Caractérisation de la réponse au stress chez une espèce invasive Drosophila suzukii

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Résumé

Les changements globaux accélérés par l'impact anthropique sont responsables de multiples évènements d'introductions d'organismes dans de nouvelles aires géographiques dont ils étaient absents auparavant. La capacité de certaines espèces à survivre, se reproduire et se disperser dans ces nouveaux environnements pose la question des mécanismes d'adaptation de ces espèces invasives. En effet, dans bien des cas, le processus d'invasion commence avec un petit nombre de propagules et donc une possible réduction de la variation génétique dans la population. La plasticité phénotypique devrait jouer un rôle crucial dans le succès invasif d'une espèce et il est généralement supposé que les populations invasives devraient avoir une plus grande plasticité que les populations non invasives. Au niveau moléculaire, le rôle des mécanismes liés aux éléments transposables sont de plus en plus proposés mais peu d'études ont été effectuées dans le contexte invasif. Par ailleurs, le rôle des mécanismes non-génétiques héritables comme les modifications épigénétiques peuvent aussi expliquer la capacité à répondre à différents environnements sur une échelle de temps courte. Les espèces invasives offrent donc la possibilité d'étudier ces mécanismes d'adaptations rapides.

Au travers de ce doctorat, nous avons cherché à décrire la diversité de la réponse phénotypique à différentes contraintes environnementale en fonction du statut de la population, et ensuite les mécanismes moléculaires impliqués dans cette réponse chez une espèce récemment invasive *Drosophila suzukii*. Cette espèce originaire d'Asie, a été introduite en 2008 de façon concomitante aux États-Unis et en Europe; elle est maintenant présente du nord au sud du continent américain, et en Europe où elle est détectée jusqu'en Russie. L'objectif a été d'étudier la réponse aux stress thermique et chimique de diverses populations d'une aire native et des aires envahies (U.S.A et France), pour ensuite identifier au niveau moléculaire les mécanismes impliqués dans ces résistances. Nous avons donc cherché à caractériser la plasticité du phénotype et du transcriptome des populations étudiées, en tenant compte de la diversité en éléments transposables et leurs conséquences sur la stabilité génomique.

Nous avons observé que les populations présentaient des différences dans les réponses vis à vis des stress étudiés, au niveau phénotypique comme moléculaire. Contrairement aux populations natives, les populations françaises et américaines présentent une espérance de vie beaucoup plus importante et une capacité de résistance au froid accrue. Cependant, la réponse au stress chimique montre des profils différents entre France et U.S.A. Nous avons observé au niveau moléculaire de la variabilité du transcriptome associée majoritairement aux génotypes plus qu'à l'environnement, démontrant une différentiation génétique rapide entre génotypes qui pourrait refléter de l'adaptation locale malgré l'invasion récente. Contrairement à ce qui est suggéré dans la littérature, le stress n'induit pas une expression accrue des éléments transposables chez cette espèce, mais de nombreux gènes, spécifiques à chaque génotype, présentent des insertions d'éléments transposable dans leur voisinage, offrant des candidats à l'adaptation locale dans chaque pays.

Abstract

Global changes accelerated by anthropogenic impact are responsible for multiple events of introductions of organisms into new geographical areas from which they were previously absent. The ability of certain species to survive, reproduce and disperse in these new environments raises the question of the mechanisms of adaptation of these invasive species. Indeed, in many cases, the invasion process begins with a small number propagules and thus a possible reduction in genetic variation in the population. Phenotypic plasticity should play a crucial role in the invasive success of a species and it is generally assumed that invasive populations should have greater plasticity than non-invasive populations. At the molecular level, the role of mechanisms related to transposable elements is increasingly proposed but few studies have been carried out in the invasive context. Furthermore, the role of heritable non-genetic mechanisms such as epigenetic modifications may also explain the ability to respond to different environments on a short time scale. Invasive species, therefore, offer the opportunity to study these rapid adaptation mechanisms.

Through this PhD, we sought to describe the molecular mechanisms involved in the response to environmental stresses in a recent invasive species *Drosophila suzukii*. This species, native from Asia, was introduced concomitantly in 2008 in the United States and Europe; it is now present from the north to the south of the American continent, and in Europe, it is detected as far as Russia. The aim was to study the phenotypic response to thermal and chemical stresses of diverse populations in native and invaded areas (U.S.A. and France) and then, to identify at the molecular level the mechanisms involved in these resistances. We therefore sought to characterise the phenotypic and the transcriptome plasticity of the populations studied, taking into account the diversity in transposable elements and their consequence on genome stability.

