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Bastien Quaglietti

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Bastien Quaglietti. Impact de la consanguinité et de l'hybridation chez quatre auxiliaires de lutte biologique. Sciences agricoles. Université Côte d'Azur, 2017. Français. NNT: 2017AZUR4048 . tel-01618119

HAL Id: tel-01618119

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École doctorale 85 Sciences de la Vie et de la Santé
Unité de recherche : Institut Sophia-Agrobiotech, UMR 1355

Thèse de doctorat

Présentée en vue de l'obtention du
grade de docteur en Sciences de l'
Université Côte d'Azur

par
Bastien Quaglietti

Impact de la consanguinité et de l'hybridation chez quatre auxiliaires de lutte biologique

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RESUME

La consanguinité et l'hybridation sont deux processus génétiques pouvant avoir des effets antagonistes dans les élevages d'auxiliaires de lutte biologique. L'hybridation peut minimiser les risques de dépression de consanguinité (diminution de fitness des individus consanguins), et favoriser l'apparition de phénotypes avantageux. Bien que les mécanismes de la dépression de consanguinité et des conséquences de l'hybridation soient bien connus, très peu d'études ont été réalisées dans le cadre de la lutte biologique. Il convient d'évaluer les effets réels de la consanguinité et de l'hybridation sur la fitness des auxiliaires pour orienter les pratiques de l'industrie de la lutte biologique.

Dans le cadre d'une collaboration public-privé, des données ont été produites sur les effets de la consanguinité chez les quatre auxiliaires *Allotropa burrelli*, *Chrysoperla near comanche*, *Cryptolaemus montrouzieri* et *Macrolophus pygmaeus*. La consanguinité a notamment provoqué une baisse de 30% du succès reproducteur chez *M. pygmaeus*. Une étude approfondie des conséquences de l'hybridation intra-spécifique a alors été réalisée en utilisant quatre populations de *M. pygmaeus*. Trois groupes génétiques séparés par un fort isolement reproducteur ont été mis en évidence. Néanmoins, un avantage de fitness pour les individus issus de croisements entre parents génétiquement distants a été mis en évidence au sein de chaque groupe génétique.

Ce travail de thèse apporte des données utilisables à court-terme par les entreprises partenaires. Il contribue par ailleurs à créer un corpus de données pour mieux évaluer l'importance réelle des effets génétiques dans les élevages d'auxiliaires.

Mots-clés : lutte biologique, collaboration public-privé, consanguinité, hybridation, recherche et développement

ABSTRACT

Inbreeding and hybridization are two genetic processes that may have antagonistic effects in biological control agents (BCAs) rearing. Hybridization can minimize the risk of inbreeding depression (decrease in fitness of inbred individuals), and favor the emergence of advantageous phenotypes. Although the mechanisms of inbreeding depression and the consequences of hybridization are well known, very few studies have been carried out in the context of biological control. The actual effects of inbreeding and hybridization on the fitness of BCAs should be assessed to guide the practices of the biocontrol industry.

In a public-private collaboration, data were generated on the effects of inbreeding in the four BCAs *Allotropa burrelli*, *Chrysoperla near comanche*, *Cryptolaemus montrouzieri* and *Macrolophus pygmaeus*. Inbreeding resulted in a 30% decrease in reproductive success in *M. pygmaeus*. A thorough study of the consequences of intra-specific hybridization was then carried out using four populations of *M. pygmaeus*. Three genetic groups separated by strong reproductive isolation were identified. Nevertheless, a fitness advantage for individuals from crosses between genetically distant parents has been highlighted within each genetic group.

This work brings data usable in the short term by the partner companies. It also helps to create a corpus of data to better evaluate the real importance of genetic effects in BCAs rearing.

Keywords: biological control, public-private collaboration, inbreeding, hybridization, research & development

REMERCIEMENTS

Je tiens tout d'abord à remercier **Thibaut Malausa** et **Xavier Fauvergue** de m'avoir offert l'opportunité d'effectuer cette thèse sous leur supervision. Leur encadrement pendant ces 4 années m'a permis de grandir scientifiquement, professionnellement, humainement. Merci à Thibaut pour sa disponibilité, son humour, sa patience et ses encouragements au quotidien. Je souhaite à tous les doctorants d'avoir un encadrant qui les valorise à ce point. Merci à Xavier pour sa bonne humeur également, sa grande disponibilité, et à ses soucis du détail pour toujours améliorer ce qui peut l'être.

Je remercie **Tolis Pekas** et **Felix Wäckers** de Biobest, **Oswaldo Farias** et **Paul Amouroux** de Xilema pour leur confiance et leur écoute.

Je remercie également **Jérôme Moreau**, **Alexandre Robert** et **Ferran Palero** d'avoir accepté de participer à mes trois comités de thèse. Leurs nombreux conseils et encouragements m'ont été d'une grande aide. Je tiens également à remercier **Patrick de Clercq** pour les discussions très enrichissantes sur mon travail.

Un grand merci à tous les membres et anciens membres de l'équipe BPI : **Aurélie, Laurent, Didier, Elodie, Aline, Isabelle Y., Marjorie, Eric, Thomas, Alexandra, Emeline, David, Isabelle L.G., Thibaut M-J., Margarita, Sophie, Chloé** et **Gérald**. J'ai grandi scientifiquement et humainement à leur contact.

Je tiens à dire un grand merci à tous les stagiaires, **Lucie Tamisier, Teddy Urvois, Seydou Diabaté, François Hervi, Agnès Bailly-Maitre** et **Silène Lartigue** pour leur travail et de m'avoir permis d'effectuer mes premières périodes d'encadrement si enrichissantes.

Je remercie également les amis qui ont participé activement à l'élaboration de cette thèse en me permettant de l'oublier de temps en temps, **Ben, Elodie, Seb, Kelly, Clémence, Géraldine, Alex, Hugo, Angela, Fran, Jérémy** et évidemment '**Rito, Pablo** et **Jacob**. Un merci tout particulier aux membres du groupe Cap Soul, **Jay-Bee, Rom's, Mouss**, et **Benoît**.

Merci à ma famille pour son soutien et sa confiance pendant ces très nombreuses années.

Je souhaite enfin remercier les personnes qui ont gentiment accepté de faire partie de mon jury, **Sara Magalhães, Laurence Mouton, Marc Kenis** et **Richard Stouthamer**.

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- Manuscrit 2** - Quaglietti, B., Vega Carvajal M-V., Lartigue, S., Bailly-Maitre, A., Baeza Morales, R., Le Goff, I., Hervi, F., Roman Gajardo C., Pekas, A., Wäckers, F., Amouroux, P., Fauvergue, X. & Malausa, T. Effects of inbreeding on the three biocontrol agents *Chrysoperla* near *comanche*, *Cryptolaemus montrouzieri* and *Macrolophus pygmaeus* in rearing conditions. *Soumis à Evolutionnary applications*. 83
- Manuscrit 3** - Quaglietti, B., Malausa, T., Fauvergue, X., Crochard, D., Wäckers, F., Pekas, A. & Palero, F. Genetic determinants of hybridization and fitness in mass-rearing of the biocontrol agent *Macrolophus pygmaeus* using genotyping-by-sequencing. *Redaction en cours*. 121
- Manuscrit 4** - Quaglietti, B., Gautier, P., Groussier, G., Fleisch, A., Kreiter, P., Ris, N. & Malausa, T., (2017) Pre-release host-range determination of the parasitoid *Allotropa burrelli* for the biocontrol of *Pseudococcus comstocki* in France, *Journal of Applied Entomology* 141(8), 665-668 165

CHAPITRE 1 : Contexte général – La lutte biologique.

1.1. Définition et généralités

Depuis plusieurs décennies, l'utilisation massive de produits chimiques de synthèse pour la protection des cultures (Stephenson, 2003) est remise en question du fait de leurs effets néfastes sur l'environnement et la santé (Hallenbeck and Cunningham-Burns, 2012; Jeyaratnam, 1990), de leur efficacité variable (Georghiou, 1972; Ghekiere et al., 2006; Hanazato, 2001; Srinivas et al., 2004; van Lenteren, 2012) et de leur impact économique réel sur la société (Bourguet and Guillemaud, 2016; Pimentel et al., 1992). Une volonté de mettre en place de nouvelles méthodes de protection des cultures est donc apparue à la fin du XXe siècle. Pour faire face aux ravageurs de culture tout en prenant en compte les conséquences sanitaires et écologiques des méthodes de lutte, la protection des cultures s'est dirigée vers la lutte intégrée. Celle-ci est définie comme une approche systémique visant à coordonner les méthodes de lutte disponibles pour limiter le développement des organismes nuisibles en prenant en compte les intérêts et impacts économiques et écologiques sur les producteurs, la société ou l'environnement (Ehler, 2006; Kogan, 1998). La lutte biologique est une des méthodes mobilisables dans la lutte intégrée (**Figure 1**) et consiste en l'utilisation d'organismes vivants (prédateurs, parasites, pathogènes) pour diminuer la densité de population ou l'impact d'organismes nuisibles et particulièrement des ravageurs de cultures (Eilenberg et al., 2001; IOBC, 2012).

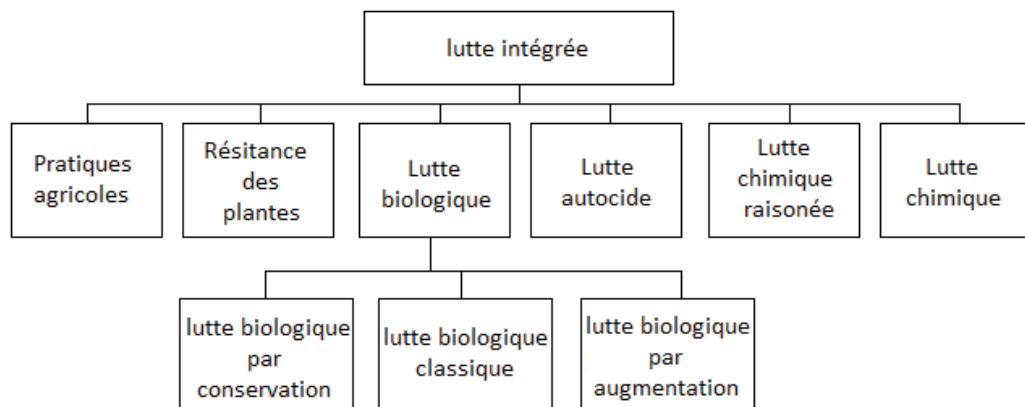


Figure 1 : Diagramme replaçant la lutte biologique dans le contexte de la lutte intégrée (d'après Eilenberg et al., 2001)

1.2. Les méthodes de lutte biologique

La lutte biologique comprend plusieurs méthodes pouvant s'accorder à cette définition mais qui divergent dans leur application si bien qu'Eilenberg et al. (2001) ont proposé de définir trois catégories de lutte biologique (**Figure 1**) dont deux impliquent des lâchers d'auxiliaires :

- 1) La lutte biologique par acclimatation consiste en l'introduction intentionnelle d'un auxiliaire exotique (ayant préférentiellement co-évolué avec le ravageur) en vue de l'établissement et le contrôle à long terme d'un ravageur de culture exotique.
- 2) La lutte biologique par augmentation consiste en l'introduction intentionnelle d'un auxiliaire pour effectuer un contrôle temporaire voir immédiat de la cible. L'auxiliaire peut être natif de l'environnement ou exotique, mais dans ce cas, son établissement n'est pas recherché et de nouveaux auxiliaires devront être lâchés lors de la saison suivante.

Selon la méthode employée, on distingue parfois les luttes biologiques par inoculation et par inondation. Par inoculation, le contrôle est effectué par les auxiliaires lâchés et leurs descendants pendant plusieurs générations. Par inondation, le contrôle sera effectué uniquement par les organismes relâchés.

- 3) La lutte biologique par conservation n'implique pas de lâchers d'auxiliaires dans l'environnement. Elle consiste à modifier l'environnement afin de protéger et favoriser le développement des populations d'un auxiliaire spécifique déjà présent dans l'environnement. Pour la distinguer des « bonnes pratiques agricoles », Eilenberg et al. (2001) suggèrent de nommer lutte biologique par conservation uniquement les pratiques visant à favoriser un ou plusieurs auxiliaire(s) spécifique(s) dans le but de contrôler un ou plusieurs nuisible(s) spécifique(s).

Dans cette thèse, je m'intéresse uniquement aux méthodes de lutte biologique par acclimatation, par inondation et par inoculation qui impliquent des lâchers d'auxiliaires.

1.3. Historique

La première introduction d'un ennemi naturel recensée fut réalisée dans les années 1860, lorsque *Dactylopius ceylonicus* (Green) fut introduit au Sri Lanka où il contrôla les populations

invasives de figuier de barbarie, *Opuntia vulgaris* (Rao et al., 1971; Syrett et al., 2000). En 1864 la cochenille *Icerya purchasi* a été identifiée dans un parc en Californie avant de coloniser les cultures d'agrumes. Des entomologistes ont alors cherché l'origine de ce ravageur et ont découvert en Australie de petites populations dont les ennemis naturels semblaient empêcher la prolifération. L'un de ces ennemis naturels, la coccinelle *Rodalia cardinalis*, fut introduit en 1887 en Californie et participa au contrôle des populations nuisibles d'*Icerya purchasi* (Caltagirone and Douth, 1989). Cette introduction est l'une des premières recensées et constitue pour de nombreux auteurs l'évènement fondateur de la lutte biologique par acclimatation (Hill and Greathead, 2000). La lutte biologique par acclimatation est souvent dite « lutte biologique classique » puisqu'elle fait référence à la méthode utilisée lors des premiers cas référencés. Les premières espèces utilisées dans le cadre de la lutte biologique par augmentation furent l'hyménoptère *Metaphycus lounsburyi* et le coléoptère *Chilocorus circumdatus* introduits en Australie en 1902 pour lutter contre les cochenilles *Saissetia oleae*, et *Diaspidiotus perniciosus* et *Aonidiella aurantii* (Bartlett, 1978; Houston, 1991; van Lenteren, 2012).

1.4. Etat des lieux

1.4.1. Lutte biologique par acclimatation

La très grande majorité des ennemis naturels utilisés en lutte biologique par acclimatation sont des arthropodes et notamment des insectes. Entre 1880 et 2000, plus de 5000 introductions d'arthropodes comprenant 2384 espèces d'insectes ont été effectuées pour contrôler plus de 500 espèces nuisibles dans 148 pays (Cock et al., 2016; Hill and Greathead, 2000) et plus de 1000 introductions sont référencées pour le contrôle de plantes invasives (Hill and Greathead, 2000; Syrett et al., 2000). Parmi les 6158 introductions d'insectes référencées par Cock et al., (2016), 2007 (32.6%) ont mené à l'établissement de l'auxiliaire dans le nouvel environnement et 620 (10.1% des introductions, 30.9% des auxiliaires établis) ont permis le contrôle de la cible. En outre, près de 25% des 1150 introductions d'auxiliaires pour le contrôle de 133 plantes invasives ont mené au contrôle de la cible (Julien, 1989; Julien and Griffiths, 1998). D'un point de vue historique, le nombre d'introductions par décennie a été en croissance constante depuis la fin du 19^e siècle jusqu'à la fin des années 1970, à l'exception d'un pic entre 1930 et 1940 (**Figure 2**) et a commencé à diminuer par la suite. Néanmoins, depuis 1950, une

proportion croissante des auxiliaires s'est établie et a contribué au contrôle de leur cible (**Figure 3**).

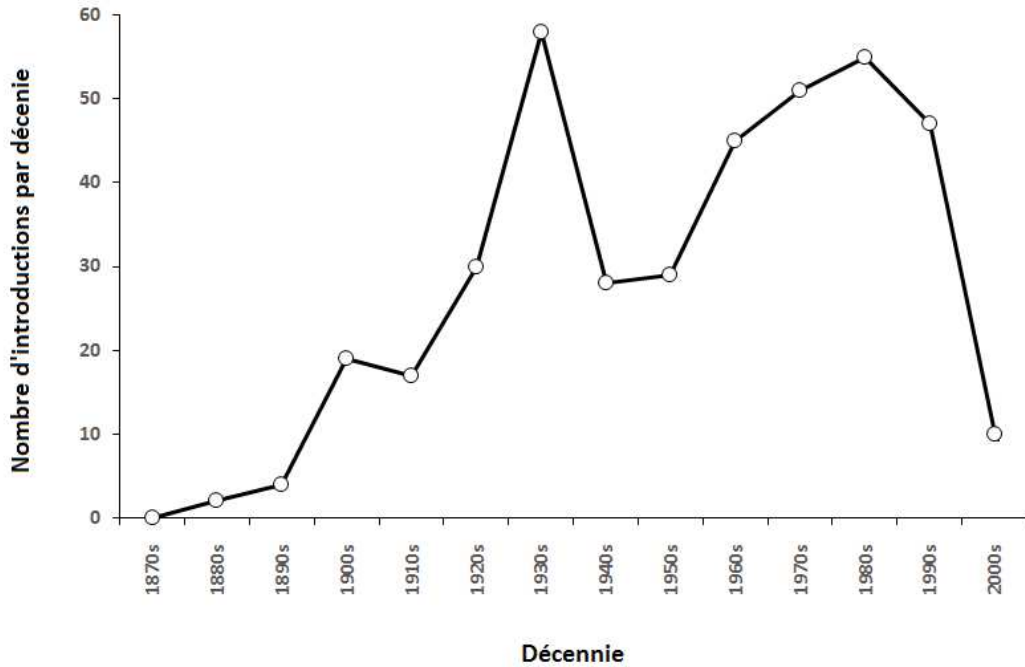


Figure 2 : Nombre d'introductions d'auxiliaires de lutte biologique classique par décennie (d'après Cock et al., 2016).

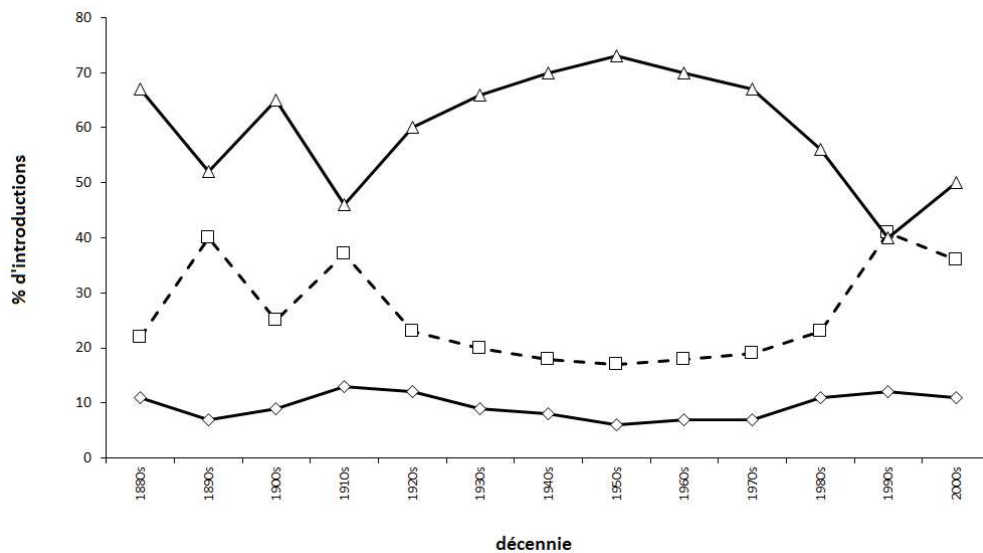


Figure 3 : Pourcentage d'introductions n'ayant pas mené à l'établissement (triangles), ayant mené à l'établissement sans contrôle de la cible (Carrés), et ayant mené à l'établissement et au contrôle du ravageur (losanges) (d'après Cock et al., 2016).

1.4.2. Lutte biologique par augmentation

Bien que l'élevage massif d'ennemis naturels existe depuis plus d'un siècle, la lutte biologique par augmentation s'est développée dans les années 1970 et connaît une forte croissance depuis quelques années (van Lenteren, 2012). En 2003, environ 85 entreprises produisaient plus de 150 espèces d'ennemis naturels (Van Lenteren and Bueno, 2003) pour un marché mondial de 25 million de dollars en 1997 et de 50 millions de dollars en 2000 (Bolckmans, 1999; IOBC, 2006). En 2010, c'est environ 500 entreprises qui produisaient plus de 230 espèces d'ennemis naturels invertébrés représentant un marché supérieur à 200 millions de dollars (van Lenteren, 2012). Comme pour la lutte biologique par acclimatation, la très grande majorité des auxiliaires sont des arthropodes (95,2%) parmi lesquels quatre groupes taxonomiques fournissent la plupart des ennemis naturels : les Hyménoptères (52,2%), les Acariens (13,1%), les Coléoptères (12,2%) et les Hétéroptères (8,3%) (**Figure 4**).

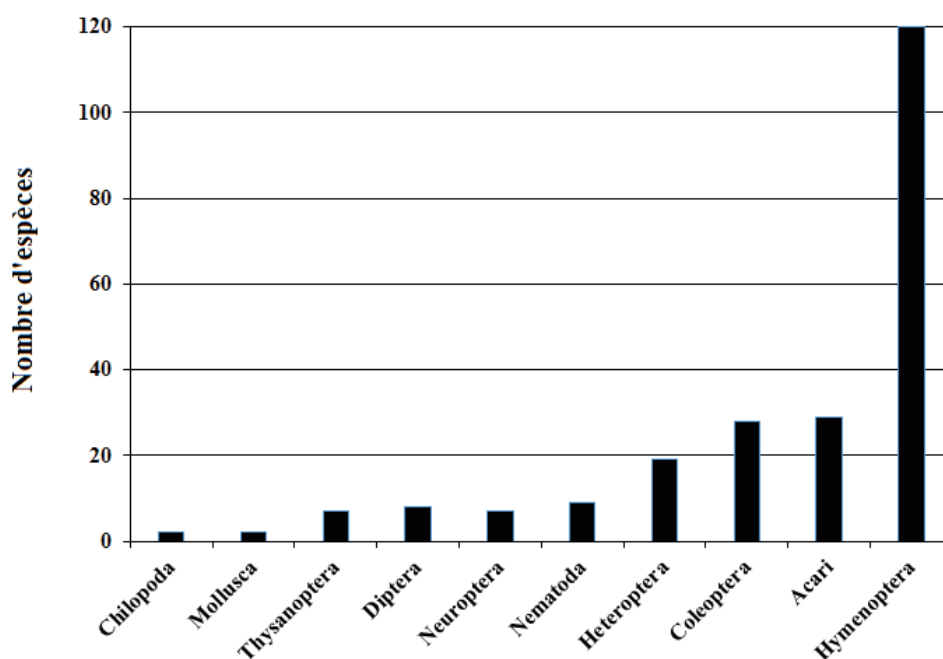


Figure 4 : Groupes taxonomiques des auxiliaires utilisés en lutte biologique par augmentation entre 1900 et 2010 (d'après van Lenteren, 2012).

1.5. Limites et développement

Malgré la croissance importante de l'utilisation de la lutte biologique par augmentation, cette dernière n'est appliquée que sur une minorité des surfaces agricoles et la lutte chimique est encore la méthode majoritairement utilisée en protection des cultures (IOBC, 2006; Naranjo et al., 2015; van Lenteren, 2012). Une des principales raisons expliquant les limites de l'expansion de la lutte biologique est sa rentabilité à court terme pour les agriculteurs (van Lenteren, 2012; Wilson and Tisdell, 2001). En effet, bien que la lutte biologique soit globalement plus efficace que la lutte chimique et plus rentable pour la société en termes économique, écologique et sanitaire (Naranjo et al., 2015; van Lenteren, 2012), sa rentabilité économique directe pour les agriculteurs n'encourage pas ces derniers à changer leur modèle de production. Néanmoins, la pression des consommateurs et des pouvoirs publics encourage depuis plusieurs années la recherche et développement dans le domaine de la lutte biologique pour maximiser sa rentabilité à court terme et ainsi convaincre les agriculteurs de s'orienter vers ces méthodes.

1.6. Potentiel d'amélioration de la lutte biologique

Entre l'émergence d'un ravageur de culture et l'introduction d'un auxiliaire dans l'environnement pour son contrôle, plusieurs phases successives de développement sont nécessaires. Elles comprennent généralement une phase de recherche des ennemis naturels potentiellement efficaces, leur collecte et importation, l'évaluation de leur efficacité et des risques potentiels sur l'environnement, et l'élevage de masse des candidats retenus dans des conditions sécurisées (Cronk and Fuller, 1995; Sforza, 2010).

Les productions de masse d'auxiliaires de lutte biologique peuvent atteindre un rendement de plusieurs dizaines de milliers d'individus par semaine (Naranjo et al., 2015; Van Lenteren and Woets, 1988). Selon que l'auxiliaire soit un ennemi naturel d'une plante invasive ou d'un phytophage, sa production peut nécessiter l'élevage simultané de plusieurs niveaux trophiques (plante hôte, phytophage, auxiliaire). Ces phases d'élevage peuvent donc impliquer des ressources importantes en termes de logistiques, de personnel, de matériel ou d'énergie.

Ainsi, le potentiel d'amélioration de la lutte biologique quant à son rendement économique et son efficacité réside principalement dans l'optimisation de ces phases d'élevage. Plusieurs facteurs parmi lesquels l'optimisation des procédures et du matériel utilisé dans les

élevages, le développement de nourritures artificielles permettant de s'affranchir des différents niveaux trophiques, ou la gestion de la génétique des populations d'auxiliaires sont des leviers potentiels visant à l'optimisation de la phase d'élevage (Leppä et al., 2014).

Les processus génétiques agissant dans les populations d'élevage sont susceptibles d'impacter positivement et négativement le rendement des élevages ou la qualité des auxiliaires produits (Hopper et al., 1993; Hufbauer and Roderick, 2005; Mackauer M., 1976). Dans cette thèse, je me suis en particulier intéressé à l'impact de la consanguinité et de l'hybridation dans les populations d'élevage de lutte biologique. En effet, la dépression de consanguinité est le principal processus génétique redouté par les professionnels de la lutte biologique et l'hybridation est une conséquence attendue des pratiques mises en place par ces mêmes professionnels pour limiter les problèmes de consanguinité et de diminution de diversité génétique. Par exemple, les industriels de lutte biologique ont souvent recours à des introductions dans les élevages, d'échantillons de populations collectés dans la nature, ce qui peut conduire à une hybridation entre des populations présentant des niveaux de divergence génétique variables.

CHAPITRE 2 : Background sur la génétique des populations d'élevage

2.1. Risques liés à la consanguinité dans les populations

2.1.1. Définition et généralités

Parmi les processus pouvant impacter les populations isolées ou de petite taille, la consanguinité et ses potentiels effets néfastes constituent une des préoccupations majeures dans la gestion des populations. La consanguinité (*i.e.* la reproduction entre individus apparentés) est un processus génétique qui peut se produire naturellement dans les populations à l'équilibre démographique (Crnokrak and Roff, 1999; Keller and Waller, 2002). On parle de consanguinité systématique pour laquelle les individus vont se reproduire non-aléatoirement avec des individus apparentés (Malécot, 1969). Elle peut résulter de processus d'adaptations évolutives ayant favorisé ce mode d'appariement et les individus y sont donc en général bien adaptés (Crnokrak and Roff, 1999). En revanche, quand la reproduction entre individus apparentés a lieu de manière aléatoire on parle alors de consanguinité panmictique (Malécot, 1969). Cette dernière est favorisée dans les populations isolées ou de petite taille ou celles ayant subi un ou plusieurs évènements démographiques appelés goulots d'étranglement, menant à une diminution drastique des effectifs et de la diversité génétique (England et al., 2003; Fauvergue et al., 2012; Whitehouse and Harley, 2001; Wisely et al., 2002). Particulièrement dans les cas de consanguinité panmictique, on peut observer une diminution de la fitness des individus consanguins, par rapport à celle des individus non-consanguins. On parle alors de dépression de consanguinité.

2.1.2. Mécanismes

La consanguinité, provoque une augmentation de l'homozygotie qui dans le cas de la consanguinité panmictique est susceptible de provoquer de la dépression de consanguinité qui se traduit par une diminution de la fitness des individus consanguins comparée à celle des

individus non-consanguins (**Figure 5**) (Charlesworth, B, Charlesworth, 1999; Charlesworth and Willis, 2009). Celle-ci peut alors survenir via deux mécanismes :

- 1) L'augmentation de l'homozygotie à des loci pour lesquels l'hétérozygotie est un avantage (hypothèse de surdominance).

Dans ce cas, la fréquence des allèles surdominants va être maintenue à un niveau intermédiaire via sélection équilibrante (Charlesworth and Willis, 2009) et la fitness moyenne de la population va décroître en même temps que la fréquence des hétérozygotes (Barrett and Charlesworth, 1991; Lande and Schemske, 1985; Roff, 2002). Le rétablissement de l'hétérozygotie à ces loci aura pour effet d'augmenter la fitness moyenne de la population à son niveau initial dans le meilleur des cas.

- 2) L'augmentation de l'homozygotie pour des allèles récessifs délétères dont les effets étaient précédemment masqués à l'état hétérozygote (hypothèse de dominance partielle).

L'intensité de la dépression de consanguinité provoquée par la dominance partielle va dépendre du fardeau génétique de la population. Ce dernier correspond à la proportion d'allèles délétères dans le pool génétique et leurs effets sur la fitness.

Bien que certains cas de dépression de consanguinité due à de la surdominance aient été démontrés (Charlesworth and Charlesworth, 1990; Hedrick, 2012; Karkkainen et al., 1999; Li et al., 2001), l'hypothèse de dominance partielle est généralement considérée comme la cause majeure de la dépression de consanguinité observée (Barrett and Charlesworth, 1991; Charlesworth, B, Charlesworth, 1999; Dudash and Carr, 1998; Lande and Schemske, 1985; Roff, 2002).

2.1.3. Fardeau génétique d'une population

Dans une population panmictique et démographiquement stable, le fardeau génétique va dépendre de l'équilibre mutation-sélection-dérive agissant sur les allèles délétères (Crnokrak and Barrett, 2002; García-Dorado et al., 2007; Glémin, 2003; Roff, 2002). Plusieurs facteurs, tels que le taux de mutation, la magnitude des effets des allèles délétères, leur degré de dominance, la taille de population, le niveau de ploïdie des organismes et leur système de reproduction vont contribuer à établir cet équilibre (Charlesworth et al., 1990; Charlesworth

and Willis, 2009; Glémin, 2007; Glémin and Galtier, 2012; Otto and Gerstein, 2008). Bien que les mutations génétiques puissent être quelquefois neutres, et dans de rares cas bénéfiques, elles sont principalement délétères (Drake et al., 1998). Les effets de la sélection vont se traduire par un processus de purge de ces mutations délétères (**Figure 5**) dont la vitesse et l'efficacité dépendront de la magnitude des effets des mutations délétères sur la fitness et de leur degré de dominance à l'état hétérozygote (Charlesworth and Charlesworth, 1987; Crnokrak and Barrett, 2002; Glémin, 2003; Lande and Schemske, 1985). Les mutations ayant des effets fortement délétères voir létaux de même que les mutations dont le degré d'expression à l'état hétérozygote sera le moins élevé seront rapidement éliminés (Charlesworth et al., 1990; Crnokrak and Barrett, 2002; Glémin, 2003; Hedrick, 1994; Lande and Schemske, 1985; Roff, 2002; Schultz and Willis, 1995; Wang et al., 1999). On estime que le fardeau génétique responsable de la dépression de consanguinité est majoritairement constitué d'allèles partiellement récessifs et partiellement délétères et de rares allèles fortement récessifs et plus fortement délétères (Charlesworth, B, Charlesworth, 1999; Charlesworth and Willis, 2009; Crow, 1993; Wang et al., 1999, 1998). La dérive génétique est la variation des fréquences alléliques due à la ségrégation aléatoire des gamètes au moment de la reproduction. Elle peut ainsi provoquer l'augmentation ou la diminution de la fréquence d'un allèle délétère (Kimura, 1983; Kimura and King, 1979).

La rupture de l'équilibre mutation-sélection-dérive d'une population peut survenir avec la modification du régime de reproduction ou de la taille de la population. Ainsi, plusieurs générations de consanguinité imposées à une population panmictique intensifieront le phénomène de purge du fardeau génétique aboutissant à un regain progressif de fitness pouvant dépasser la fitness moyenne de la population initiale (Ávila et al., 2010; Ballou, 1997; Barrett and Charlesworth, 1991; Crnokrak and Barrett, 2002; Szövényi et al., 2014). L'intensité du regain de fitness dépendra de la magnitude des effets des allèles délétères éliminés (Hedrick, 1994; Wang et al., 1999; Willis, 1999). En outre, la taille des populations a également un effet sur l'évolution du fardeau génétique. Bien que les effets de la dérive soient relativement négligeables dans les grandes populations, ils peuvent surpasser ceux de la sélection dans les petites populations (Fauvergue et al., 2012). Par conséquent, dans des populations de petite taille ou ayant récemment subi un goulot d'étranglement, la variation des fréquences alléliques peut provoquer la fixation d'un allèle délétère, ou son élimination, participant ainsi au phénomène de purge (**Figure 5**) (Fauvergue et al., 2012; Leberg and Firmin, 2008; Luque et al., 2016; Whitlock and Bürger, 2004). Par ailleurs, la croissance rapide d'une population après

un goulot d'étranglement pourra favoriser la contre-sélection des allèles délétères (Facon et al., 2011; Kirkpatrick and Jarne, 2000; Pujol et al., 2009). Ainsi, la dépression de consanguinité dans les populations de petite taille ou récemment goulotées dépendra de l'intensité et de la durée du goulot d'étranglement et semble donc difficile à prédire.

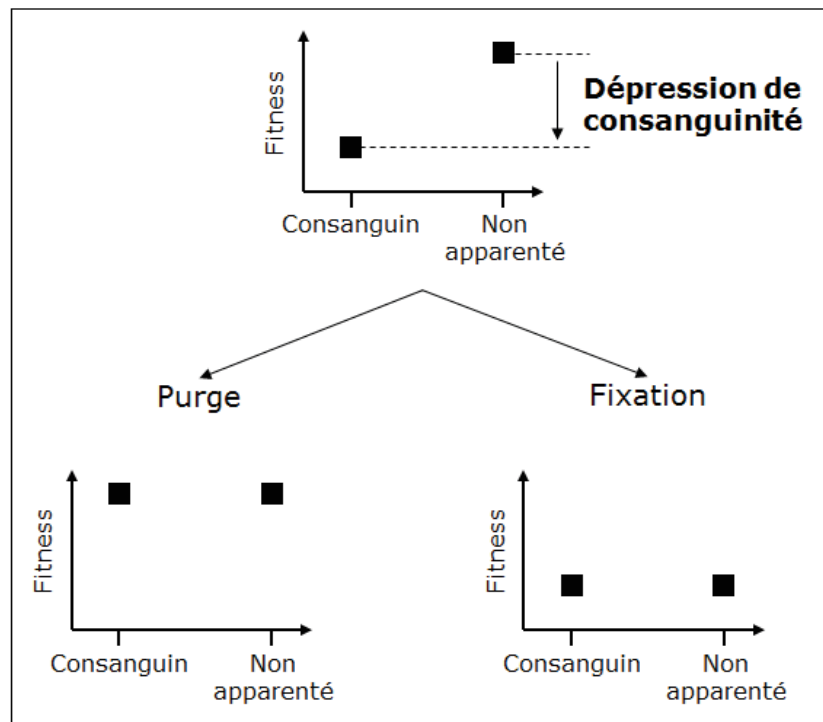


Figure 5 : Description de l'évolution possible du fardeau génétique et de ses effets sur la fitness des individus

2.1.4. Le cas des espèces haplodiploïdes

Une grande proportion d'acariens et surtout d'hyménoptères haplodiploïdes comptent parmi les auxiliaires de lutte biologique (van Lenteren, 2012) (**Figure 4**). Or le niveau de ploïdie des organismes joue un rôle primordial dans l'évolution du fardeau génétique. En effet, chez la plupart des espèces haplodiploïdes, les femelles sont diploïdes issues d'œufs fécondés, et les mâles sont haploïdes, issus d'œufs non fécondés. Les mutations délétères sont par conséquent nécessairement exprimées chez les mâles et soumises à la sélection favorisant ainsi le processus de purge (Crozier, 1985; Werren, 1993). Néanmoins, des approches théoriques par modélisation ont montré que bien que le fardeau génétique devait en effet être plus efficacement purgé chez les organismes haplodiploïdes il n'était toutefois pas totalement éliminé (Glémin, 2003;

Werren, 1993). De plus, des mutations délétères affectant uniquement des traits chez les femelles ne seront pas purgées chez les mâles haploïdes (Brekke et al., 2010; Gallardo et al., 2004; Losdat et al., 2014; Tien et al., 2014). Une méta-analyse réalisée par Henter (2003) a cherché à comparer les effets de la consanguinité entre insectes diploïdes et haplodiploïdes et a démontré que bien que les haplodiploïdes étaient moins sensibles que les diploïdes, ils n'étaient cependant pas immunisés à la dépression de consanguinité et pouvaient parfois souffrir d'effets substantiels corroborant ainsi les hypothèses des modèles prédictifs (Gerloff and Schmidhempel, 2005; Greeff et al., 2009; Henter, 2003).

