal	15	2 (2/1)	3	1
NC	Clonal	6	0	0	0
M7	Sexual	29	13 (11/4)	109	23
M3-F	Sexual	14	6 (6/0)	51	0
M6	Sexual	3	0	0	0
Total		117	34 (31/11)	212	33

Names and locations of collection sites are identical to those in the study by Foucaud *et al*., 2007.

Genetic analyses

Following Fournier *et al*. (2005b), we extracted DNA from all reproductives produced in our experimental nests, together with at least eight workers (pupae, adults or both). When possible, we extracted the DNA from their mother queen as well as the spermathecal content of the latter to directly obtain the genotypes of the mother and of the fathering male. In some cases, the mother queen could not be collected because it had died and was not found again. For these nests, however, we could easily infer the mother and father genotypes from the genotypes of the workers (see Results section).

A total of 616 specimens were genotyped at 12 microsatellite loci, as described in the study by Fournier *et al*., 2005b). The numbers and types of genotyped specimens (queens, males and workers) are detailed in Table 2. The PCR products were separated on a MegaBace DNA sequencer (GE Healthcare Bio-Sciences, Uppsala, Sweden) and gel files were analyzed using GENETIC PROFILER (GE Healthcare Bio-Sciences).

Statistical analysis and interpretation of data

To infer the reproduction systems of our laboratory families, the relationship between parents and offspring genotypes was investigated both visually and with the help of a personal programme identifying identical multilocus genotypes (can be made available by the authors on request). The identification of the modes of production of each caste and sex was straightforward. For a female offspring, if her multilocus genotype is identical to the genotype of the parental female genotype, then parthenogenetic (thelytokous) production is supported. If the genotype is a combination of half of male and female parental genotypes at each locus, then sexual production is supported. For a haploid male offspring, if his multilocus genotype is identical to the parental male genotype, then clonal production is supported. If it is a combination of one of the queen's alleles at each locus, then arrhenotokous parthenogenesis (that is, 'sexual' production, according to our previous definition) is supported. It should be noted that workers are sterile in this species (Ulloa-Chacon and Cherix, 1988), so that males could only be produced by the queen.

The co-occurrence of parthenogenetic and sexual modes of female offspring production (that is, queen and workers) found in the clonal populations of *W. auropunctata* provides an opportunity to test whether the caste is genetically determined in this species. If the caste is genetically determined, we expect that (i) the parthenogenetic females produced by a thelytokous mother queen will be all targeted toward the queen caste and (ii) most sexually produced female offspring will be targeted to the worker caste. This is because the genetic combination needed for the queen phenotype is likely to be broken by recombination and the male's genetic contribution. On the contrary, if the caste is environmentally determined, we expect no significant relationship between the mode of production and caste fate for the female offspring. Using the data we obtained from the 14 clonal laboratory-settled nests that produced both female reproductives (that is, F1 gynes) and workers, we have statistically tested the association between the mode of offspring production (parthenogenetic or sexual) and the offspring caste (queen or worker) using a Fisher's exact test. The total amount of data for this test was 265 individuals (52 gynes and 213 workers). Our approach is similar to that used in previous studies dealing with genetic caste determination in social insects (for example, see Fraser *et al*., 2000; Smith *et al*., 2008).

We also searched for some evidences of a genetic caste determination mechanism in the sexual population samples by testing for a genetic differentiation at our 12 microsatellite loci between gynes and workers. Following Schwander *et al*. (2007a), we considered in each sexual population the sampled gynes and workers as two genetic groups and tested the significance of F_{ST} values computed at each microsatellite locus and overall loci. Significance of the overall loci and locus-by-locus F_{ST} values were tested by randomizing genotypes among genetic groups (10 000 randomizations) using FSTAT (Goudet, 2001).

It is worth pointing that, in some rare cases, parthenogenetic production of females is not strictly synonymous with identical genotypes between queen and daughter gynes in *W. auropunctata* depending on the type of thelytokous parthenogenesis occurring (Foucaud *et al*., 2006). This survey allows the computation of the rate of locus homogenization (that is, the conversion of a heterozygous locus into a locus homozygous for one of the two maternal alleles) during thelytokous parthenogenesis (Foucaud *et al*., 2006; Pearcy *et al*., 2006). This rate allows, at least theoretically, the discrimination between the types of thelytoky displayed by parthenogenetic organisms. Basically, automictic organisms (that is, whose parthenogenesis involve meiosis) commonly

Table 2 Reproduction system for the gynes, males and workers produced in laboratory nests, inferred from multilocus genotypic data

Putative reproduction system	*Number of laboratory-settled nests*	*Number of nests with produced reproductives (gynes/males)*	*Number of produced gynes*		*Number of produced males*		*Number of produced workers*		*Total*
			Clonal	*Sexual*	*Clonal*	*Sexual*	*Clonal*	*Sexual*	
Clonal	71	15 (14/7)	51	1	7	3	10	203	275
Sexual	46	19 (17/4)	0	160	0	23	0	158	341
Total	117	34 (31/11)	51	161	7	26	10	361	616

All reproductives and only a subset of workers were genotyped.

exhibit high rates of locus homogenization (for example, from 1/3 for loci independent from the centromeres to 1.0 for a gamete duplication mechanism; see Oldroyd *et al*., 2008), whereas apomictic organisms (that is, whose parthenogenesis do not involve meiosis) usually exhibit low rates of locus homogenization (typically $r = 0.0$; but see Baudry *et al*., 2004). Here we computed the rate of locus homogenization independently for each locus. We averaged the number of homogenization events over the number of thelytokous individual heterozygous for each particular locus ($n = 1$–61 individuals depending on the investigated locus).

Results

Production of gynes

The F0 queens (that is, mother queens) in 31 of our 117 experimental nests produced F1 gynes (14 and 17 queens from putatively clonal and sexual populations, respectively; Tables 1 and 2). A total of 51 gynes of the 52 produced by a queen originating from a putatively clonal population displayed a multilocus genotype identical to the genotype of their mother (except one homogenization event, see below), and did not bear any allele of the male mated to their mother (see Supplementary Table S1 for an illustration). This result demonstrates unambiguously the occurrence of thelytokous parthenogenesis in a subset of *W. auropunctata* populations. We also found that a single gyne was produced sexually by a queen that also produced two parthenogenetic gynes (Table 2 and see Supplementary Table S3 for an illustration). Therefore, although the vast majority of gyne offspring is produced through thelytokous parthenogenesis, at least some of the thelytokous queens retain the ability to produce gynes sexually. In agreement with indirect evidences from field studies, we observed a low rate of locus homogenization during the thelytokous production of new parthenogenetic queens (mean $r = 0.13\%$ overall loci with single locus r values ranging from 0 to 1.64%).

We found that all 160 gynes laid by queens originating from putatively sexual populations were sexually produced (Table 2 and see Supplementary Table S2 for an illustration). No parthenogenetic reproduction event was identified for those queens. This result demonstrates that gynes are exclusively produced sexually in another set of *W. auropunctata* populations.

Interestingly enough, four of the 44 isolated F1 virgin queens originating either from clonal or sexual populations produced few to many eggs; none of these eggs could develop into pupae (worker, gyne or male), and this during more than four months of experiment.

Production of males

On 117 experimental nests, the F0 queens of 11 nests produced F1 males during our experiment (seven and four queens from putatively clonal and sexual populations, respectively; Table 1). A majority of males produced by queens isolated from putatively clonal populations displayed a multilocus genotype identical to that of their father and did not bear any allele of their mother ($n = 7$; Table 2 and see Supplementary Table S1 for an illustration). This pattern clearly demonstrates the occurrence of male clonality in some *W. auropunctata* populations. However, one parthenogenetic queen also produced three males through classical arrhenotokous parthenogenesis (in addition to one clonal male; Supplementary Table S3). The production by parthenogenetic queens of new males through classical arrhenotoky is therefore feasible.