En outre, chez les hyménoptères parasitoïdes, le système de détermination du sexe (*CSD* pour « Complementary Sex Determination ») (Asplen et al., 2009; Heimpel and de Boer, 2008) est le système ancestral de détermination du sexe. Dans le cas du *CSD*, le sexe d'un individu dépend de sa composition allélique au locus (single locus-*CSD*) ou loci (multiple loci-*CSD*) du *CSD*. Les individus hétérozygotes à ce ou ces loci se développeront en femelle. En revanche, les homozygotes et les haploïdes hemizygotes se développeront en mâles. Les diploïdes homozygotes au(x) gène(s) du *CSD* se développeront le plus souvent en mâles stériles ou non viables provoquant ainsi de la dépression de consanguinité via surdominance pouvant également mener à l'extinction des populations (Duchateau et al., 1994; Harper et al., 2016; Harpur et al., 2013; Vayssade et al., 2014; Zayed and Packer, 2005; Zhou et al., 2007).

2.2. Potentiel et risques liés à l'hybridation dans les populations d'élevage.

L'hybridation est définie par Harrison (1990) comme la reproduction entre individus provenant de populations ou souches différenciées sur des caractères héritables. Bien qu'on puisse différencier l'hybridation inter-spécifique (reproduction entre individus d'espèces différentes) de l'hybridation intra-spécifique (reproduction entre individus d'une même espèce issus de populations ou souches différentes) leurs conséquences évolutives sont régies par les mêmes mécanismes. Ces conséquences ont été sujettes à débat depuis plusieurs années en biologie évolutive (e.g. Arnold and Hodges, 1995; Barton, 2001; Burke and Arnold, 2001). En effet, les individus hybrides peuvent arborer des phénotypes intermédiaires (fitness moyenne de celle des parents) mais également supérieurs (vigueur hybride) ou inférieurs (dépression hybride) à ceux de leurs parents.

2.2.1. Effets sur la fitness des descendants

2.2.1.1. *Vigueur hybride*

La vigueur hybride correspond à l'augmentation de la fitness des individus hybrides relativement à la celle de leurs parents. Elle peut résulter de deux choses :

1) La diminution de la dépression de consanguinité via l'effet heterosis (Carlson et al., 2014; Frankham, 2015). Il est consécutif à l'augmentation du taux d'hétérozygotie dans la population suite à l'augmentation de la diversité génétique due à l'hybridation (Charlesworth et al., 1993).

2) Des évolutions adaptatives consécutives au processus d'hybridation. Plusieurs processus génétiques peuvent provoquer ce genre d'adaptations (Burke and Arnold, 2001; Lippman and Zamir, 2007):

- L'accumulation (sans relation épistatique) des effets positifs de l'augmentation de l'hétérozygotie à des loci ayant un avantage à l'hétérozygotie (hypothèse de surdominance).
- L'accumulation chez les descendants hybrides, d'allèles favorables dominants initialement présents dans l'une ou l'autre des deux populations parentales.
- La création de nouvelles relations épistatiques entre allèles initialement présents dans chacune des deux populations parentales

2.2.1.2. *Dépression hybride*

La dépression hybride est une diminution de la fitness des individus hybrides relativement à celle de leurs parents. Elle s'explique par plusieurs mécanismes (Burke and Arnold, 2001; Lynch, 1991; Schierup and Christiansen, 1996; Tallmon et al., 2004) :

- La diminution des adaptations locales de la population par la dilution, par les allèles « migrants », des allèles qui procurent un effet positif sur la fitness dans les conditions locales.

- L'expression à l'état hétérozygote d'allèles dominants ayant un impact négatif sur la fitness (hypothèse de sous-dominance).
- La rupture de complexes de gènes co-adaptés provoquée au moment de la recombinaison, par l'atténuation des relations épistatiques positives sélectionnées au sein de chaque population.
- Les incompatibilités des variations génomiques sélectionnées au sein de chaque population.

2.2.2. Effets sur la reproduction

En outre, les conséquences de l'hybridation ne se limitent pas à la fitness des individus hybrides. De l'isolement reproducteur total ou partiel peut être également observé entre individus issus de populations différenciées. Cela peut être la conséquence d'un isolement pré-accouplement (Schluter, 2001; Servedio and Noor, 2003; Turelli et al., 2001) ou post-accouplement avec des incompatibilités pré-zygotiques (Edmands, 2002; Servedio and Noor, 2003) ou post-zygotiques (Orr and Presgraves, 2000). Ces incompatibilités peuvent résulter de l'incompatibilité de reproduction due à la différenciation génomique trop importante entre les partenaires ou les ruptures de complexe de gènes co-adaptés (Orr and Turelli, 2001; Turelli and Orr, 2000).

Par ailleurs, des facteurs extérieurs au génome de l'espèce au sens strict, mais néanmoins héréditaires sont susceptibles d'avoir un effet sur la reproduction. En effet, les bactéries endosymbiotiques des genres *Wolbachia*, *Cardinium* et *Rickettsia* notamment (Duron et al., 2008), sont connues pour pouvoir manipuler la reproduction particulièrement chez les arthropodes (Bourtzis and O'Neill, 2010; Breeuwer and Jacobs, 1996; Johanowicz and Hoy, 1998; Stouthamer et al., 1999). Elles peuvent notamment induire la parthénogénèse, provoquer la « féminisation » de mâles génétiques, et influencer sur la production de descendants. Néanmoins, l'effet le plus connu est le phénomène d'incompatibilité cytoplasmique induit lors de l'association des gamètes. Une altération des chromosomes des mâles infectés va entraîner des conséquences lors de l'accouplement (**Figure 6**) :

- Lors de l'accouplement d'un mâle et d'une femelle infectés par la même souche bactérienne, la présence des bactéries dans le cytoplasme de l'œuf entraînera un phénomène de « rescousse » des chromosomes paternels (**Figure 6a**).

- L'accouplement d'un mâle sain avec une femelle infectée n'engendrera pas de manipulation particulière de la reproduction (**Figure 6a**).
- Si un mâle est porteur d'une souche bactérienne, l'accouplement avec une femelle saine (incompatibilité unidirectionnelle) ou infectée par une autre souche de la bactérie (incompatibilité bidirectionnelle) ne permettra pas la formation d'un zygote viable (**Figure 6b et 6c**).

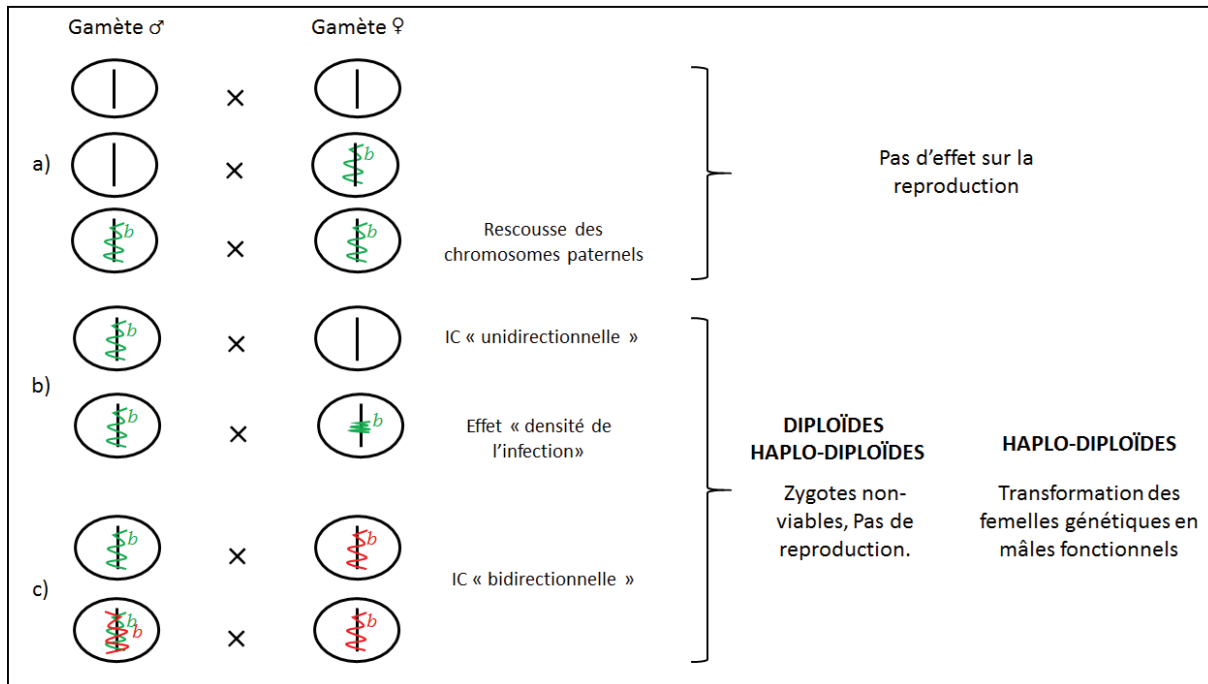


Figure 6 : Description simplifiée des mécanismes d'incompatibilité cytoplasmique (IC) pouvant être provoqués par les endosymbiontes.

L'incompatibilité cytoplasmique pourra être totale ou partielle selon l'espèce hôte, mais également selon le niveau de l'infection de chacun des partenaires (Bourtzis and O'Neill, 2010) (**Figure 6b**). Par ailleurs, chez les haplodiploïdes, les individus mâles étant issus d'œufs non fécondés, si un mâle est porteur d'une souche bactérienne alors l'accouplement avec une femelle saine ou infectée par une autre souche de la bactérie n'engendrera pas d'impact sur les mâles mais uniquement sur la descendance femelle. Le phénomène d'incompatibilité cytoplasmique pourra provoquer la non-viabilité du zygote mais pourra également entraîner la transformation des femelles génétiques en mâles fonctionnels. Ainsi, ces endosymbiontes sont susceptibles de provoquer des phénomènes rapides de différenciation entre populations,

particulièrement dans le cas d'incompatibilités cytoplasmiques bidirectionnelles (Bordenstein et al., 2001; Breeuwer and Werren, 1990; Shoemaker et al., 1999).

2.2.3. Effets de l'exogamie sur la fitness

On peut donc craindre une diminution de fitness due à la trop grande similarité génomique des partenaires d'une part (dépression de consanguinité), mais également dans le cas d'une trop grande divergence génétique entre les partenaires (dépression hybride). Il semble établi que le risque de dépression hybride augmente avec les distances génétique, géographique et écologique (Edmands, 2007; Frankham et al., 2011; Pekkala et al., 2012). Ainsi, il existe très certainement un niveau optimal d'exogamie qui permet d'une part d'éviter les effets néfastes de la consanguinité par effet heterosis (sans dilution des adaptations locales) et de maximiser le potentiel adaptatif des populations, tout en minimisant les risques de dépression hybride (Edmands, 1999; Frankham et al., 2011; Lynch, 1991; Tallmon et al., 2004).

2.3. Approches méthodologiques – état de l'art

2.3.1. Comparaison de la fitness par traitement expérimental

L'évaluation de la dépression de consanguinité a le plus souvent été réalisée en comparant la fitness d'individus ayant des coefficients de consanguinité différents. Ce coefficient de consanguinité est le plus justement évalué grâce aux analyses de pédigrées des individus testés (e.g. Boakes et al., 2007; Cassell et al., 2003; Johnston and Schoen, 1994; Mc Parland et al., 2008). Cependant, bien qu'établir le pédigrée d'un individu soit aisé dans des populations où les individus participant à la reproduction sont contrôlés, cela peut s'avérer compliqué voire impossible dans des populations d'élevage de masse où les accouplements se font sans contrôle. Dès lors, l'évaluation de la dépression de consanguinité est généralement effectuée en comparant la fitness d'individus provenant de croisements ayant différents degrés de consanguinité (« selfing », croisement frère/sœur, croisement inter-familles, croisements inter-populations)(Domingue and Teale, 2007; Luna and Hawkins, 2004; Radwan and Drewniak, 2001; Rye and Mao, 1998; Zhou et al., 2007). L'objectif de cette approche « par

traitement » est d'obtenir des catégories pour lesquels les individus vont exposer différents taux d'homozygotie. Néanmoins, lorsque la diversité génétique initiale et le taux de consanguinité des populations ne sont pas connus, le contraste entre les taux moyens d'homozygotie de chaque niveau de traitement peut ne pas être suffisant pour permettre la détection d'une potentielle dépression de consanguinité.

2.3.2. Corrélations hétérozygotie – fitness au niveau individuel

Une autre approche consiste à observer la relation entre fitness individuelle et l'hétérozygotie individuelle directement mesurée à l'aide de marqueurs moléculaires. Plusieurs études ont montré des corrélations significativement et fortement positives entre hétérozygotie et fitness (en anglais « Heterozygosity-fitness correlations » 'HFCs') suggérant une mise en évidence de dépression de consanguinité (Britten, 1996; Chapman et al., 2009; Hansson and Westerberg, 2002; Szulkin et al., 2010). Par ailleurs, Reed & Frankham (2003), ont démontré une corrélation faiblement positive entre diversité génétique d'une population et fitness moyenne dans une méta-analyse et également suggéré l'importance de la dépression de consanguinité sur ce résultat. Néanmoins, les papiers de revues bibliographiques et méta-analyses effectués sur les HFCs ont montré que bien que la corrélation hétérozygotie-fitness était la plupart du temps faible ou non significative (Chapman et al., 2009) une tendance générale montrait une corrélation positive, démontrant ainsi que celle-ci était probablement due aux exemples arborant des effets forts. Ces résultats suggèrent que la corrélation hétérozygotie-fitness varie selon l'espèce étudiée, mais doit également dépendre de l'historique des individus, et donc des populations, souches ou échantillons étudiés (Chapman et al., 2009). De nombreuses limites des études visant à démontrer des HFCs ont néanmoins été soulevées.

D'abord, la pertinence des marqueurs moléculaires utilisés dans ce genre d'études est remise en question. En effet, la plupart des études visant à démontrer une corrélation hétérozygotie-fitness ont été réalisées en utilisant des allozymes ou des marqueurs microsatellites pour évaluer l'hétérozygotie individuelle (Coltman and Slate, 2003; David, 1998; Pemberton, 2004). Compte-tenu de la nature de ces marqueurs (neutres ou sous-sélection) ainsi que leur nombre pour chaque étude (la plupart du temps inférieur à 30), la détection d'une HFC pourrait résulter de la détection d'un effet direct des marqueurs observés (allozymes) ou local via déséquilibre de liaison avec des loci sous sélection (microsatellites) et pas de l'effet

de l'hétérozygotie moyenne à travers le génome (Pemberton, 2004; Slate et al., 2004; Slate and Pemberton, 2002).

2.3.3. Apport des nouvelles méthodes de génomique moléculaire

Ces dernières années ont vu l'essor des nouvelles technologies de séquençage (Metzker, 2010) parmi lesquelles le génotypage par séquençage (en anglais « Genotyping-by-sequencing » 'GBS'). Le GBS est une méthode de séquençage haut-débit qui permet d'obtenir des données génomiques pour un coût par échantillon relativement faible. Il est basé sur le séquençage du génome réduit (portions de génome) obtenu via des enzymes de restriction et permet la découverte de nombreux sites nucléotidiques polymorphes (en anglais « single nucleotide polymorphism » 'SNP') pouvant être utilisés pour l'étude la diversité génétique ou de la structure des populations, la cartographie génétique ou des études à l'échelle du génome (Elshire et al., 2011; Metzker, 2010; Thomson, 2014). La méthode du GBS est également particulièrement pertinente pour les études portant sur des espèces ayant des petits génomes ou sur l'ADN microbien. Par conséquent, l'analyse des données génomiques obtenues via GBS apporte une opportunité pour 1) évaluer le rôle de la génétique et des endosymbiontes dans le succès de reproduction et 2) l'étude de la corrélation entre hétérozygotie et fitness dans les populations d'élevage permettant de fournir une alternative plus fiable à l'approche « par traitement » qui impliquent des protocoles expérimentaux lourds et coûteux (Hoffman et al., 2014; Miller et al., 2014; Szulkin et al., 2010). Pour pouvoir effectuer une comparaison entre les deux méthodes, la méthode GBS a été utilisée sur une des espèces étudiées pour laquelle la méthode classique par traitement a montré des variations dues aux effets génétiques (**chapitre 3 et 4**).

2.4. Contexte et stratégie de la thèse

Cette thèse a été réalisée dans le cadre d'un projet financé par la commission européenne (Programme Marie-Curie «Industry-Academia Partnerships and Pathways »). Ce projet nommé « COLBICS » (*Intersectoral Collaborations to Boost Research and Development Dynamics in Biological Control of Agricultural pests*) avait pour objectif principal de favoriser la collaboration entre les secteurs privé et public afin d'encourager la recherche et le

développement pour l'amélioration de la lutte biologique (www.colbics.eu). Mon travail de thèse a donc été réalisé au sein de trois organisations différentes : deux entreprises privées, Biobest (Belgique) et Xilema-Anasac (Chili), et un organisme public, l'Institut National de Recherche Agronomique (France) :

- Le laboratoire « Institut Sophia Agrobiotech » (INRA, CNRS, Université Côte d'Azur) possède notamment une expertise dans le développement de programmes de lutte biologique par acclimatation allant de la recherche d'ennemis naturels potentiels jusqu'à l'évaluation, l'élevage et l'introduction d'auxiliaires. Mon travail s'est déroulé dans le cadre d'un programme de lutte biologique par acclimatation visant au contrôle des populations de la cochenille *Pseudococcus comstocki* en France à l'aide du parasitoïde *Allotropa burrelli*.
- Xilema-Anasac est une entreprise chilienne spécialisée dans la production d'auxiliaires de lutte biologique et le développement de méthodes d'élevage. Durant huit mois, j'ai travaillé sur deux prédateurs, la coccinelle *Cryptolaemus montrouzieri* et la chrysope *Chrysoperla near comanche* produits pour la lutte biologique par augmentation contre les cochenilles et pucerons.
- Biobest est un des leaders mondiaux dans la production d'auxiliaires de lutte biologique et le développement de méthodes de lutte intégrée. Parmi les auxiliaires produits par Biobest, la punaise *Macrolophus pygmaeus* est l'un des dix auxiliaires les plus commercialisés dans le monde.

L'un des principaux enjeux de cette thèse fut d'élaborer une stratégie permettant à la fois de répondre à des interrogations des partenaires industriels, assurer un transfert de connaissances et savoir-faire entre secteur public et secteur privé, et de produire des résultats originaux en termes de recherche fondamentale. Les choix de modèles biologiques utilisés, la durée des expériences, le nombre et la provenance des souches utilisées résultent ainsi de la prise en compte des intérêts des partenaires et des opportunités et contraintes dans chaque contexte de collaboration public-privé.

2.5. Objectifs de la thèse

Au cours de cette thèse j'ai voulu atteindre 3 objectifs principaux :

1) Dans un premier temps j'ai voulu évaluer les risques de déclin de la fitness ou des effectifs des populations d'élevage dus à la dépression de consanguinité sur des espèces d'intérêt pour les organisations dans lesquelles j'ai travaillé. J'ai donc appliqué une approche « par traitements » de comparaison de fitness entre individus issus de différents types de croisements (croisements frère-sœur vs croisements exogamiques) pour évaluer les effets de la consanguinité dans des populations d'élevage de *A. burrelli*, *C. near comanche*, *C. montrouzieri* et *M. pygmaeus*.

2) Le second objectif de cette thèse consistait à évaluer les effets de l'hybridation sur la fitness individuelle de *M. pygmaeus*, espèce pour laquelle des effets de la consanguinité ont été observés. À l'image des expériences effectuées sur les effets de la consanguinité, j'ai également appliqué une approche « par traitement » de comparaison de fitness entre individus issus de différents types de croisements (croisements intra-population vs croisements inter-populations).

3) Enfin, le troisième objectif a été de documenter finement la relation entre hétérozygotie (et plus généralement caractéristiques du génome et du microbiome) et fitness en laboratoire chez *M. pygmaeus*. Ceci permettant à la fois d'évaluer l'apport potentiel de la génomique moléculaire dans l'évaluation des processus génétiques étudiés et d'évaluer les bénéfices et risques potentiels d'une manipulation de la diversité intra-spécifique chez cette espèce.

2.6. Plan de thèse

Dans le **chapitre 3**, je présenterai une introduction spécifique à l'étude des effets de la consanguinité dans les populations d'élevage. Je présenterai également les résultats obtenus au cours des travaux concernant les effets de la consanguinité effectués avec une approche classique « par traitements » sur le parasitoïde haplodiploïde *A. burrelli* et sur les trois prédateurs diploïdes *C. near comanche*, *C. montrouzieri*, et *M. pygmaeus* (objectif 1). En outre, je présenterai également les résultats issus des données de séquençage du génome de

Macrolophus pygmaeus ayant permis d'établir la relation entre hétérozygotie et fitness à l'échelle individuelle, (objectif 3).

Dans le **chapitre 4**, j'ai choisi de présenter l'étude traitant des effets de l'hybridation intra-spécifique chez *Macrolophus pygmaeus* (objectif 2). Les résultats présentés seront issus de l'approche classique « par traitement » ainsi que de l'utilisation de données de séquençage du génome et du microbiome de *M. pygmaeus* (objectif 3).

Enfin, dans le **chapitre 5**, je propose une discussion générale sur les risques liés à la consanguinité et l'hybridation dans les élevages d'auxiliaires de lutte biologique. Je discuterai également de la pertinence des différentes approches pour étudier et gérer ces processus génétiques dans le contexte de la lutte biologique.

CHAPITRE 3 : EFFETS DE LA CONSANGUINITE SUR LA FITNESS DE QUATRE AUXILIAIRES DE LUTTE BIOLOGIQUE

3.1. Introduction

Comme je l'expliquais précédemment, la dépression de consanguinité peut avoir un impact sur des traits phénotypiques particuliers comme la prédation, la recherche d'un hôte ou d'une proie, la fécondité qui peuvent avoir une répercussion sur la dynamique des populations jusqu'à les mener à l'extinction (Bijlsma et al., 2000; Brook et al., 2002; Frankham, 1995; Liao and Reed, 2009; Nieminen et al., 2001; Saccheri et al., 1998). En outre, les effets de la dépression de consanguinité, notamment sur le risque d'extinction, sont d'autant plus perceptibles dans les environnements changeants et stressants (Armbruster and Reed, 2005; Bijlsma et al., 1999; Fox and Reed, 2010; Kristensen et al., 2008; Liao and Reed, 2009). Ainsi, l'étude des effets de la consanguinité représente un intérêt majeur pour la persistance des populations menacées (d'autant plus dans un contexte de changement global), ou l'élevage d'organismes d'intérêt commercial.

Ainsi, depuis plusieurs décennies, de très nombreuses études se sont intéressées aux effets de la consanguinité dans le cadre de la gestion de populations, que ce soit en biologie de la conservation (e.g. Grueber et al., 2011; Hedrick and Kalinowski, 2000; Jamieson et al., 2006), la biologie de l'invasion (e.g. Facon et al., 2011; Hardesty et al., 2012; Schmid-Hempel et al., 2007), la gestion des populations captives (e.g. Lacy, 1993; Lacy et al., 1993; Templeton and Read, 1984) mais également pour les productions de masse:

- L'élevage de bétail (e.g. Casellas et al., 2009; Croquet et al., 2006; Leroy, 2014)
- L'aquaculture (e.g. Gallardo et al., 2004; Gjerde et al., 1983; Kincaid, 1983)
- Les cultures de plantes et céréales (e.g. Abdelgadir et al., 2009; Basamma et al., 2009; Jain and Bharadwaj, 2014)

En lutte biologique, la dépression de consanguinité est susceptible d'avoir un impact sur la qualité des auxiliaires produits si elle porte sur des traits phénotypiques tels que la capacité de prédation, la recherche d'un hôte ou d'une proie, la fécondité, etc. Par ailleurs, elle pourrait

également constituer un risque pour le rendement des élevages ou le succès des introductions si elle impactait la dynamique des populations. Néanmoins, bien que l'importance de la dépression de consanguinité sur l'efficacité de la lutte biologique ait été souvent discutée (Fauvergue et al., 2012; Hopper et al., 1993; Hufbauer and Roderick, 2005; Mackauer M., 1976), très peu d'études empiriques ont été réalisées pour des résultats variables. Par exemple, aucun effet de la consanguinité n'a pu être identifié malgré 30 générations de consanguinité imposée au prédateur *Podisus maculiventris* (Say ; Heteroptera: Pentatomidae) (de Clercq et al., 1998). En revanche, une diminution constante de longévité, la fécondité et des capacités de prédation a été montrée sur différentes souches de la coccinelle prédatrice *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) (Al-Khateeb et al., 2013, 2012).

La plupart des organismes utilisés en lutte biologique sont des parasitoïdes hyménoptères haplodiploïdes. Néanmoins, bien que plusieurs études aient été réalisées sur les effets de la consanguinité chez des organismes haplodiploïdes (Henter, 2003; Peer and Taborsky, 2014; Perrot-Minnot et al., 2004; Tien et al., 2014; Vayssade, 2014), peu l'ont explicitement été dans le cadre de la lutte biologique (Luna and Hawkins, 2004; Nakamura, 1996; Sorati et al., 1996). Une forte diminution de fitness due à la consanguinité pouvant même mener à l'extinction des populations a pu être identifiée chez *Exorista japonica* (Nakamura, 1996). Par ailleurs, une augmentation de fitness chez des individus obtenus via des croisements exogamiques suggère également de la dépression de consanguinité chez *Nasonia vitripennis* (Luna and Hawkins, 2004). Ces exemples révèlent particulièrement la difficulté de prévoir les effets de la consanguinité dans les populations d'auxiliaires, notamment chez les organismes haplodiploïdes censés être moins sensibles à la dépression de consanguinité.

Dans le cadre de cette thèse, les effets de la consanguinité ont été étudiés chez quatre espèces utilisées en lutte biologique. L'objectif principal a été de documenter la sensibilité de populations d'intérêt pour les partenaires de cette thèse et de contribuer à la production de données empiriques sur un panel d'espèces d'intérêt en lutte biologique. Cette étude n'avait cependant pas pour prétention de comparer les effets de la consanguinité entre espèces ou types d'espèces, puisque le travail n'a été effectué que sur quatre modèles.

3.2. Matériel biologique

3.2.1. Allotropia burrelli

3.2.1.1. Généralités

A. burrelli (Muesebeck ; Hymenoptera: Platygasteridae) est un hyménoptère haplodiploïdes (**Figure 8B**): les femelles sont diploïdes, issues d'œufs fécondés alors que les mâles sont haploïdes, issus d'œufs non fécondés. Cet endoparasitoïde grégaire est un ennemi naturel spécialiste de la cochenille farineuse *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae). Originnaire d'Asie de l'Est, cette dernière a envahi de nombreux territoires à travers le monde (Ferris, 1937; Takahashi, 1942) et a récemment été identifiée en Europe de l'Ouest en Italie et en France en 2005 (Kreiter and Germain, 2005; Pellizzari, 2005). Bien qu'elle soit associée à une trentaine de genres botaniques différents (Meyerdirk and Newell, 1979), elle s'attaque en France aux vergers de pommiers et de poiriers sur lesquels elle provoque de fortes pertes économiques (Villa et al., 2011). Sur la base des précédentes introductions d'*A. burrelli* en ex-URSS et aux USA (Kobakhidze, 1965; Meyerdirk and Newell, 1979) (**Figure 7A**) un programme de lutte biologique par acclimatation a été initié par l'INRA en 2008. Des populations natives d'*A. burrelli* ont été prélevées au Japon et importées en France dans l'optique d'effectuer des introductions dans l'environnement (**Figure 7B**). Cette importation a nécessité une évaluation de risques et une étude visant à déterminer la gamme d'hôte d'*A. burrelli* sur le territoire français a été réalisée dans cette optique. Cette étude est présentée dans une publication en **Annexe I**.

3.2.1.2. Cycle de vie

Le cycle de vie d'*A. burrelli* dure en moyenne 31 jours à 28°C et 70% d'humidité (Clancy, 1944 ; observations personnelles) (**Figure 9A**). Après l'oviposition dans l'hôte, les œufs se développent en moyenne 9,5 jours avant l'éclosion. Les larves vont ensuite se nourrir de leur hôte et entraîner sa mort progressive jusqu'à sa momification (durcissement de la cuticule de la cochenille morte) environ 25 jours après l'oviposition. Le développement des larves dure en moyenne 6,5 jours avant la pupaison. La métamorphose dure en moyenne 15 jours avant l'émergence des adultes.

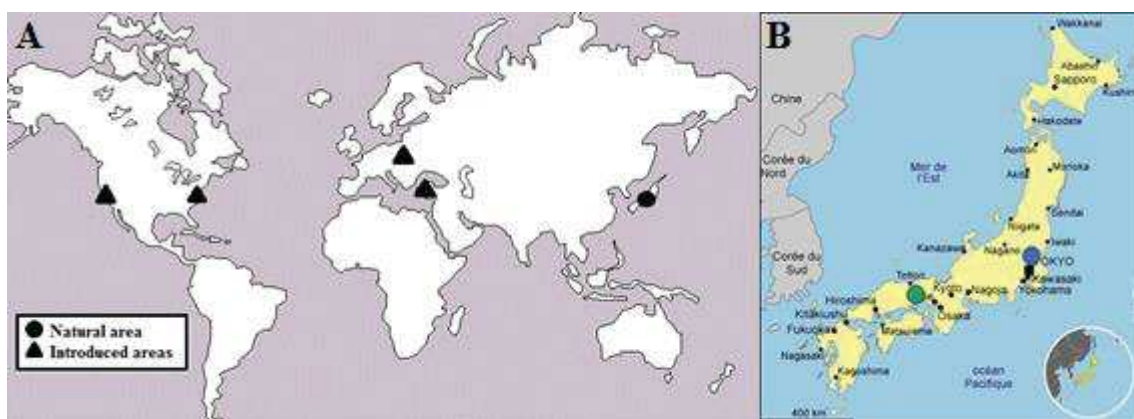


Figure 7 : (A) Distribution connue de l'espèce *A. burelli* et (B) origine géographique des populations d'origine des élevages (**Tottori**, **Utsunomiya**).

3.2.2. *Chrysoperla near comanche*

3.2.2.1. Généralités

Les espèces du genre *Chrysoperla* sont des névroptères prédateurs distribués à travers le monde aussi bien dans la nature que dans les cultures (New, 1975). Les larves sont des prédateurs généralistes s'attaquant notamment aux pucerons, cochenilles, psylles, thrips, aleurodes, lépidoptères sur de nombreux type de végétaux (Albuquerque et al., 1994; Danel and Yokota, 1997; Güven and Göven, 2003; Pappas et al., 2011). Très voraces, les larves constituent un bon auxiliaire potentiel. Bien que de très nombreuses espèces soient référencées, seulement quelques-unes ont pour l'instant été utilisées en lutte biologique pour la protection de vergers, des vignobles ou des cultures céréalières (Pappas et al., 2011; Senior et al., 2001; van Lenteren, 2012). Néanmoins, avec la multiplication des espèces du genre *Chrysoperla*, il est très difficile de différencier les espèces les plus proches selon des critères morphologiques ou écophysiologiques (Brooks, 1994; Henry, 1985). L'espèce présente au Chili et utilisée dans cette thèse n'a pas d'identification taxonomique définitive. Elle présente une séquence d'ADN mitochondrial absente des principales bases de données internationales. Je l'identifierai sous l'appellation *Chrysoperla near comanche* (**Figure 8A**) sur la base d'identifications morphologiques effectuées par M. Jean-Claude Streito (INRA CBGP, Montferriez-sur-Lez) et M. Bruno Michel (Cirad CBGP, Montferriez-sur-Lez).

3.2.2.2. *Cycle de vie*

Le cycle biologique de *Chrysoperla* near *comanche* tel que décrit ici est basé sur mes observations personnelles et sur la bibliographie concernant un espèce proche *C. externa* (Nunez, 1989). Ce cycle biologique dure 30 jours à 25°C et comporte 3 stades larvaires et un stade pupal avant la mue en imago (**Figure 9B**). Les œufs éclosent environ 4 jours après la ponte et le développement des trois stades larvaires dure environ 11 jours avant la mise en pupe. La métamorphose dure environ 10 jours jusqu'à l'émergence de l'adulte. Une période de pré-oviposition dure en moyenne 5 jours avant le début de la ponte des femelles.

3.2.3. *Cryptolaemus montrouzieri*

3.2.3.1. *Généralités*

C. montrouzieri (**Figure 8D**) est une coccinelle prédatrice généraliste de cochenilles. Originnaire d'Australie elle a été introduite dans plus de 40 pays à travers le monde à des fins de lutte biologique par augmentation (Clausen et al., 1978). Elle compte aujourd'hui parmi les 15 espèces les plus utilisées en lutte biologique par augmentation et est le premier prédateur utilisé contre les cochenilles (van Lenteren, 2012). Les stades larvaires aussi bien que le stade adulte sont prédateurs et peuvent contribuer au contrôle des populations de cochenilles (Clausen et al., 1978).

3.2.3.2. *Cycle de vie*

Les trois souches utilisées dans les expériences ont un cycle de vie similaire. Ce dernier, décrit ci-après, est basé sur nos observations personnelles pour une température de 25°C, une humidité relative de 70%, et chez des coccinelles nourris avec des œufs d'*Ephestia kuehniella*. De la ponte à l'émergence de l'adulte, le développement dure environ 26 jours et inclut cinq stades larvaires, un stade de pré-nymphé et une nymphe (**Figure 9C**). Après la ponte, le développement des œufs va durer en moyenne 4 jours. Après l'éclosion, les larves vont se développer en moyenne 13 jours et les pré-nymphe et nymphe pendant en moyenne 10 jours

avant l'émergence de l'adulte (Ghorbanian et al., 2011). Une période de pré-oviposition va durer en moyenne 6 jours avant le début de la ponte des femelles.

3.2.4. Macrolophus pygmaeus

3.2.4.1. Généralités

M. pygmaeus (**Figure 8B**) est une punaise prédatrice originaire du bassin méditerranéen où elle peut être trouvée sur de nombreuses espèces végétales (Alomar et al., 1994). *M. pygmaeus* est une des 10 espèces les plus utilisées à travers le monde dans le cadre de la lutte biologique par augmentation et le premier prédateur utilisé contre les aleurodes (van Lenteren, 2012). Principalement utilisée pour la protection des cultures de Tomate par le contrôle des populations des aleurodes *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) et *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) (Alomar et al., 2006; Malausa, 1989; Millot, 1994; Sampson and King, 1996; Trottin-Caudal et al., 1992) elle est également utilisée face aux acariens, thrips, mineuses et mites (e.g. Fauvel et al., 1987; Foglar et al., 1990; Riudavets and Castañé, 1998).

3.2.4.2. Cycle de vie

Le cycle de vie de *M. pygmaeus* nourri avec *Ephestia kuehniella* dure environ 32 jours à 25°C et comprend cinq stades nymphaux (Hansen et al., 1999) (**Figure 9D**). L'accouplement peut s'effectuer dès l'émergence néanmoins la femelle ne peut pondre qu'après une période de pré-oviposition de 5 jours en moyenne. Les premières nymphes vont éclore après 12 jours en moyenne et se développer pendant en moyenne 20 jours avant la métamorphose en adulte. Au dernier stade nymphal des ailes juvéniles apparaissent.

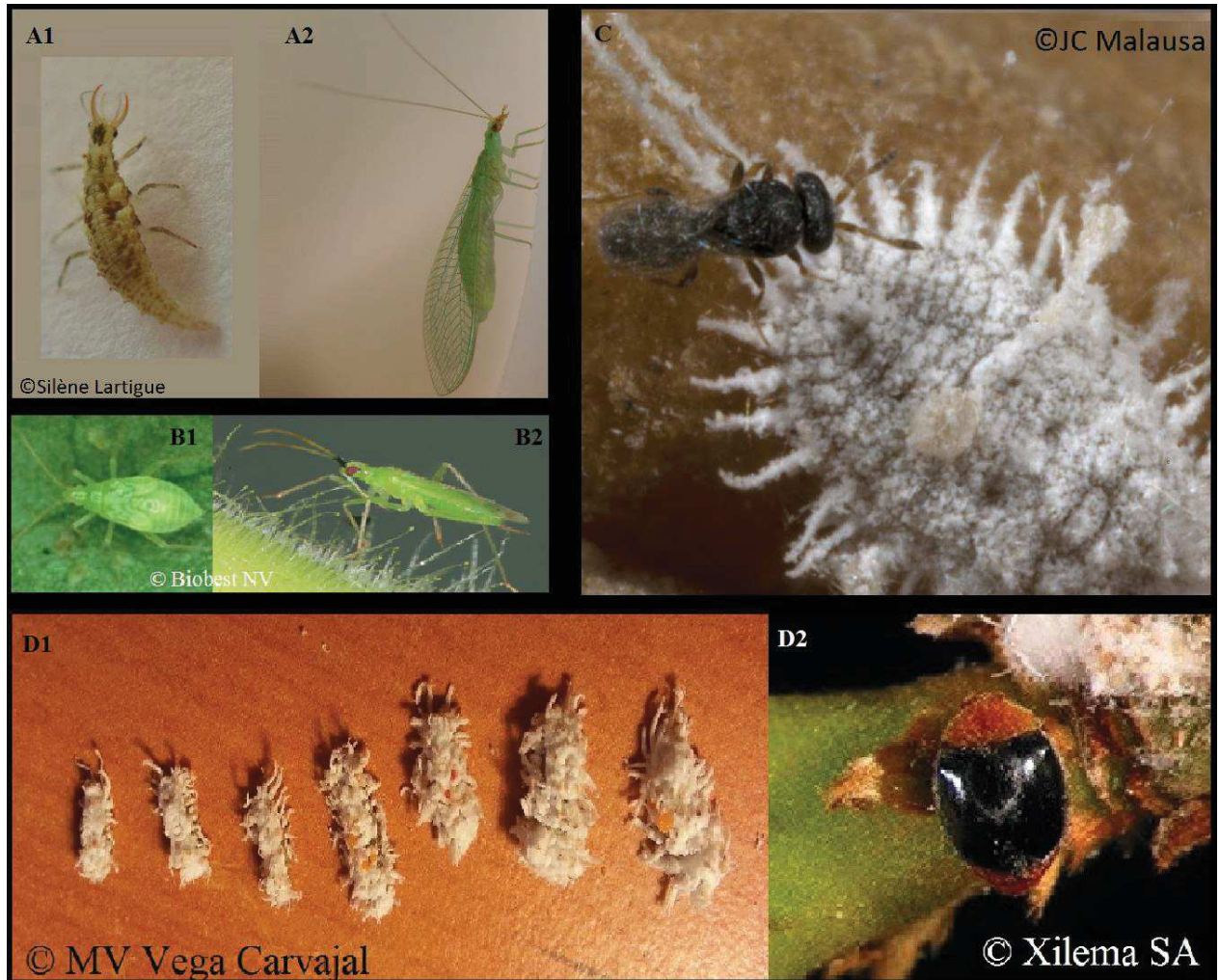


Figure 8: Photographies de chacune des espèces utilisées au cours de la thèse : *Chrysoperla near comanche* larve (A1) et adulte (A2), *Macrolophus pygmaeus* nymphe (B1) et adulte (B2) *Allotropa burrelli* (C), et *Cryptolaemus montrouzieri* larves (D1) et adultes (D2).

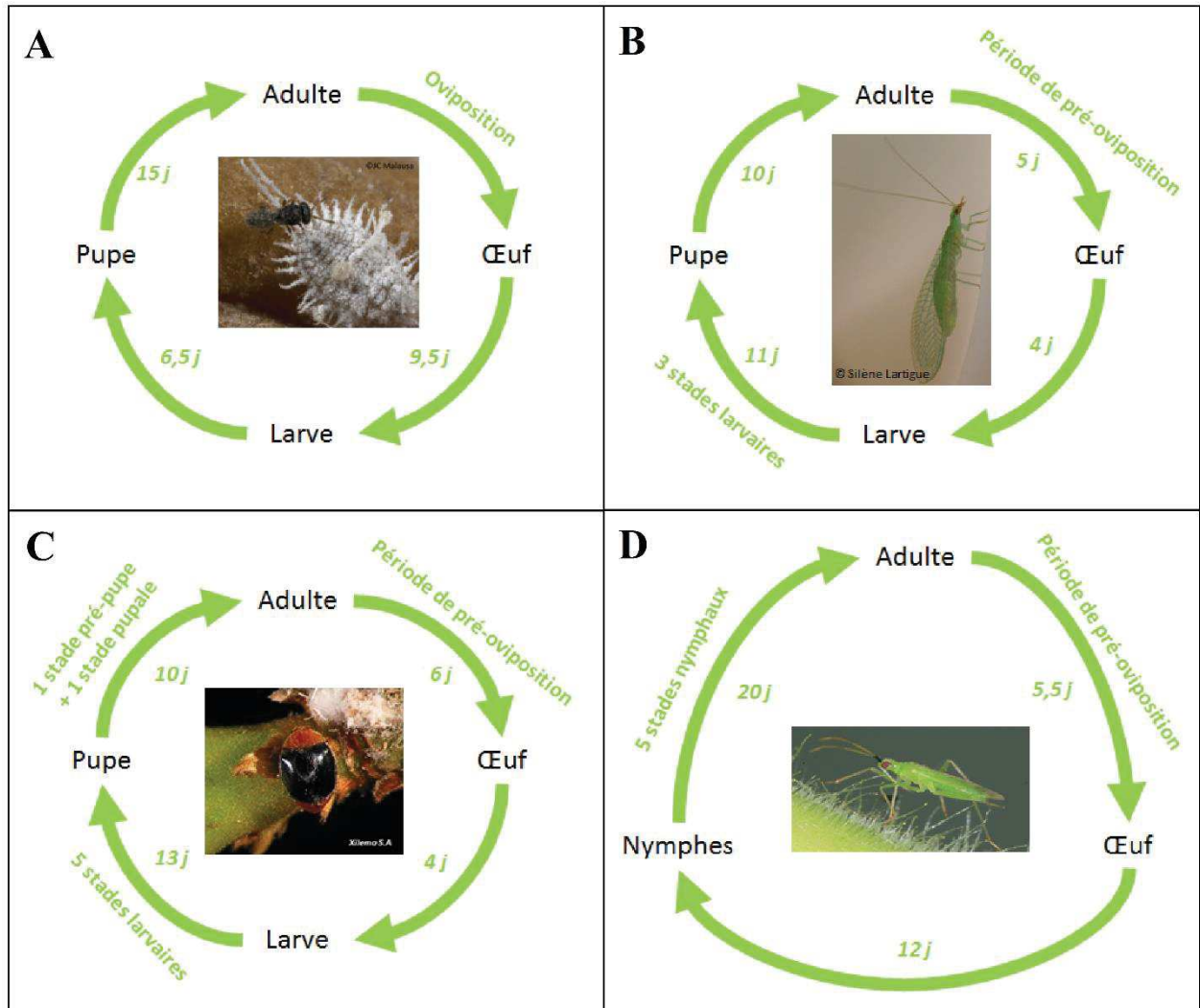


Figure 9 : Cycles de vie de (A) *A. burrelli* à 28°C, (B) *C. near comanche* à 25°C, (C) *C. montrouzieri* à 25°C et (D) *M. pygmaeus* à 25°C.

Tableau 1 : Description des caractéristiques biologiques et des populations des quatre espèces utilisées lors des expériences sur les effets de la consanguinité

Modèle biologique	ploïdie	souches utilisées	Nombre de générations en élevage
<i>Allotropa burrelli</i>	haplodiploïde	<u>Japon (x2)</u> Utsunomiya Tottori	>20
<i>Chrysoperla near comanche</i>	diploïde	<u>Chili</u>	>20
<i>Cryptolaemus montrouzieri</i>	diploïde	<u>Chili (x2)</u> sauvage élevage <u>Souche commerciale</u> <u>tierce</u>	1 >20 >20
<i>Macrolophus pygmaeus</i>	diploïde	<u>Belgique</u> <u>France</u>	2

3.3. Protocole standard

Pour chacune des expériences, j'ai employé une approche « par traitement » consistant à comparer la fitness d'individus issus de croisements frère-sœur à celle d'individus issus de croisement non-consanguins (croisements aléatoires effectués hors fratries). Pour chacun des organismes, des couples de génération « G₀ » ont été créés en prélevant aléatoirement des individus dans les élevages. Dans le cas où plusieurs souches étaient disponibles, des couples G₀ ont été créés à partir d'individus provenant de différentes souches pour maximiser l'hétérozygotie initiale de l'expérience. Les descendants de ces couples G₀, les individus G₁, ont ensuite été utilisés pour former des croisements intra-famille (frère – sœur) ou des croisements inter-familles. Les mesures de fitness ont enfin été effectuées sur les individus G₂ consanguins (provenant des croisements intra-famille) et non-consanguins (provenant des croisements inter-familles) (**Figure 10**). Bien que le protocole utilisé fut relativement similaire dans sa conception, j'ai pris soin de l'adapter aux caractéristiques biologiques des chacun des modèles biologiques et des souches disponibles (**Tableau 1**).

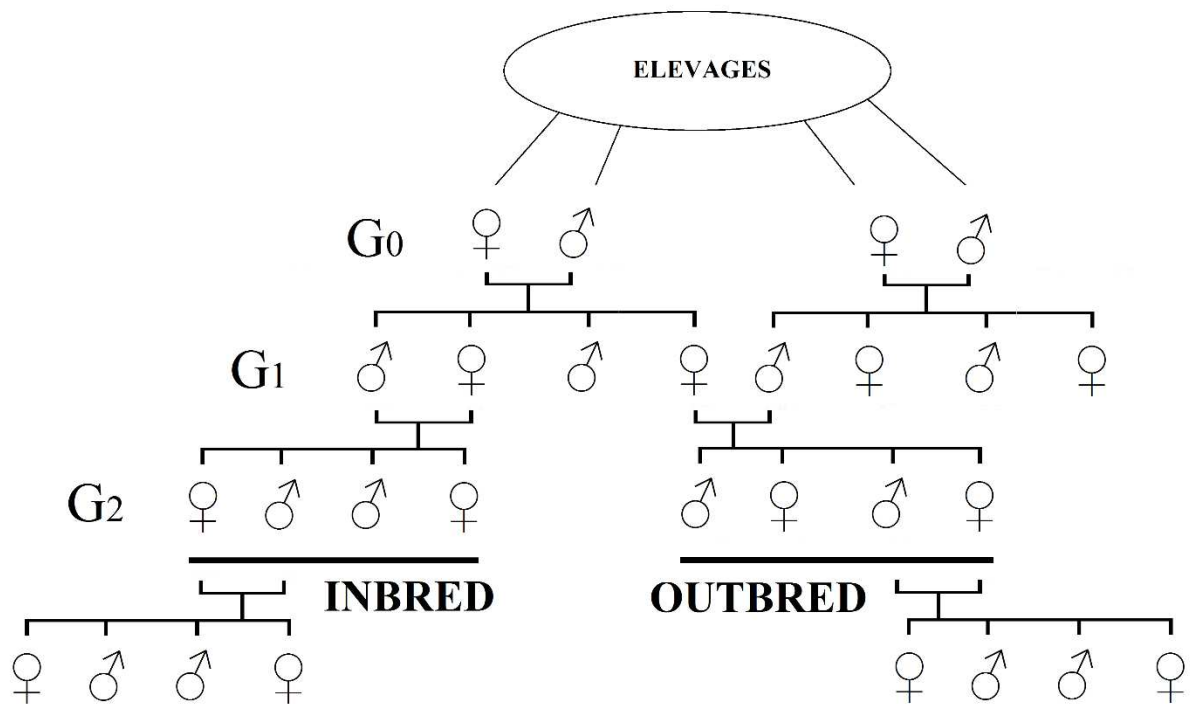


Figure 10: Design expérimental simplifié utilisé pour chacune des expériences réalisées sur les quatre modèles biologiques. Selon le modèle biologique concerné, le design a été adapté pour permettre l'utilisation de toutes les souches disponibles.

Nous avons observé des effets importants de la consanguinité chez l'espèce *M. pygmaeus* pour laquelle il a donc semblé pertinent d'évaluer les effets de l'hybridation pouvant minimiser la dépression de consanguinité (voir articles et paragraphe ci-après). Par conséquent, nous avons également utilisé la méthode du GBS sur *M. pygmaeus* pour permettre une comparaison entre les approches « par traitement » et l'approche corrélative grâce aux données génomiques. La méthode GBS repose sur plusieurs étapes expérimentales et informatiques pour permettre l'obtention des SNPs. Dans un premier temps nous avons effectué l'extraction de l'ADN au sein de l'Institut Sophia Agrobiotech. Les solutions d'ADN ont ensuite été placées dans 8 plaques 96 puits et envoyées au centre de diversité génomique de l'institut de biotechnologie à l'Université de Cornell (NY, Etats-Unis) (www.biotech.cornell.edu/brc/genomic-diversity-facility). Sur place, le protocole a été le même pour chaque plaque. L'ADN a été coupé grâce à des enzymes de restrictions et des codes-barres spécifiques à chaque individu ont été liés à chaque portion d'ADN. L'ADN de tous les individus a ensuite été regroupé dans une même solution pour effectuer une PCR et enfin le séquençage effectué avec la méthode Illumina haut débit. L'université de Cornell nous a fourni un fichier de séquences pour chacune des plaques

séquencées. Grâce à un pipeline informatique nous avons filtré, regroupé et comparé les séquences pour identifier les SNPs. Grâce au programme TASSEL, nous avons enfin pu extraire le taux d'hétérozygotie individuelle et la distance génétique interindividuelle du fichier de SNPs. Nous avons donc cherché à mettre en évidence une corrélation entre hétérozygotie et fitness chez cette espèce qui corroborerait les résultats observés avec l'approche par traitement.

Le protocole et les résultats de l'étude des effets de la consanguinité chez *A. burrelli* sont détaillés dans l'article suivant :

Quaglietti, B., Tamisier, L., Groussier, G., Fleisch, A., Le Goff, I., Ris, N., Kreiter, Ph., Fauvergue, X. & Malausa, T. (2017). No inbreeding depression in laboratory-reared individuals of the parasitoid wasp *Allotropa burrelli*. *Ecology and Evolution*, 7(3), 964-973. **(Manuscrit 1)**.

Le protocole et les résultats de l'étude des effets de la consanguinité chez *C. near comanche*, *C. montrouzieri* et *M. pygmaeus* sont détaillés dans l'article suivant :

Quaglietti, B., Vega Carvajal M-V., Lartigue, S., Bailly-Maitre, A., Baeza Morales, R., Le Goff, I., Hervi, F., Roman Gajardo C., Pekas, A., Wäckers, F., Amouroux, P., Fauvergue, X. & Malausa, T. Effects of inbreeding on the three biocontrol agents *Chrysoperla near comanche*, *Cryptolaemus montrouzieri* and *Macrolophus pygmaeus* in rearing conditions. *Soumis à Evolutionnary applications*. **(Manuscrit 2)**

Le protocole et les résultats visant à établir la relation entre hétérozygotie et fitness à l'échelle individuelle chez *M. pygmaeus* sont détaillés dans l'article suivant :

Quaglietti, B., Malausa, T., Fauvergue, X., Crochard, D., Wäckers, F., Pekas, A. & Palero, F. Genetic determinants of hybridization and fitness in mass-rearing of the biocontrol agent *Macrolophus pygmaeus* using genotyping-by-sequencing. *Redaction en cours*. **(Manuscrit 3)**

3.4. Synthèse des résultats obtenus

Les quatre expériences réalisées ont mis en évidence des résultats contrastés (**Tableau 2**). D'une part, aucune diminution de fitness due à la consanguinité n'a pu être mise en évidence sur les traits phénotypiques mesurés chez *A. burrelli* et *C. near comanche*. En revanche, nous avons pu observer une diminution de 6.7 % de la probabilité de survie des mâles chez *C. montrouzieri*. Par ailleurs, nous avons également pu mettre en évidence une diminution

substantielle (35.4%) du succès reproducteur chez *M. pygmaeus*. Globalement, nous avons pu observer plus de variation à la consanguinité chez *M. pygmaeus* que chez les trois autres espèces (**Figure 11**). Par ailleurs, j'ai pu mettre en évidence une corrélation directe entre la fitness (nombre de descendants produits au cours de la vie) chez les femelles *M. pygmaeus* et leur taux d'hétérozygotie individuelle obtenu grâce aux données génomiques issues du GBS (**Figure 12**). Dans le **chapitre 5**, je discuterai de ces résultats au regard des caractéristiques démographiques de chacune des espèces et populations étudiées et de leur conséquences vis-à-vis de l'efficacité de la lutte biologique. Je discuterai ces résultats dans le **chapitre 5**.

Tableau 2 : Récapitulatif des résultats obtenus lors des expériences visant à évaluer les effets de la consanguinité chez les quatre auxiliaires de lutte biologique

Modèle biologique	pléidie	souches utilisées	Nombre de générations en élevage	Traits phénotypique mesurés	Effet de la consanguinité
<i>A. burrelli</i>	haplodiploïde	<u>Japon (x2)</u> Utsunomiya Tottori	>20	Succès de parasitisme	NS
				Taux de parasitisme	NS
				Succès reproducteur	NS
				Fécondité réalisée ♀	NS
				Sex-ratio de la descendance	NS
				Temps de développement	NS
				Taille de l'abdomen ♀	NS
				Longévité ♀	NS
<i>C. near comanche</i>	diploïde	<u>Chili</u>	>20	Temps de développement	NS
				Succès de développement	NS
				Prédation	+ 40%
				Succès reproducteur	NS
				Fécondité ♀	NS
				Survie ♀	NS
<i>C. montrouzieri</i>	diploïde	<u>Chili (x2)</u> sauvage élevage <u>Souche commerciale tierce</u>	1 >20 >20	Succès de développement	NS
				Temps de développement	NS
				Sex-ratio	NS
				Prédation ♂	NS
				Fécondité	NS
				Survie ♀	NS
				Survie ♂	- 6.7%
<i>M. pygmaeus</i>	diploïde	<u>Belgique</u> <u>France</u>	2	Prédation	NS
				Succès reproducteur	- 35.4%
				Fécondité	NS
				Survie	NS

Indices de Dépression de Consanguinité + IC 95%

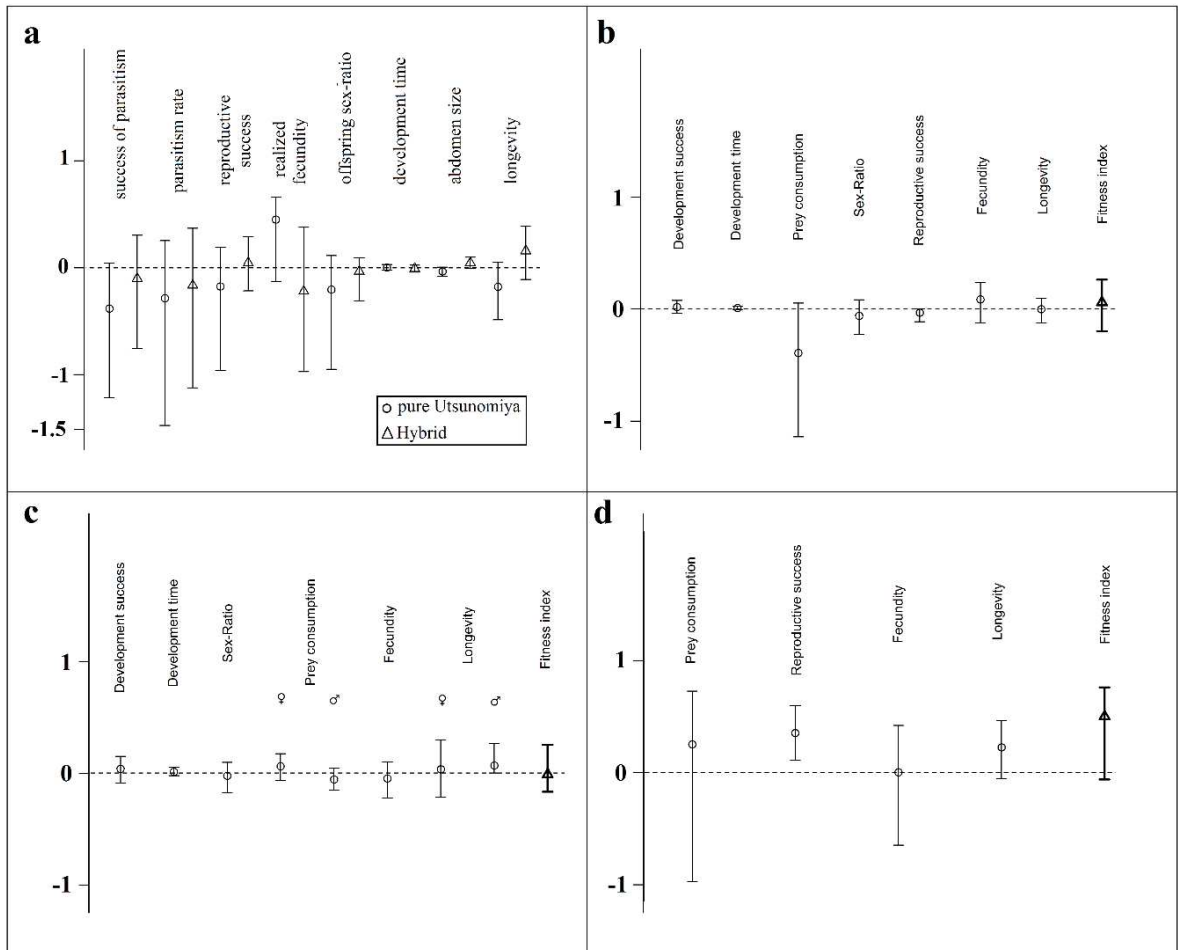


Figure 11 : Indices de dépression de consanguinité et intervalles de confiance à 95% calculés pour (a) *Allotropa burrelli*, (b) *Chrysoperla near comanche*, (c) *Cryptolaemus montrouzieri* et (d) *Macrolophus pygmaeus*.

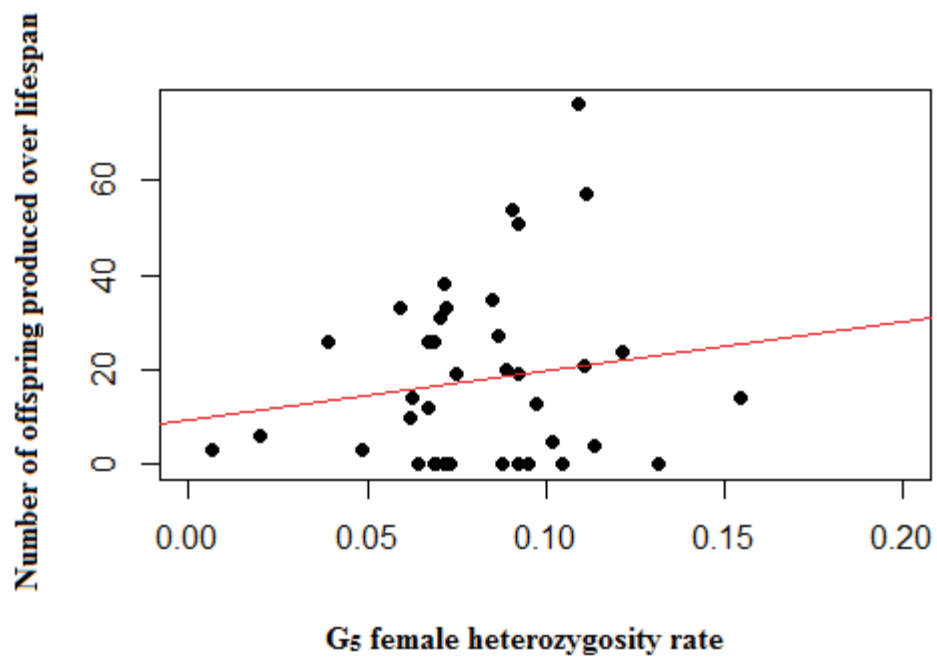


Figure 12 : Corrélation entre le nombre de descendants produits au cours de la vie et le taux d'hétérozygotie des femelles

CHAPITRE 4 : CONSEQUENCES DE L'HYBRIDATION SUR LA FITNESS INDIVIDUELLE DE *M. PYGMAEUS*.

4.1. Introduction

Diverses méthodes potentielles d'améliorations impliquant l'utilisation de processus génétiques sont appliquées dans de nombreux contextes d'élevages et de productions d'organismes ayant un intérêt économique. Parmi ces méthodes, on trouve des exemples de sélection de traits phénotypiques d'intérêt est appliquée pour maximiser le rendement et la qualité :

- des productions végétales (e.g. Dirlewanger et al., 2004; Munns et al., 2002).
- des élevages de bétails (e.g. Morley, 1955; Shook, 1993).
- des élevages d'aquaculture (e.g. Gjerdem, 1983; Hulata, 1995).
- et également des élevages d'auxiliaires de lutte biologique (e.g. Hoy et al., 1983; Roush and Hoy, 1981a, 1981b; Segal and Glazer, 2000).

La plupart des processus sélectifs sont dirigés vers des traits d'intérêts économiques liés à la croissance des populations ou du rendement des productions, mais également vers la sélection des phénomènes de résistance aux conditions extrêmes, aux maladies ou aux pesticides.

En outre, l'hybridation intra-spécifique est aujourd'hui une méthode particulièrement appliquées dans ces nombreux domaines. A l'instar de la consanguinité et de la sélection, l'hybridation intra-spécifique a reçu beaucoup d'attention dans le cadre des productions végétales (e.g. Briggles et al., 1967; Luan et al., 2010; Riday and Brummer, 2002) et les élevages de bétail ou d'aquaculture (e.g. Franke, 1980; Hulata, 1995; McGuirk et al., 1978; Rege et al., 1994). L'hybridation a donc déjà montré sa pertinence pour l'augmentation du rendement économique de certaines productions et constitue une méthode applicable à la production d'auxiliaires de lutte biologique.

Dans le cadre de la lutte biologique, on peut différencier les études liées à l'hybridation intra-spécifique en fonction du type de lutte biologique concerné. Dans le contexte de la lutte biologique classique, les études se sont intéressées à la relation potentielle entre hybridation

intra-spécifique et succès d'établissement des auxiliaires exotiques dans leur nouvel environnement (Cheyppé-Buchmann et al., 2011; Malausa et al., 2010; Szucs et al., 2011). Dans le contexte de la lutte biologique par augmentation et inondation, l'amélioration génétique des auxiliaires de lutte biologique via l'hybridation a été largement débattue depuis plusieurs décennies (Hopper et al., 1993; Hoy, 1992, 1985; Mackauer, 1972; Mackauer M., 1976; Roderick and Navajas, 2003). En effet, la variabilité phénotypique et génétique observée à un niveau intra-spécifique est un support potentiel de l'amélioration génétique des élevages d'auxiliaires de lutte biologique (Baker et al., 2003; Gebiola et al., 2016; Salame et al., 2010; Shapiro et al., 1997; Wu et al., 2004). L'hybridation est par ailleurs induite par une pratique courante supposée réduire les problèmes de dépression de consanguinité ou d'adaptation non contrôlée aux conditions industrielles : l'introduction de matériel biologique échantillonné dans la nature dans les populations d'élevage. Ces introductions sont cependant effectuées sans que les distances génétiques et écologiques entre les individus introduits et ceux de la population d'élevage soient parfaitement connues. Ainsi les effets de l'hybridation de ces individus est difficile à prédire. Les études s'intéressant à l'hybridation dans le cadre de la lutte biologique par augmentation ont cherché à mettre en évidence l'apport potentiel de l'hybridation sur la qualité des auxiliaires produits via le transfert de phénotypes préexistants ou l'apparition de nouveaux phénotypes pouvant avoir un impact sur le rendement des productions ou l'efficacité des auxiliaires sur le terrain. L'objectif est d'évaluer les effets de l'hybridation (parfois en amont d'un processus sélectif) pour maximiser les valeurs de traits impactant directement le rendement des productions ou l'efficacité des auxiliaires sur le terrain (Bai et al., 2005; Hoffmann et al., 2003; Mathenge et al., 2010; Mukuka et al., 2010; Shapiro-Ilan et al., 2005; Wu et al., 2004). Par exemple, Shapiro et al. (1997) ont pu induire une résistance aux températures élevées chez une souche commerciale du nématode entomopathogène *Heterorhabditis bacteriophora* auxiliaire de lutte biologique via l'hybridation avec une souche sauvage arborant ce caractère. Par ailleurs, une expérience a évalué les effets de l'hybridation sur la spécificité de deux haplotypes de *Dactylopius opuntiae*, utilisés comme auxiliaires spécifiques de deux espèces de cactus du genre *Opuntia* (Hoffmann et al., 2003). L'hybridation de deux haplotypes a constitué un avantage substantiel pour les hybrides de 1^{ère} génération leur permettant de s'attaquer et de se développer sur les deux plantes hôtes. En revanche, compte tenu de l'haplodiploïdie de *D. opuntiae*, certains descendants mâles de seconde génération élevés sur la plante hôte de leur mère ne pouvaient survivre avec leur génotype « parental » hérité de leur père. Les conséquences de l'hybridation pouvant être aussi bien positives que

négatives, celles-ci doivent être évaluées avant le mélange de souches différenciées dans les populations d'élevage.

Lors d'une expérience visant à décrire et prédire les conséquences de l'hybridation entre sept souches de l'auxiliaire *Trichogramma chilonis*, Benvenuto et al. (2012) ont pu mettre en évidence une variabilité dans l'isolement reproducteur allant d'un isolement reproducteur asymétrique à complet entre les différentes souches. Une grande variabilité phénotypique a pu être mise en évidence avec de la dépression de consanguinité pour une souche et de l'hétérosis lors de certains croisements. Plus généralement, une relation quadratique a pu être mise en évidence entre la fitness des individus hybrides et la distance génétique entre les souches parentales. Ces résultats suggèrent la pertinence de l'étude des effets de l'hybridation intra-spécifique pour développer des outils de diagnostic et à terme prédire les conséquences de l'hybridation dans l'optique de l'amélioration génétique des auxiliaires de lutte biologique. Bien qu'il apparaisse aujourd'hui encore difficile d'appliquer ces méthodes d'évaluation à tous les auxiliaires de lutte biologique, il apparaît pertinent d'éprouver ces outils sur des auxiliaires principaux ayant un intérêt économique majeur à travers le monde.

L'utilisation potentielle de l'hybridation intra-spécifique en lutte biologique nécessite plusieurs prérequis pour évaluer ses bénéfices et ces risques potentiels. D'abord, l'évaluation de la structure des populations et de leurs distances génétique et écologique au sein des espèces concernées. En effet, les variations génétiques, écologiques, comportementales des auxiliaires ou des endosymbiontes qui y sont associés peuvent avoir des effets sur leur gamme d'environnements ou des proies ou hôtes (Heraty, 2009; Hunter, 1999). L'interaction de nombreuses espèces cryptiques au sein des espèces d'auxiliaires peut également provoquer des phénomènes d'incompatibilités de reproduction ayant un effet néfaste sur le rendement des élevages ou sur l'efficacité sur le terrain (McEwen et al., 2007; Stouthamer et al., 2000). Les bactéries endosymbiotiques connues pour affecter la reproduction des arthropodes sont également à prendre en considération dans le cadre de la lutte biologique. Aebi & Zindel (2013) ont évalué à 40% la proportion d'auxiliaires de lutte biologique porteurs d'endosymbiontes pouvant altérer leur reproduction pour près de la moitié desquels des effets ont déjà été démontrés. Il apparaît donc très important de prendre en compte l'impact de ces endosymbiontes et de leurs effets potentiels sur la reproduction ou les paramètres de fitness des auxiliaires dans le cadre de la gestion des populations, à des fins d'amélioration génétique ou

d'évaluation des conséquences de l'incorporation dans les élevages industriels d'individus provenant de la nature.

L'essor des nouvelles méthodes de séquençage et notamment du « Génotyping by Sequencing » (GBS) fournit un outil méthodologique pouvant permettre d'explorer la structure des populations d'auxiliaires (paragraphe 2.3.3.). Ces méthodes peuvent permettre de mettre en évidence des espèces cryptiques ou des taxa différenciés au sein d'une même espèce et d'explorer la relation entre la distance génétique et le succès de reproduction des individus et la fitness des hybrides. De plus les méthodes de génotypage par séquençage étant susceptibles de caractériser une partie des génomes bactériens présents dans les échantillons, elles pourraient permettre d'identifier les principaux endosymbiontes et prédire leur effet sur l'hybridation.

Dans le cadre de cette thèse, j'ai voulu étudier les facteurs pouvant impacter les conséquences de l'hybridation chez *M. pygmaeus*, via une approche classique « par traitement » et également en utilisant la méthode GBS. L'objectif principal était d'explorer les effets de la diversité génétique et endosymbiotique sur la reproduction et la fitness pour évaluer le potentiel d'amélioration génétique pour cet auxiliaire d'intérêt majeur à la fois pour un des partenaires de la thèse mais également pour la lutte biologique par augmentation en général.

4.2. Matériel biologique – *Macrolophus pygmaeus*

Pour cette étude, nous avons utilisé quatre populations européennes de *M. pygmaeus* prélevées dans des espaces cultivés (serres ou champs ouverts) sur tomate ou souci (**Figure 13**). Chacune des populations a été maintenue en élevage séparément des autres pendant deux générations avant le début de l'expérience.



Figure 13 : Origines géographiques des populations de *Macrolophus pygmaeus* utilisées lors de l'expérience d'hybridation (Belgique, Espagne, France, et Grèce).

La taxonomie du genre *Macrolophus* a été fondamentalement modifiée au cours des dernières années. *Macrolophus caliginosus* a pendant longtemps été identifiée comme une espèce homogène, avant que les deux taxa différenciés *M. melanotoma* et *M. pygmaeus* ne soient récemment identifiés (Castañé et al., 2012; Martinez-Cascales et al., 2006; Perdikis et al., 2003). Depuis, la structure des populations européennes de *M. pygmaeus* a été explorée et l'existence de deux voire trois haplotypes a pu être mise en évidence sur la base de l'analyse d'ADN mitochondrial et de microsatellites (Sanchez et al., 2012; Streito et al., 2016). Bien que ces haplotypes aient été trouvés en sympatrie dans les cultures, un flux de gènes asymétrique a pu être mis en évidence suggérant un isolement reproducteur au moins partiel. Par ailleurs, une étude de la communauté microbienne du genre *Macrolophus* a pu mettre en évidence la présence de bactéries des genres *Wolbachia* et *Rickettsia* (Machtelinckx et al., 2012) suspectées de provoquer des incompatibilités cytoplasmiques entre les espèces *M. pygmaeus* and *M. melanotoma* (Machtelinckx et al., 2009). Compte tenu de l'importance économique de *Macrolophus sp.* en lutte biologique, des doutes concernant sa structure des populations et de la présence de bactéries pouvant provoquer des incompatibilités cytoplasmiques entre ses populations, l'étude des conséquences de l'hybridation chez cette espèce apparait particulièrement pertinente.