We found that all 23 males laid by queens isolated from putatively sexual populations were produced by arrhenotokous parthenogenesis (Table 2 and see Supplementary Table S2 for an illustration). We did not observe any clonal reproduction events for those queens. This result demonstrates the occurrence of only classical sexual production of males in some other *W. auropunctata* populations.

It is worth pointing that all clonal males ($n = 7$) were produced by queens also producing parthenogenetic gynes. Male clonality was thus strictly associated to thelytokous parthenogenesis.

Production of workers

We observed that a very large majority of workers (361 workers from all genotyped nests) were produced sexually regardless of the mode of production of gyne and male offspring (Table 2). In all nests for which the genotypes of both the F0 queen and her spermathecal content could be directly obtained, workers displayed a genotype demonstrating their sexual production (that is, at each locus, one of the two alleles of the worker genotype was present in the F0 queen genotype and the other one present in the spermathecal content genotype; see Supplementary Tables S1 and S2 for illustrations). Monoandry (that is, a single fathering male for each queen) was also obvious from these data. This important observation allowed us to safely infer the parental genotypes from worker genotypes, in those experimental nests for which the queen or male genotypes could not be directly determined.

Interestingly, we found some worker genotypes that did not fit the above reproduction rules in a single clonal nest (Table 2 and see Supplementary Table S1 for an illustration). In this nest, the large majority of adult

workers were sexually produced but most pupae, as well as two adults, displayed a multilocus genotype identical to the genotype of their mother and did not bear any allele of their mother's male mate. This small set of workers, mostly composed of pupae, was thus produced by thelytokous parthenogenesis.

Caste determination

Within clonal populations, we genotyped a total of 52 gynes (51 parthenogenetic and 1 sexual) and 213 workers (10 parthenogenetic and 203 sexual; Table 2). Parthenogenetic diploid eggs were hence significantly more directed toward the royal caste than sexual diploid eggs (Fisher's exact test: $P<10^{-6}$). This result holds when considering independently each set of parthenogenetic queens originating from different populations and that were characterized by different microsatellite genotypes (all $P<10^{-6}$). It is also worth stressing that the only parthenogenetic queen laying clonal eggs directed toward the worker caste produced adult workers at a very low rate (cf. most worker larvae did not complete their development). Although most pupal workers laid by this particular queen were parthenogenetic, most of the few adult workers were sexually produced. In clonal populations, we thus observed a clearly disproportionate allocation of individuals between castes depending on production mode, together with a high parthenogenetic worker mortality during development.

Regarding sexual populations, we found that gynes and workers directly sampled within two field populations were genetically different, as evidenced by low but significantly different from zero F_{ST} values computed overall microsatellite loci (population M7: $F_{ST}=0.017\pm0.005$, $P=0.006$; population M3-F: $F_{ST}=0.026\pm0.005$, $P=0.006$). Single locus F_{ST} values were significantly different at four (M3-F) and six (M7) loci, with only one locus showing significant differences in common for both populations.

Discussion

This study provides, to the best of our knowledge, the first direct experimental evidence of the co-occurrence of thelytokous parthenogenesis and male clonality within some populations of *W. auropunctata* (that is, 'clonal' populations). Thelytokous parthenogenesis is relatively common in insects (Normark, 2003), and has already been observed in seven ant species (see references in the study by Himler *et al*., 2009). On the contrary, the male clonality system demonstrated here is extremely rare (McKone and Halpern, 2003). Alternative variants of androgenesis have only been evidenced twice in insects and four times in all lifeforms (in *Vollenhovia emeryi*, Ohkawara *et al*., 2006; in *Bacillus* stick insects, Mantovani *et al*., 2001; in *Corbicula* clams, Komaru *et al*., 1998; and in the desert tree *Cupressus dupreziana*, Pichot *et al*., 2001). In these cases, androgenesis occurred through a variety of mechanisms, such as through the syngamy of two spermatozoa in *Bacillus* (Tinti and Scali, 1996), or unreduced triploid spermatozoa in *Corbicula* (Komaru *et al*., 1998). The androgenetic system found in *W. auropunctata* is original in that clonal males remain haploid (as measured by flow cytometry; Fournier *et al*., 2005a) and that workers are still produced through standard sexuality. Although sterile, workers are crucial to the fitness of queens and males. Interestingly enough, the joint occurrence of thelytoky and androgenesis in queens and males would theoretically avoid inbreeding depression and enable specific worker genomic combinations to be maintained over time. This system might enable workers to experience an increased niche breadth, as suggested for other ant species displaying separate male and female gene pools, through different hybridization mechanisms (Umphrey, 2006; Anderson *et al*., 2008b).

A particular finding, not to be overlooked, is the rare production of sexual gynes and arrhenotokous males in otherwise clonal populations. This result echoes indirect evidence obtained from an extensive population genetic survey of *W. auropunctata* in New Caledonia (Foucaud *et al*., 2006). Even at low frequency, these events are most likely of evolutionary importance, especially in the case of geographically isolated invasive populations such as those in New Caledonia. Such rare events might indeed disrupt the putative advantages of clonality by increasing genetic drift, inbreeding depression and diploid male load, and by breaking genomic combination potentially adapted to the new invaded environments (Foucaud *et al*., 2006).

Our experimental results do not permit to favor any current hypothesis on the mechanisms of the clonal reproduction system. We found low rates of single locus homogenization during female parthenogenesis (single locus rates ranging from 0 to 1.64%). Although estimated from a relatively low number of individuals (from 1 to 61 individuals depending on the locus), such low rate values favor an apomictic mechanism associated with rare gene conversion events rather than automixis with central fusion (our 12 microsatellite loci are indeed unlikely to be all close to centromeres). This result echoes that found in the study on *Platythyrea punctata* (Schilder *et al*., 1999), but contrasts with the automixis mechanism inferred on another thelytokous ant species, *Cataglyphis cursor* (Pearcy *et al*., 2006) and to the Cape honeybee (Baudry *et al*., 2004; Oldroyd *et al*., 2008). Our results regarding the male clonality system do not allow to discard either of the two current mechanical hypothesis (that is, 'maternal genome elimination' and 'anucleate ovules'; Fournier *et al*., 2005a; Foucaud *et al*., 2007). We are currently running some reciprocal crossing experiments using sexual and clonal males and females to investigate this issue. Cytological investigations of *W. auropunctata* gametogenesis are also required to get a clearer view on the mechanisms involved in both clonal reproduction systems. Beside the above mechanical issues, *W. auropunctata* represents a promising biological model to unravel the genetic bases of male clonality and thelytokous parthenogenesis using large-scale genomic tools.

Our 30 isolated virgin queens originating from clonal populations did not produce any pupa of worker, queen or male. This result suggests that the parthenogenetic production of queens may require mating to occur. Alternative hypotheses, such as the age of the gynes, the resources intake or the necessity of flying cannot be discarded, but seem, however, unlikely due to our experimental procedure and previous observations (see Materials and methods section for details). Some (alated) gynes produced a large amount of eggs indicating that dealation is not necessary to lay eggs as in *Lasius niger*

queens (Jemielity *et al*., 2006). None of those eggs could reach the pupal stage, however, indicating a developmental rather than a resource issue. Consequently, unmated gynes from clonal populations seem unable to directly benefit from thelytokous parthenogenesis to achieve some reproductive success.