4.3. Protocole standard

Des individus ont été prélevés aléatoirement dans chacune des populations et des couples de génération « G₀ » ont été créés en effectuant toutes les combinaisons possibles entre et au sein des quatre populations (**Figure 114**). Le succès de reproduction et le nombre de descendants a été mesuré pour chacun des couples formés. Pour chaque combinaison, des croisements aléatoires entre individus « G₁ » ont ensuite été réalisés pour obtenir la génération « G₂ ». Pour comparer la fitness des individus hybrides et des individus issus des populations parentales, quatre traits phénotypiques (le succès reproducteur, la fécondité, la prédation, et la survie) ont été mesurés chez les descendants « G₁ » et « G₂ ». Nous avons séquencé l'ADN génomique des individus « G₀ » et « G₁ » via GBS pour permettre l'évaluation de la structure des populations et l'identification des endosymbiontes bactériens.

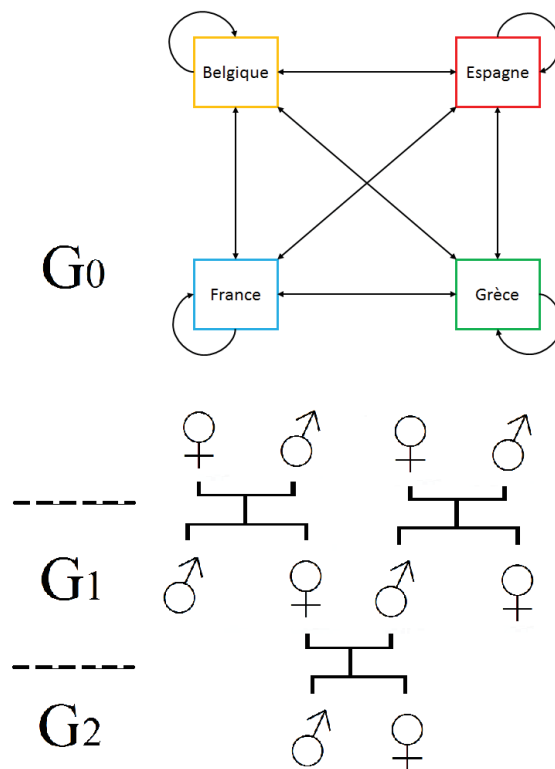


Figure 14: Design expérimental simplifié utilisé pour l'expérience visant à évaluer les conséquences de l'hybridation sur *M. pygmaeus*.

Le protocole et les résultats visant à établir la relation entre hétérozygotie et fitness à l'échelle individuelle chez *M. pygmaeus* sont détaillés dans l'article suivant :

Quaglietti, B., Malausa, T., Fauvergue, X., Crochard, D., Wäckers, F., Pekas, A. & Palero, F. Dissecting the determinants of reproductive isolation and fitness in mass-rearing of the biocontrol agent *Macrolophus pygmaeus*, using genotyping-by-sequencing. *Redaction en cours*. (Manuscrit 3)

4.4. Synthèse des principaux résultats

Nous avons pu identifier trois clusters génétiques à l'intérieur des populations européennes. Des variations importantes de succès reproducteur dépendantes des combinaisons de populations ou de clusters génétiques entre les partenaires sexuels ont pu être observées, avec notamment un isolement reproducteur quasi-total entre les trois clusters génétiques (Figure 15) et lié à la distance génétique entre les partenaires (Figure 16).

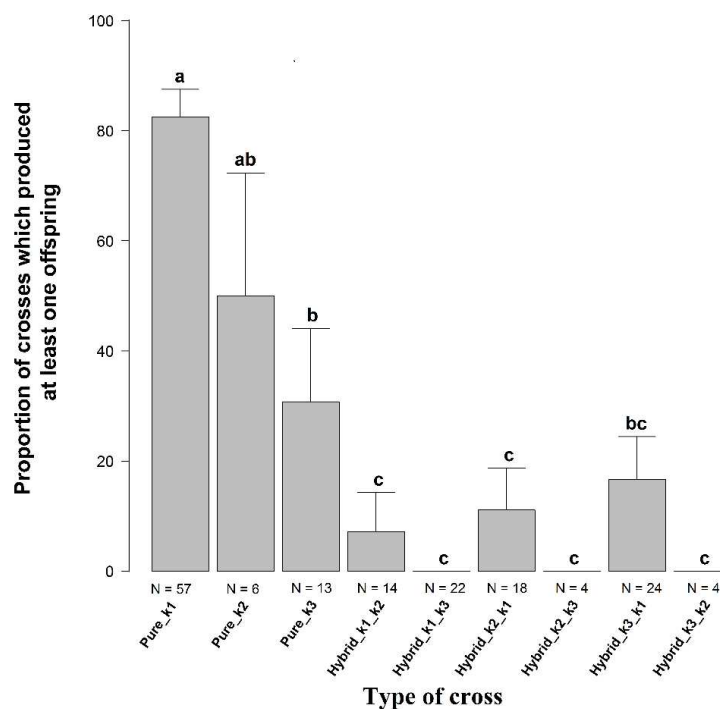


Figure 15 : Proportion de croisements ayant produit au moins un descendant en fonction de la combinaison de clusters de la femelle et du mâle.

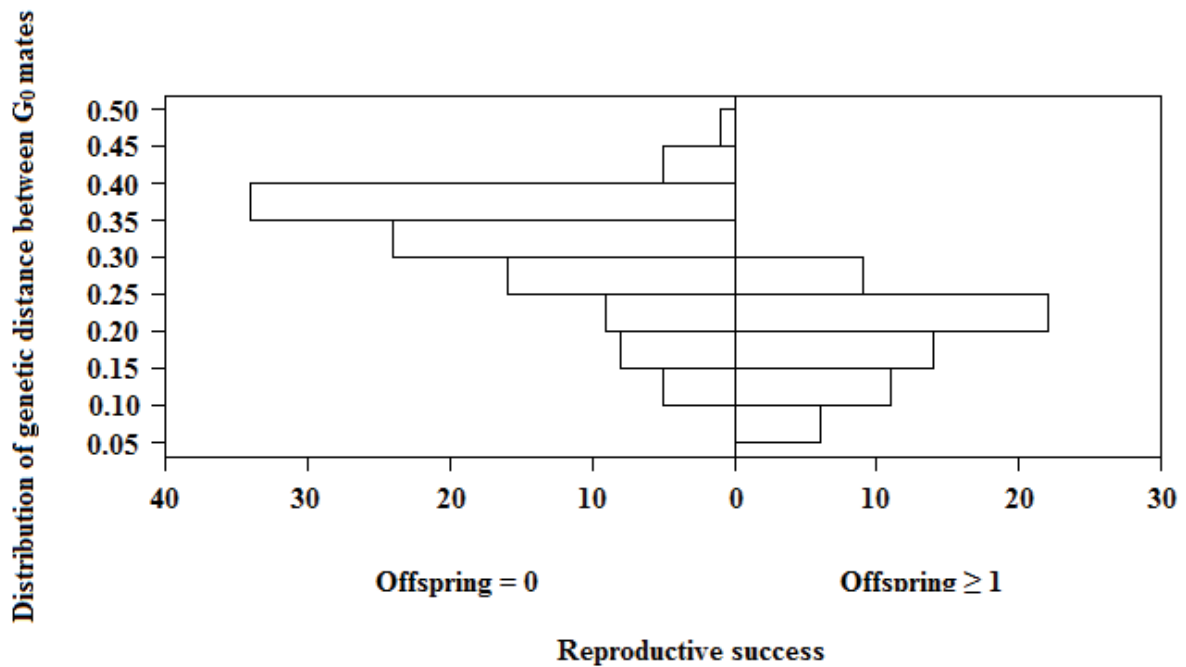


Figure 16 : Distribution de la distance génétique (en %) entre mâles et femelles G_0 (in %) ayant produit ou non des descendants.

Par ailleurs, nous avons également pu mettre en évidence une relation positive entre la distance génétique des parents G_0 et la prédation (**Figure 17**) et le succès reproducteur des femelles G_1 (**Figure 18**). Je discuterai tous ces résultats dans le **chapitre 5**.

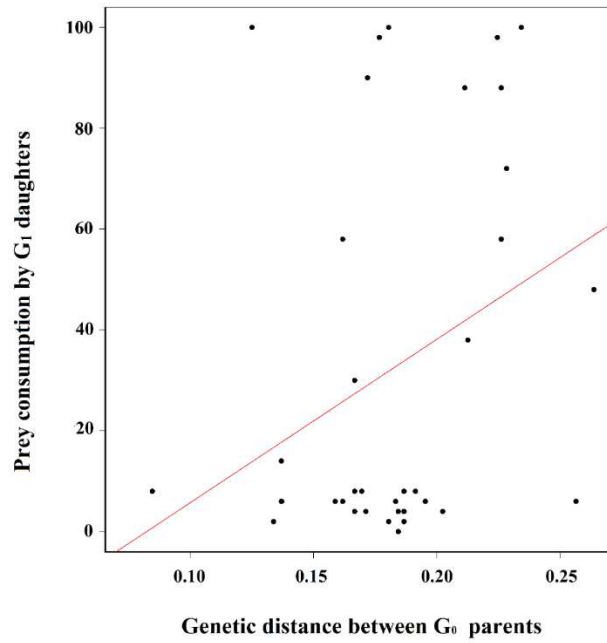


Figure 17 : Relation entre la distance génétiques des parents G₀ et le nombre de proies consommées par les femelles G₁.

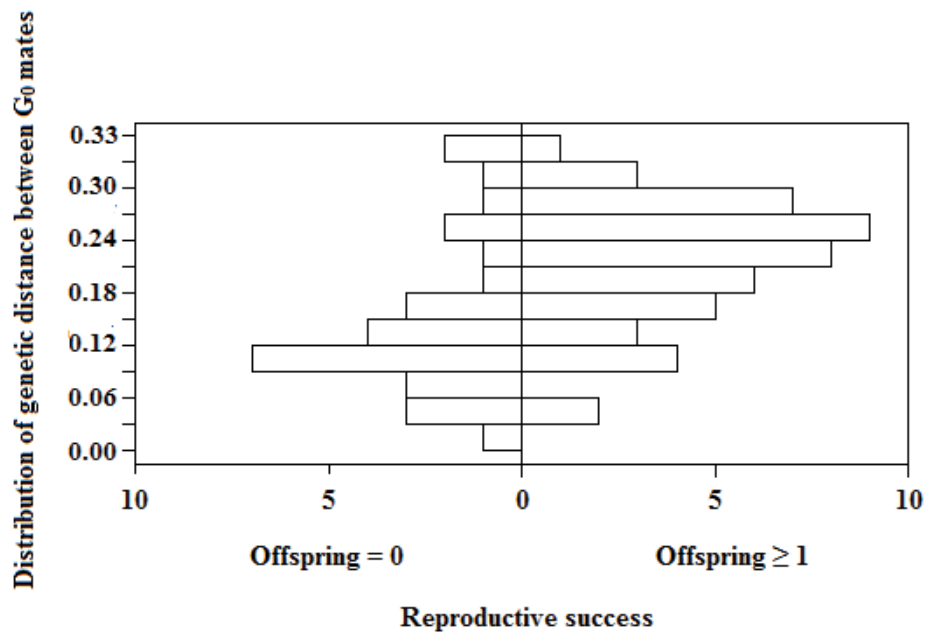


Figure 18 : Distribution des distances génétiques entre parents G₀ pour les femelles G₁ ayant produit ou non des descendants.

CHAPITRE 5 : DISCUSSION GENERALE

Je m'étais fixé trois objectifs principaux au cours de cette thèse :

- D'abord, en collaboration avec les partenaires industriels des projets auxquels j'ai participé, évaluer les effets de la consanguinité dans les élevages de plusieurs auxiliaires de lutte biologique à l'aide d'une approche expérimentale classique « par traitement » visant à comparer la fitness d'individus issus de croisements intra-famille (croisements frère – sœur) à celle d'individus issus de croisement inter-familles (croisement aléatoires hors fratries).

- Ensuite, évaluer les effets de l'hybridation intra-spécifique chez *M. pygmaeus* pour son amélioration génétique dans le cadre de la lutte biologique par augmentation.

- Enfin, évaluer la pertinence, dans le contexte de la R&D en lutte biologique, des nouvelles méthodes de séquençage pour évaluer les effets des processus génétiques au sein des populations d'élevage d'auxiliaires.

5.1. Effets de la consanguinité et implications pour la lutte biologiques

L'étude des effets de la consanguinité sur les quatre auxiliaires de lutte biologique étudiés ont montré des résultats contrastés. Nous avons pu observer deux types de réponses au protocole expérimental mis en place :

- D'une part, nous n'avons pu mettre en évidence aucun effet néfaste majeur de la consanguinité sur la fitness des individus des populations étudiées d'*Allotropa burrelli*, et *Chrysoperla near comanche* et qui pourraient avoir un impact négatif pour la lutte biologique impliquant ces auxiliaires (**Tableau 2**). Bien que l'augmentation de près de 40% du nombre de proies consommées chez les larves de *C. near comanche* pourrait traduire de la dépression de consanguinité sur des voies métaboliques impliquant un besoin accru de consommation de ressources, cela pourrait aussi se traduire de manière positive dans le cadre de la lutte biologique en induisant la consommation d'un plus grand nombre de ravageurs. De manière générale, l'approche quantitative via la mesure des indices de dépression de consanguinité a montré peu de variations des traits phénotypiques liées à la consanguinité chez ces deux espèces (**Figure 11**).

- D'autre part, nous avons pu mettre en évidence une diminution de la probabilité de survie de 6.7% chez les mâles consanguins de *Cryptolaemus montrouzieri* (**Tableau 2**). Cet effet avait déjà été observé lors d'études précédentes sur des souches Egyptiennes et Syriennes de *C. montrouzieri* suggérant que ce trait est sensible à la consanguinité (Al-Khateeb et al., 2013, 2012). Bien que n'entraînant pas une diminution majeure de fitness, cette diminution pourrait impacter l'efficacité de la lutte biologique car la longévité des adultes est importante lors des phases de conditionnement, stockage et transport préalables aux lâchers sur le terrain. Par ailleurs, nous avons mis en évidence de la dépression de consanguinité sur le succès reproducteur des femelles consanguines chez *Macrolophus pygmaeus*. L'effet observé est significatif et conséquent puisqu'il montre une diminution de la valeur du trait de 35.4% chez les femelles consanguines. Cet effet de la consanguinité peut constituer une préoccupation majeure dans le cadre de la lutte biologique puisqu'il pourrait provoquer une diminution drastique du rendement des productions de masse mais également affecter l'établissement temporaire nécessaire à l'efficacité du contrôle des ravageurs après l'introduction sur le terrain. La mesure des indices de dépression de consanguinité a également montré une variation plus importante des valeurs de traits mesurés due à la consanguinité chez *M. pygmaeus*, en comparaison aux autres espèces étudiées (**Figure 11**).

Les effets de la consanguinité sont dépendants du fardeau génétique et donc de l'historique démographique des populations et varieront selon les espèces, souches et populations utilisées (Fauvergue et al., 2012; Kirkpatrick and Jarne, 2000). Lors de l'initiation d'une population d'élevage, le goulot d'étranglement que subira la population va favoriser la consanguinité et l'expression des allèles délétères. Après l'élimination rapide des allèles très délétères voir létaux, la fitness moyenne de la population se stabilisera, soit parce que les allèles délétères auront été soit purgés, soit fixés (Glémin, 2003). Ainsi, les effets néfastes du fardeau génétiques sont le plus à craindre dans les premières étapes d'établissement des populations d'élevage mais ne risquent pas d'occasionner un déclin des populations à moyen terme. Cependant, dans le cas de la fixation d'allèles délétères dans les populations, le rendement des productions et la qualité des auxiliaires produits peuvent s'avérer sous-optimaux. Il appartient donc à chaque producteur de prendre des mesures pour maximiser la fitness de ces élevages et l'hybridation constitue alors une opportunité pour diminuer les effets du fardeau génétique.

5.2. Effets de l'hybridation sur *M. pygmaeus* et implications pour la lutte biologique

Nous avons pu identifier trois clusters au sein des populations européennes de *M. pygmaeus*, en cohérence avec les études publiées récemment à l'aide de données microsatellites (Sanchez et al., 2012; Streito et al., 2016). Nous avons pu mettre en évidence une forte incompatibilité de reproduction entre ces différents clusters puisque seulement 8% des croisements entre clusters ont mené à la production de descendants. Ce résultat est congruent avec les études montrant un isolement reproducteur par la distance dans le cadre d'hybridation intra-spécifique (Benvenuto et al., 2012; Edmands, 2002; Frankham et al., 2011). Ce résultat apparaît important pour la lutte biologique. D'une part, l'incorporation de matériel biologique pour maximiser la diversité génétique des élevages doit être fait en connaissance des clusters dans les populations d'élevage et celles prélevées dans la nature. Dans le cas contraire, le mélange de clusters différents pourrait provoquer une diminution brutale du nombre d'individus produits, occasionnant une perte de rendement des populations d'élevage et un goulot d'étranglement qui diminuerait la diversité génétique et pourrait provoquer des effets génétiques néfastes. Ce résultat ne permet pas pour l'instant d'envisager l'amélioration génétique de *M. pygmaeus* via l'hybridation entre ces clusters. Le faible nombre d'individus hybrides produit n'a pas permis d'évaluer leur fitness et leur potentiel pour la lutte biologique sans pour autant l'éliminer totalement. En effet, nos données en l'état n'ont pas permis d'évaluer les effets des endosymbiontes sur le succès reproducteur ou sur la fitness individuelle.

En considérant uniquement les croisements à l'intérieur d'un même cluster, nous avons pu mettre en évidence une relation positive entre la distance génétique des parents et des traits de fitness tels que le nombre de proies consommées et le succès reproducteur de leurs descendants. Nos résultats pourraient s'avérer intéressants pour l'optimisation de la production et de l'efficacité sur le terrain de *M. pygmaeus*. Ils suggèrent que de nombreux types d'améliorations peuvent être envisagés, pouvant aller d'actions très simples utilisant la diversité observée jusqu'à des programmes d'amélioration génétique plus complexes. Parmi les actions simples, l'élevage parallèle de plusieurs populations correspondant aux différents clusters pourrait permettre de relâcher sur le terrain des individus correspondant au cluster déjà présent pour favoriser la reproduction et le contrôle des ravageurs. Ensuite, nos résultats suggèrent que la maximisation des croisements allogamiques pourrait permettre une augmentation de fitness des populations du moment que les individus proviennent d'un même cluster. En fonction des

mécanismes sous-jacents, il pourrait être possible de capturer certains traits phénotypiques d'intérêt en créant une ou plusieurs lignées fixées au niveau génétique et les utiliser dans les agrosystèmes dans lesquels leur efficacité est optimale (Bai et al., 2005; Ballou, 1984). La diversité génétique et phénotypique observée permet par ailleurs d'envisager de réels programmes d'amélioration génétique basés sur la mise au point de méthodes de phénotypage utilisables pour sélectionner les phénotypes sur des traits conférant un avantage en termes de production ou d'efficacité de terrain. Pour toutes les pistes d'amélioration, le défi majeur est sans doute de faire le lien entre les traits phénotypiques mesurés au laboratoire et les caractéristiques clés du succès attendu dans chaque agrosystème. Avant d'engager des programmes d'amélioration lourds, des preuves de concept pourraient être visées, en utilisant des populations ou lignées présentant des valeurs extrêmes à certains traits et en caractérisant leur dynamique d'établissement et leur capacité de contrôle des ravageurs dans des agrosystèmes ciblés par la lutte biologique. La pertinence des différentes voies de R&D dépend avant tout de critères technico-économiques, *Macrolophus pygmaeus* étant un auxiliaire largement utilisé mais générant des revenus sans commune mesure par rapport aux organismes faisant traditionnellement l'objet de recherches en amélioration génétique (plantes cultivées, bétail, etc.). Se pose également la question de la protection des innovations chez les macro-organismes de lutte biologique : les entreprises développant des souches plus efficaces n'ont pour l'instant aucun moyen évident d'empêcher une autre entreprise de l'utiliser et de la commercialiser (Cock et al., 2010).

La mise en évidence de nombreuses espèces cryptiques au sein des taxa utilisés comme auxiliaires de lutte biologique (Mound et al., 2010; Paterson et al., 2016; Sha et al., 2007; Tosevski et al., 2011) suggère que de nombreux cas pourraient être similaires à celui de *M. pygmaeus*. L'identification des populations ou sous-espèces est donc primordial avant de procéder à la gestion des populations d'auxiliaires, pour éviter les incompatibilités de reproduction ou la dépression hybride qui pourraient survenir.

5.3. Approches expérimentales

Les conséquences des processus génétiques qu'ils soient consanguinité ou hybridation sont très spécifiques des espèces et des populations étudiées et paraissent donc difficilement généralisables. Les questions concernant les effets de ces processus génétiques se poseront donc

pour chaque nouvel auxiliaire mis en production. Le développement de méthodes d'étude de ces effets génétiques est donc primordial pour leur prise en compte en lutte biologique. Grâce à une approche expérimentale classique « par traitement » nous avons pu mettre en évidence des effets génétiques dans certaines des populations étudiées. Bien que ce type d'approche se fasse à moindre coût elle est néanmoins très chronophage et peut mobiliser des ressources matérielles et logistiques importantes. Chacune des expériences menées au cours de cette thèse a nécessité plusieurs mois de préparation et d'expérimentation pour permettre l'obtention des données. Par rapport à l'approche expérimentale par traitements, l'obtention des données génomiques pour l'approche corrélatrice a imposé des coûts supérieurs en termes de fonctionnement (prestations de séquençage principalement), mais inférieurs en termes de besoins en ressources humaines et en infrastructures. L'utilisation de données génomiques nous a permis de mettre en évidence la structure des populations étudiées, une incompatibilité de reproduction et l'effet de la distance génétique sur la fitness de manière fiable et rapide.

5.4. Conclusions et perspectives

Nous avons pu démontrer au cours de cette thèse l'importance que pouvait avoir l'étude des effets de la consanguinité et de l'hybridation dans les élevages d'auxiliaires de lutte biologique. Les institutions partenaires se sont montrées très concernées par la gestion des effets génétiques dans leur production à l'avenir. Toutefois, la mise en place de protocoles expérimentaux lourds et contraignants pouvant prendre plusieurs mois constitue un obstacle dans des entreprises disposant de budgets R&D limités et en attente des résultats rapides ayant un impact majeur sur leur rendement économique. L'utilisation des nouvelles méthodes de séquençage peut alors constituer une alternative fiable et rapide pour réduire les besoins en personnel et infrastructures. Néanmoins, il apparaît aujourd'hui difficile d'imaginer une généralisation de l'utilisation de ces méthodes dans l'industrie de lutte biologique. D'abord parce qu'elles restent liées à un coût relativement élevé de séquençage et que les résultats ne garantissent pas des bénéfices majeurs ou garantis. Ensuite parce que les analyses des séquences obtenues nécessitent des ressources matérielles (informatiques et laboratoires) et des compétences dont ne disposent généralement pas les entreprises produisant des auxiliaires. L'utilisation de ces méthodes risque alors de se limiter à des auxiliaires d'intérêt majeur et dans le cadre de collaborations entre secteurs public et privé.

Les partenaires du projet ont néanmoins pris conscience de l'intérêt que pouvait avoir la gestion de la génétique des populations sur l'efficacité de la lutte biologique. Plusieurs d'entre eux ont notamment débuté le développement d'élevages de lignées consanguines dans le but de stabiliser certains traits phénotypiques ou de favoriser la purge des allèles délétères et ainsi provoquer un effet hétérosis lors des lâchers. D'autres ont également mis en place des populations parallèles élevées dans des conditions différentes pour favoriser l'adaptation à des proies, hôtes, ou conditions environnementales différentes et ainsi développer des produits particulièrement adaptés à la demande. Sur ce plan, je considère que cette thèse a permis un transfert de compétences mutuelles entre les secteurs privé et public et une meilleure compréhension des contraintes et des intérêts de chacun qui s'avèreront salutaires pour les collaborations futures.

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Manuscrit 1

No inbreeding depression in laboratory-reared individuals of the parasitoid wasp *Allotropa burrelli*

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Funding information

French Agence Nationale de la Recherche,
Grant/Award Number: ANR-10-JCJC-1708;
European Union Seventh Framework
Programme FP7, Grant/Award Number: FP7-
IAPP #324475

Abstract

Inbreeding depression is a major concern in almost all human activities relating to plant and animal breeding. The biological control of pests with natural enemies is no exception, because populations of biocontrol agents experience a series of bottlenecks during importation, rearing, and introduction. A classical biological control program for the Comstock mealybug *Pseudococcus comstocki* (Hemiptera: Pseudococcidae) was initiated in France in 2008, based on the introduction of an exotic parasitoid, *Allotropa burrelli* Mues. (Hymenoptera: Platygasteridae), a haplodiploid parasitoid imported from Japan. We evaluated the sensitivity of *A. burrelli* to inbreeding, to optimize rearing and release strategies. We compared several morphological and life-history traits between the offspring of siblings and the offspring of unrelated parents. We took into account the low level of genetic variability due to the relatively small size of laboratory-reared populations by contrasting two types of pedigree: one for individuals from a strain founded from a single field population, and the other generated by hybridizing individuals from two strains founded from two highly differentiated populations. Despite this careful design, we obtained no evidence for a negative impact of inbreeding on laboratory-reared *A. burrelli*. We discussed the results in light of haplodiploid sex determination and parasitoid mating systems, and classical biological control practices.

KEYWORDS

Allotropa burrelli, biological control, captive populations, genetic load, haplodiploidy, inbreeding depression

1 | INTRODUCTION

Bottlenecked populations, whether natural or artificially reared, may display panmictic inbreeding, when genetically related individuals mate despite the occurrence of random mating (Malécot, 1969). Severe consequences may occur such as inbreeding depression, defined as a lower fitness of inbred than of outbred individuals (Charlesworth & Charlesworth, 1987; Keller & Waller, 2002), which itself increases the risk of population extinction (Bijlsma, Bundgaard, & Boerema, 2000;

Fauvergue, Vercken, Malausa, & Hufbauer, 2012; Frankham, 2005; Nieminen, Singer, Fortelius, Schöps, & Hanski, 2001; Wright, Tregenza, & Hosken, 2007).

Inbreeding depression arises when the increase in homozygosity in inbred individuals leads to at least one of the following mechanisms: (1) the expression of detrimental recessive alleles (partial dominance) or (2) a general advantage for heterozygosity which translates into lower relative fitness for highly homozygous inbred individuals (overdominance; Charlesworth & Willis, 2009). As partial dominance

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is the most pervasive mechanism (Charlesworth & Charlesworth, 1987; Crnokrak & Roff, 1999), the intensity of inbreeding depression thus depends on the population “genetic load.” It is defined as the frequency of detrimental recessive alleles in the genetic pool (Charlesworth & Charlesworth, 1987; Charlesworth & Willis, 2009; Glémin, 2003) which is mainly influenced by purge, that is, the elimination of detrimental alleles by drift or by selection (Crnokrak & Barrett, 2002; Glémin, 2003; Swindell & Bouzat, 2006). The strength of purge is influenced by many factors, including the effect size of detrimental alleles, their degree of recessiveness, and population size.

Population size has a complex effect on the purging of detrimental alleles. Inbreeding depression is most likely to occur in small populations, which have higher levels of genetic relatedness, and in recently bottlenecked populations in which genetic drift may overcome selection and favor an increase in the frequency of deleterious mutations, or even their fixation (Fauvergue et al., 2012; Luque et al., 2016). By contrast, a rapid increase in population size after a bottleneck may purge the genetic load, leading to a decrease in inbreeding depression (Facon et al., 2011; Kirkpatrick & Jarne, 2000; Pujol, Zhou, Sanchez, & Pannell, 2009). Thus, inbreeding depression in small populations may be either stronger or weaker than that in populations at demographic equilibrium, depending on the magnitude and duration of the bottlenecks experienced.

Ploidy (diploid vs. haplodiploid) may also affect the purging process. Haplodiploid organisms should be less sensitive to inbreeding, because recessive deleterious alleles are expressed in haploid males and purged through selection (Crozier, 1985; Werren, 1993). However, theoretical models suggest that haplodiploids should nevertheless suffer from inbreeding depression, because the proportion of deleterious alleles is decreased, but these alleles are not completely eliminated (Glémin, 2003; Werren, 1993). In addition, genes involved in female-specific traits would not be purged through haploid males, so inbreeding depression is more likely for such traits (Werren, 1993). Secondly, in many gregarious or quasi-gregarious species (which deposit more than one egg per host or per host patch), males mate locally with their sisters emerging from the same host individual or host patch. This behavior induces regimes of high inbreeding, which could accelerate purging. Gregarious organisms would therefore be expected to display lower levels of inbreeding depression than solitary species.

Henter (2003) performed a meta-analysis to test these predictions in insects, by comparing inbreeding depression between diploid and haplodiploid species and between solitary and gregarious species. As expected, diploids were more strongly affected by inbreeding than haplodiploids, although significant inbreeding depression was observed in some haplodiploids (Gerloff & Schmid-hempel, 2005; Greeff, Jansen van Vuuren, Kryger, & Moore, 2009; Henter, 2003). Nevertheless, the authors found no difference between solitary and gregarious species. Finally, a difference in sensitivity to inbreeding between female and male traits was reported in one study, on the mite *Tetranychus urticae*, in which inbreeding affected oviposition rate but not traits common to males and females (Tien, Sabelis, & Egas, 2014).

Inbreeding depression has been studied in the context of live-stock breeding (Croquet, Mayeres, Gillon, Vanderick, & Gengler,

2006; Dorostkar et al., 2012), plant breeding (Basamma, Kajjidoni, Salimath, & Malagouda, 2009; Jain & Bharadwaj, 2014), conservation biology (Hedrick & Kalinowski, 2000), invasive species management (Facon et al., 2011), and biological control (Facon et al., 2011; Fauvergue et al., 2012; Henter, 2003). In biological control, inbreeding depression may be an issue during laboratory and industrial rearing (de Clercq, Vandewalle, & Tirry, 1998) as well as during field releases (Fauvergue & Hopper, 2009), leading to a decrease in biocontrol performance and consequent chance of extirpation of the control species. Although concerns have frequently been raised about inbreeding depression in biological control (Hopper, Roush, & Powell, 1993; Mackauer, 1976), experimental data are scarce and diverse patterns have been observed. For example, inbreeding was found to have no effect on phenotypic traits in the predator *Podisus maculiventris* (Say; Heteroptera: Pentatomidae) during the monitoring of inbred lines and mass-reared populations for 30 generations (de Clercq et al., 1998). By contrast, decreases in adult emergence and host mortality were observed after two generations of inbreeding in the fly *Exorista japonica* (Nakamura, 1996). Inbred lines even went extinct after six generations of inbreeding, whereas conventionally bred lines continued to thrive. Similarly, a laboratory study on *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) reported the rapid development of differences in fecundity, longevity, and predation capacities between sib-mating lines and lines displaying random allogamy (Al-Khateeb, Asslan, El-Heneidy, & Basheer, 2013).

We investigated the effect of inbreeding in the parasitoid wasp *Allotropa burrelli* Muesebeck (Hymenoptera: Platygasteridae). As a specialist natural enemy of the Comstock mealybug *Pseudococcus comstocki* (Hemiptera: Pseudococcidae), it was used as a biological control agent. *A. burrelli* originates to Northeast Asia and has been introduced into Central Asia (DeBach & Rosen, 1991) and North America (Meyerdirk & Newell, 1979), for the biological control of *P. comstocki*, and is currently used in a biological control program in France.

Allotropa burrelli has several features likely to render it insensitive to inbreeding depression. It is a gregarious endoparasitoid, and the females lay several eggs per host. The males generally emerge before the females and stay on the host, waiting for the females to emerge (Clancy, 1944). As in other species of the order Hymenoptera, the males hatch from unfertilized eggs and are haploid. This combination of systematic inbreeding and haplodiploidy should have facilitated the purging of deleterious alleles over the species' evolutionary history (Crnokrak & Barrett, 2002; Glémin, 2003; Malécot, 1969). However, this general expectation may be modified by the demography of *A. burrelli*: Strong bottlenecks resulting from host-parasitoid dynamics may cause genetic drift that may either eliminate or fix deleterious alleles (Boakes, Wang, & Amos, 2007; Kirkpatrick & Jarne, 2000). Furthermore, as mentioned above, haplodiploidy does not preclude inbreeding depression for female traits.

We thus evaluated the sensitivity of *A. burrelli* to inbreeding depression, by comparing fitness-related traits between inbred (crosses between siblings) and outbred (random mating) individuals. This study confirms the predicted absence of inbreeding depression for this

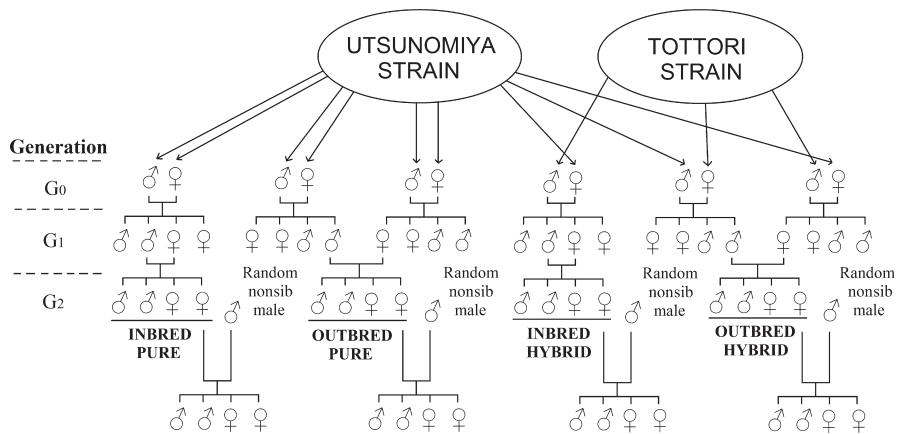


FIGURE 1 Crossing design. We established the families by crossing two G₀ individuals from Utsunomiya (“pure families”), or from Utsunomiya and Tottori (“hybrid families”). At generation G₁, we performed sib crosses (inbred crosses) and nonsib crosses (outbred crosses). Morphological and life-history traits were measured on G₂ individuals

species and highlights an interesting way to overcome a problem that often limits inferences in similar studies. Inbreeding depression may go undetected in laboratory-reared populations, because the deleterious alleles have been either eliminated or been fixed through genetic drift. We thus used two different populations and an ad hoc crossing design to detect putative deleterious alleles: The individuals tested (G₂) were descended from siblings or from unrelated parents (G₁), the parents of which (G₀) originated either from the same strain or from two different strains (the G₁ individuals were therefore either “pure” or “hybrid”). If the two strains used had lost or fixed different detrimental alleles at different loci, then inbreeding depression would be expected to be weak or absent in the progeny of pure parents. Inbreeding depression might nevertheless be detected in the progeny of hybrid parents, because these alleles would occur in the heterozygous state more frequently in outbred than in inbred progeny. The absence of inbreeding depression in the two crossing schemes made it possible to draw robust conclusions about the frequency of strong-effect deleterious alleles in the gene pool.

2 | MATERIALS AND METHODS

2.1 | Biological material

We used *P. comstocki* individuals sampled from a mass rearing founded with individuals collected in the field in southern France in 2008. We reared *P. comstocki* on sprouted potatoes in controlled conditions (25°C, 60% ± 10% R.H. and total darkness). Two laboratory strains of *A. burrelli* were founded in 2010 from natural populations sampled in Japan. We collected seven hundred individuals from the Utsunomiya District and 99 individuals from the Tottori District. The sex of specimens can be determined on the basis of a specific dimorphism of the antennae. Males have long, hirsute, and moniliform antennae, whereas females have shorter, sparsely pubescent, and distinctly club-shaped antennae. Development lasts 30 days at 28°C. The two strains (“Utsunomiya” and “Tottori”) were mass-reared in plastic boxes containing second- and third-instar larvae of *P. comstocki*. We smeared honey on the box lids as a food source for the parasitoids. We carried out rearing at 24 ± 3°C, 70% ± 10% R.H., and 12-hr light/12-hr dark cycle.

2.2 | Experimental design

We measured the impact of inbreeding on *A. burrelli*, by carrying out crosses between individuals from the two strains (Figure 1). We first established families from males and females sampled from the two strains (generation G₀), crossed G₁ individuals within and between families (sibling vs. outbred crosses), and measured phenotypic traits in the progeny (G₂). Environmental stresses, such as extreme temperature, are known to increase sensitivity to inbreeding (Kristensen, Barker, Pedersen, & Loeschcke, 2008). Therefore, we chose to increase temperature and duration of the light period relatively to the rearing conditions in order to make experiment conditions more extreme. The experiment was carried out at 28 ± 3°C, 70% ± 10% R.H., and with a 14-hr light/10-hr dark cycle.

2.2.1 | Establishment of families

At generation G₀, we set up two types of families, using individuals collected from mass-reared strains: (1) “pure families,” with one virgin female and one male from the Utsunomiya strain, and (2) “hybrid families,” with one virgin female from one of the two strain and a male from the other. We performed all crosses with the same protocol. We isolated parasitized mealybugs in tubes and monitored the emergence of adult *A. burrelli* every morning. We immediately isolated all emerging wasps in separate tubes. We recorded the date and time of emergence and determined the sex of each individual under a microscope. Only females emerging more than 15 min before or after a male were considered to be virgin. Then, we selected individuals for crosses according to the design described above. We placed each couple in a tube with honey for 24 hr for mating. We then released the couple into a plastic box (diameter = 10 cm) containing honey for food and a sprouted potato infested with 10 third-instar mealybugs, in which we kept them for the rest of their lives.

2.2.2 | Inbred versus outbred crosses

At generation G₁, we mated virgin females by either a brother, in within-family crosses, or with an unrelated male from another family in between-family crosses (Figure 1). We used a 2 × 2 factorial design

with two types of family (pure vs. hybrid) and two levels of inbreeding (inbred vs. outbred). As well, we used several (up to 11) females and males from each G_0 mating, so each G_0 pair was considered as a random block factor. We collected mealybugs that had been parasitized by G_0 females, and selected emerging adults for mating according to the crossing design for G_1 adults. Emergence, mating, and the exposure of mealybug hosts to G_1 adults were as described above.

Twenty days after G_1 oviposition, we counted the parasitized mealybugs from each box and transferred them to a tube for monitoring of G_2 emergence. We recorded the number of emerging wasps, and isolated five females from each tube in separate tubes for the estimation of phenotypic traits. We selected one of these females for the measurement of G_2 realized fecundity by mating with a male randomly selected from another family (this approach made it possible to treat inbred and outbred females similarly). We carried out mating and host exposure as for the G_0 and G_1 generations. We kept all the offspring of G_2 females reaching adulthood in 96% ethanol at 4°C for counting and sex determination.

Phenotypic traits

We measured eight life-history and morphological traits on second-generation parasitoids (G_2 ; Figure 1): we measured development time on males and females, whereas we measured abdomen size, longevity, reproductive success, parasitism rate, success of parasitism, and realized fecundity only for females. We also determined the sex ratio for the G_2 progeny.

We approximated average development time by the time between the release of the G_1 parents in the box and the emergence of G_2 adults. Longevity was the lifetime of one G_2 female between emergence and death, each female being isolated in a tube with honey and monitored daily. We determined female abdomen size as the mean abdomen size (calculated from three measurements per female) of two females, measured with a binocular microscope coupled to Axiovision® software. Success of parasitism was a binary variable (1 when the female

generated at least one parasite mealybug, 0 otherwise). Parasitism rate was the number of mealybugs, of the 10 supplied to a parasitoid female for 48 hr, transformed into a mummy. Reproductive success was a binary variable, with a value of 1 corresponding to the emergence of adults from the parasitized host generated by a female and 0 otherwise. Female realized fecundity was the number of adult offspring emerging from the mealybugs parasitized by a female producing at least one offspring. Since only females can parasitize the host and lay eggs, we considered a high proportion of female as an advantage for biocontrol production and control of the pest. Thus, offspring sex ratio was expressed as the proportion of females among the offspring.

Inbreeding depression coefficients

As a quantitative approach for the effect of inbreeding on fitness, we estimated the inbreeding depression coefficient (IDC) for each phenotypic trait (Basamma et al., 2009) as:

$$IDC_W = \frac{\bar{W}_{outbred} - \bar{W}_{inbred}}{\bar{W}_{outbred}},$$

where \bar{W} is the mean value for the phenotypic trait considered. Therefore, the IDC is not different from zero in the absence of inbreeding depression, but increases to one as the relative mean performance of inbred individuals decreases. If relative mean performance of inbred individuals is higher than outbred individuals, IDC will decrease from zero to theoretically minus infinite. Note that for the trait "development time," we considered lower values as a fitness advantage, and thus we multiplied the IDC value by (-1) for this trait (so that higher values correspond to higher fitness). For each IDC, we estimated confidence intervals by a bootstrap method: For each trait and each family type (pure vs. hybrid), we created 1,000 new datasets by resampling with replacement from the raw data for outbred and inbred crosses. From each new dataset, we recalculated means and IDCs. Thus, we obtained 1,000 IDCs and after sorting, we considered values of the 25th and 975th IDCs as boundaries for the 95% confidence intervals.

TABLE 1 Mean values (\pm standard errors) of life-history and morphometric traits measured in *Allotropa burrelli* from different type of families

Traits	Unit	IP		OP		IH		OH	
		N	Mean \pm SE	N	Mean \pm SE	N	Mean \pm SE	N	Mean \pm SE
Success of parasitism	%	30	76.67 \pm 7.85	27	55.56 \pm 9.75	29	62.07 \pm 9.17	30	56.67 \pm 9.20
Parasitism rate	%	30	22.33 \pm 3.91	27	17.41 \pm 3.89	29	18.62 \pm 3.77	30	16.00 \pm 3.31
Reproductive success	%	23	78.26 \pm 12.60	15	66.67 \pm 8.79	18	83.33 \pm 9.04	17	88.24 \pm 8.05
Female realized fecundity	offspring	18	29.78 \pm 5.10	8	47.00 \pm 8.62	14	47.21 \pm 8.67	15	40.07 \pm 7.68
Offspring sex ratio	% females	18	80.78 \pm 2.99	10	69.18 \pm 11.18	15	82.90.10 \pm 2.02	15	80.61 \pm 6.04
Development time	days	67	33.86 \pm 0.29	58	33.72 \pm 0.36	46	34.33 \pm 0.36	46	34.73 \pm 0.44
Female abdomen size	mm	39	0.427 \pm 6.9 $\times 10^{-3}$	33	0.413 \pm 6.0 $\times 10^{-3}$	26	0.407 \pm 1.1 $\times 10^{-2}$	29	0.435 \pm 5.5 $\times 10^{-3}$
Longevity	days	40	13.73 \pm 0.99	27	11.67 \pm 1.11	24	12.08 \pm 1.35	27	13.44 \pm 1.41

N is the number of replicates. IP, inbred pure; OP, outbred pure; IH, inbred hybrid; OH, outbred hybrid.

TABLE 2 Results of generalized linear model for (a) success of parasitism (b), parasitism rate, (c) reproductive success, (d) female realized fecundity, (e) offspring sex ratio, (f) development time, (g) female abdomen size, and (h) female longevity

Model	df = 1	F	p (>F)
(a) Success of parasitism (binary errors, P VC = 0 [$N_p = 30$], M VC = 0.64 [$N_M = 25$])			
Family type		0.46	.509
Level of inbreeding		3.69	.074
Family type × level of inbreeding		0.14	.714
(b) Parasitism rate (binomial errors, P VC = 0.19 [$N_p = 30$], M VC = 0.40 [$N_M = 25$])			
Family type		0.25	.627
Level of inbreeding		2.83	.113
Family type × level of inbreeding		0.00	.951
(c) Reproductive success (binary errors, P VC = 0 [$N_p = 20$], M VC = 0.44 [$N_M = 18$])			
Family type		1.66	.234
Level of inbreeding		0.08	.790
Family type × level of inbreeding		0.49	.506
(d) Female realized fecundity (Poisson errors, P VC = 0.11 [$N_p = 16$], M VC = 0.41 [$N_M = 15$])			
Family type		0.06	.822
Level of inbreeding		0.99	.393
Family type × level of inbreeding		1.35	.330
(e) Offspring sex ratio (binomial errors, P VC = 0 [$N_p = 16$], M VC = 0 [$N_M = 15$])			
Family type		1.60	.957
Level of inbreeding		0.004	.303
Family type × level of inbreeding		2.42	.120
(f) Development time (gamma errors, P VC = 8.3×10^{-5} [$N_p = 63$], M VC = 3.8×10^{-3} [$N_M = 59$])			
Family type		0.61	.438
Level of inbreeding		0.44	.510
Family type × level of inbreeding		1.54	.219
(g) Female abdomen size (normal errors, P VC = 1.3×10^{-3} [$N_p = 33$], M VC = 4.4×10^{-4} [$N_M = 28$])			
Family type		0.07	.794
Level of inbreeding		0.08	.779
Family type × level of inbreeding		4.69	.053
(h) Female longevity (lognormal error, P VC = 0 [$N_p = 30$], M VC = 0.21 [$N_M = 28$])			
Family type		0.20	.661
Level of inbreeding		0.00	.985
Family type × level of inbreeding		1.30	.272

Details are given in parentheses: error distribution, variance components (VC) for the random effects selected (M, maternal G_0 family; P, paternal G_0 family), number of levels for random effects (N_M , number of maternal families; N_p , number of paternal families).

Fitness

If inbreeding significantly affects traits related to survival or reproduction, it should have an impact on individual fitness. We evaluated the effect of inbreeding on fitness by two different methods, both using the number of females produced per female as a proxy for fitness. We used this proxy because both theoretical models and experimental data suggest that, in species with local mating, females ovipositing alone maximize the number of daughters (Hardy, 1994).

We first analyzed the individual data, using raw data for the numbers of daughters produced per G_2 female as the dependent variable. We then analyzed the group data (four groups: pure inbred, pure outbred, hybrid inbred, hybrid outbred), estimating the same proxy by combining the mean values of several traits for each group. We calculated a fitness index as follows: [success of parasitism] × [reproductive success] × [mating success] × [realized fecundity] × [proportion of females among offspring] (Moreau, Benrey, & Thiery, 2006). This combination cannot be calculated at the individual level, because several of the traits are binary. We took into account success of parasitism as the ability of females to parasitize the host. Reproduction success represented the ability to bypass the immune defences of the host and then to produce offspring. Mating success was a binary variable with a value of 1 when at least one daughter was present in the offspring, which makes sure that a mating event took place. We considered realized fecundity (number of offspring) as an overall fitness assessment. We considered a high proportion of females in the offspring as an advantage for biological control purposes. For comparisons between groups, we estimated 95% confidence intervals via bootstrap resampling as described above for IDCs.

2.3 | Statistical analysis

We fitted generalized linear mixed models (GLMM) to assess the effects of family type (pure vs. hybrid) and inbreeding level ("inbred" vs. "outbred") on the variations of all measured phenotypic traits. Both the family of origin of the mother and the family of origin of the father were implemented as random effects. We used a binomial distribution for the analysis of parasitism, reproductive success, and offspring sex ratio. We used a Poisson distribution to analyze realized fecundity, the number of parasitized hosts, and the total number of daughters produced. We also used a lognormal distribution for female longevity. We used a gamma distribution for the analyses of development time. Finally, we used a normal distribution to analyze female abdomen size. We performed type III tests of fixed effects. We used the `glmer` and `ANOVA` functions from packages `lme4` and `car` to perform all analyses (Bates, Maechler, Bolker, & Walker, 2015; Fox & Weisberg, 2011) on R version 3.3.4 (R Core Team, 2016). We assessed the power of the statistical analyses as follows. We created 20,000 new datasets by simulating data within the four groups (inbred Utsunomiya, outbred Utsunomiya, inbred hybrid, and outbred hybrid) for each phenotypic trait. For each simulated dataset, we simulated data within each group by using the same sample size and variance as in the observed data, and draw a mean at random within a range corresponding to a 15% deviation around the mean estimated on observations (we considered

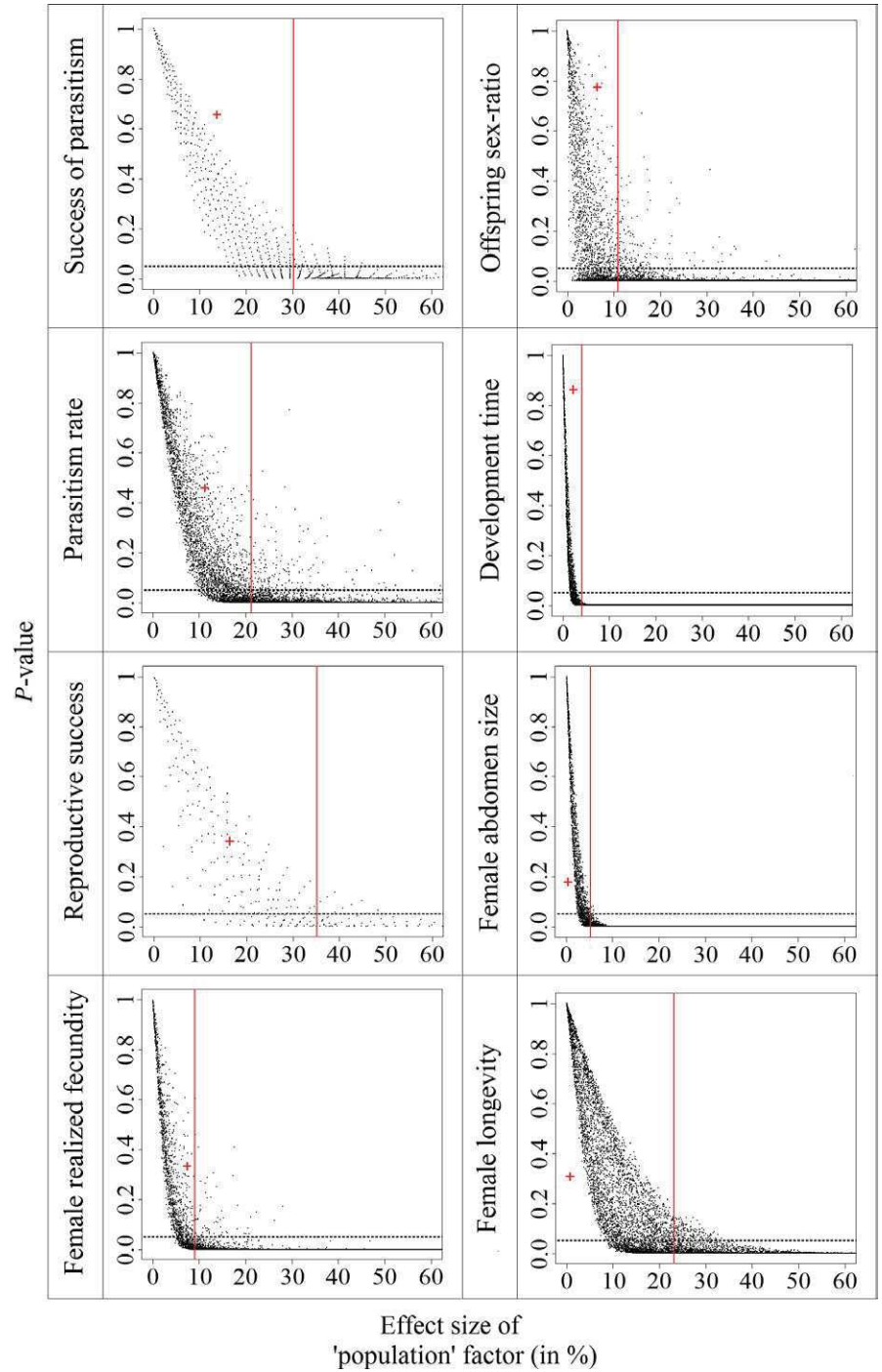


FIGURE 2 Distribution of *p*-values depending on effect size (in %) of “family type” factor obtained from simulations for all phenotypic traits. The cross in red shows the obtained data from the experiments. The red line marks the minimum effect size for which the 80% statistical power threshold is obtained. Dashed horizontal line shows the .05 threshold for *p*-value significance

this range as biologically realistic). We then calculated the difference between means of inbred and outbred groups (referred to as effect size of “level of inbreeding” factor) as well as between Utsumiya and hybrid groups (referred to as effect size of “family type” factor). We finally performed GLMM analyses for each generated dataset and calculated the proportion of *p*-values under .05 in a range of effect size from 0% to 60% (iterations for superior effect sizes were not considered). We performed data simulations with `rnorm`, `rpois`, and `rbinom` functions of package `stats` in R software (R Core Team, 2016). We performed GLMM analysis with `glmer` function of package `lme4` (Bates et al., 2015).

3 | RESULTS

3.1 | Phenotypic traits

For each trait, the number of replicates, mean, and standard error are summarized in Table 1. Generalized linear models revealed no effect of the level of inbreeding ($p > .074$), family type ($p > .234$), or their interaction ($p > .120$) on any of the life-history traits studied (details in Table 2). Inbred and outbred females had similar development times (34.1 ± 0.2 days), abdomen sizes ($0.421 \pm 3.7 \times 10^{-3}$ mm), longevity (12.9 ± 0.6 days), parasitism rates ($18.6\% \pm 1.9\%$), success

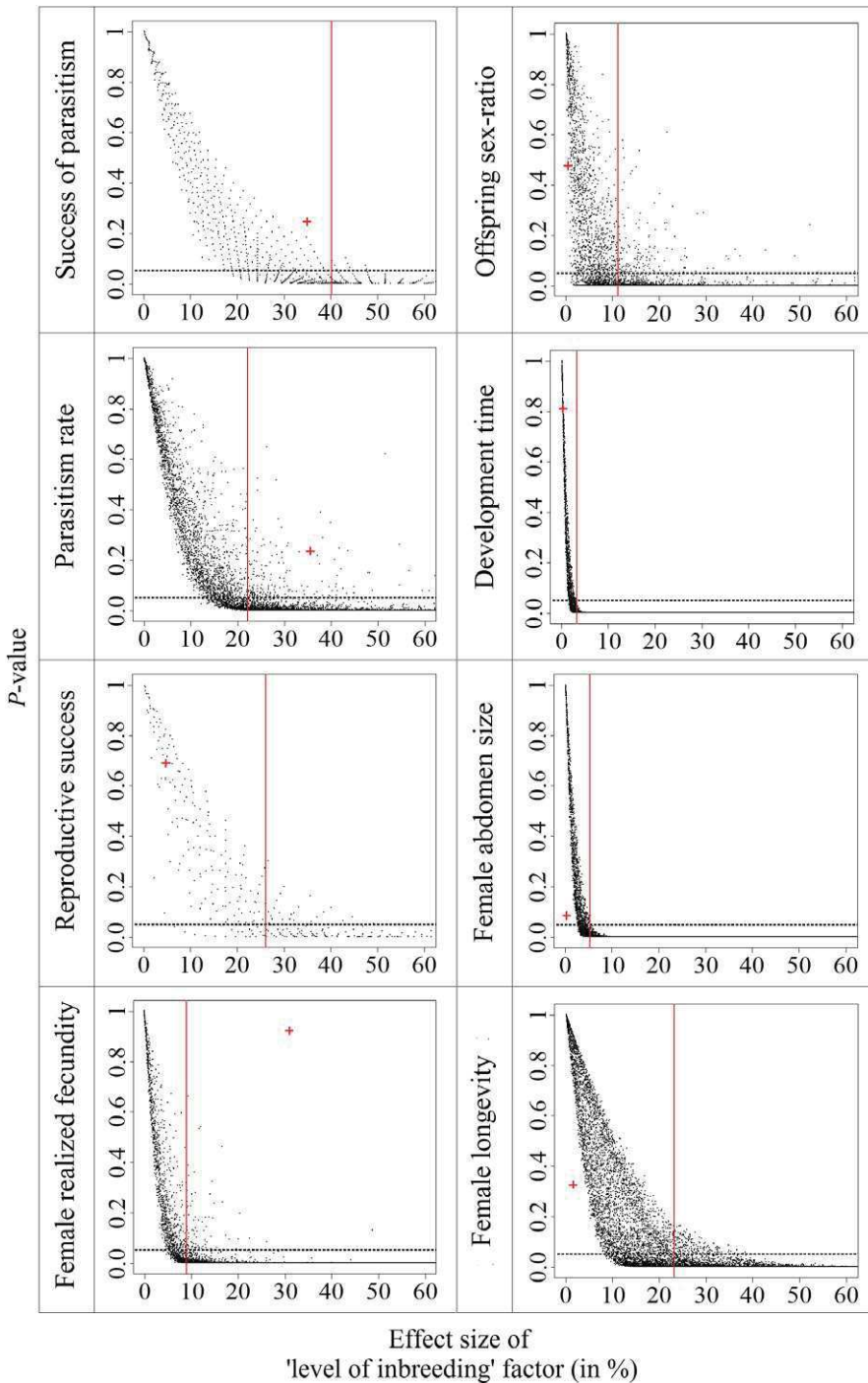


FIGURE 3 Distribution of p -values depending on effect size (in %) of “level of inbreeding” factor obtained from simulations for all phenotypic traits. The cross in red shows the obtained data from the experiments. The red line marks the minimum effect size for which the 80% statistical power threshold is obtained. Dashed horizontal line shows the .05 threshold for p -value significance

of parasitism ($62.9\% \pm 4.5\%$), reproductive success ($79.5\% \pm 4.8\%$), and realized fecundity (44.8 ± 4.7 offspring), regardless of the type of family (pure or hybrid) from which they originated. The sex ratio of the offspring was strongly biased toward females ($20.7\% \pm 2.8\%$ males) and, like other traits, was unaffected by inbreeding. Variances of original paternal and maternal families as random effects approximate 0 and thus suggest no effects from original pure family Utsunomiya or Tottori on male fitness.

Overall analysis of phenotypic traits thus does not reveal evidence of inbreeding depression in *A. burrelli*. Analyses of statistical power revealed that, for “family type” factor, 80% power threshold is reached

for 30%, 21%, 35%, 9%, 11%, 4%, 5%, and 23% effect sizes for success of parasitism, parasitism rate, reproductive success, female realized fecundity, offspring sex ratio, development time, female abdomen size, and female longevity, respectively (Figure 2). For “level of inbreeding” factor, 80% power threshold is reached for 30%, 21%, 35%, 9%, 11%, 4%, 5%, and 23% effect sizes (Figure 3).

3.1.1 | Inbreeding depression coefficients

Inbreeding depression coefficients and their 95% confidence intervals were estimated for eight traits, for both pure and hybrid families. For

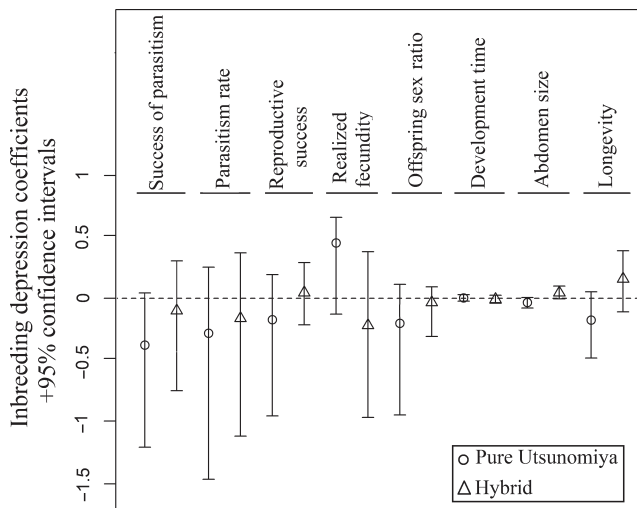


FIGURE 4 Inbreeding depression coefficients (IDC) and 95% confidence intervals for the eight fitness traits measured in G_2 individuals

pure families, the IDCs for the eight traits had a confidence interval including zero and were considered to be nonsignificant (Figure 4). For hybrid families, abdomen size had a small but significant IDC (0.07 [95% CI = 0.02–0.12]), whereas the coefficients of the other traits were not significantly different from zero. IDCs thus revealed an absence of inbreeding depression for most fitness traits, the only exception being abdomen size in hybrid families. However, the inbreeding depression for abdomen size was extremely small.

3.1.2 | Fitness

The number of daughters produced per female was not affected by the level of inbreeding (p -value = .644), in either pure or hybrid families (interaction inbreeding \times family type: p -value = .145; main effect of family type: p -value = .266). On average, G_2 females produced 31.3 ± 3.06 daughters in 2 days (Figure 5a). These results are consistent with the fitness index estimated from a combination of traits. The

estimates and 95% CI obtained suggested that fitness was not dependent upon inbreeding status (Figure 5b).

4 | DISCUSSION

We evaluated the effect of inbreeding on fitness traits in the parasitoid wasp *A. burellii*. Specifically, we assessed possible inbreeding depression by comparing phenotypic traits between inbred and outbred individuals. Since we hypothesized that detrimental alleles might have been both purged and fixed in the genetic pool, we established an experimental design to restore heterozygosity to test these two assumptions. Therefore, we carried out the analysis for both pure and hybrid families.

Overall, the obtained results provide no evidence for inbreeding depression in *A. burellii*. When traits were analyzed independently, with generalized linear models, we found no effect of inbreeding in either pure or hybrid families. The power analysis we performed reveals that the experiment could have led to the detection of significant effects of the “level of inbreeding” on parasitism rate and female realized fecundity, even with effect sizes lower than those observed (35.5% and 31.0%, respectively). Hence, the absence of significant effect of “level of inbreeding” on these two traits in the GLMM analysis is likely caused by random variations, rather than an insufficient statistical power. Moreover, although our statistical power is rather low for some other phenotypic traits, the very small effect size observed suggests no occurrence of a biologically relevant pattern at these traits. However, the “level of inbreeding” effect size observed for parasitism success (34.8%) is slightly lower than the threshold at which statistical power reaches 80% (Figure 5), and yet could correspond to a biologically important difference between inbred and outbred individuals. However, at this trait, even if the 34.8% effect size was found significant in the GLMM, it would correspond to a higher trait value in inbred individuals. Hence, this result would not challenge the main conclusion that no major evidence for inbreeding depression was detected. In addition, the same trend was observed for IDCs for which seven out of the eight IDCs calculated (the exception being abdomen size in hybrid families) show no evidence for inbreeding

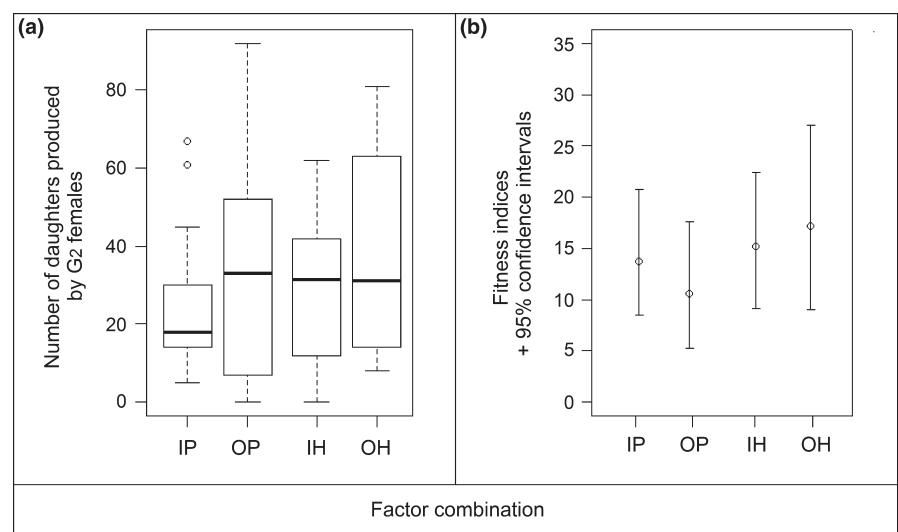


FIGURE 5 (a) Number of females produced per G_2 female for each type of family and (b) fitness indices and 95% confidence intervals calculated for each type of family. IP, inbred pure; OP, outbred pure; IH, inbred hybrid; OH, outbred hybrid

depression in either pure Utsunomiya or hybrid families, because the confidence intervals included zero. Moreover, overall fitness evaluation through the calculation of fitness indices or the assessment of daughter production showed no effect of family type or inbreeding level. The obtained results agree with published findings concerning the effects of inbreeding in haplodiploids (Charlesworth & Willis, 2009; Henter, 2003; Werren, 1993). The genetic load of *A. burellii* had probably been purged through haploid males on one hand and by the systematic inbreeding resulting from its gregarious behavior on the other.

One key finding of this work was that the mass-reared populations of *A. burellii* used for the biological control program in France are unlikely to be strongly affected by inbreeding, at least in laboratory conditions. As a consequence, no specific measures to prevent inbreeding are required in mass-rearing protocols, other than trying to ensure that the population size remains large, to avoid losses of genetic diversity, which could lead to a decrease in adaptability (García-Dorado, 2015). The present findings suggest the possibility to create inbred lines of *A. burellii*. Therefore, we generated and maintained highly inbred lines by crossing brothers and sisters over several generations, to capture unique genotypes that may be needed to reintroduce into the population in the future if there is a loss of genetic diversity from the main mass-reared populations. This method has already proved effective in *Chiasmia assimilis* Warren (Lepidoptera: Geometridae), in which genetic diversity is similar in conventionally reared insects and in accumulated isofemale lines (Wardill et al., 2004).

ACKNOWLEDGMENTS

This work has received funding from the French Agence Nationale de la Recherche (grant ANR-10-JCJC-1708 "BICORAMICS") and from the European Union Seventh Framework Programme FP7 (grant agreement FP7-IAPP #324475 "COLBICS"). We thank the Biology of Introduced Populations "BPI" and Research and Development in Biological Control "RDLB" teams of the Institut Sophia Agrobiotech for technical and material support. Special thanks go to Didier Crochard and Thierry Dumbardon.

CONFLICT OF INTEREST

None declared.

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How to cite this article: Quaglietti, B., Tamisier, L., Groussier, G., Fleisch, A., Le Goff, I., Ris, N., Kreiter, P., Fauvergue, X. and Malausa, T. (2017), No inbreeding depression in laboratory-reared individuals of the parasitoid wasp *Allotropa burrelli*. *Ecology and Evolution*, *7*: 964–973. doi: 10.1002/ece3.2643

Manuscrit 2

1 **Effects of inbreeding on the three biocontrol agents *Chrysoperla near comanche*,**
2 ***Cryptolaemus montrouzieri* and *Macrolophus pygmaeus* in rearing conditions.**

3
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19 **Abstract**

20 Inbreeding depression is a major concern for various human activities including animal and
21 plant breeding, the conservation of endangered species, and the biological control of pests. In the
22 latter, routine practices are often based on the assumption that inbreeding impacts the performance
23 of biocontrol agents. However, strong evidence for inbreeding depression is scarce, and many
24 practices appear disconnected from scientific grounds. In this study, we evaluated the impacts of
25 inbreeding on three biocontrol agent species, with the twofold aim of (1) supporting the biocontrol
26 industry with knowledge specific to their reared populations and (2) assessing the importance of
27 inbreeding depression in several non-model biological control agents. We studied three species
28 belonging to genera widely used as generalist biocontrol agents: *Chrysoperla* near *comanche*
29 (Neuroptera: Chrysopidae), *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) and
30 *Macrolophus pygmaeus* (Hemiptera: Miridae). Specifically, we measured life-history traits likely
31 impacting rearing performance in inbred or outbred individuals. We did not detect inbreeding
32 depression in *C. near comanche* and *C. montrouzieri* but observed a decrease in reproductive
33 success (35%) in inbred *M. pygmaeus*. These results are directly applicable for the management of
34 the populations studied and highlight one case where inbreeding really matters. More generally,
35 our study highlights the idiosyncrasy of single-species estimations of inbreeding depression. Our
36 approach on several species with contrasted life-histories increases the corpus of data on inbreeding
37 in biological control context and is a first step towards generic interpretations.

38 **Keywords:** inbreeding depression, biological control, *Chrysoperla* near *comanche*,
39 *Cryptolaemus montrouzieri*, *Macrolophus pygmaeus*, multi-species approach.

40

41 **1. Introduction**

42 In recently bottlenecked population, panmictic inbreeding, defined as the random
43 reproduction of genetically related individuals (Malécot, 1969), may occur and lead to inbreeding
44 depression (ID). ID is the relative decrease in fitness in inbred individuals compared to that of
45 outbred individuals (Charlesworth and Willis, 2009; Crnokrak and Roff, 1999; Keller and Waller,
46 2002). ID is underpinned by an increase in homozygosity that causes a decrease in fitness through
47 two mechanisms (Charlesworth and Charlesworth, 1987; Charlesworth and Willis, 2009) : (1) the
48 expression of recessive deleterious alleles that were hidden in heterozygous state (partial
49 dominance); (2) the decrease in fitness at loci for which heterozygosity is advantageous
50 (overdominance). Although both mechanisms may lead to inbreeding depression, previous studies
51 suggest that partial dominance is the most pervasive mechanism (Charlesworth and Charlesworth,
52 1987; Crnokrak and Roff, 1999; Hedrick, 2012).

53 Intensity of ID due to partial dominance depends on the genetic load, defined as the
54 frequency of recessive deleterious alleles in the genetic pool (Charlesworth and Charlesworth,
55 1987; Charlesworth and Willis, 2009; Crow, 1993). This frequency depends on the history of
56 populations and genetic processes ongoing upon these alleles. At population equilibrium in infinite
57 populations, the frequencies of deleterious alleles are expected to be maintained thanks to a balance
58 between mutation, selection and drift (García-Dorado et al., 2007). Selection purges these alleles
59 depending on their effect size and degree of expression (Crnokrak and Barrett, 2002; Glémin, 2003;
60 Swindell and Bouzat, 2006). Genetic drift acts randomly and therefore may both decrease (up to
61 elimination) or increase (up to fixation) the frequency of deleterious alleles.

62 Decreases in population size have a strong impact on the selection-drift balance and
63 consequently, on the genetic load (Glémin, 2003; Hedrick and Garcia-Dorado, 2016; Kirkpatrick

64 and Jarne, 2000). First, if rare deleterious alleles are not eliminated by drift, their frequency may
65 increase as a result of decreasing genetic diversity and therefore, increase the risk of ID. Further,
66 genetic drift may overcome selection and provoke strong negative and positive variations in the
67 frequency of deleterious alleles. Populations may rebound rapidly after a bottleneck, toning down
68 purging and favouring selection against deleterious alleles. These processes are so complex that
69 the frequency of deleterious alleles and consequent risk of ID is hardly predictable in recently
70 bottlenecked populations.

71 Inbreeding depression yields fitness decreases that range from barely perceptible to
72 dramatic, sometimes driving small population toward threatening declines or even extinctions
73 (Bijlsma et al., 2000; Fauvergue et al., 2012; Frankham, 2005, 1998; Nieminen et al., 2001).
74 Therefore, inbreeding and inbreeding depression have been the focus of many scientific fields with
75 interests in endangered or captive populations. In conservation biology, high levels of inbreeding
76 are often observed and risks of ID have to be carefully assessed and managed to avoid population
77 declines or extinction (Brook et al., 2002; Edmands, 2007; Hedrick and Kalinowski, 2000;
78 Woodworth et al., 2002). In food industry, plant and animal breeding, severe selection and dramatic
79 variations in population size may yield high levels of inbreeding (Croquet et al., 2006; Dorostkar
80 et al., 2012; Gallardo et al., 2004; Jain and Bharadwaj, 2014; Leberg and Firmin, 2008). Inbreeding
81 is also central in invasion biology, as it may sometimes explain the demographic success and failure
82 of invasive populations (e.g., Facon et al., 2011).

83 Populations of biological control agents experience a series of bottlenecks during
84 importation, lab rearing, and introduction. These bottlenecks likely result in (1) the loss of genetic
85 diversity and consequent ID through overdominance, and (2) strong variations in selection-drift
86 balance affecting the genetic load with, as argued, unpredictable outcomes. A common belief

87 among biocontrol manufacturers is that ID reduces performance during mass-rearing and that
88 decreased genetic diversity jeopardize adaptability and efficiency on the field (Charlesworth, 2003;
89 Reed and Frankham, 2003). Despite decades of heated debates on the importance of genetic
90 processes, including inbreeding, on the success of biological control (Hopper et al., 1993; Hufbauer
91 and Roderick, 2005; Mackauer M., 1976; Nunney, 2003), published studies are rare and generally
92 concern haplodiploid species (parasitoid wasps) for which inbreeding depression is not expected
93 (de Clercq et al., 1998; Henter, 2003; Luna and Hawkins, 2004; Sorati et al., 1996; Zhou et al.,
94 2007). Therefore, no generalized conclusions was proposed about the risks associated with
95 inbreeding in reared-populations of biocontrol agent. Therefore, determine the real importance of
96 inbreeding and its potential adverse effects in their reared populations is a major challenge for
97 biocontrol stake-holders.