This experimental study provides direct evidence that a standard sexual reproduction system is fully effective in some other *W auropunctata* populations (that is, the so-called sexual populations). We therefore unambiguously confirmed the polymorphism of reproduction system inferred indirectly from previous genetic studies on *W. auropunctata* population samples taken in the field at the same locations (Fournier *et al*., 2005a; Foucaud *et al*., 2006, 2007). In other species showing such a reproduction system polymorphism, the balance between the sexual and clonal reproduction system is usually best explained by environmental conditions, whether abiotic (for example, seasonal climatic variation in *Myzus persicae*; Vorburger *et al*., 2003) or biotic (for example, prevalence of parasites; Lively *et al*., 1990). As *W. auropunctata* is an invasive species found in both natural and human-altered habitats (Tennant, 1994; Wetterer and Porter, 2003; Orivel *et al*., 2009), it would be worth studying the ecology of clonal and sexual populations to gain insight into both the evolution of reproduction systems diversity and of invasiveness.

Our experimental laboratory study provides evidence for a genetic basis for caste determination in *W. auropunctata*, at least in clonal populations. Genetic caste determination (most notably in dependent lineages of *Pogonomyrmex*; Julian *et al*., 2002; Volny and Gordon, 2002; Helms Cahan *et al*., 2004; Schwander *et al*., 2007a), or at least some genetic influence on caste fate (Fraser *et al*., 2000; Hughes *et al*., 2003), have been demonstrated several times in Formicidae (for review, see Anderson *et al*., 2008a). In the case of the clonal populations of *W. auropunctata*, only 1.9% of all *W. auropunctata* adult females did not comply with expectations based on a purely genetic determination of caste (one adult queen on 52 sampled and two adult workers on 104 sampled). This figure is similar to the observed proportion of *Pogonomyrmex* adult females not complying with a purely genetic caste determination (1.9%; three adult queens on 80 sampled and zero adult worker on 75 sampled; Helms Cahan and Keller, 2003). Alternatively to genetic caste determination, one could also consider that these results are the consequence of a cyclical parthenogenesis mechanism with some environmental conditions favoring the development of queens or workers only. However, all *W. auropunctata* workers and gynes were sampled and hence produced simultaneously in each individual laboratory nest. The occurrence of both parthenogenetic and sexual worker pupae in the only nest in which thelytokous adult workers were sampled also confirms that clonal and sexual eggs are produced simultaneously by parthenogenetic queens. As a consequence, a cyclical parthenogenesis mechanism seems highly unlikely to account for the observed partition of genotypes in each caste. As we showed here, certain types of genotypes are largely constrained in their development and caste fate, and thus caste determination can be considered to be genetically hardwired in *W. auropunctata*. The correlation between genotype and caste observed in clonal populations was also indirectly detected, although much less clearly, in sexual populations, in which we observed a low but significant genetic differentiation between gynes and workers. No cyclical parthenogenesis mechanism could account for this latter result because all gynes and workers are sexually produced. One explanation could be that queens undergo multiple matings and that different patrilines show a bias in which female caste they produced, as inferred for *Pogonomyrmex badius* (Smith *et al*., 2008), *Acromyrmex echinatior* (Hughes *et al*., 2003) or *Pogonomyrmex rugosus* (Schwander and Keller, 2008). However, our results clearly indicate that *W. auropunctata* queens mate only once. An alternative explanation could be that, at least in sexual populations, different queens invest differently in queen and worker castes (that is, reproductive skew), as inferred for many ant species, such as *Formica exsecta* (Kummerli and Keller, 2007) or *Formica fusca* (Bargum and Sundstrom, 2007). This hypothesis deserves further investigations.

It is worth noting that strong genetic effects over caste determination in social Hymenoptera (see Anderson *et al*., 2008a) has only been demonstrated in species displaying unusual breeding patterns, which prevented normal segregation of genetic markers. This includes hybridization in the *P. rugosus–P. barbatus* complex (Anderson *et al*., 2006; Schwander *et al*., 2007a, b) and in the *Solenopsis geminata* × *S. xyloni* complex (Helms Cahan and Vinson, 2003), parthenogenesis in *Vollenhovia emeryi* (Ohkawara *et al*., 2006) and in *W. auropunctata* (this study). This feature might actually simply reflect the fact that microsatellite markers are traditionally used in studies dealing with reproduction systems. For *W. auropunctata* as for other species displaying 'strong' genetic caste determination (GCD; see Anderson *et al*., 2008a), hybridization or thelytokous parthenogenesis, in preventing gene flow between the male and female gene pools, necessarily linked all female genes (including the one(s) responsible for GCD) to a large part of microsatellite alleles at each locus, hence the 'strong' signal of GCD (and its detection even using low numbers of microsatellite loci randomly located on the genome; Ohkawara *et al*., 2006; Schwander *et al*., 2007a). In contrast, other breeding systems that include sexual recombination of male and female gene pools necessarily lead to 'weaker' correlations between microsatellite genotypes and caste (as in *W. auropunctata* sexual populations, or in so-called 'weak' GCD species, Anderson *et al*., 2008a). Thus, these 'strength' differences in GCD may only reflect different breeding systems and not necessarily different mechanisms of caste determination, which could be under the control of various amounts of genes (see Anderson *et al*., 2008a). In the case of *W. auropunctata*, there is hence no evidence that the GCD could be 'weaker' or different in sexual compared with clonal populations (sexual populations are most probably ancestral to clonal ones; Foucaud *et al*., 2007). This study as well as previous ones advocate for specific studies of caste determination in social Hymenoptera using appropriate large-scale genomic tools (for example, AFLP), especially in non-hybrid, sexually reproducing species.

Together with recent studies (for example, Matsuura *et al*., 2009), our study suggests that eusocial insect species displaying mixed modes of reproduction associated with different castes are likely to help us

understand the pros and cons of sexual and asexual reproduction systems, and unravel the basis of caste determination.

Conflict of interest

The authors declare no conflict of interest.

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ANNEXE 3:

ARTICLE 8: Anthropogenically-Induced Adaptation to Invade (AIAI): Contemporary adaptation to human-altered habitats within the native range can promote invasions

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PERSPECTIVE

Anthropogenically induced adaptation to invade (AIAI): contemporary adaptation to human-altered habitats within the native range can promote invasions

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Abstract

Adaptive evolution is currently accepted as playing a significant role in biological invasions. Adaptations relevant to invasions are typically thought to occur either recently within the introduced range, as an evolutionary response to novel selection regimes, or within the native range, because of long-term adaptation to the local environment. We propose that recent adaptation within the native range, in particular adaptations to human-altered habitat, could also contribute to the evolution of invasive populations. Populations adapted to human-altered habitats in the native range are likely to increase in abundance within areas frequented by humans and associated with human transport mechanisms, thus enhancing the likelihood of transport to a novel range. Given that habitats are altered by humans in similar ways worldwide, as evidenced by global environmental homogenization, propagules from populations adapted to human-altered habitats in the native range should perform well within similarly human-altered habitats in the novel range. We label this scenario 'Anthropogenically Induced Adaptation to Invade'. We illustrate how it differs from other evolutionary processes that may occur during invasions, and how it can help explain accelerating rates of invasions.

Introduction

The increasing rate at which species are invading new ranges is fundamentally linked to the expansion of international trade (Carlton and Geller 1993; Cohen and Carlton 1998; Levine and D'Antonio 2003; Pysek et al. 2010). Policies have been implemented to minimize new introductions *via* trade (McAusland and Costello 2004; Olson and Roy 2010), yet rates of invasion continue to increase, suggesting that other additional processes might play a role. We propose that human alteration of habitats within the native range induce evolutionary changes that could promote invasion into novel ranges. We employ a broad definition of invasion, encompassing successful establishment and spread in a new range with or without particular environmental or economic impacts.

The facilitating role of evolution in invasions, particularly rapid adaptive evolution during invasions, has recently become a major subject of research (e.g., Carroll and Dingle 1996; Reznick and Ghalambor 2001; Lee 2002; Lambrinos 2004; Wares et al. 2005; Facon et al. 2006; Prentis et al. 2008). Much of this research has a temporal and geographic focus on evolutionary shifts that occur following introduction into a (usually remote) new location, rather than a focus on evolution within the native

range. This perspective is logical, given that introduction into a new environment is likely to impose a novel selection regime, making rapid evolution probable. Striking examples of evolution following introduction include reduced size at reproduction in fish (Bohn et al. 2004), increased size or reproductive capacity of invasive plants (e.g., Blair and Wolfe 2004), rapid evolution of physiologic tolerance to fresh water (Lee et al. 2003, 2011), and increased dispersal distance in toads (Phillips et al. 2006; Phillips et al. 2010).

However, evolution within the native range, prior to introduction to a remote and novel range, can also promote biological invasions (Di Castri 1989; Lee and Gelembiuk 2008). This process has been referred to as 'preadaptation', in the invasion literature. However, the term preadaptation already has a widely recognized and well-established meaning in the evolutionary literature, that of exaptation (Bock 1959; Gould and Vrba 1982). In Box 1, we discuss the two different meanings of preadaptation and how they each might contribute to invasion. To differentiate between the two meanings, we use the terms 'exaptation' and 'prior adaptation' (Box 1), where prior adaptation is simply evolution of traits in the native range, prior to introduction to a new range, that enhance success of introduced populations without a change in function. Both exaptation and prior adaptation have the potential to promote successful invasion, but prior adaptation is likely to be more important.

Box 1: Preadaptation: exapatation and prior adaptation

Evolution within the native range can lead to traits that confer higher fitness, i.e., are adaptive, within a novel habitat. Generally, this has been called 'preadaptation'. The term encompasses two distinct processes, however: exaptation and what we call here prior adaptation. Exaptation occurs when a trait that has evolved under one selection regime is co-opted by chance for a different function (Bock 1959; Gould and Vrba 1982; see also Grant 1977; Futuyma 2005). This is the original meaning of the term preadaptation, from the evolutionary biology literature. The classic example of exaptation is feathers in dinosaurs. Their original function is thought to have been thermoregulation, and then they were co-opted for use in movement and eventual flight. While we know of no clear example from invasion biology whereby a trait acquired a truly new adaptive function that enhanced its invasiveness in the new range, theoretically it is possible. Thus, exaptation constitutes one mechanism by which traits could evolve in the native range that would facilitate invasion of a novel environment.

To avoid confusion with exaptation, we prefer to distinguish this second meaning of preadaptation as 'prior adaptation'. Prior adaptation denotes the case in which adaptation to one or more facets of the environment within the native range facilitates invasions to similar environments in the novel range (Parker and Gilbert 2004; Dietz and Edwards 2006; Bossdorf et al. 2008; Fausch 2008; Treier et al. 2009). Thus, with prior adaptation, there is not a change in function as there is with exaptation. Prior adaptations can be associated with an evolutionary history in fluctuating environments in the native range (Lee and Gelembiuk 2008), which might select for organismal flexibility or evolvability, both of which could facilitate invasion into a wide range of habitats. This appears to be the case in the copepod *Eurytemora affinis* Lee et al. (2003).

Alternatively, prior adaptation can occur via local adaptation, which can facilitate the founding and spread of new populations if those populations happen to be introduced to a region with a similar environment, and thus, traits that conferred high fitness in the native environment do so in the novel environment as well (Sax and Brown 2000; Blumenthal 2006; Dietz and Edwards 2006). One example of this mechanism appears to be found in *Senecio inaequidens* (Bossdorf et al. 2008; Lachmuth et al. 2010). Within its native southern African range, it is able to use a variety of habitats, while in parts of the introduced range in Europe this species invades rocky railroad tracks and motorways. Common garden comparisons reveal that invasive populations are phenotypically most similar to native populations originating from rocky slopes and dry riverbeds of mountainous regions in Southern Africa. Bossdorf et al. (2008) thus hypothesize that populations in the native range found on (and presumably adapted to) rocky slopes are the source of the populations in Europe invading, which have prior adaptations to similarly disturbed and open environments of the introduced range.

We introduce here another mechanism leading to prior adaptation called Anthropogenically Induced Adaptation to Invade (AIAI), which we detail in the main text. Fundamentally, AIAI begins with local adaptation, but rather than adaptation being to the native habitat, it is to new human-altered habitat. Thus, other forms of prior adaptation facilitate invasion when traits that are adaptive in the native range are, essentially by chance, adaptive in the introduced range as well. In contrast, because AIAI starts with adaptation to human-altered environments, it directly facilitates invasions into human-altered environments. As such, it may contribute to ever increasing rates of invasion.

Either local adaptation to a stable environment or particular disturbance regime and an evolutionary history in fluctuating environments (Lee and Gelembiuk 2008) can lead to prior adaptation to a novel environment (Box 1). The evolutionary processes leading to local adaptation to native environments can span many generations and often act over the long-term evolutionary history of the species within its native range and continue up to the present day. These longstanding evolutionary processes in the native range cannot fully help us understand the ever-increasing rate of biological invasions, because contemporary invasive populations are increasingly facing anthropogenic change within their native ranges, often marked by sudden, dramatic, and episodic impacts. Such impacts impose selection for new adaptative states that may create populations within the native ranges that are primed to become invasive. Thus, we argue here that anthropogenic change introduces a unique set of circumstances that warrants a separate category.

We propose that contemporary adaptation to human-altered environments within the native range is a central means by which prior adaptations to invasion evolve. Because the rate at which humans alter environments is increasing, the process we outline might aid in understanding mechanisms underlying the ever-increasing rate of invasions. We call this process Anthopogenically Induced Adaptation to Invade (AIAI). AIAI is summarized in Fig. 1. Briefly, species are exposed to human-altered habitats in their native range, and commonly become adapted to those habitats. This process leads to an increase in abundance within close proximity to human transportation systems, increasing the likelihood that they will be transported to a new range. Furthermore, the very adaptations that confer advantages within the human-altered habitat in the native range will also confer advantages in remote, similarly-altered habitats, facilitating successful establishment of new populations and subsequent invasions.