98 This study has three major objectives: (1) support the biocontrol industry with knowledge
99 on inbreeding effects specific to the populations they rear; (2) assess the importance of inbreeding
100 depression on non-model organisms in biocontrol conditions (in private companies); (3) propose
101 novel strategies for the assessment of inbreeding depression and the subsequent management of
102 organisms that are bred for biological control.

103 This work was performed in close collaboration between research institutions and industry,
104 and all experiments were performed in industrial premises. We chose to work on three predator
105 species which are representative of widely-used biocontrol agents worldwide: *Chrysoperla* near
106 *comanche* (Neuroptera: Chrysopidae), *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) and
107 *Macrolophus pygmaeus* (Hemiptera: Miridae). For each species, we compared the fitness of
108 individuals obtained from one generation of brother-sister mating (drastically increasing
109 homozygosity of the progeny) and that of individuals obtained from outbred mating. The origin

110 and history of the populations used, for each species, was known. *M. pygmaeus* populations
111 originated from field samples collected in southern France and Belgium and maintained in rearing
112 conditions for 3 generations before the start of the experiment. For *C. montrouzieri*, we used a
113 population created from a mix of individuals from two populations reared in laboratory conditions
114 for many years and one population sample from the wild. Finally, we used *C. near comanche*
115 individuals originating from one population reared in laboratory conditions for more than ten
116 generations. Our intent to create mixed populations (which was eventually not possible for *C. near*
117 *comanche*) was to increase genetic diversity, and in turn heterozygosity rates to improve the power
118 of detection of our study (which is proportional to the difference between the average
119 heterozygosity rate of populations and the lower heterozygosity rate imposed by the sibling-mating
120 treatment).

121

122 **2. Materials & methods**

123 **2.1. Biological material**

124 *C. near comanche* life cycle lasts 25 ± 2.5 days at 25°C and includes egg, larva, nymph and
125 adult stages. A sexual dimorphism of the abdomen extremity enables to distinguish males from
126 females: in females, an ovipositor can be observed instead of the last two segments visible in males.
127 We reared adults in plastic cylinders (inner diameter = 29 cm, height = 21 cm) and fed them on
128 artificial diet supplied *ad libitum*. We collected the eggs and maintained larvae in plastic boxes
129 (diameter = 10 cm, height = 6 cm), fed on *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs
130 (hereafter referred to as “*Ek* eggs”) supplied *ad libitum*. Around 15 days after eggs collection, we
131 isolated pupae in a new plastic box, and used emerging adults to initiate the next generation.

132 *C. montrouzieri* life cycle lasts around 26 days at 25°C and includes egg, larva, pre-nymphal
133 and nymphal stages before the adult. We can distinguish male and female thanks to a sexual
134 dimorphism. The first pair of legs of males is black while that of female is orange. We used three
135 strains of *C. montrouzieri* for the experiment. Two strains originated from mass-rearings and we
136 collected the third strain from the wild in Chile (strains hereafter referred to as “R1”, “R2” and “C”
137 respectively). Before the experiment, each strain was kept in a large rearing cage and individuals
138 were fed with *Pseudococcus viburni* (Hemiptera: Pseudococcidae) supplied *ad libitum*. We reared
139 *P. viburni* populations on sprouted potatoes at 25°C ± 3°C, 60 ± 10% R.H. and complete darkness.

140 *M. pygmaeus* life cycle last about 30 days at 25°C and includes egg, nymph and adult stages.
141 We can distinguish the female which has bigger abdomen with an ovipositor. We reared two strains
142 collected from the wild in Belgium and southern France. We kept both strains separated, in rearing
143 cages containing Tomato plants (*Solanum lycopersicum*) and *Ek* eggs supplied *ad libitum*. We
144 maintained the adults in plastic boxes so that females could lay eggs in stems and leaves of tomato
145 plants. After around 30 days, we collected next generation adults and released them in a new box
146 to perpetuate the rearings.

147 We carried out rearings of both *C. near comanche* and *C. montrouzieri* at 25 ± 3°C,
148 60 ± 10% R.H. and 18h light/6h dark, while we reared *M. pygmaeus* at 25 ± 1°C, 75 ± 5% R.H.
149 and 16h light/8h dark.

150

151 **2.2. Experimental design**

152 For each species, we similarly compared phenotypic traits of inbred and outbred
153 individuals. For this, we first established families created by picking individuals from the available

154 population samples or strains (generation G_0). Then, we crossed individuals from their progeny
155 (generation G_1) either within and between families (sibling vs. outbred crosses). Finally, we
156 measured phenotypic traits in the progeny of these sibling or outbred crosses (generation G_2), i.e.
157 in individuals expected to display high or low homozygosity.

158 Although we used very similar protocols for the three species, procedures were adapted to
159 the biological material and equipment available (see below). Temperature, hygrometry and light
160 conditions were identical to that of the above-mentioned mass-rearing.

161 2.2.1. Experimental arenas

162 For *C. near comanche*, the experimental arena was a plastic cylinder (height = 21 cm,
163 diameter = 11 cm) containing a sheet of paper on the inner wall and closed with fine mesh. We
164 provided water and artificial diet *ad libitum*. For *C. montrouzieri*, it was a round plastic box (height
165 = 5cm, diameter = 10 cm) in which we provided water and *P. viburni* as prey, *ad libitum*. Finally,
166 for *M. pygmaeus*, it was a plastic goblet (height = 16 cm, diameter = 7 cm) closed with fine mesh
167 in which a 3-week tomato plant was rooted and *Ek* eggs supplied *ad libitum*.

168 2.2.2. Establishment of families (generation G_0)

169 2.2.2.1. *Chrysoperla near comanche*

170 We randomly isolated pupae from the mass-rearing in Petri dishes. When adults emerged,
171 we determined the sex and created families by mating a virgin female with a virgin male (Figure
172 1a). For each family, we kept both adults in the experimental arena until their death to allow
173 reproduction and oviposition.

174

175

176 2.2.2.2. *Cryptolaemus montrouzieri*

177 In order to increase initial heterozygosity, we created families of generation G₀ by crossing
178 individuals from the three available strains (R1, R2, C) which resulted in three types of G₀ families
179 in equal proportions: R1-R2, R1-C and R2-C (Figure 1b). We performed crosses in both direction
180 (female × male and vice-versa) so that a total of 48 G₀ families were created, with 16 families of
181 each type. To perform all crosses, we isolated pupae from each strain until adult emergence. We
182 determined the sex of each emerging adult and created each G₀ couple accordingly to the design
183 described above. We released each couple in an experimental arena and maintained both adults
184 until death to allow reproduction and oviposition.

185 2.2.2.3. *Macrolophus pygmaeus*

186 At generation G₀, we created three types of families by crossing individuals collected from
187 the French (FR) and Belgian (BE) populations (Figure 1c): BE x BE (N=20), FR x FR (N=11) and
188 BE x FR (N=24). In the BE x FR crosses, we performed both BE♂ × FR♀ and FR♂ × BE♀
189 combinations in equal proportions. These crosses were carried out by isolating last-instar nymphs
190 from each strain rearing in Petri dishes. We then mated a virgin female with a virgin male,
191 following the design described above and released each couple in an experimental arena. We
192 maintained each couple until female death to allow reproduction and oviposition.

193 2.2.3. Inbred versus outbred crosses (generation G₁)

194 In each species and family type created at G₀, we isolated last immature stages to make sure
195 all individuals used to create families were virgin. At the emergence of adults, we mated a virgin
196 female with either a brother (sibling mating treatment), or with an unrelated male randomly picked
197 from another family (outbred mating treatment) (Figure 1d). For *M. pygmaeus*, outbred crosses

198 were performed with males picked from another family of the same family type. We then released
199 each couple in an experimental arena and maintained both adults to allow reproduction and
200 oviposition. In total, we performed 43 inbred crosses and 43 outbred crosses for *C. near comanche*,
201 60 inbred crosses and 60 outbred crosses for *C. montrouzieri*, and 25 inbred crosses and 30 outbred
202 crosses for *M. pygmaeus*.

203 2.3. Phenotypic traits (generation G₂)

204 We measured seven phenotypic traits on *C. near comanche*: development success, development
205 time, prey consumption, sex-ratio, and female reproductive success, fecundity and longevity. For
206 each family created at G₁, we isolated a cohort of G₂ individuals by changing the sheet of paper of
207 the experimental arena five days after the release of G₁ adults and collected 20 G₂ eggs the next
208 day. We recorded the date of oviposition and isolated each egg in a Petri dish. We used the first six
209 hatching larvae. We used the first larva to measure prey consumption. To do so, we starved the
210 larva for 24h and then provided it with eight 2nd-instar larvae of *P. viburni* for four hours after
211 which we counted the number of preys consumed. We monitored daily the other five larvae and
212 recorded development success, defined as a binary variable (1 if the individual became adult, 0
213 otherwise) and the date of adult emergence to calculate development time from the oviposition
214 date. After adult emergence, we determined the sex of the adults and calculated sex-ratio. We then
215 created brother/sister couples, and released them in an experimental arena to assess fecundity over
216 15 days. Finally, we used another adult female and monitored it daily to determine longevity.

217 Concerning *C. montrouzieri* we measured six life-history and morphological traits on both male
218 and female: development success, development time, larva prey consumption, sex-ratio, fecundity,
219 and longevity. We measured larva prey consumption and survival on both male and females. For
220 each G₁ couple we recorded the date of crossing and counted the total number of 2nd instar larvae

221 in their progeny after 13 days of development (because smaller larvae can hardly be counted as
222 they develop within *P. viburni* ovisacs). We counted the number of G₂ adults to obtain development
223 success. We recorded the date of emergence of the first 15 adults to obtain development time and
224 determined their sex to assess sex-ratio. For each family, we measured prey consumption with one
225 female and male, separately. Prey consumption was the number of 3rd-instar *P. viburni* larvae
226 consumed over 24 hours, out of 40 initially provided to the individual. Longevity was measured
227 by daily monitoring another adult female and another adult male, individually maintained with
228 food *ad libitum*. Finally, for each family, we measured fecundity as the number of larvae counted
229 after 13 days in the progeny of a G₂ female, after a sibling-mating and oviposition for six days.

230 We measured four phenotypic traits on *M. pygmaeus*: female prey consumption, reproductive
231 success, fecundity, and female survival. For each family, we isolated five G₂ last-instar nymphs in
232 Petri dishes to obtain virgin adults. We then determined the sex of each individual and mated one
233 female with a brother before release in an experimental arena. We removed the male and assessed
234 female prey consumption four days after the release. To do so we first starved the female for 24h
235 and then provided 50 *Ek* eggs. We then counted the number of eggs consumed after 24h. We kept
236 the same female in the arena for oviposition for a maximum of 20 days and daily monitored it to
237 check on longevity (if female was still alive after 20 days we removed it from the experimental
238 arena and considered the data as censored). We counted the number of nymphs produced to assess
239 fecundity 20 days after female death or removal (small larvae were hardly visible before). We
240 calculated reproductive success as a binary variable: 0 = no offspring produced by the G₂ female,
241 1 = at least one offspring produced.

242

243

244 2.4. Statistical analysis

245 We fitted generalized linear models on all measured phenotypic traits to assess the effects
246 of inbreeding ('inbred' vs. 'outbred'). We used a binomial distribution for the analyses of
247 development success, sex-ratio, and reproductive success. We used a Poisson distribution for the
248 analyses of predation and fecundity in *C. near comanche* and *C. montrouzieri* (we performed this
249 analysis on both male and female for *C. montrouzieri*). We used a negative binomial distribution
250 and a Gamma distribution for the analyses of predation and daily fecundity in *M. pygmaeus*,
251 respectively. We also used a Gamma distribution for the analysis of development time in the three
252 species. We performed survival analyses by fitting Cox regression models on longevity data (we
253 performed this analysis on both male and female for *C. montrouzieri*). For analyses concerning *C.*
254 *montrouzieri* and *M. pygmaeus* we performed analyses with a random effect to take into account
255 the different types of G0 families used in the experiments. Therefore, we added both maternal and
256 paternal G1 family types as random effect for the analyses concerning *C. montrouzieri*. For analyses
257 concerning *M. pygmaeus* we added family type of the tested individual as random effect.

258 All analyses were performed with the R software version 3.2.3 (R core Team, 2016). We
259 performed GLMs with the *glm* function from package {stats}. We fitted GLMMs with the *glmer*
260 function from package {lme4} (Bates et al., 2015). We used the *coxph* and *coxme* functions from
261 packages {survival} and {coxme} to fit Cox regression simple and mixed models, respectively
262 (Therneau, 2015a, 2015b; Therneau and Grambsch, 2000). We applied type III tests with the Anova
263 function from package {car} (Fox and Weisberg, 2011) to extracted Chi² and *p* values for each
264 model.

265

266

267 2.5. Fitness index and inbreeding depression coefficient (IDC)

268 For each species we calculated a fitness index by combining the mean values (except for
269 longevity, for which we preferred to use the median because censored data and extreme values may
270 have had overweighed impact on the index) of several traits for both inbred and outbred groups
271 (Moreau et al., 2006). We calculated fitness index as follows: *C. near comanche*: [reproductive
272 success] × [longevity] × [fecundity]; *C. montrouzieri*: [female longevity] × [fecundity]; *M.*
273 *pygmaeus*: [reproductive success] × [daily fecundity] × [longevity]. We chose to calculate fitness
274 index and its components at a treatment scale (inbred *vs* outbred) because as a binary variable,
275 reproductive success would have overexpress 0 values.

276 As a quantitative approach, we estimated the effect size of inbreeding by calculating
277 inbreeding depression coefficients for all phenotypic traits and fitness indices (Basamma et al.,
278 2009; Quaglietti et al., 2016):

279
$$IDC = \frac{\overline{W}_{outbred} - \overline{W}_{inbred}}{\overline{W}_{outbred}}$$

280 where \overline{W} is the mean value of the phenotypic trait. We then estimated 95% confidence intervals
281 for each IDC by a bootstrap method: we created 1,000 new datasets by resampling with
282 replacement from the raw data for outbred and inbred crosses. From each new dataset, means,
283 medians, fitness index, and IDCs were recalculated. We thus obtained 1,000 IDCs, sorted them and
284 used the 25th and 975th IDCs as the boundaries for the confidence intervals. The IDC ranges from
285 minus infinite (theoretically) to 1. Positive and negative values corresponds to inbreeding and
286 outbreeding depression, respectively. If IDC's confidence interval contains zero it means that we
287 did not detect a significant effect of inbreeding. Note that for development times of *C. near*

288 *comanche* and *C. montrouzieri*, lower values were considered as a fitness advantage, thus we
289 multiplied IDC values by (-1) so that higher values correspond to higher fitness.

290

291 **3. Results**

292 3.1. Phenotypic traits

293 Summary statistics for all traits and species are compiled in Table 1. GLMM revealed no
294 effect of the level of inbreeding on traits measured for *C. montrouzieri* (Table 2). For *C. near*
295 *comanche*, we found no effect of the level of inbreeding in all trait ($p > 0.159$) but larval prey
296 consumption ($p = 0.029$) (Table 3). Inbred larvae consumed significantly more preys (2.78 ± 0.35
297 preys consumed) than outbred larva (2.00 ± 0.32 preys consumed) (Figure2). Concerning *M.*
298 *pygmaeus*, we found no effect of the level of inbreeding in both prey consumption ($p = 0.306$) and
299 daily fecundity ($p = 0.978$) (Table 4), but a significant effect on reproductive success ($p = 0.015$).
300 Inbred couples had lower reproductive success ($56.00 \pm 10.13\%$) than outbred couples ($86.67 \pm$
301 6.31%) (Figure 3). For both *C. montrouzieri* and *M. pygmaeus* variances associated to random
302 factors were close to zero for each phenotypic trait tested.

303 Analyses of survival performed with Cox regression models showed no effect of the level
304 of inbreeding on survival probabilities in *M. pygmaeus* and *C. near comanche* (Table 5). However,
305 we observed an effect of inbreeding on male survival probability in *C. montrouzieri* ($p = 0.023$)
306 (Figure 4) but not in female (Table 5). As for mixed models, variances associated to random effects
307 in survival analyses for both *C. montrouzieri* and *M. pygmaeus* were close to zero.

308

309 **Table 1: Mean values (\pm standard errors) of phenotypic traits measured for each level of inbreeding in**
 310 **the three species. *N* is the number of replicates.**

Traits	Unit	inbred		outbred	
		N	mean \pm se	N	mean \pm se
<i>Chrysoperla near comanche</i>					
development time	days	41	24.50 \pm 0.11	36	24.23 \pm 0.07
development success	%	41	93.50 \pm 2.39	36	95.37 \pm 1.95
predation capacity	preys consumed	41	2.78 \pm 0.35	36	2.00 \pm 0.32
sex-ratio	proportion of females	41	0.54 \pm 0.03	36	0.51 \pm 0.03
reproductive success	%	40	100 \pm 0	34	97.06 \pm 2.94
female fecundity	offspring	39	295.54 \pm 19.62	34	323.88 \pm 24.60
longevity	days	32	9 \pm 0.23	20	9 \pm 0.29
<i>Cryptolaemus montrouzieri</i>					
Development success	%	41	72.61 \pm 2.94	41	75.39 \pm 3.61
Development time	days	41	27.71 \pm 0.33	41	27.42 \pm 0.39
Sex-Ratio	proportion of females	40	0.52 \pm 0.02	40	0.51 \pm 0.03
predation capacity	preys consumed	♀ 36	14.5 \pm 0.56	♀ 36	15.42 \pm 0.73
		♂ 36	16.83 \pm 0.68	♂ 36	15.92 \pm 0.53
fecundity	offspring	33	48.82 \pm 2.91	32	46.56 \pm 2.35
longevity	days	♀ 36	14.5 \pm 0.83	♀ 37	15 \pm 0.09
		♂ 36	14 \pm 0.66	♂ 37	15 \pm 0.09
<i>Macrolophus pygmaeus</i>					
predation capacity	preys consumed	15	7.13 \pm 3.17	22	9.55 \pm 2.87
reproductive success	%	24	56.00 \pm 10.13	30	86.67 \pm 6.31
daily fecundity	offspring/day	14	0.51 \pm 0.11	26	0.51 \pm 0.08
longevity	days	24	20.5 \pm 1.44	30	26.5 \pm 1.25

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Table 2: Results of generalized linear mixed models for (A) development success (B) development time (C) sex-ratio, (D) adult predation capacity, and (E) fecundity in *Cryptolaemus montrouzieri*. Details are given for variance components (VC) and number of levels (N) of random effects (M = maternal G₁ family type; P = paternal G₁ family type) and residuals (res).

Model	Df = 1	Chisq	P (>Chisq)
A. Development success			
Binomial errors			
M VC = 0.012 (N _M = 3), P VC = 0.64, (N _P = 3)			
level of inbreeding		0.901	0.343
B. Development time			
Gamma errors			
M VC = 0 (N _M = 3), P VC = 0.64 (N _P = 3), res VC = 0.007			
level of inbreeding		0.211	0.646
C. Sex-ratio			
Binomial errors			
M VC = 0 (N _M = 3), P VC = 0.64, (N _P = 3)			
level of inbreeding		0.021	0.884
D. Prey consumption			
Poisson errors			
♀ : M VC = 0 (N _M = 3), P VC = 0 (N _P = 3)			
♂ : M VC = 0 (N _M = 3), P VC = 0.001 (N _P = 3)			
level of inbreeding	♀	0.966	0.326
	♂	0.923	0.337
E. Fecundity			
Poisson errors			
M VC = 0 (N _M = 3), P VC = 0.64 (N _P = 3)			
level of inbreeding		1.705	0.192

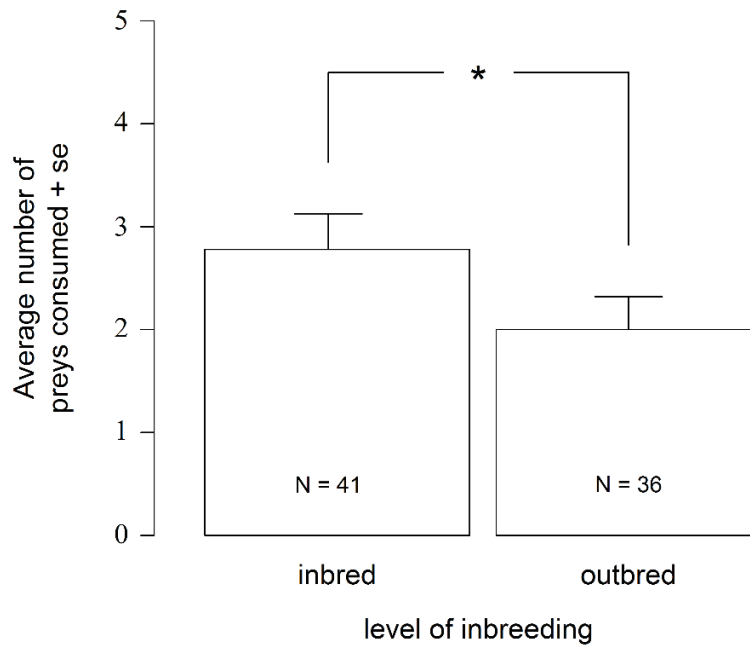
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Tableau 3: Results of generalized linear models for (A) development success (B) development time (C) larval prey consumption, (D) sex-ratio, (E) reproductive success and (F) female fecundity in *Chrysoperla* near *comanche*.

Model	Df = 1	Chisq	P (>Chisq)
A. Development success			
Binomial errors			
level of inbreeding		0.011	0.916
D. Development time			
Gamma errors			
level of inbreeding		1.981	0.159
C. Larval prey consumption			
Poisson errors			
level of inbreeding		4.791	0.029*
D. Sex-ratio			
Binomial errors			
level of inbreeding		0.043	0.836
E. Reproductive success			
Binomial error			
level of inbreeding		0.000	0.997
F. Female fecundity			
Poisson errors			
level of inbreeding		0.833	0.362

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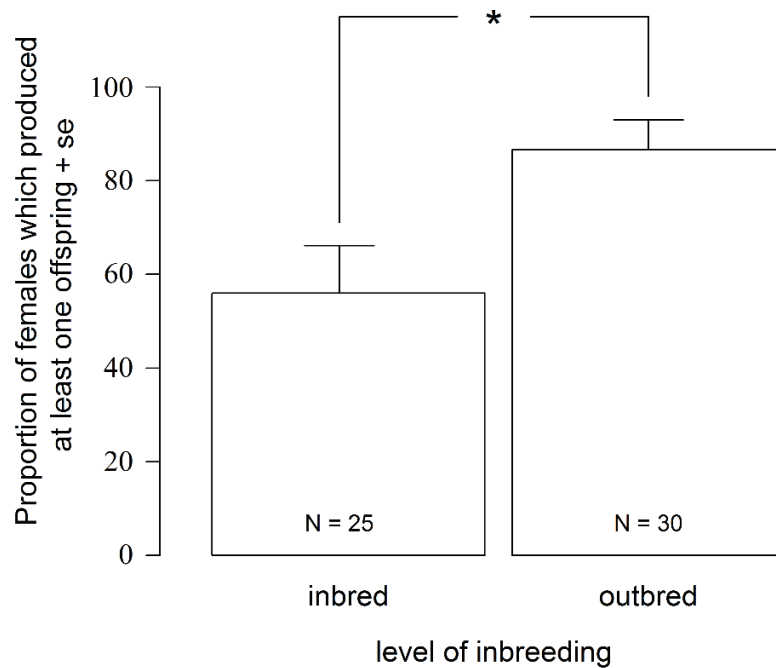
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337 **Figure 2: Average number of prey consumed by *C. near comanche* larvae depending on their level of**
 338 **inbreeding**

339

340 **Table 4: Results of generalized linear mixed models for (A) development success (B) development time**
 341 **(C) sex-ratio, (D) adult prey consumption, and (E) fecundity in *Macrolophus pygmaeus*. Details are given**
 342 **for variance components (VC) and number of levels (N) of the population random effects and residuals**
 343 **(res).**

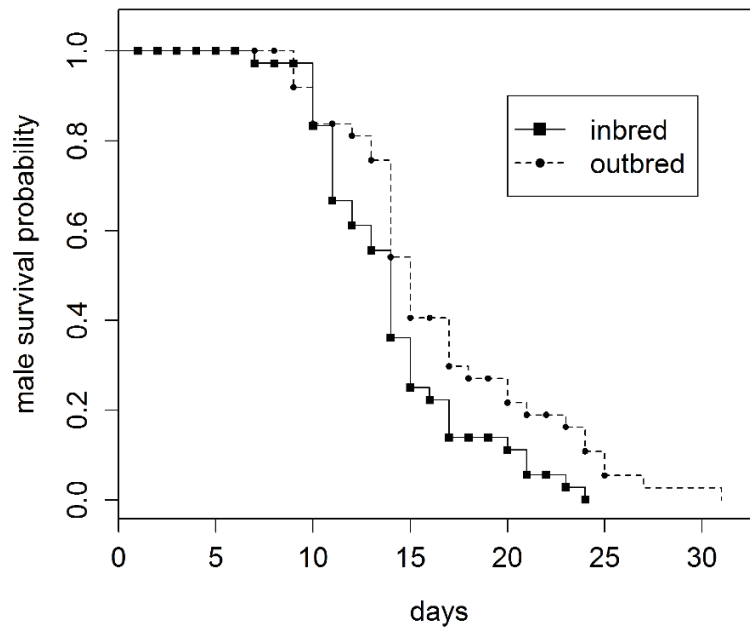
Model	Df = 1	Chisq	P (>Chisq)
A. Adult prey consumption			
Negative binomial errors population VC = 0.108 (N _M = 3)			
level of inbreeding		1.047	0.306
B. Reproductive success			
Binomial errors population VC = 0 (N _M = 3)			
level of inbreeding		5.898	0.015*
C. Daily fecundity			
Gamma errors population VC = 0 (N _M = 3), res VC = 0.386			
level of inbreeding		0.001	0.978



344
 345 **Figure 3: Proportion of *M. pygmaeus* females which produced at least one offspring (in%) depending**
 346 **on their level of inbreeding.**

347
 348 **Table 5: Results of Cox regression models for survival analysis of (A) *Chrysoperla near comanche* (B)**
 349 ***Cryptolaemus montrouzieri* and (C) *M. pygmaeus*. Number of observations for each level of inbreeding**
 350 **(N_{obs}) and details are given for variance components (VC) and number of levels (N) of random effects (M**
 351 **= maternal G_1 family type; P = paternal G_1 family type for *Cryptolaemus montrouzieri*; population for *M.***
 352 ***pygmaeus*).**

Species	Df = 1	Chisq	P (>Chisq)
A. <i>Chrysoperla near comanche</i>			
$N_{obs\ inbred} = 32, N_{obs\ outbred} = 20$			
level of inbreeding		0.280	0.597
B. <i>Cryptolaemus montrouzieri</i>			
♀ : $N_{obs\ inbred} = 36, N_{obs\ outbred} = 37, M\ VC = 3 \times 10^{-4} (N_M = 3), P\ VC = 0 (N_P = 3)$			
♂ : $N_{obs\ inbred} = 36, N_{obs\ outbred} = 37, M\ VC = 4 \times 10^{-4} (N_M = 3), P\ VC = 0.04 (N_P = 3)$			
level of inbreeding	♀	1.443	0.230
	♂	5.143	0.023*
C. <i>Macrolophus pygmaeus</i>			
$N_{obs\ inbred} = 24, N_{obs\ outbred} = 30, population\ VC = 0.108 (N_M = 3)$			
level of inbreeding		2.010	0.156



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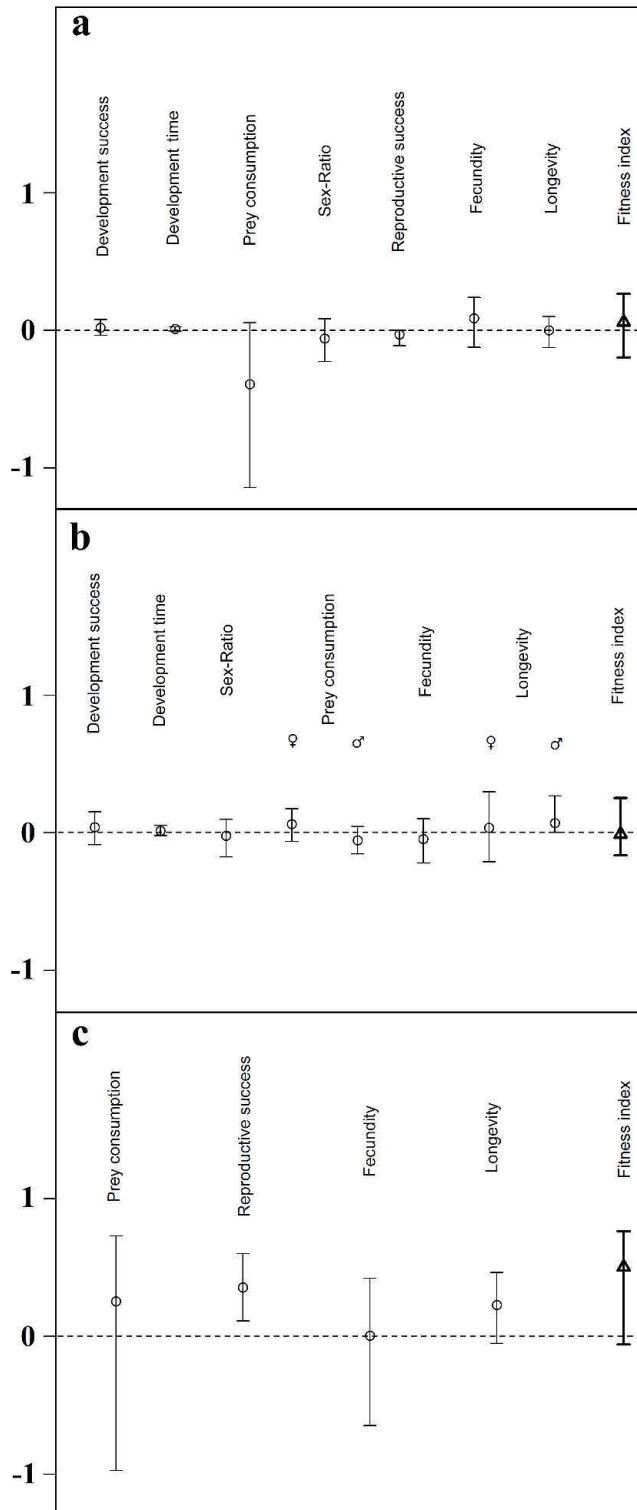
355 **Figure 4: Survival curve for inbred and outbred males on *C. montrouzieri*.**

356

356 3.2. Inbreeding depression coefficients

357 Inbreeding depression coefficients and estimated 95% confidence intervals were consistent
 358 with generalized linear modelling (Figure 5). In *M. pygmaeus* we found an IDC of 35% in
 359 reproductive success for which the confidence interval did not include zero. Moreover, the IDC's
 360 confidence interval calculated for longevity in *C. montrouzieri* barely included zero, showing the
 361 same trend as Cox regression model. However, IDC's confidence interval for larval predation
 362 capacity of *C. near comanche* included zero which is not congruent with the GLMM analysis. For
 363 all other traits, IDCs' confidence intervals all included zero. Overall, inbreeding depression
 364 coefficients calculated for all traits and fitness indices of *C. near comanche* and *C. montrouzieri*
 365 are very close to zero. On the contrary, we observe greater effect sizes for phenotypic traits
 366 measured in *M. pygmaeus*, particularly for the fitness index even if its confidence intervals barely
 367 include zero.

Inbreeding Depression Coefficient + 95% CI



368

369

370

Figure 5: Inbreeding depression coefficient calculated for each phenotypic trait and the fitness index in (a) *C. near comanche*, (b) *C. montrouzieri* and (c) *M. pygmaeus*.

371 4. Discussion

372 The aim of this study was to assess the effect of inbreeding on phenotypic traits in three
373 biocontrol agent *C. near comanche*, *C. montrouzieri* and *M. pygmaeus*. We thus compared traits of
374 individuals originating from one generation of sibling and outbred mating, and performed analyses
375 using GLMM and Cox regression on individual fitness traits, as well as inbreeding depression
376 coefficients (IDC) on average trait values at the level of the treatments (including a fitness index
377 calculated with the average values of traits supposedly related to fitness).

378 Two different patterns were observed. On the one hand, evidence for inbreeding depression
379 was weak in both *C. montrouzieri* and *C. near comanche*. IDC indices revealed small effect sizes
380 for all phenotypic traits and fitness indices for both species (Figure 5). However, GLMM analyses
381 on individual data revealed that prey consumption in *C. near comanche* was 40% higher in inbred
382 individuals. Higher prey consumption is difficult to interpret in terms of impacts on fitness or
383 applied interest in biological control. In the absence of any other detected fitness advantage for
384 inbred individuals, higher food consumption may actually be a cost in fitness. In terms of biological
385 control, it may be an advantage if higher prey consumption is also observed in field conditions but
386 it may also be detrimental if larvae need more food to in industrial mass-rearing conditions.
387 Furthermore, Cox mixed regression analyses shows an effect of inbreeding on survival
388 probabilities of males in *C. montrouzieri* (Table 5). Similar patterns have been shown on Syrian
389 and Egyptian strain of *C. montrouzieri* in which male longevity constantly decrease with
390 generations of inbreeding (Al-Khateeb et al., 2013, 2012). This could impact biological control as
391 adult *C. montrouzieri* survival is an important parameter since they are usually maintained several
392 days before field releases and their expected field efficacy is likely proportional to their survival
393 after release. All in all, these results suggest that although inbreeding does not dramatically impact

394 these two species, some traits may be affected. Moreover, only a few traits were measured in this
395 study and important traits for field efficacy may be affected. Hence, although we did not propose
396 to the companies any specific counter-measure (such as systematic recollection of wild individuals
397 or maintenance of several genetically differentiated lines to be periodically mixed, etc.), we
398 considered that avoiding strong demographic bottlenecks during low commercial activity seasons
399 is relevant for these two species.

400 On the other hand, both GLMM analyses on individual data and IDC on average values per
401 treatment highlighted a major effect of inbreeding in *M. pygmaeus* with a decrease of 35% in
402 reproductive success of inbred females. Moreover, IDC analyses revealed greater effects of
403 inbreeding in *M. pygmaeus* than in the two other species (Figure 5). Notably, the fitness index for
404 *M. pygmaeus* displayed a decrease of 50%, even if its confidence included zero at its extremity. As
405 a consequence, this study provides evidence that *M. pygmaeus* is likely quite impacted by
406 inbreeding and that specific countermeasures could be adopted to improve its performance as
407 biological control agent, be it in terms of industrial production capacity or pest control efficiency
408 after field-release.

409 We implemented similar experimental designs on all three species. We thus argue that
410 differences observed among the three species cannot be explained by discrepancies among
411 experiments. These contrasted patterns may rather be explained by interspecific differences or by
412 differences in genetic load between the studied populations (or an interaction between these two
413 factors). Inter-populations differences in terms of genetic load is likely. Indeed, population rearing
414 and demographic history are very different among the three species studied. *C. near comanche* and
415 *C. montrouzieri* populations completely or partially originated from populations reared for many
416 generations before the experiment while we used only recently wild-sampled populations of *M.*

417 *pygmaeus*. Long-term laboratory-reared populations likely face repeated bottlenecks which can
418 result in the elimination or fixation of deleterious alleles (Fauvergue et al., 2012; Kirkpatrick and
419 Jarne, 2000) which both prevent our experimental treatment to reveal differences between inbred
420 and outbred individuals (although we can assume as little probable that the three populations of *C.*
421 *montrouzieri* crossed in this experiment all eliminated or fixed the same deleterious alleles).