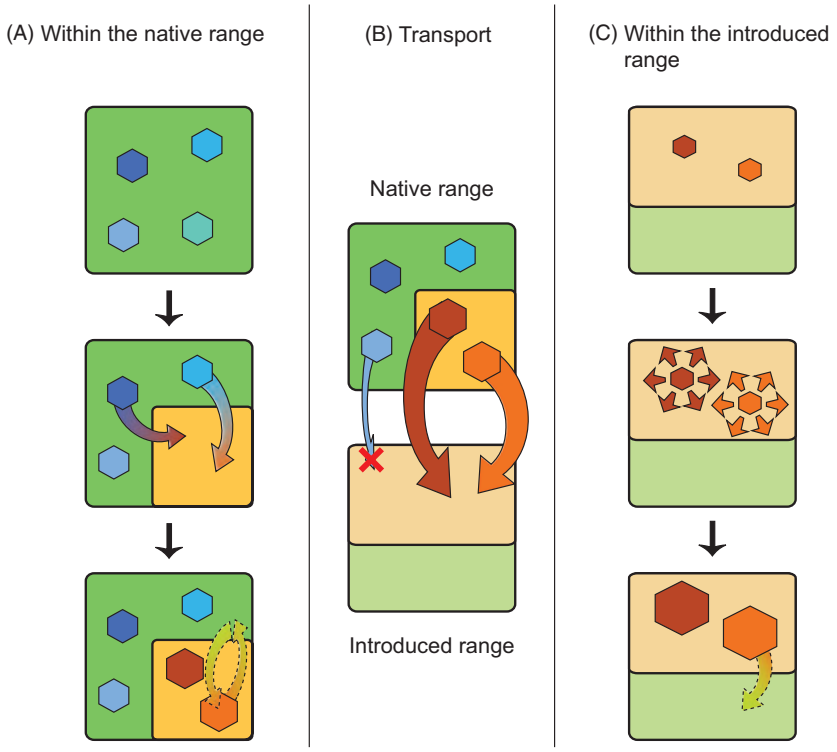


Figure 1 Schematic representation of the AIAI scenario. (A) Within the natural habitats (in green) of the native range, local populations (blue hexagons) are exposed to human-altered habitat (in light orange). Some populations adapt to this new type of habitat becoming either generalists able to use both habitat types, or specialists on the human-altered habitat (orange and brown hexagon, respectively; see Box 2). Generalist populations are more likely to have substantial flow of movement and genes across habitat boundaries (dashed arrow). (B) Most long-distance transport happens between two human-altered habitats (hence the large arrows). The presence of adapted populations in the human-altered habitat of the native range results in increased transport probability and a diminished need for further adaptation in the human-altered habitat of the introduction range. In contrast, populations from natural habitats of the native range are expected to suffer both from rare introduction events and lack of necessary adaptations to start a population in the introduced range (red cross). C. The introduced populations are expected to invade rapidly, because of previous adaptation to a similar habitat. Generalist phenotypes may also further cross human-altered habitat boundaries (dashed arrow) without the additional adaptations needed by specialist phenotypes.

Accounting for the effects of anthropogenic change on invasive success is critical because anthropogenic changes can fundamentally alter the rate of invasions and the type of species that are likely to invade. AIAI represents a unique series of ecological and evolutionary processes in the native and then introduced range. First, the adaptation is to human-altered habitats in particular, rather than to natural environments. Second, anthropogenic change fundamentally alters the landscape nearly instantaneously, such that the evolutionary response that enhances subsequent invasion is strictly concurrent with the anthropogenic change rather than a longstanding response occurring over historic and contemporary time scales. Third, an increase in prevalence of human-altered habitat increases the likelihood of adaptation and transport to a new range. As such, we argue that the conceptual framework of AIAI is important to distinguish from other types of evolutionary change in the native range because it fills in an important gap in our understanding of factors that promote invasive success and helps us recognize and understand the rapidity and increasing pace of biological invasions. It also highlights that contemporary adaptation facilitating invasions is not restricted to the introduced ranges of invasive species.

In support of our ideas, it has long been noted that adaptation to human-altered habitats occurs (Wet and Harlan 1975). Additionally, Crosby (1986) and Di Castri (1989) suggested that the dominance of European species among invaders might be due to their longstanding association with human disturbances. Jeschke and Strayer (2006) found that affiliation with humans per se (not just the increased propagule pressure that comes with such an affiliation) is associated with increased success of invasion. Additionally, in a recent study, Foucaud et al. (2009) introduced a process similar to AIAI, calling it a 'two-step' invasion, without detailing the evolutionary factors at play, the expected phenotypic outcomes and the general implications for biological invasions. To fill in these gaps and develop this idea more fully, we (i) describe the AIAI process in detail and review some basic evolutionary principles underlying it, (ii) outline the evidence necessary to illustrate it and present several systems that are candidates for AIAI, and (iii) conclude by further discussing its importance.

Anthropogenically induced adaptation to invade

As is well documented, humans are altering the environment at an increasing pace, driven by many factors (Sala et al. 2000; Daily et al. 2001; Pereira et al. 2004; Scharlemann et al. 2004; Jetz et al. 2007). Indeed, the most common and widespread environmental perturbations today are those caused by humans. Similar types of alterations can be found on different continents. Many of the same agricultural crops grown in the same fundamental ways are found essentially worldwide. For example, maize culture and other cropping systems offer a relatively homogeneous habitat throughout the world, from Africa to Asia, North and South America and Europe (Anonymous 1993). Likewise, forests are harvested, forest edges are created, roadsides are mown, and nutrients and other pollutants are added to terrestrial and aquatic ecosystems in a similar manner on different continents. Indeed, there is general agreement that habitats and biota are becoming more homogeneous worldwide (McKinney and Lockwood 1999; Tilman et al. 2001; Olden et al. 2004).

These newly altered habitats represent novel environments that impose strongly altered selection regimes. They might select for a wide variety of traits including an increased or altered host range of parasites (sensu Price 1980), increased tolerance to physiologic stressors (such as reduced humidity associated with edge effects) or a faster 'r-selected' life history. The potential for adaptive evolution in response to the novel selection regime is likely to be high within the native range because of greater effective population sizes, genetic variation, and propagule pressure than might be expected in the introduced range. Box 2 provides additional theoretic background for these processes. In particular, it details when an outcome of adaptation to human-altered habitats within the native range is likely, and under what conditions local adaptation to only the novel human-altered habitat is expected (the evolution of habitat specialists), and under what conditions adaptation of high performance in both the natural and the human-altered habitat is expected (the evolution of habitat generalists). Populations that adapt, either as specialists or generalists, to human-altered habitats within the native range, may increase in size or become more abundant in those habitats (Kawecki 2008). Human alteration of habitat is typically associated with human transportation systems. Thus, when a species becomes abundant in human-altered habitats, the likelihood that propagules will be taken up by various modes of long-distance transportation will increase (Lockwood et al. 2007). This will favor the species reaching a new range in numbers substantial enough to establish a new (invasive) population. It is well known that many introduced species are associated with agriculture (including both cropping systems and rangeland) and urbanization, both because they increase resource availability (or fluctuation in resource ability; Davis et al. 2000) and are associated with high propagule pressure (Lockwood et al. 2007). We argue that, in addition, many of these species may have adapted to anthropogenic modifications in the native range prior to introduction to a novel range. Given the global nature of

human–habitat alterations, the likelihood that similar human-altered habitat will be found in the region of introduction is increased and the adaptations from the human-altered native range should be advantageous in similarly altered habitats in the introduced range. These newly introduced species themselves will then contribute to the increased homogenization of habitats worldwide (Mack et al. 2000), which can then further facilitate additional invasions (Simberloff and Von Holle 1999).

Trade-offs in the ability to locally adapt are central to the theory of adaptation to heterogeneous environments. Trade-offs are constraints on the set of possible fitness values (also called fitness sets, Levins 1968) such that when at the optimum in the natural habitat, better adaptation to human-altered conditions translates to a loss of adaptation to natural conditions. Figure 1 of Box 2 presents the local fitness sets for the natural (black curves)

Box 2: Factors affecting the potential for adaptation to human-altered habitats within the native range and outcomes expected in term of life-history strategies

Anthropogenically induced adaptation to invade occurs in a transition zone between natural and human-altered habitats within the native range; thus, ample genetic variation can be maintained more easily than in populations introduced to a new range. In such a setting, adaptation to human-altered habitats is not always possible. When adaptation does occur, it can lead to habitat generalists or habitat specialists (or both, Abrams 2006). Here, we provide readers with the fundamental theory underlying which outcome is expected.

From a management perspective, whether a generalist or specialist evolves can be important. Generalists may be slow to establish initially, but might readily invade habitats within their new range that are not human-altered. Specialists on the human-altered habitat, in contrasts, may immediately exhibit high population growth rates upon introduction to comparable human-altered habitats in a new range, but their spread from those habitats may be constrained.

Adaptation to human-altered habitats not possible

A population might fail to adapt to the newly available human-altered habitat for three main reasons. The first reason is lack of adequate genetic variation. For instance, habitat alteration might be so drastic that the variation required to adapt is simply not available. Second, adaptation may not occur if there is too much migration from the natural habitat to the new human-altered one, hindering adaptation to the new conditions (underlying this pattern are gene swamping, as well as hard selection leading to differences in the number of individuals produced and subsequent migrational meltdown; Dempster 1955; Kawecki 2000; Ronce and Kirkpatrick 2001; Lenormand 2002; Travis et al. 2005; Bridle and Vines 2007; Kawecki 2008; Ravigné et al. 2009).