422 Inbreeding depression may be due to both large fitness-effect alleles at a few loci or to the
423 accumulated effects of mildly deleterious alleles (Bataillon and Kirkpatrick, 2000; Charlesworth
424 and Willis, 2009). Highly detrimental and lethal alleles are more likely to be eliminated while
425 mildly deleterious alleles may be maintained longer in the genetic pool (Barrett and Charlesworth,
426 1991; Crnokrak and Barrett, 2002; Glémin, 2003; Kirkpatrick and Jarne, 2000; Swindell and
427 Bouzat, 2006). Therefore, if ID is due to mildly deleterious alleles, adverse effect of inbreeding
428 could have been observed only after accumulated generation of inbreeding (Al-Khateeb et al.,
429 2013, 2012; Ávila et al., 2010; Franco et al., 2011; Geden et al., 1992; Latter and Mulley, 1995;
430 Shabalina et al., 1997).

431 Our results illustrate that the cross control experimental design we used – which is very
432 commonly used to study inbreeding effects as an alternative of pedigree comparison (e.g. Luna &
433 Hawkins, 2004; Peer & Taborsky, 2014; Quaglietti et al., 2016; Vayssade et al., 2014) – displays
434 several intrinsic limitations. Firstly, it is highly labour-intensive: for example, the experimental
435 work carried out on each species spread over at least five months and was particularly tedious.
436 Secondly, unless experiments are performed on very different populations for each species, results
437 are population-specific because of possible large differences in genetic load, possibly underpinned
438 by different demographic history. Thirdly, depending on the characteristics of the genetic load, the
439 experimental design may not be powerful enough to detect significant difference in phenotypic

440 traits. Finally, inbreeding may affect any phenotypic traits in certain environmental conditions only
441 (Armbruster and Reed, 2005; Fox and Reed, 2010), so that lab experiments cannot predict the
442 absence of inbreeding depression with a high level of confidence. Consequently, such approaches
443 suffer a low ratio “information / feasibility or cost”, especially when considering the resources they
444 need in terms of time, space, material, money, and staff to obtain reliable and sufficient data.
445 Biocontrol manufacturers may not be ready or able to afford such expense of resource for
446 unguaranteed benefits. Therefore, we strongly advocate to focus on developing new, more efficient
447 and simpler methods for assessing inbreeding and its effects in reared-populations.

448 For instance, the recent rise of genomic technologies brings new perspectives for the estimation
449 of inbreeding and inbreeding depression when pedigrees are unavailable (Kardos et al., 2016;
450 Kristensen et al., 2010; Paige, 2010; Vanraden, 2007). As coefficient of inbreeding is assumed to
451 be negatively correlated to genome-wide heterozygosity (Keller and Waller, 2002), many studies
452 suggest that inbreeding depression is the main reason for observed heterozygosity-fitness positive
453 correlations (HFCs) (Britten, 1996; Chapman et al., 2009; Hansson and Westerberg, 2002).
454 However, HFCs are weakened because of the small number of DNA marker used to estimate
455 genome-wide heterozygosity (Coltman and Slate, 2003; DeWoody and DeWoody, 2005; Miller et
456 al., 2014). High-throughput sequencing now allow the identification of large number of DNA
457 markers (Single Nucleotide Polymorphism = “SNPs”) at relatively low cost. In addition, as SNPs
458 are widely distributed in the genome, they would allow better estimation of genome-wide
459 heterozygosity and consequently HFCs. The use of such molecular markers to highlight HFCs
460 would allow the detection of inbreeding depression without involving large experimental crossing-
461 design. However, once would maximize the range of individual heterozygosity in the population
462 to increase the chance of detecting HFC (Grueber et al., 2008). An alternative approach would be

463 to investigate the underlying molecular basis of inbreeding depression. Sequencing coding regions
464 of the genome may enable the detection of genes associated with inbreeding depression (e.g.
465 Ayroles et al., 2009; Charlier et al., 2008). Therefore, it would allow to compare the genetic load
466 of sampled populations (e.g. wild populations vs several mass-rearings), by using indices such as
467 the synonymous to non-synonymous mutation (a substitution of a base in the DNA that leads to
468 the production of the transcript) ratio, or methods taking into account the type of non-synonymous
469 mutation (e.g. leading to stop-codons or changing the class of amino-acid) (Bi et al., 2012; Henn
470 et al., 2015; Kardos et al., 2016; Lohmueller et al., 2008; Paige, 2010).

471 We therefore advocate the use of genomic data to assess inbreeding depression in biological
472 control agents such as it has already been started in ecology and population management for
473 livestock, aquaculture or conservation (Allendorf et al., 2010; Bjelland et al., 2013; Hedrick and
474 Garcia-Dorado, 2016; Kristensen et al., 2010; Leroy, 2014; Leutenegger et al., 2003; Wenne et al.,
475 2007).

476 **Acknowledgments**

477 This work has received funding from the European Union Seventh Framework Programme
478 FP7, grant agreement FP7-IAPP #324475 “COLBICS”, and by the INRA program
479 “Métaprogramme SMACH”, project #P10225. Special thanks go to Nancy Lenaerts, Simon Van
480 den Bergh and Sergio Salvador.

481

482 **5. References**

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Manuscrit 3

1 **Genetic determinants of hybridization and fitness in mass-rearing of the biocontrol agent**
2 ***Macrolophus pygmaeus* using genotyping-by-sequencing**

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20 **Abstract**

21 The biocontrol industry is in need for research and development to improve its capacity to
22 produce at lower cost biological control agents (BCAs) displaying higher quality. One way to
23 improve production efficiency and BCA quality is genetic improvement, using for example
24 selection or hybridization programmes. Other simple practices currently used in biocontrol consist
25 in increasing genetic diversity in mass-reared populations by adding field-collected individuals, in
26 order to limit the negative impacts of inbreeding depression or loss of diversity. Carryout such
27 activities require a comprehensive knowledge on the available intraspecific genetic variability in
28 the species to be improved and data about the outcomes of hybridization between populations or
29 taxa available *in natura*. *Macrolophus pygmaeus* is one of the most used BCA worldwide. Its
30 population structure in Europe has recently been investigated with mitochondrial DNA and
31 microsatellite markers and several taxa, morphologically similar, have been identified.
32 Documenting the outcomes of hybridization between these taxa and more generally the relationship
33 between the genomic characteristics of individuals coming from various populations or crosses
34 would make it possible to evaluate of the interests of manipulating the genetic diversity of this
35 species for biocontrol applications. In this work, we sampled field populations of *M. pygmaeus*
36 from Belgium, Greece, France and Spain, and compared phenotypic traits and genomic data (using
37 Genotyping by Sequencing) obtained from individuals created by intra- and inter-population
38 crosses over several generations. We observed high genetic diversity within and between
39 populations and a strong effect of genetic distance between parents on both reproductive
40 compatibility and fitness of the progeny. These results reveal the potential of working on
41 intraspecific diversity to improve BCAs but also highlight the risks, in terms of induced
42 reproductive isolation, of mixing industrially reared populations with field-collected populations.

43 **Keywords:**

44 Hybridization, Heterozygosity-fitness-correlations, Genotyping-by-Sequencing, biological
45 control, *Macrolophus pygmaeus*

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62 1. Introduction

63 Biological control is a crop pest management method which mainly relies on the release of
64 natural enemies called biological control agents (BCAs) to control pest populations (Eilenberg et
65 al., 2001). Success of biocontrol notably depends on the cost-effective production of quality BCAs
66 (Naranjo et al., 2015; van Lenteren, 2012). In this context, the management of the genetic
67 improvement of reared populations is a challenge for the global improvement of biological control
68 (Hopper et al., 1993; Leppla et al., 2014).

69 One main concern of the biocontrol industry is the loss of genetic diversity and inbreeding
70 depression, the decrease in fitness in inbred individuals compared to those outbred. Such genetic
71 processes may provoke a decrease in production yield and a loss of adaptability in the field. In
72 addition, the development and selection of more efficient BCAs appears as a potential key to
73 improve their production as well as their efficiency once released in the field.

74 A simple way to use of intra-specific variability is through intraspecific hybridization. Adding
75 genetic variability via hybridization may mitigate the effect of from inbreeding depression (ID) in
76 reared populations. ID occurs when homozygosity increases due to inbreeding and leads to the
77 expression of recessive deleterious alleles previously masked at heterozygous state (Charlesworth
78 and Charlesworth, 1987; Charlesworth and Willis, 2009). Therefore, the incorporation of genetic
79 variability in reared populations may decrease the frequency of deleterious mutation and alleviate
80 inbreeding depression. Second, hybridization may also favour the creation of new favourable gene
81 interactions or the accumulation of non-interacting advantageous alleles in hybrid individuals
82 (Arnold, 1992; Burke and Arnold, 2001; Lippman and Zamir, 2007).

83 However, although benefits from intra-specific hybridization has been considered has a
84 powerful evolutionary process to explain the adaptation to changing environment (Arnold, 1992;

85 Barton, 2001; Lewontin and Birch, 1966; Rieseberg et al., 1999), it often leads to fitness decrease.
86 Indeed, hybridization may lead to outbreeding depression via the breakdown of co-adapted gene
87 complexes, or genome incompatibilities (Burke and Arnold, 2001; Orr and Turelli, 2001; Tallmon
88 et al., 2004). Therefore, an optimal and intermediate level of hybridization may exist (Krystkowiak
89 et al., 2009; Li et al., 2008; Stelkens and Seehausen, 2009). Furthermore, the success of
90 reproduction between individuals from differentiated strains or populations may be influenced by
91 several factors as genome incompatibilities (Arnold, 1992; Edmands, 2002; Servedio and Noor,
92 2003; Turelli et al., 2001) or non-genetic factors as interactions between mates' endosymbionts.
93 Indeed, endosymbiotic incompatibilities between mates is known to manipulate reproduction in
94 many arthropods (Breeuwer and Jacobs, 1996; Johanowicz and Hoy, 1998; Poinot et al., 1998;
95 Stouthamer et al., 1999) and 40% of BCAs are known to carry such endosymbionts for which
96 impacts on reproduction have already been reported in 50% of cases (Aebi and Zindel, 2013).

97 Intra-specific hybridization has received a lot of attention and been proven effective in crop
98 production (e.g. Briggie et al., 1967; Luan et al., 2010; Riday and Brummer, 2002) and livestock
99 or aquaculture rearing (e.g. Franke, 1980; Hulata, 1995; McGuirk et al., 1978; Rege et al., 1994).
100 This and the fact that the biocontrol industry is growing (and has increasing R&D capacities) makes
101 hybridization-based procedures a credible source of improvement for BCA. Although several
102 reviews and opinion articles suggest a potential role of hybridization for the improvement of BCA
103 populations for decades (Hopper et al., 1993; Hufbauer and Roderick, 2005; Mackauer M., 1976;
104 Roderick and Navajas, 2003), experimental data have been produced only in recent years
105 (Benvenuto et al., 2012a, 2012b; Cheyppé-Buchmann et al., 2011; Gebiola et al., 2016; Hoffmann
106 et al., 2003; Malausa et al., 2010; Mathenge et al., 2010; Mukuka et al., 2010; Shapiro et al., 1997;
107 Szucs et al., 2011; Wu et al., 2004). These studies have shown variable results, with sometimes
108 reproductive isolation between strains or populations but in other cases the appearance of beneficial

109 phenotypes of interest for biological control. For instance, it has been shown that tolerance to
110 extreme conditions could be transferred through hybridization in entomopathogenic nematodes
111 (Mukuka et al., 2010; Shapiro-Ilan et al., 2005; Shapiro et al., 1997). Furthermore, Hoffmann et al.
112 (2003) and Mathenge et al. (2010) showed that hybridization could have an impact on fitness and
113 host specificity which could be either beneficial or detrimental for biological control. Although it
114 is appears difficult to make general rules about the interests of hybridization in biocontrol, it seems
115 very relevant to conduct investigation at least on major BCAs of worldwide economic interest in
116 order to provide proofs of concept and propose new practices to the industry. In this perspective, a
117 study carried out on the BCA *Trichogramma chilonis* assessed the mating success between several
118 strains of the BCA as well as the fitness of hybrids (Benvenuto et al., 2012b). A great variation in
119 the mating success between strains has been shown as well as a quadratic relation between the
120 hybrid fitness and the genetic distance of their parental lines.

121 Evaluating the interest of intraspecific hybridization in a biocontrol agent species can be
122 performed by answering two main questions: (i) is there a substantial extent of genetic and
123 phenotypic variation in the species considered, and (ii) what are the consequences of the
124 manipulation of the genomic composition via more or less complex processes of hybridization
125 (which leads to the investigation of the relationship between the distance between parents – or the
126 genetic composition of the progeny – and the fitness of the progeny).

127 While investigation of the genetic diversity available can be carried out with numerous types
128 of genetic markers, experimental assessment of the effects of hybridization represents a major
129 bottleneck for R&D. This can be performed via experimental crossing designs aiming to compare
130 the fitness in individuals coming from crosses between parents with various extents of relatedness
131 (sibling, within-population, or between-population) (e.g. Luna & Hawkins, 2004; Peer & Taborsky,
132 2014; Quaglietti et al., 2016; Vayssade et al., 2014) but such approaches request time, space and

133 material to obtain reliable and sufficient data and therefore suffer a low ratio “information vs
134 feasibility/cost”. One way to avoid complex experimental designs is the use of individual
135 phenotypic and genomics individual data to investigate the relationship between inter-parent
136 distance or heterozygosity and fitness. Such approaches have been performed for a few decades
137 (often referred to as heterozygosity-fitness correlations “HFCs”) (Britten, 1996; Chapman et al.,
138 2009; David, 1998; Grueber et al., 2008; Reed and Frankham, 2003). HFCs in animals are
139 commonly weak or absent but there is a general positive trend mainly due to a very few examples
140 with very strong effect. This suggests that HFCs may highly depend on species, populations or
141 samples (Chapman et al., 2009). In addition, it appears that studies on HFCs have been much
142 constrained and maybe biased by the limited availability of abundant and informative molecular
143 markers (Coltman and Slate, 2003; DeWoody and DeWoody, 2005; Miller et al., 2014). However,
144 new DNA sequencing technologies now alleviate this constrain. Hence, studies relying on
145 individual phenotypic and genomic data now appear as a serious alternative to experiments
146 involving large crossing designs (Hoffman et al., 2014; Miller et al., 2014; Szulkin et al., 2010).

147 *Macrolophus pygmaeus* is a Mirid bug which originates to the Mediterranean basin and is one
148 of the most used BCA worldwide. Inbreeding depression has recently been detected on the mating
149 success of females in an experiment comparing fitness of individuals from sib-mating to that of
150 outbred individuals (Quaglietti et al., submitted). Moreover, taxonomy of *Macrolophus pygmaeus*
151 has been recently debated and subject to changes. Long referred to *Macrolophus caliginosus*, *M.*
152 *melanotoma* and *M. pygmaeus* were recently separated as two different taxa (Castañé et al., 2012;
153 Martínez-Cascales et al., 2006; Perdikis et al., 2003). In addition, recent population genetic studies
154 performed with microsatellites and mitochondrial markers revealed that *M. pygmaeus* is composed
155 of at least two subgroups differentiated as “commercial” and “wild” haplotypes with asymmetric
156 and limited gene flow (Sanchez et al., 2012; Streito et al., 2016). Finally, *Wolbachia sp.* and

157 *Rickettsia sp.* have been recently identified in the microbial community of the genus *Macrolophus*
158 (Machtelinckx et al., 2012) and may be responsible for reproductive isolation via cytoplasmic
159 incompatibilities between *M. pygmaeus* and *M. melanotoma* (Machtelinckx et al., 2009). Seen its
160 economic relevance in biocontrol, its documented sensitivity to inbreeding, the doubts regarding
161 its population structure in Europe, and the occurrence of endosymbionts in its populations, *M.*
162 *pygmaeus* appears as the ideal candidate to characterize intraspecific diversity, detect potential
163 HFCs and investigate the outcomes of intra-specific hybridization.

164 In this study, we sampled four European populations of *M. pygmaeus* in the field and performed
165 controlled crosses within and between populations. To obtain a wide range of individual
166 heterozygosity rates, we created inbred and outbred lines and maintained them until over five
167 generations. We measured fitness traits and performed Genotyping-By-Sequencing (GBS) on each
168 individual in generations G₀, G₁ and G₅. GBS is a high-throughput sequencing method which
169 provides genomic data for low per-sample-cost without heavy optimisation methods. It is based on
170 the sequencing of genome subsets obtained via restriction enzymes and allow to discover large
171 numbers of single nucleotide polymorphisms markers (SNPs) for exploring genetic diversity,
172 population structure, trait mapping or genome-wide association studies (GWAS) (Elshire et al.,
173 2011; Metzker, 2010; Thomson, 2014). Hence, the study could be performed by using phenotypic
174 and genomic data, both at the treatment (inter-population or intra-population crosses) and
175 individual levels. To investigate the factors influencing fitness and reproductive isolation, we used
176 the following strategy. First, we used the GBS data to investigate the genetic structure of the field-
177 sampled populations and distinguish between genetically differentiated clusters. Based on the
178 assignment of individuals to genetic clusters, we addressed four questions. (1) Is the genetic
179 structure of European populations of *M. pygmaeus* based on SNP data consistent with previous

180 studies performed with a few microsatellite or mitochondrial markers? (2) Can we use genomic
181 data to disentangle the role of genetics on the hybridization success in *M. pygmaeus*? (3) Can we
182 highlighted a heterozygosity-fitness correlation in *M. pygmaeus*?

183

184 **2. Material & methods**

185 *2.1. Macrolophus pygmaeus*

186 *M. pygmaeus* (Hemiptera: Miridae) is a predaceous bug which originates to the
187 Mediterranean basin. Females lay their eggs in the stem or leaves rib of host plant and nymphs
188 emerged around ten days after oviposition. Juvenile development lasts around 20 days at 24°C with
189 five nymphal instar (Hansen et al., 1999). The last instar can be easily recognized with the
190 appearance of juvenile wings. Sexual dimorphism allows the differentiation of males and females
191 which have a larger abdomen with an ovipositor.

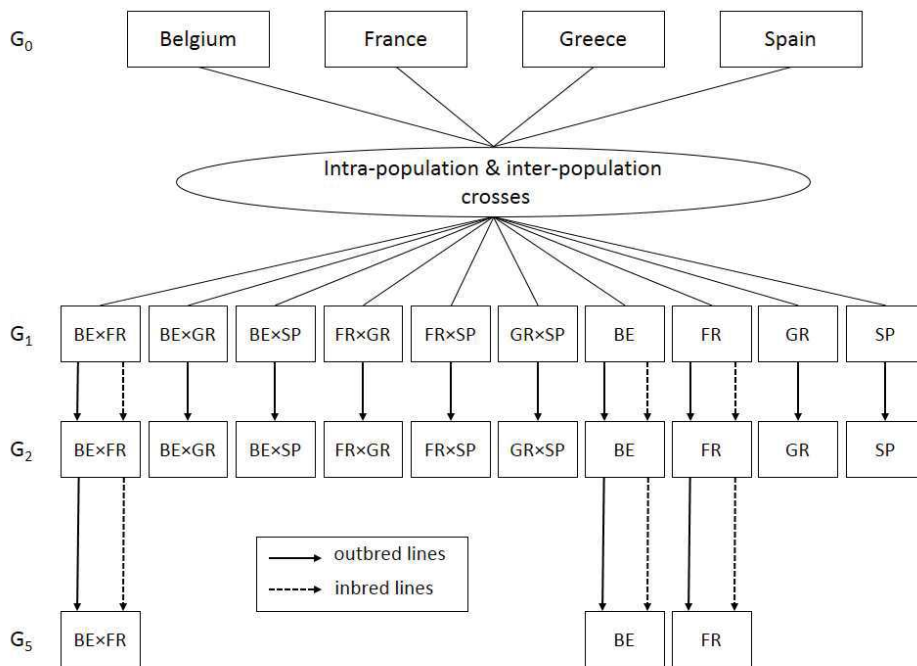
192 *2.2. Sampling and rearing*

193 We collected 48 and 122 individuals from Belgium and France on Pot Marigold (*Calendula*
194 *officinalis*) and 17 and 143 individuals from Greece and Spain on Tomato (*Solanum lycopersicum*).
195 We reared the four populations in plastic boxes (30 x 20 x 15 cm) closed with lid pierced and
196 covered with fine mesh. We kept the adults on five weeks-old root-poached Tomato plants and fed
197 on *Ephestia kuehniella* (*Ek*) eggs and cotton rolls soaked in sweetened water provided *ad libitum*.
198 When nymphs emerged after around 10 days, we removed the adults and provided a new Tomato
199 plant to allow the nymphs to finish their development. Populations were maintained this way for 3
200 generations before the experiment.

201

202 2.3. Experimental design

203 We aimed to check the heterozygosity-fitness correlation and disentangle the effects of genetic
 204 factors and endosymbionts on the reproductive success of *M. pygmaeus*. To do so, we performed
 205 controlled crosses over six generations to test potential effects of outbreeding and inbreeding
 206 (Figure 1). After creating couples within and between the four populations (generation “G₀”), we
 207 created inbred and outbred lines (generation “G₁”) and maintained some of them for four
 208 generations (until generation “G₅”) to obtain a wide-range of individual heterozygosity rates (figure
 209 1). To get proxy of fitness, we measured several traits: couple reproductive success, and female
 210 daily fecundity (at generations G₀, G₁, and G₅), and female survival probability and female prey
 211 consumption (at generations G₁ and G₅).



212
 213 **Figure 1: Experimental design used to perform crosses within and between populations**
 214 **and to create inbred and outbred lines.**

215 *2.3.1. Intra-specific hybridization (generation “G₀”)*

216 We collected fifth-instar nymphs from the four reared populations and isolated them in Petri
217 dishes (= generation “G₀”). Each Petri dish contained a piece of tomato stem, a disk of cotton
218 soaked in sweetened water and was closed with fine mesh (hereafter referred to as “isolation dish”).
219 We daily monitored all individuals, determined the sex of new adults, and randomly paired a male
220 and a 4-days-old female in an isolation dish. We created 26, 28, 28, and 29 within-population
221 couples for Belgium, France, Greece and Spain, respectively. We made all possible crosses
222 between populations, with 28 couples for the following crosses: Belgium x France; Belgium x
223 Spain; France x Greece; France x Spain; and Greece x Spain (with 14 couples ♀_{population a} × ♂
224 population b and 14 couples ♀_{population b} × ♂_{population a}). We created 29 couples between Belgium and
225 Greece, (14 couples ♀_{France} × ♂_{Greece} and 15 couples ♀_{Greece} × ♂_{France}). After 24h, we released each
226 couple in a plastic goblet (height = 16 cm, diameter = 7 cm) closed with fine mesh in which a 3-
227 weeks-old tomato plant was rooted and *Ek* eggs supplied *ad libitum* (hereafter referred to as
228 “experimental arena”). We monitored the female of each couple for 20 days (or until its death).
229 The 20-days period corresponds to two 10-days periods spent in two experimental arenas to avoid
230 cannibalism of younger nymphs by the older ones. We removed the male after four days to avoid
231 potential harassment on the female while we removed the female after 20 days even in some cases
232 when it was still alive on the plants. We kept all individuals in 96% ethanol at -20°C.

233 We counted the number of offspring in each experimental arena, 20 days after we released the
234 female. We obtained two variables. First, the reproductive success, a binary variable for which “1”
235 was given to couples which produced at least one offspring, “0” otherwise. Second, for couples
236 which produced at least one offspring, we measured the daily fecundity by dividing the overall
237 fecundity by the number of days the female spent in the experimental arenas.

238 At generation F₁, we performed crosses between individuals from different families of the same
239 family type (Figure 1). To do so, we collected fifth-instar G₁ nymph offspring for each G₀ couples,
240 and isolated them as done at generation G₀. We paired each female with a random male from
241 another family of the same family type and released and monitored them following the same
242 protocol as for generation G₀. In addition to mating success, female fecundity and survival we also
243 measured female prey consumption. To do so, we starved the female for 24h four days after the
244 release of G₁ couples. We then provided 50 *Ek* eggs and counted the number of eggs consumed
245 after 24h. All G₁ couples were kept in 96% ethanol as for generation G₀. We created 13, 3, 6, and
246 4 G₁ families with individuals coming from within-population crosses from Belgium, France,
247 Greece, and Spain, respectively; and 7, 2, 4 and 2 families with individuals coming from between-
248 population crosses Belgium × France, Belgium × Greece, Belgium × Spain, and France × Greece,
249 respectively (because of the small number of G₁ individuals produced by some types of G₀ crosses,
250 we could not create G₁ families for crosses France × Spain and Greece × Spain). In total we
251 therefore created 41 G₁ families.

252 2.3.2. *Generating a range of individual heterozygosity rates*

253 In order to produce individuals displaying a wide range of heterozygosity rates, we created and
254 maintained inbred lines for four generations, in parallel to the above-mentioned 41 outbred lines
255 generated at G₁ (also maintained for four generations). Inbred lines were created and maintained
256 by crossing, at each generation, brothers and sisters in the available families. However, in practice,
257 a sufficient number of lines could be maintained only for families of the family types originating
258 to Belgium and France. We created 11 and 13 inbred lines with individuals coming from each
259 within-population crosses from Belgium and France, respectively; and 20 inbred lines with
260 individuals coming from between-population crosses Belgium × France. In total we therefore

261 created 44 inbred lines. All inbred and outbred lines were monitored until generation G₅ and
262 measures of the four above-mentioned traits were performed at the last generation.

263 *2.4. Analysis of genomic data*

264 *2.4.1. DNA extraction, genotyping-by-sequencing*

265 We performed genomic DNA extractions at Institute Sophia Agrobiotech (France) using the
266 QIAGEN DNeasy Tissue Kit (QIAGEN, Hilden, Germany). We crushed bugs before extraction
267 and cell lysis was done for ~4h. We performed two elution steps (2 × 40µl of AE buffer) to increase
268 DNA concentration.

269 Genotyping-by-sequencing was performed at Cornell University, genomic diversity facility, on
270 all individual DNAs randomly distributed in seven 94-plex plates. Genomic DNA was digested
271 with the restriction enzyme PstI (CTGCAG). Adapters with single barcodes were ligated to
272 individual samples before pooling and PCR amplification. DNA fragments were sequenced over
273 100bp on a single lane of Illumina HiSeq 2500. The detailed protocol is described by Elshire et al.
274 (2011). DNA analysis was performed on 363 individuals from G₀ (Belgium: 83, France: 89,
275 Greece: 95, and Spain: 84), 140 individuals from G₁ (Belgium: 21, France: 20, Greece: 16, Spain:
276 24, Belgium × France: 24, Belgium × Greece: 8, Belgium × Spain: 5, France × Greece: 11, France
277 × Spain: 5 and Greece × Spain: 6) and 86 individuals from G₅ (Belgium: 38, France: 16, and
278 Belgium × France: 32)

279 *SNP calling*

280 We ran the TASSEL 5.0 GBS discovery pipeline (www.maizegenetics.net/tassel) to perform
281 SNP calling (Glaubitz et al., 2014). As no reference genome is available for *Macrolophus*
282 *pygmaeus* we used the Universal Network Enabled Analysis Kit (UNEAK) pipeline which builds

283 false reference chromosomes directly with tag pairs (a tag is a sequence corresponding to a similar
284 reads in the libraries) (Lu et al., 2013). Tag networks are created from reciprocal tag pairs (1bp
285 diverging tags) and UNEAK filters complex networks of repeats, paralogs to identify suitable tag
286 pairs to construct false reference chromosomes. To obtain tag counts from libraries, we set
287 TASSEL parameters as follows: for the 300,000,000 for the maximum number of good reads per
288 lane, PstI for the restriction enzyme used for DNA digestion, 10 for the minimum number of tags
289 per lane to be output, and 10 for the minimum number of tags in all lanes to be output in Merged
290 tag count files. For the construction of false chromosomes, we set to 0.05 the network error
291 tolerance rate in the UNEAK plugin.

292

293 *Blast, genetic distance and population structure*

294

295 We used the blastN tool to differentiate microbial DNA from insect DNA sequenced by GBS
296 and build three different datasets. First, we blasted the tags containing SNPs on a microbial DNA
297 database obtained from microbial reference genome build from a review of microbial community
298 of Mirid bugs (unpublished data). We kept SNP sequences with no correspondence on this database
299 as ‘*Macrolophus*’ DNA dataset. Then, to create ‘*Wolbachia*’ and ‘*Rickettsia*’ datasets, we blast
300 SNP sequences which had a correspondence on microbial DNA on *Wolbachia* and *Rickettsia*
301 nucleotide reference databases from NCBI website.

302 On each dataset, we extracted the genetic distance matrix from TASSEL graphical
303 interface. TASSEL calculates distance as 1 - IBS (identity by state) similarity, with IBS defined as
304 the probability that alleles drawn at random from two individuals at the same locus are the same.
305 For clustering, the distance of an individual from itself is set to 0. The calculation is based on the
306 following definition. For a bi-allelic locus with alleles A and B, probability $IBS(AA,AA) = 1$,

307 $p_{\text{IBS}}(\text{AA},\text{BB}) = 0$, $p_{\text{IBS}}(\text{AB}, \text{xx}) = 0.5$, where xx is any other genotype. For two taxa, p_{IBS} is
308 averaged over all non-missing loci. Distance is $1 - p_{\text{IBS}}$ ([https://bitbucket.org/tasseladmin/tassel-](https://bitbucket.org/tasseladmin/tassel-5-source/wiki/UserManual/DistanceMatrix/DistanceMatrix)
309 [5-source/wiki/UserManual/DistanceMatrix/DistanceMatrix](https://bitbucket.org/tasseladmin/tassel-5-source/wiki/UserManual/DistanceMatrix/DistanceMatrix)). We then performed clustering
310 analyses based on Multi-Dimensional Scaling (MDS) (on two axes) for each dataset with the
311 *cmdscale* and *hclust* functions from the package {stats} in R software version 3.2.3 (R core Team,
312 2016). We used the *pamk* function from the package {fpc} to assess the optimal number of clusters.
313 to $k = 2$ for microbial DNA and

314
315 *Data analyses*

316 We performed all analyses with generalized linear models (GLMs) with binomial, Gamma,
317 Poisson and Normal families to assess the determinant factors of couples reproductive success,
318 female daily fecundity, female prey consumption and female longevity, respectively. To check on
319 the consequences of hybridization in G_0 , we used two approaches. First, a “treatment” approach by
320 using the combination of male and female original population and clusters as categorical
321 explanatory variables. Second a correlative approach at the individual level by using genetic
322 distance between mates as a continuous explanatory variable. To check on hybrid fitness in
323 generation G_1 , we only used the correlative approach by using genetic distance between parents as
324 explanatory variables for variations of prey consumption, survival, reproductive success and daily
325 fecundity. Finally, to study the HFC in individuals from Belgian, French and their hybrids at
326 generations G_5 we used the female individual heterozygosity rate as explanatory variables of the
327 number of offspring produced in the lifespan (this variable is the best approximation of fitness we
328 had because it took into account the reproductive success, the fecundity and the longevity of the
329 female). For each model, we extracted Chi square and p values from anova tables obtained by
330 performing type III tests. We fitted GLMMs and CRMM with the *glmer* and *coxme* functions from

331 packages {lme4} and {coxme}, respectively (Bates et al., 2015; Therneau, 2015; Therneau and
332 Grambsch, 2000). We did type III tests with the *Anova* function from package {car} (Fox and
333 Weisberg, 2011).

334 **3. Results**

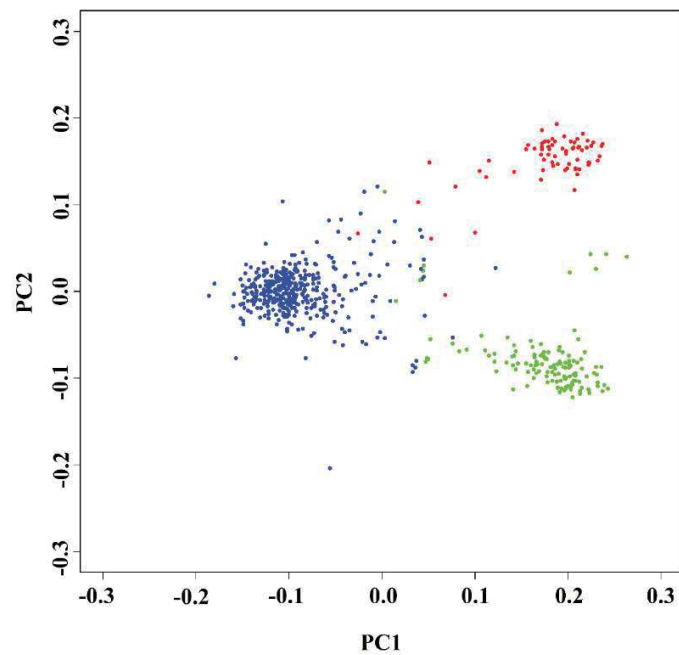
335 *SNPs calling, Blast, and population structure*

336 We initially identified 39530 SNPs from extracted DNA. After blasting those sequences on our
337 specific microbial sequences of Mirid we identified 4204 SNPS from microbial DNA. We then
338 used the remaining 35326 SNPs as *Macrolophus* genomic DNA. Out of 4204 microbial SNPS only
339 206 and 244 SNPS were identified as *Wolbachia* and *Rickettsia*, respectively, after we performed
340 the blast on ncbi databases so that cluster analysis could not be performed because of the large
341 amount of missing data.

342 Cluster analyses performed on *M. pygmaeus* dataset revealed an optimal for k=3 clusters which
343 tended to confirm the hypothesis for the existence of three sub-populations of *M. pygmaeus* in
344 Europe (Figure 2) and revealed variations in the proportion of individuals from the three Cluster in
345 the four sampled populations (Figure 3). Out of 93 individuals from Belgium, 70 (75.3%) and 23
346 (24.7%) individuals were assigned to clusters k1 and k3, respectively. Out of 89 individuals from
347 France, 87 (97.8%) and 2 (2.2%) individuals were assigned to clusters k1 and k2 respectively. Out
348 of 97 individuals from Greece, 44 (45.4%), 51 (52.6%), and 2 (2.1%) individuals were assigned to
349 clusters k1, k2 and k3 respectively. Out of 84 individuals from Spain, 12 (14.3%), 1 (1.2%), and
350 71 (84.5%) individuals were assigned to clusters k1, k2 and k3 respectively. As a consequence, we
351 found out that 57 crosses we initially performed were pure crosses in cluster k1 (mainly (39) from
352 crosses within and between Belgian and French populations, 6 were pure crosses in cluster k2 (only
353 from crosses within Greek population), and 13 were pure crosses in cluster k2 (mainly (8) from

354 crosses within Spanish population). Finally, 86 crosses were crosses between clusters.
355 Unfortunately, probably due to the high number of missing, no cluster was identified for microbial
356 DNA so that we did not take into account the bacterial DNA in the analyses.

357



358

359 **Figure 2: multi-dimensional scaling and cluster assignments of all sequenced individuals**
360 **based on genetic distance between individuals (blue = cluster k1, red = cluster k2, green =**
361 **cluster k3).**

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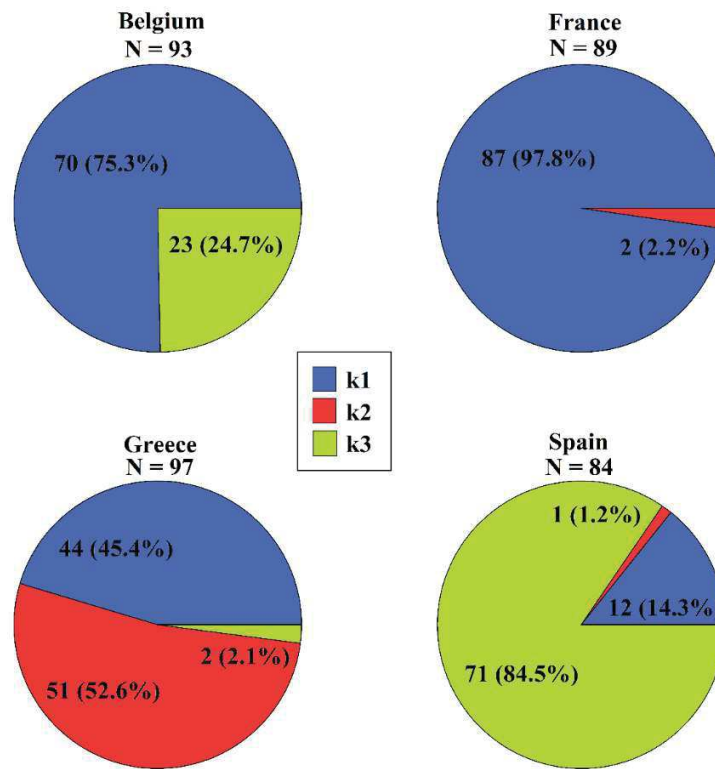


Figure 3: Distribution of G₀ individuals among the three cluster in the four sampled European populations used in the experiment.