The third reason for failure to adapt to human-altered habitat is because of selective processes, that lead the population to remain 'trapped' around the source optimum (i.e., that of the natural habitat). This can happen if the traits under selection evolve through small mutation steps (as expected when such traits are determined by many loci of small effect) and the trade-off between adaptation to both habitats is very strong (Holt and Gaines 1992; Kawecki 2000; Ronce and Kirkpatrick 2001; Rueffler et al. 2004; Ravigné et al. 2009). Technically, the trade-off curve is said to be convex (Fig. 1A). In this case, the adaptive valley that separates both optima is so steep that intermediate evolutionary steps are strongly selected against.

Adaptation to human-altered habitats possible producing either generalists or specialists

Generalist phenotype

A generalist phenotype is expected to evolve when the trade-off between adaptation to both habitats is weak, so that the fitness of the intermediate phenotype is greater than the mean fitness of any mixture of specialists (e.g., Levins 1968; Brown 1990; van Tienderen 1991, 1997; Wilson and Yoshimura 1994; Egas et al. 2004; Rueffler et al. 2004; Ravigné et al. 2009). Technically, the trade-off curve is said to be concave (Fig. 1B). This outcome is favored by a combination of high migration rate and small mutational effects, two conditions that tend to hamper the differentiation between habitats (Kawecki 2000, 2004, 2008; Ronce and Kirkpatrick 2001). In many instances, generalist species, although less fit than habitat specialists within their preferred habitats, may be fit enough to be invasive [e.g., potentially exhibiting 'jack-of-all-trades' phenotypic plasticity sensu Richards et al. (2006)]. This is expected when trade-offs

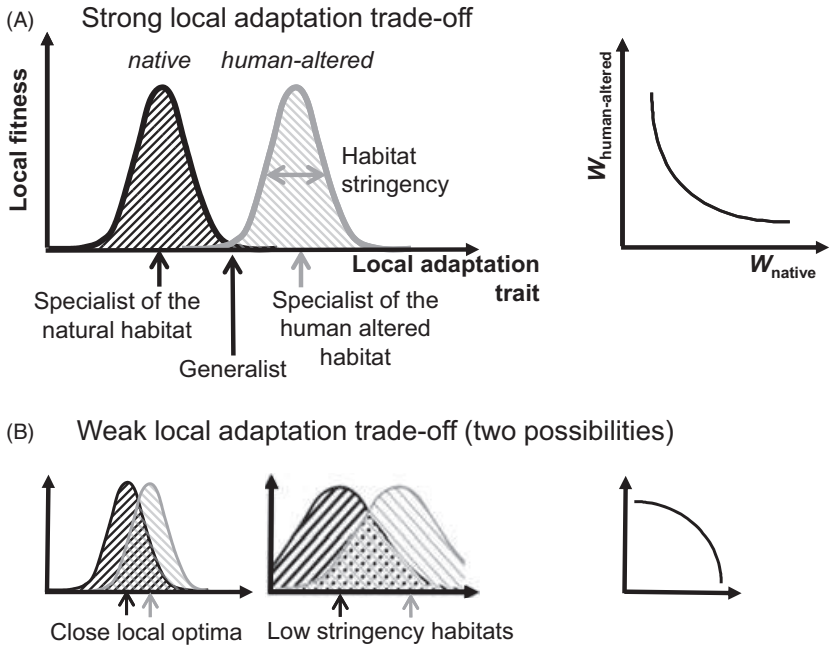
are weak or when habitats are subject to important temporal variability under which a generalist strategy may be advantageous.

Specialist phenotype

A true specialist of the human-altered habitat, with high fitness (and thus particularly high potential for invasiveness upon introduction to similarly human-altered habitats) will tend to emerge more readily when migration is low and the traits under selection evolve through large mutation steps (few loci with large effects) (e.g., Levene 1953; Dempster 1955; Maynard Smith 1966; Hedrick 1990; Kawecki 2008). In contrast, if the traits under selection evolve through small mutation steps (many loci with small effects), then adapting to the human-altered habitat additionally requires a moderate trade-off in adaptation to both habitats (slightly convex trade-off curve) as well as some independent density-regulation in both habitats (i.e., soft selection; e.g., van Tienderen 1997; Kisdi and Geritz 1999; Ronce and Kirkpatrick 2001; Ravigné et al. 2009).

Complex theory and missing data

From a theoretical perspective, the effects of all factors cited earlier on the outcome of adaptation to a new habitat are now widely documented in a vast number of models, only a small subset of which was cited here. It has now become quite clear that no single factor by itself can determine the outcome. For instance, a weak trade-off may select for either specialization or generalization depending on the level of migration and the genetic architecture of traits underlying adaptation. Understanding these factors as deeply as possible will aid in predicting the risk that invasive populations emerge through adaptation to human-altered habitats. Although some factors may be very tricky to document (e.g., trade-off strength or the mode of density-regulation), others, though not trivial, may be feasible to estimate (e.g., habitat frequencies, the existence of a strong dissymmetry in habitat productivities, sexual vs. asexual mating system, whether adaptation is likely to evolve through small or large mutation steps). To improve our ability to forecast and to prevent biological invasions, better integration of empirical and theoretical research is hence much needed. Future models should explore more thoroughly the relative importance of the various factors at play, and how they interact (e.g., Kawecki 1994, 1996 for another view on trade-offs), while empirical studies should explicitly measure those factors already agreed to be critical in determining outcomes.



Box Figure 1. Local adaptation trade-offs (See Box 2 text for details)

and human-altered (gray curves) habitats. Narrow fitness curves lead to highly stringent habitats (gray horizontal arrow) in which only a narrow range of trait values produces individuals with high relative fitness. Wide fitness curves produce less stringent habitats in which a wide range of trait values can produce individuals with high relative fitness. (i) Strong trade-offs in the ability to locally adapt. The generalist phenotype, which is intermediate between both specialist phenotypes, has low fitness in both habitats. A strong trade-off (also called a convex trade-off) tends to hamper adaptation to the conditions in human-altered habitats if mutation effects are small. In contrast, moderately strong trade-offs may favor the emergence of specialists. (ii) Weak trade-offs with the ability to locally adapt. Weak trade-offs (also called concave trade-offs) may exist if the optimum trait values for both natural and human-altered habitat are close or if habitat stringencies are low (or any combination of the two). Weak trade-offs enable the generalist phenotype to achieve good fitness in both habitats. Weak trade-offs may favor the evolution of a generalist population when migration is high or specialists when migration is low enough for differentiation to occur.

Thus, the combination of high evolutionary potential and strong selection in human-altered habitats within the native range is likely to lead to rapid adaptation prior to introduction elsewhere, and furthermore to increase the probability of successful introduction into a novel location. Upon introduction to a new location, such altered populations are indeed likely to perform well, particularly when introduced to habitats affected by the environmental homogenization occurring globally. It is worth pointing out that the AIAI scenario is evolutionarily parsimonious because rather than requiring that rapid adaptive evolution occur multiple times when organisms are introduced to multiple different places around the globe, the critical adaptations need to evolve only once. At the same time, however, given that human-altered habitats are often created in multiple places within one species' range, there is the opportunity for different populations of a species to adapt separately to those habitats. Finally, the AIAI process by no means precludes either continued adaptive evolution in the new range (e.g., Blair and Wolfe 2004) or a role for hybridization and outcrossing in the new range (Kolbe et al. 2004; Lavergne and Molofsky 2007; Facon et al. 2008).

The necessary evidence

Documenting the AIAI scenario is not a trivial task and requires both ecological and genetic approaches. Briefly, it should be demonstrated that contemporary adaptive evolution has occurred within the native range, and that it leads to native populations with higher fitness in a new, human-altered habitat relative to a naïve native population that has not experienced that habitat. This adaptive evolution would also need to confer higher fitness than a naïve native population would have upon introduction to the new range. Additionally, data from neutral genetic markers, appropriately analyzed (Keller and Taylor 2008; Estoup and Guillemaud 2010), should provide evidence that the populations from human-altered habitat within the native range were the actual source for populations found in human-altered habitat in the introduced range. Table 1 outlines in detail evidence required for unambiguous support for this process.

Table 1. Evidence required to support conclusively the anthropogenically induced adaptation to invade scenario.

	Evidence needed
Native range	
Habitat	Documentation that the species is in a habitat that is human-altered relative to historical habitat of species Altered habitat presents a known and measurable challenge (e.g., change in salinity)
Organism	Quantitative genetic evidence that the population within the altered habitat has adapted in response to anthropogenic change
Population genetic structure	Populations are structured at neutral loci within the native range, making it possible to identify areas of origin of the invasive populations
Introduced range	
Habitat	Habitat documented to be similar to the altered habitat within the native range (e.g. comparable salinity)
Organism	Evidence that introduced populations grew to large size in the human-altered habitat similar to native-range human-altered habitat. Quantitative genetic evidence that the introduced populations show similar adaptations to those found for native populations within the altered habitat.
Population genetic structure	Evidence that introduced populations originated directly (primary introduction) or indirectly (secondary intro duction) from population(s) located in the human altered habitat within the native range.

Candidate systems

We are not aware of any study system for which robust and complete data sets supporting each separate point from Table 1 are available. At this point in time, some of the most clear-cut examples of adaptation to human-altered habitat occurring prior to invasion come from crop pests. One likely example is *Leptinotarsa decemlineata*, the Colorado potato beetle, which is a pest of many solanaceous crops. Its original geographic distribution includes Mexico and parts of the western and central USA, and its original host range is thought to span only three species of the genus Solanum, *Solanum rostratum*, *S. angustifolium*, and *S. elaeagnifolium* (Forister et al. 2007). The human alteration of habitat comes in the form of potato farming. The potato, *Solanum tuberosum*, was introduced from South America into North America and Europe for intensive cropping in the 18th century (Glendinning 1983) and the beetle *L. decemlineata* started to use it as a host in the 1830s and 1840s within its native range (the central US). Subsequently, the beetle spread throughout both North America and Europe as a major pest. Evidence suggests that adaptation was involved in the use of potato as a host plant. Studies illustrate that potato, rather than the native hosts, is the most suitable host for US pest populations of *Leptinotarsa decelimneata* (Hare 1990). In contrast, potato elicits only weak oviposition and feeding in populations associated with the original hosts *S. angustifolium* and *S. elaeagnifolium* (Hsiao 1978, 1985; Harrison and Mitchell 1988; Lu and Logan 1994a,b,c). Furthermore, Forister et al. (2007) conducted quantitative genetic experiments showing genetic variation in many traits associated with host use, suggesting that a dietary shift itself might have evolved as a distinct trait in *L. decelimneata*. After the inclusion of potato in its diet, the beetle was hence able to spread far beyond its original geographic range, both to contiguous areas and to other continents where potatoes are grown, notably Europe, via human-aided long-distance dispersal.

As biological invasions are generally considered a contemporary phenomenon, most candidate systems have spread recently (e.g., neophytes and neozoa introduced to a new range within the last 2000 years). However, there were human-altered habitats much earlier than that, and thus, older examples also may fit this pattern. One possibility is the ascomycete *Mycosphaerella graminicola*, one of the most damaging fungal pathogens of wheat. Phylogeographic studies located at the center of origin of *M. graminicola* in the Middle East (Banke et al. 2004). In addition, using Bayesian inference on DNA sequence data, Stukenbrock et al. (2007) have provided evidence that the divergence between *M. graminicola* and its congeners (sampled on noncultivated grasses in the Middle East) occurred approximately 10 500 years ago, coincident with the beginning of agriculture and the domestication of wild grasses in the Fertile Crescent. The timing of divergence strongly suggests that *M. graminicola* originated from populations of pathogens associated with wild grasses that then adapted to wheat during its domestication. The invasive populations of *M. graminicola* are specific to wheat (Eyal et al. 1973, 1985; van Ginkel and Scharen 1987; Saadaoui 1987), and following their divergence, spread throughout the world on cultivated wheat crops.

The AIAI process is not restricted to agricultural pests. The little fire ant, *Wasmannia auropunctata,* is a species originating from Central and South America that has been successfully spreading over the World tropics and parts of the Mediterranean zone since the beginning of the last century (Wetterer and Porter 2003; Vonshak et al. 2009). As yet, the precise geographic origin of the introduced populations within the native range is still unknown, but one possibility is that introductions occurred in association with food products shipped from plantations to markets worldwide. In natural areas of its native range (primary forests), low density, mostly sexually reproducing populations are found. The human-altered habitat consists of forest edges and plantations. In these areas, the little fire ant occurs at high density and has become ecologically dominant (Orivel et al. 2009). A clear shift toward clonal reproduction is associated with the human-altered habitat (Foucaud et al. 2009), and that shift appears to be genetically based (Foucaud et al. 2010a). Additionally, populations in the human-altered habitat exhibit greater tolerance to stressful temperature and humidity conditions than populations from the natural forest habitats (J. Foucaud, O. Rey, A. Estoup, B. Facon, unpublished data). These life-history and physiologic changes appear likely to be adaptations to the human-altered habitat within the native range. Populations in the introduced range are most common in human-altered habitats, and are most similar with respect to life-history and physiology traits to populations in human-altered habitats in the native range; that is to say, they are clonal, and characterized by a high tolerance of stressful temperature and humidity conditions. Mikheyev and Mueller (2007) and Foucaud et al. (2010b) show that the main vector of *W. auropunctata* long-distance dispersal is human trade.

Animal and human diseases may also follow an AIAI scenario. Take, for example, AIDS, one of the most fatal infectious diseases facing humankind. Human immunodeficiency virus-type 1 (HIV-1) group M is responsible for the great majority of all HIV infections in humans and has infected more than 50 million individuals worldwide (Hahn et al. 2000). Current evidence indicates that HIV-1 moved to human hosts in west equatorial Africa, and arose via transmission from a simian lentivirus (SIVcpz)

infecting chimpanzees (*Pan troglodytes troglodytes*) (Keele et al. 2006). A fundamental part of most human-altered habitats is an increased density of humans themselves, which represents a large unused pool of potential hosts. Increased human population density may also have increased opportunities for contact with the original host and hence the likelihood of the initial transmission event. Molecular data show that the introduction of SIVcpz into humans, giving rise to HIV-1 group M, most likely occurred in the early part of the 20th century. It is thought that differences in selection pressures in the two hosts have led to differentiation of the viruses (Hahn et al. 2000), and that viral adaptation to the new human host contributed to the outbreak of AIDS as an epidemic by the 1980s (Worobey et al. 2008). In association with dense and highly mobile human populations (e.g., migrant workers) transmission rates would be high, favoring further adaptation leading to the evolution of increased virulence.

Conclusion and implications

We describe a mechanism promoting biological invasion that we label as AIAI. We argue that the combination of high evolutionary potential provided by high effective population size and strong novel selection imposed by human-altered habitats within the native range is likely to lead to rapid adaptation prior to introduction elsewhere, and simultaneously increased probability of introduction. Upon introduction to a new location, propagules adapted to human-altered habitats are likely to perform well, particularly when they are introduced into habitats that have been modified in a manner similar to that of their native range. This phenomenon is likely, given the environmental homogenization that is occurring globally. We argue that AIAI is fundamentally distinct from other mechanisms leading to prior adaptation primarily because the evolution is strictly contemporary rather than longstanding, and because the AIAI scenario emphasizes the central role of humans in imposing selective pressures within the native range and in enhancing dispersal via global trade.