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Consequences of hybridization in G₀

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The two approaches of GLM analyses performed on the *M. pygmaeus* dataset to disentangle

373

the genetic effects of hybridization showed consistent results (Table1):

374

On the one hand, categorical approach showed that the variation of reproductive success

375

between crosses could be explain by the cross combination between populations ($\chi = 46.8$, $df = 9$,

376

$p = 1.1^{-08}$) and clusters ($\chi = 60.4$, $df = 8$, $p = 2.8^{-12}$). Post hoc tests for cross combinations between

377

populations revealed that crosses within and between Belgian and French populations had higher

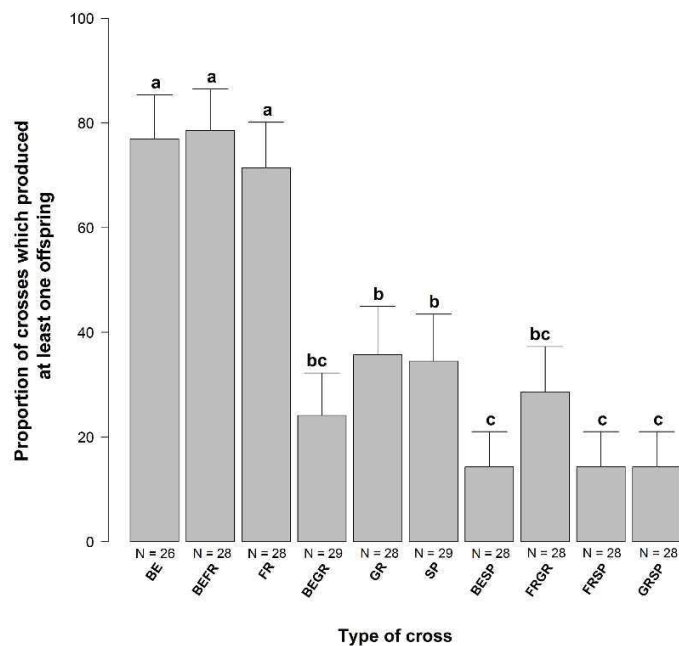
378

reproductive success than other combinations (Figure 4). Post hoc tests for cross combinations

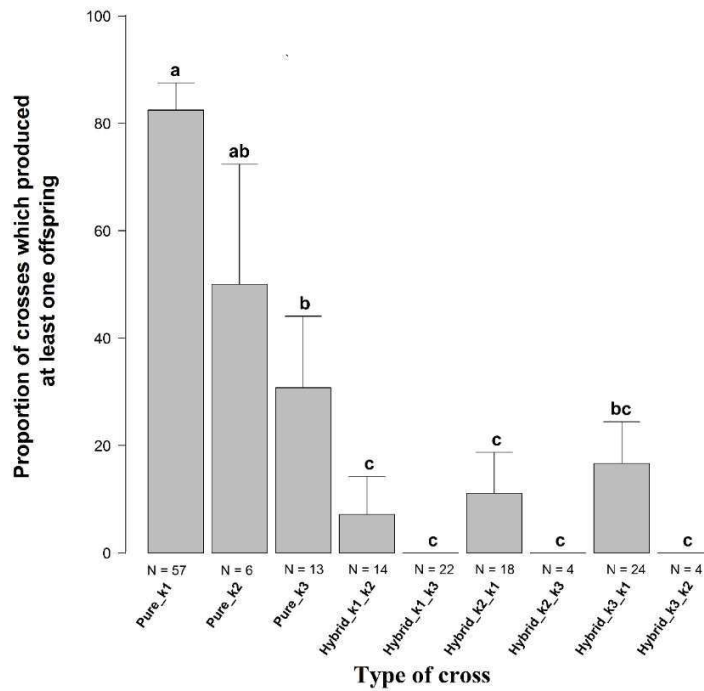
379

between clusters revealed that hybrid crosses between clusters rarely produced offspring (8.1 ± 3

380 %) whatever the clusters involved in the cross. To the contrary, a higher and significant proportion
 381 of pure crosses within cluster produced at least one offspring particularly for cluster k1 (82.5 ± 5.1
 382 %) which mainly corresponds in French, Belgian within and between population crosses. An
 383 important proportion of crosses within clusters k2 (50.0 ± 22.4 %) and k3 (30.8 ± 13.3 %)
 384 surprisingly failed to produce offspring (Figure 5). On the second hand, the continuous approach
 385 highlighted a significant ($\chi = 6.64$, $df = 1$, $p = 0.01$) effect of genetic distance between mates on
 386 the reproductive success (Table1). Indeed, we found out that both small and high genetic distance
 387 between mates were more likely to provoke reproductive failure (Figure 6). However, we found no
 388 effect of populations ($\chi = 10.4$, $df = 9$, $p = 0.07$) and cluster ($\chi = 2.3$, $df = 5$, $p = 0.48$) combinations
 389 nor genetic distance ($\chi = 0.11$, $df = 1$, $p = 0.67$) on the variation of daily fecundity whatever the
 390 approach we used (Table 1).



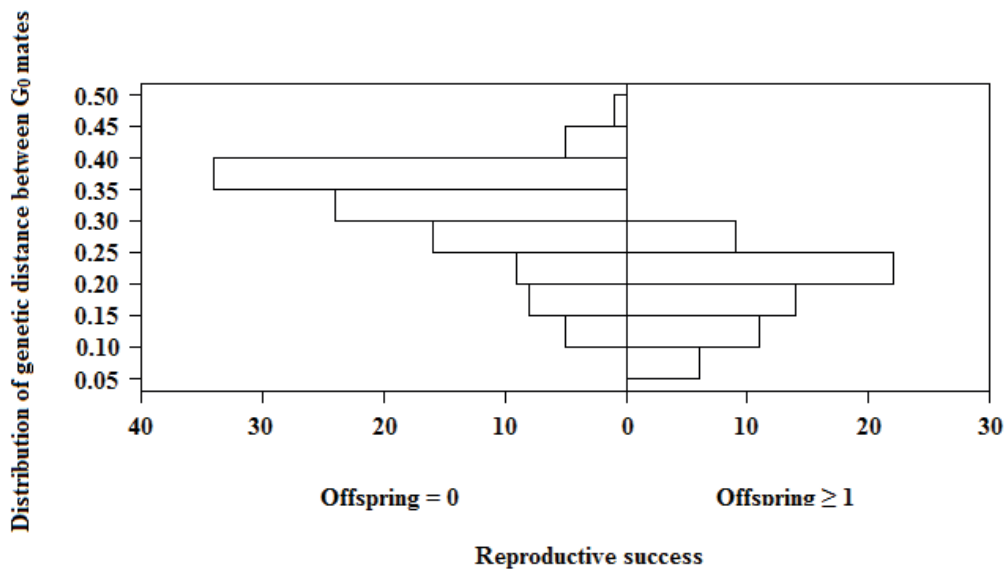
391
 392 **Figure 5: Proportion of crosses which successfully produced at least one offspring**
 393 **depending on *M. pygmaeus* population combinations.**



395

396 **Figure 4: Proportion of crosses which successfully produced at least one offspring**
 397 **depending on *M. pygmaeus* DNA cluster combinations.**

398



399

400 **Figure 6: Distribution of genetic distance (in %) between G₀ mates in couples having**
 401 **produced no offspring or at least one offspring.**

402

403 **Table 1: GLM analyses for (A) reproductive success and (B) Daily fecundity in generation**
 404 **G₀.**

Model	Df	Chisq	P (>Chisq)
A. Reproductive success			
Binomial error			
Model 1: population combination	9	46.8	1.1 ⁻⁰⁸
cluster combination	8	60.4	2.8 ⁻¹²
Model 2: genetic distance between mates	1	6.64	0.01
B. Daily fecundity			
Gamma error			
Model 1: population combination	9	10.4	0.07
cluster combination	5	2.3	0.48
Model 2: genetic distance between mates	1	0.11	0.67

405

406 *Fitness of hybrids*

407 Given the small proportion of between-cluster crosses which successfully produced at least one
 408 offspring, we only considered the continuous approach to investigate the determinant of hybrid
 409 fitness.

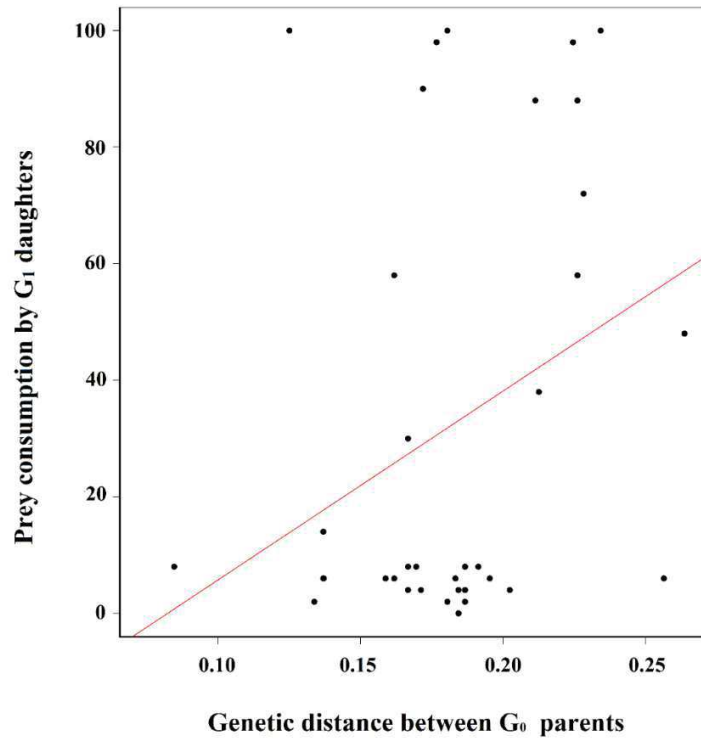
410 GLM analyses revealed a positive relationship between “genetic distance between parents” and
 411 “prey consumption by daughters” ($\chi = 147.78$, $df = 1$, $p = 0.05$) (Table 2, figure 7). A positive
 412 relationship was found between “genetic distance between parents” and reproductive success of
 413 daughter” ($\chi = 6.5$, $df = 1$, $p = 0.01$) (Figure 8). We found no relationship between “distance
 414 between parents” and “daughter longevity” ($\chi = 0.4$, $df = 1$, $p = 0.15$) or daily fecundity ($\chi = 0.3$,
 415 $df = 1$, $p = 0.43$) (Table 2).

416

417 **Table 2: GLM analyses for (A) Prey consumption, (B) Longevity, (C) Reproductive**
 418 **success and (D) Daily fecundity in daughters (generation G₁).**

Model	Df	Chisq	P (>Chisq)
A. Prey consumption			
Poisson error			
Genetic distance between parents	1	147.8	0.05
B. Longevity			
Normal error			
Genetic distance between parents	1	0.4	0.15
C. Reproductive success			
binomial error			
Genetic distance between parents	1	6.5	0.01
Genetic distance between daughter and its G ₁ mate	1	3.7	0.06
Interaction	1	3.6	0.06
D. Daily fecundity			
Gamma error			
Genetic distance between parents	1	0.3	0.43
Genetic distance between daughter and its G ₁ mate	1	0	0.99
Interaction	1	0.97	0.12

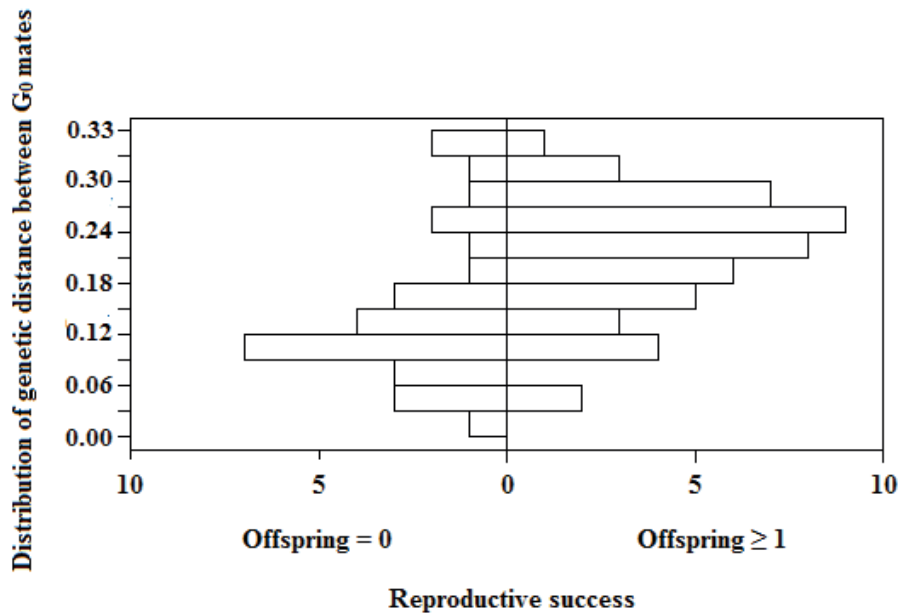
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Figure 7: relationship between “genetic distance between G_0 parents” and “prey consumption by G_1 daughters”.



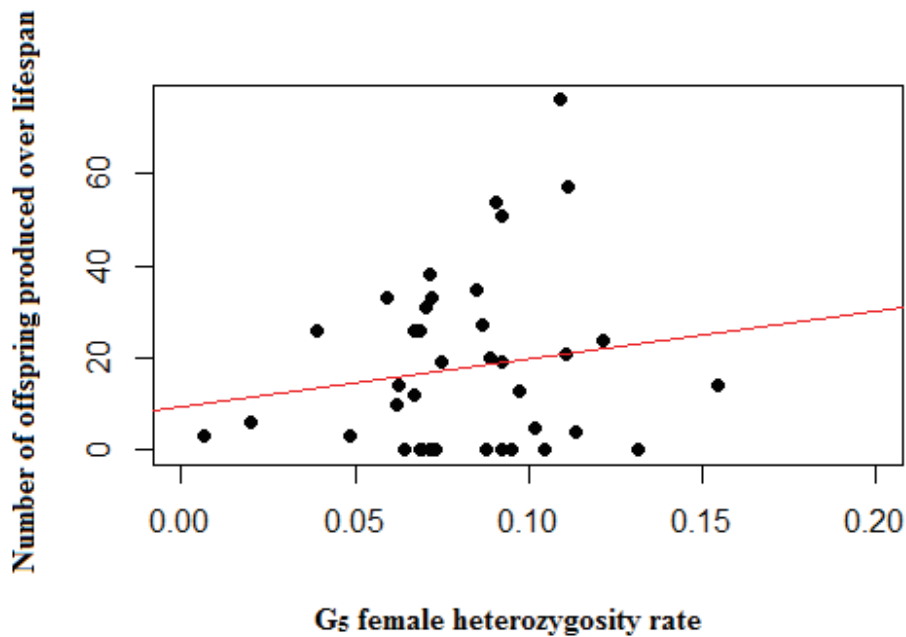
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Figure 8: Distribution of genetic distance between G_0 parents for G_1 daughters producing or not producing progeny.

426 *Heterozygosity-fitness correlation*

427 Using the females from generation G₅, we found a clear evidence ($\chi = 18.7$, $df = 1$, $p = 1.6^{-05}$)
428 of a positive relationship between female genome-wide heterozygosity and overall fitness
429 variations (Figure 9).



430

431 **Figure 9: Relationship between the number of offspring produced in lifespan and G₅**
432 **female heterozygosity rate.**

433

434 **4. Discussion**

435 In this study we investigated the genetic variations occurring in four field-sampled European
436 populations of the BCA species *Macrolophus pygmaeus* and studied the effect of within- and
437 between-population hybridization on fitness-related traits. To do so, we coupled phenotypic traits
438 measurements and genotyping by sequencing (GBS) of individuals, in a design consisting of

439 crosses between and within populations. In addition, we investigated the overall relationship
440 between individual heterozygosity rate and fitness-related trait values.

441 Clustering of *M. pygmaeus* populations based on SNP data showed consistent results with
442 recent literature on population structure of *M. pygmaeus* in Europe (Sanchez et al., 2012; Streito et
443 al., 2016). We could distinguish between three clusters occurring in sympatry but genetically
444 differentiated from each other. While Sanchez et al. (2012) had shown differentiation between
445 populations from the Greek peninsula, the Iberic peninsula and France, Streito et al. (2016) also
446 suspected that population may be differentiated between ‘wild’ and ‘commercial’ haplotypes that
447 both could be found in sympatry. The number of clusters we observed is compatible with the results
448 of both studies. The observed clustering with Multi-Dimensional Scaling consists of three clusters:
449 k1, which consists of individuals collected at sites where biocontrol releases are unlikely might
450 correspond to the so-called ‘wild’ haplotype. Clusters k2 and k3 may correspond to the so-called
451 ‘commercial’ haplotypes. Performing GBS on individuals from various commercial origins would
452 help to clarify this situation. In any case, the analysis reveals a substantial extent of genetic
453 variation to be used in the lab experiments.

454 GLM analyses revealed various reproductive incompatibilities between European
455 populations (Figure 5) which seems highly related to the genetic structure uncovered with SNPs.
456 Reproductive success in crosses between genetic clusters was much lower than within genetic
457 clusters (Figure 4). Moreover, an overall negative relationship between genetic distance and mating
458 success was observed at G_0 . Reproductive isolation between the three clusters seems relatively
459 high, since only 8.1% of all hybrid crosses succeeded in producing offspring. A surprising result is
460 that crosses within clusters k2 and k3 showed quite low reproductive success although they were
461 mainly composed of crosses within Spanish and Greek populations, respectively. Other

462 uncontrolled factors may thus induce reproduction failure in these clusters. Moreover, both Greek
463 and Spanish populations were collected on tomato and thus have not suffer any host change (when
464 compared to samples collected in Belgium and France).

465 Measures at the individual level revealed an effects of genetic distance between mates. Daughters
466 coming from couples in which parents were genetically distant tended to display higher
467 reproductive success (Figure 7; table 2), and consumed more preys in the experiment (Figure 6;
468 table 2). While it is difficult to determine whether consuming more preys is beneficial for fitness
469 in the field or for biocontrol (this could be maladaptive and not always desirable in rearing
470 conditions), the result on reproductive success suggests a positive impact of the genetic distance
471 between parents. This suggests that that there may be possibilities of enhancement of fitness at
472 certain extents of genetic distance (and heterozygosity of the progeny).

473 When examining the above-mentioned relationship between the the number of offspring
474 produced over lifespan (as an overall fitness approximation) and G_5 female individual
475 heterozygosity rate in Belgian, French and their Hybrid strains, a significant and positive trend has
476 been detected (Figure 9). It is no surprising result considering the sensitivity of the populations
477 used in this experiment to inbreeding depression (Quaglietti et al., submitted) and the relations
478 between genetic distance between parents and traits observed. Complementary analyses will be
479 conducted to further investigate both heterozygosity-fitness relationship, and determinant factor of
480 hybridization success, notably by improving the contig construction from bacterial genome with
481 sequence data obtained by shotgun sequencing of reference samples from several *Macrolophus*
482 taxa (Palero et al., in prep.), by using distinct GBS dataset analysis pipelines, by choosing
483 contrasted SNP filtering methods and by creating distinct SNP subgroups (from genomic regions
484 displaying contrasted features or belonging to bacterial genomes).

485 In brief, we found substantial genetic diversity in the four sampled European populations,
486 with the notable occurrence of genetic clusters displaying reproductive isolation between each
487 other. We detected effects of genetic distance between individuals on traits of their progeny, with
488 contrasting patterns depending on the range of distance created by the crossing design. However,
489 we failed at this stage to draw a global relationship between genetic distance of parents (or
490 heterozygosity of the progeny) and fitness-related traits because this relation is probably not linear
491 and influenced by different parameters along the whole gradient of genetic distance created by the
492 occurrence of different populations and genetic clusters. In terms of interest for biological control,
493 the current results are sufficient to address two main messages. First, the well-known BCA *M.*
494 *pygmaeus* does not consist of a genetically homogeneous genetic pool across Europe and mixes
495 between populations should be considered with extreme care because strong reproductive isolation
496 occurs between genetic clusters, even in laboratory no-choice mating conditions. This illustrates
497 the importance of the common concern about cryptic species and differentiated strains of BCAs
498 (Mound et al., 2010; Paterson et al., 2016; Sha et al., 2007; Tosevski et al., 2011). In the case of
499 *M. pygmaeus* mixing clusters in the same rearing could have harmful effects on production yield.
500 The observed genetic variations could even influence field release strategies since the genetic
501 composition of *Macrolophus* populations present in target agrosystems may influence the
502 establishment of the released biocontrol agents. The importance, in the field, of the reproductive
503 isolation patterns observed in this study are however difficult to predict. For example it will depend
504 on the mechanisms underlying the isolation (prezygotic or postzygotic?) (Orr and Presgraves,
505 2000; Orr and Turelli, 2001; Turelli et al., 2001) between the clusters and the ecological features
506 displayed by each cluster in the field: each may have distinct ecological niches, or have developed
507 assortative mating preventing hybridization *in natura*. Second, given the identified relationships
508 between fitness and heterozygosity or genetic distance between parents there is a potential to

509 maximize production yield and efficiency in the field by enhancing allogamic crosses as long as it
510 remains in the same genetic cluster.

511 **Acknowledgments**

512 This work has received funding from the European Union Seventh Framework Programme
513 FP7, grant agreement FP7-IAPP #324475 “COLBICS”, and by the INRA program
514 “Métaprogramme SMACH”, project #P10225. Special thanks go to Nancy Lenaerts, Simon Van
515 den Bergh and François Hervi for their technical support during the experiments. We also warmly
516 thank our colleagues of the genomic platform at Cornell University for their fast and efficient work
517 that enabled B. Quaglietti to include these GBS data before the end of his PhD project.

518

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756

Annexe I

Le travail sur *A. burrelli* au sein de l'INRA de Sophia-Antipolis, a été réalisé dans le cadre d'un programme de lutte biologique par acclimatation visant à l'introduction du parasitoïde dans l'environnement pour contrôler les populations de la cochenille *P. comstocki*. L'introduction d'espèces exotiques dans un nouvel environnement comporte des risques d'effets indésirables sur la biodiversité native ou endémique. En effet, le risque qu'un auxiliaire de lutte biologique classique s'attaque à des espèces non-cibles n'est pas négligeable et cela peut avoir des effets particulièrement néfastes pour ces populations. Dans ce contexte, la France parmi d'autres pays, impose une évaluation de ces risques avant l'introduction sur le territoire et dans l'environnement de tout organisme exotique. L'évaluation de la gamme d'hôte d'*Allotropa burrelli* en France a donc été effectuée. Les résultats ont fait preuve d'une évaluation de la part du ministère de l'agriculture et ont mené à l'autorisation de l'introduction du parasitoïde dans l'environnement.

Le protocole et les résultats détaillés sont détaillés dans l'article ci-après :

Quaglietti, B., Gautier, P., Groussier, G., Fleisch, A., Kreiter, P., Ris, N. & Malausa, T., (2017) Pre-release host-range determination of the parasitoid *Allotropa burrelli* for the biocontrol of *Pseudococcus comstocki* in France, *Journal of Applied Entomology*. **(Manuscrit 4)**

Manuscrit 4

Pre-release host range determination of the parasitoid *Allotropa burrelli* for the biocontrol of *Pseudococcus comstocki* in France

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Funding information

Seventh Framework Programme, Grant/Award Number: 324475; Agence Nationale de la Recherche, Grant/Award Number: ANR-10-JCJC-1708

Abstract

We performed “no-choice” tests to study the host range of the parasitoid *Allotropa burrelli* (Muesebeck) (Hymenoptera: Platygasteridae) for use against the Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae), in Southern France. We tested three Pseudococcidae species as potential non-target hosts: two species from the same genus (*Pseudococcus longispinus* and *Pseudococcus viburni*) and *Planococcus citri*. *Allotropa burrelli* did not parasitize any of the non-target mealybug species tested. No attempt of oviposition was recorded for the three species tested during the first 20 min of parasitoid release and no parasitism occurred in 6–8 hr of exposure of the mealybugs to the parasitoid.

KEYWORDS

classical biological control, parasitoids, risk assessment, specificity

1 | INTRODUCTION

Risk assessment linked to the effects on non-target species is a prerequisite before releasing a biological control agent (BCA) in the environment (Babendreier, Bigler, & Kuhlmann, 2005; van Lenteren, Bale, Bigler, Hokkanen, & Loomans, 2006; van Lenteren et al., 2003; Van Driesche & Hoddle, 1997). “No-choice” tests on both target and non-target species are usually performed to determine host range of the BCA. Non-target species are chosen on the bases of phylogenetic distance to the target, economic importance, presence in the target environment and importance for conservation biology (Babendreier et al., 2005; Kuhlmann, Schaffner, & Mason, 2005; van Lenteren et al., 2003, 2006; Wapshere, 1974).

Pseudococcus comstocki Kuwana (Hemiptera: Pseudococcidae) originates from eastern Asia and is found on fruit trees and ornamental plants. It causes economic damage in apple or pear orchards and vineyards, through sap-sucking and honeydew secretion (Villa et al., 2011). This species has been detected in western Europe in the early 2000's (Kreiter & Germain, 2005; Pellizzari, 2005), and a biological control programme was initiated in France in 2008.

The search for potential BCAs was based on previous successful biocontrol programmes in the former USSR (Kobakhidze, 1965) and the USA (Bartlett & Clancy, 1972; Haeussler & Clancy, 1944; Meyerdirk & Newell, 1979; Meyerdirk, Newell, & Warkentin, 1981), together with a worldwide survey based on DNA identification (Malausa et al., 2016). A combination of two gregarious parasitoids, *Acerophagus malinus* (Gahan) (Hymenoptera: Encyrtidae) (formerly *Pseudaphycus malinus*) and *Allotropa burrelli* (Muesebeck) (Hymenoptera: Platygasteridae), which effectively controlled *P. comstocki* populations in California (Meyerdirk & Newell, 1979), was selected for biological control in France. While faunal inventories performed in Southern France between 2007 and 2011 (Fleisch et al., 2011; Hantzberg, Gili, Giuge, & Kreiter, 2008) revealed that *A. malinus* is established in France, no *A. burrelli* populations were detected locally. Therefore a population of this last species was imported from Japan and kept in quarantine conditions at INRA Institut Sophia Agrobiotech, France (Fleisch et al., 2011).

Before releasing *A. burrelli* in France, we performed “no-choice” tests with the target species *P. comstocki*, and three other mealybug species with different phylogenetic distances from *P. comstocki*: *Pseudococcus longispinus* (Targioni-Tozzetti), *Pseudococcus viburni* (Signoret) and

Planococcus citri (Risso). We performed an analysis of (i) the oviposition behaviour of *A. burrelli* females, and (ii) the no-choice exposure of *A. burrelli* females to all species, to further record parasitism.

2 | MATERIALS AND METHODS

2.1 | Biological material

We established a rearing of *Pseudococcus comstocki* in 2008, from individuals collected in Southern France. We then collected *P. viburni*, *P. longispinus* and *P. citri* from Southern France in 2012, and used DNA markers to confirm species identification (Malausau et al., 2011). The four mealybug species were reared, isolated from each other, on sprouted potatoes in complete darkness at $25 \pm 3^\circ\text{C}$, with a relative humidity of $70\% \pm 10\%$.

The rearing of *A. burrelli* was established from about 800 individuals collected in Japan, in 2010. We reared *A. burrelli* on *P. comstocki* at $24 \pm 3^\circ\text{C}$, with a relative humidity of $70\% \pm 10\%$ and a 12-hr light/12-hr dark cycle. *Allotropa burrelli* has arrhenotokous reproduction, with unfertilized eggs producing males. Females have short, sparsely pubescent and distinctly club-shaped antennae, whereas males have longer, hirsute and moniliform antennae. Females can parasitize all instars of the Comstock mealybug, but the 3rd larval instar is the preferred host stage (Clancy 1944). About 20 days after oviposition, parasitized mealybugs can be easily identified due to their mummified appearance. The parasitoid completes its development in the host over a period of 40 days at 24°C .

2.2 | “No-choice” tests

We assessed the host specificity of *A. burrelli* females by applying the same protocol for each mealybug species. We isolated parasitized mealybugs from *A. burrelli* mass-rearing and checked the sex of the emerging parasitoids under a binocular microscope. We placed all

individuals, both male and female, in the same tube with honey drops for at least 24 hr, to allow mating.

We deposited 10 s-instar and 10 third-instar mealybugs into Petri dishes containing agarose, onto which we had placed a leaf disc from an apple tree. After 18 hr, we released a 24–48 hr old parasitoid female per Petri dish for 6–8 hr. We then removed the female parasitoid and transferred the mealybugs onto sprouted potatoes. We tested 30 female parasitoids on each mealybug species. Thirty days after the release of the parasitoids, we counted the number of parasitized mealybugs and isolated them in glass tubes (diameter = 0.7 cm, length = 7.5 cm). We then determined the number of emerging parasitoid and their sex ratio.

During the first 20 min after the release of the parasitoids, we performed behavioural observations on 15 randomly picked females of the 30 females used in the experiments for each mealybug species. We counted the number of host assessments by antennation, and the number of attempts of oviposition.

2.3 | Statistical analysis

We fitted GLM to assess the effect of mealybug species on the variations of all phenotypic traits. We used a Poisson distribution with a square root link function for all traits. We performed type III tests of effects, followed by pairwise comparisons if a significant effect of mealybug species was detected. We performed all GLM analyses and post hoc tests with the `glm()`{stats}, `Anova()`{car} and `glht()`{multcomp} (Fox & Weisberg, 2011; Hothorn, Bretz, & Westfall, 2008) functions of R version 3.2.3. (Copyright © 2014. The R Foundation For Statistical Computing) (R core Team 2016).

3 | RESULTS

The GLMs revealed a general effect of mealybug species on both the number of host assessments ($p\text{-value} = 2.649 \times 10^{-9}$) and the number

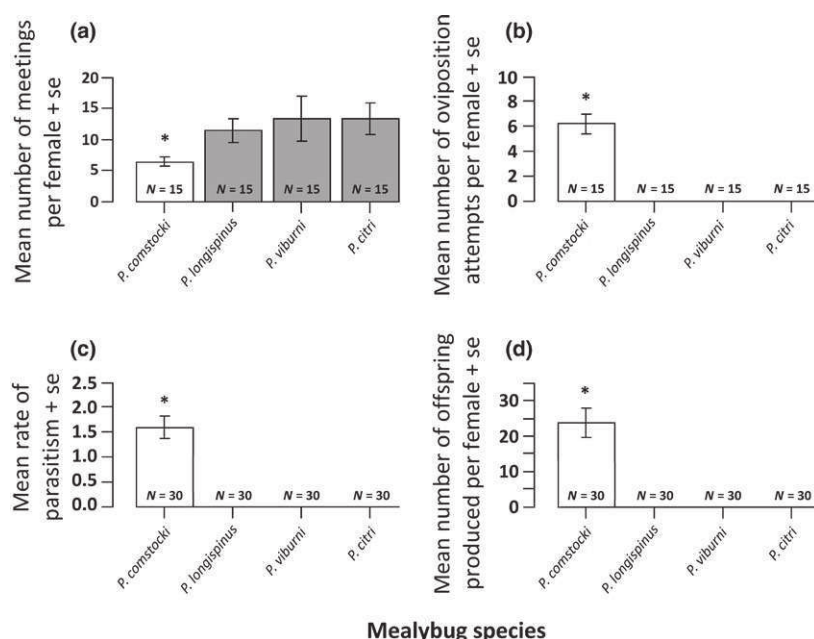


FIGURE 1 Mean + standard error (SE) of (a) the number of host assessments per female, (b) the number of attempts at oviposition events per female, (c) the rate of parasitism and (d) the number of offspring produced per female, by mealybug species (N = number of replicates). “*” marks a statistically significant difference.

of attempts of oviposition (p -value $< 2 \times 10^{-16}$). Pairwise comparisons showed that parasitoid females carried out a smaller number of host assessments (ha) when confronted with *P. comstocki* ($6.4 \pm 0.7 ha$) compared to that observed for the other mealybug species ($12.6 \pm 1.6 ha$) (p -values $< 1 \times 10^{-4}$) (Figure 1a). Similarly, we observed a clear difference in the number of attempts of oviposition (ao) between parasitoid females confronted with *P. comstocki* ($6.2 \pm 0.8 ao$) and those confronted with the other mealybug species (zero ao) (p -value $< 2 \times 10^{-16}$) (Figure 1b).

On average, *A. burellii* females parasitized 1.6 ± 0.2 *P. comstocki* larvae, corresponding to $8\% \pm 1\%$ of the hosts provided. Parasitized mealybugs produced 23.7 ± 4 offspring, $24.3\% \pm 2.6\%$ of which were males. As *A. burellii* did not parasitize *P. longispinus*, *P. viburni* and *P. citri*, we did not fit GLMs for the number of parasitized mealybugs, the number of emerging individuals and their sex ratio.

4 | DISCUSSION

Our results show that *A. burellii* is probably highly specific to *P. comstocki*, at least in France, where no mealybug species genetically more closely related to *P. comstocki* than those tested are known to occur. We found that parasitoid females of *A. burellii* performed fewer host assessments on *P. comstocki* than females, which were offered the non-target species. Parasitoids are often time-limited and must adapt their behaviour to maximize their progeny production so that a trade-off may exist between time allocation to host-searching and host-handling (Bugila, Branco, Borges da Silva, & Franco, 2014; Waage, 1983). *Allotropa burellii* females which were offered *P. comstocki* may thus have maximized the time allocated to host-handling, while females which were offered *P. longispinus*, *P. viburni* or *P. citri* maximized time allocated to host-searching and thus antennation. In addition, females attempted oviposition only in the presence of *P. comstocki*. No parasitism was detected when *A. burellii* was offered *P. longispinus*, *P. viburni* and *P. citri*. This pattern was observed both in the no-choice experiment and in several rearing boxes in which *A. burellii* females were left in the presence of mealybugs for unrestricted time (results not shown). Our results are consistent with previous studies on specialist mealybug parasitoids where such exclusive host acceptance was observed while generalists parasitized and developed successfully in various mealybug species (Bugila et al., 2014; Chong & Oetting, 2007; Dorn, Mattiacci, Bellotti, & Dorn, 2001; Roltsch, Ertle, & Meyerdirk, 2007; Sagarra, Vincent, & Stewart, 2001). According to our results, we conclude that the introduction of *A. burellii* in France represents a minor risk of non-target effects on the environment.

ACKNOWLEDGEMENTS

This work was supported by the French *Agence Nationale de la Recherche* grant ANR-10-JCJC-1708 "BICORAMICS" and by the European Union, FP7-IAPP, grant agreement #324475 "COLBICS". We thank the Biology of Introduced Populations "BPI" and Research and Development in Biological Control "RDLB" teams of the Institut Sophia Agrobiotech for technical and material support.

AUTHOR CONTRIBUTION

TM, NR, AF, GG and PK developed protocol and experimental design. GG and PG carried out the experiment. BQ wrote the manuscript and analysed the data. TM, NR, GG and AF reviewed the manuscript reviewed before submission.

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How to cite this article: Quaglietti B, Gautier P, Groussier G, et al. Pre-release host range determination of the parasitoid *Allotropa burrelli* for the biocontrol of *Pseudococcus comstocki* in France. *J Appl Entomol*. 2017;00:1–4.
<https://doi.org/10.1111/jen.12407>