Anthropogenically induced adaptation to invade is thus a contemporary phenomenon that is occurring at accelerated rates and is homogenizing the globe. As such, the AIAI paradigm sheds new insights into causes of biological invasions, as well as their ever-increasing pace. As noted, this scenario is evolutionarily parsimonious because rather than requiring rapid adaptive evolution with each introduction into a new location, the critical adaptations need only evolve once. With the AIAI scenario, the adaptive challenges are shifted to the native range where populations are less likely to have passed through bottlenecks, and variation in traits under selection is less likely to be limiting.

It is worth emphasizing, however, that once a population's invasion is facilitated by adaptation to human-altered habitats in the native range, including establishment within similar habitats in the novel range, continued ecological and evolutionary change might enable it to invade further into environments that are not strongly human-altered. As Box 2 illustrates, the outcome of evolutionary processes in the native range can either lead to habitat specialists or to habitat generalists. Upon introduction to a new range, specialists could evolve to use different habitats in the introduced range (either through evolution of a new specialist phenotype or a generalist phenotype). However, if a generalist phenotype invades, then it is likely to be immediately able to colonize further into the introduced range in environments that are not strongly human-altered, provided that there is not strong resistance from locally adapted species.

The AIAI scenario improves our understanding of some fundamental issues in invasion biology. First, it supports the idea that populations with 'invasive' behaviors (e.g. high densities or reproductive rates) can be found within native ranges (Valery et al. 2009) when they evolve to 'invade' human-altered habitats. Thus, exceptions to a strictly geographic (i.e. native/introduced) (Wilson et al. 2009) understanding of invasions may exist.

Second, AIAI may further elucidate the degree to which the Imperialist Dogma, the idea that there is a European bias to invasions (Crosby 1986; Di Castri 1989), might be true and when and why it might not be (Jeschke and Strayer 2006; Fridley 2008).

Third, the AIAI scenario provides yet an additional argument against the presumed paradox of invasion (Sax and Brown 2000; Frankham 2005; Hufbauer 2008) which suggests that adaptive evolution during invasion is constrained by low genetic variation, inbreeding and inbreeding depression. While bottlenecks can constrain evolution (e.g., Pujol and Pannell 2008), the opposite can also occur. It is now clear that reduction of variation at putatively neutral loci may not reflect variance available in quantitative genetic traits, and thus, sufficient variation may be available for adaptation even following bottlenecks (Van Buskirk and Willi 2006; Olivieri 2009). Also, even if individual groups of propagules have passed through bottlenecks, often multiple introductions can occur, which can maintain, or even increase, genetic variability in the introduced populations (Kolbe et al. 2004; Lavergne and Molofsky 2007; Roman and Darling 2007; Dlugosch and Hays 2008; Dlugosch and Parker 2008; Facon et al. 2008; Hufbauer 2008, Olivieri 2009). Bottlenecks may even be of a size that can actually purge genetic load leading to inbreeding depression (Facon et al. 2011). With the AIAI scenario, the adaptive challenges are shifted to the native range where populations are less likely to have passed

through bottlenecks, and variation in traits under selection is less likely to limit the rate and magnitude of adaptive evolution. Thus, the AIAI contributes to further resolving the initial paradox of biological invasion.

Finally, given the increase in human alteration of habitats worldwide, the AIAI scenario also may help explain why it is that rates of invasion continue to increase despite intensive efforts to prevent them. The ever-increasing alteration of natural habitats by human activities, which leads to contemporary adaptation of native populations to such altered habitats, should increase the likelihood both of being transported, and of being able to establish into similar human-altered habitats within a new geographic range.

Many species appear to conform to the AIAI scenario, requiring only a little additional evidence for verification. We hope that bringing this explicitly evolutionary perspective to how species adapt to human-altered environments in their native ranges, and how that can help us understand the increasing rates of invasion into human-altered environments in their introduced ranges, will motivate further studies to test for it.

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Résumé

Cette thèse vise à améliorer notre connaissance des processus évolutifs et écologiques liés aux invasions biologiques au travers de l'étude de populations envahissantes et non envahissantes de la petite fourmi de feu, *Wasmannia auropunctata*. Cette espèce présente un polymorphisme du système de reproduction original. Dans les populations ancestrales, les reines et les mâles se reproduisent selon le mode de reproduction sexué classique des hyménoptères (haplo-diploïde). Dans d'autres populations, les reines sont parthénogénétiques et les mâles sont produits de manière clonale via les œufs pondus par la reine. Ces reines et ces mâles produisent néanmoins des ouvrières stériles sexuellement. Ce mode de reproduction clonal semble associé indirectement au succès d'invasion des populations. Dans un premier temps nous avons identifié les mécanismes sous-jacents au système de reproduction des populations clonales. Nos résultats montrent que les reines utilisent la parthénogenèse automictique associée à une réduction du taux de recombinaison pour la production de reines, l'androgenèse pour la production des mâles et la reproduction sexuée pour la production d'ouvrières stériles. La fixation des génomes parentaux dans les descendances successives permet la reproduction entre individus d'une même cohorte en évitant la dépression de consanguinité dans la descendance ouvrière. Nous avons ensuite montré que le changement de système de reproduction de la sexualité vers la clonalité est associé à un changement adaptatif permettant aux ouvrières des populations clonales de mieux tolérer les températures stressantes caractéristiques des localités envahies, comparativement aux ouvrières des populations sexuées ancestrales. Enfin, l'utilisation d'une approche multidisciplinaire couplant des modèles de distribution d'espèces, des analyses de génétique des populations et des expériences en laboratoire, nous a permis de montrer que les changements évolutifs clefs associés au succès d'invasion des populations, ont lieu dans des habitats marginaux de l'aire native, avant la dispersion vers des localités distantes caractérisées par des conditions environnementales similaires.
Mots-clés : Invasion biologique, asexualité, parthénogenèse, androgenèse, milieu marginal, adaptation, thermotolérance, traits d'histoire de vie, marqueurs moléculaires

Abstract:

The main goal of this thesis is to provide new insights on the evolutionary processes associated to biological invasions through the study of invasive and non-invasive populations of the little fire ant, *W. auropunctata*. This species is characterised by an eccentric breeding system polymorphism. In ancestral populations, queens and males reproduce following the classical sexual reproduction system of hymenopteran species (haplo-diploid). In some other populations, queens reproduce by parthenogenesis and the males are reproduced clonally through queens' eggs. These clonal queens and males nevertheless produce sterile workers sexually. Interestingly this clonal reproduction seems indirectly associated with the invasive success of populations. In this study, we first identified the mechanisms underlying the breeding system of clonal populations. Our results indicate that queens use automictic parthenogenesis associated with a drastic reduction of meiotic recombination rate, androgenesis and sexual reproduction for the production of queens, males, and sterile workers respectively. The fixation of parental genomes in the successive generations allows individuals from the same cohort to reproduce together avoiding inbreeding depression in their worker offspring. We also found that the change of breeding system from sexuality to clonality is associated with an adaptive change that allow workers from clonal populations to better tolerate the stressing temperatures of invaded areas better than workers from ancestral sexual populations. Finally, we used a developed mutlidisciplinary approach combining niche modelling, genetic analyses and laboratory experiments, and found that the above evolutionary changes occur within the native range in marginal habitats prior to long-distance dispersal events into localities that display similar environmental conditions.
Keywords : Biological invasion, asexuality, parthenogenesis, androgenesis, marginal habitat, adaptation, thermotolerance, life history trait, molecular markers