We observed that the populations showed differences in responses to the stresses studied, both at the phenotypic and molecular levels. In contrast to native populations, French and American populations have a much longer lifespan and increased resistance to cold. However the response to chemical stress shows different profiles between France and the U.S.A. At the molecular level, we observed variability in the transcriptome expression, associated mainly with genotypes rather than with the environment, demonstrating a rapid genetic differentiation between genotypes that could reflect local adaptation despite the recent invasion. Contrary to what is suggested in the literature, stress does not induce increased expression of transposable elements in this species, but many genes, genotype-specific, show insertions in the vicinity of genes, offering candidates for local adaptation to the conditions in each country.

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A propos

Formé en biologie de l'environnement ce qui m'a conduit à m'intéresser aux interactions des organismes et leur environnement. J'ai travaillé sur le comportement des abeilles et les effets des traitements phytosanitaires.
En tant qu'ingénieur j'ai travaillé au laboratoire souterrain de Modane dans une équipe de physiciens pour étudier les conséquences des rayonnements ionisants dans un projet d'évolution expérimentale. Actuellement en doctorat, j'étudie les mécanismes de résistances chez une espèce invasive avec des approches transcriptomiques et épigénétiques.

Compétences

- Autonomie, gestion de projet
- Expérimentations biologiques
- Biologie moléculaire
- Bioinformatique (données NGS, cluster)
- Programmation (R, bash)
- Statistiques fréquentistes
- Anglais (TOEIC 655/999 en 2014)
- Statistiques bayésiennes (initiation)

(*)[Échelle de 0 (débutant) à 6 (expert)]

Cursus

2016-2020	Doctorat en sciences biologiques Université Claude Bernard Lyon 1	Lyon
2009-2012	Master Biologie et Environnement, option Microbiologie : Génomes, Ecologie et Biotechnologie Université Blaise Pascal	Clermont-Ferrand
2009-2012	Licence en Biologie, *Science de la Vie et de la Terre (SVT)* Université Blaise Pascal	Clermont-Ferrand
2009	Baccalauréat scientifique, option biologie Lycée René Descartes	Cournon d'Auvergne

Experiences

2016-2020	Etudiant en doctorat, *mécanismes moléculaires de la réponse au stress chez une espèce invasive Drosophila suzukii* Contacts : C. VIEIRA & P. GIBERT U.M.R 5558-LBBE, Lyon
2015-2016	Ingénieur d'étude, *impact à long terme des rayonnements ionisants sur le vivant* Contact : V. BRETON U.M.R 6533-LPC, Clermont-Ferrand
Déc. 2014	Ingénieur d'étude, *mise au point d'une méthode de fractionnement subcellulaire* Contact : L. BONHOMME U.M.R 1095-GDEC, Clermont-Ferrand
2014	Stage de seconde année de master, *impact comportemental d'un xénobiotique sur l'abeille domestique* Contacts : C. ARMENGAUD & D. BIRON U.M.R 5169-CRCA, Toulouse & U.M.R 6023-LMGE, Clermont-Ferrand
2013	Stage de première année de master, *effet de potentialisation de la stimulation des défenses des plantes* Contact : A. DEGRAVE U.M.R 1345-IRHS, Angers
Mai 2012	Stage volontaire, *étude des protéines intrinsèques X (XIP) aquaporines* Contact : J-S. VENISSE U.M.R 0547-PIAF, Clermont-Ferrand

Communications scientifiques

Août 2019	ESEB - *Molecular characterisation of (non) genetic mechanisms on an invasive species* Turku, Finlande	Poster
Juin 2019	EMPSEB - *Mechanisms of rapid adaptation during environmental changes, a case of an invasive species* Pedrógão Pequeno, Portugal	Poster
Juin 2019	CNET2019 - *How to deal with environmental changes: molecular characterization of mechanisms on an invasive species model* Lyon, France	Poster
Juin 2018	CNET2018 - *D. suzukii wild-type resistance to oxidative stress* Clermont-Ferrand, France	Poster

Publications

2020 [*In prep.*] Marin, P., Jaquet, A., Picarle, J., Fablet, M., Mérel, V., Delignette-Muller, M-L., Galvão Ferrarini, M., Gibert, P. & Vieira, C.. Differences in phenotypic and transcriptomic responses to stress is related to population geography in an invasive species

2020 [*In prep.*] Marin, P., Jaquet, A., Picarle, J., Mérel, V., Delignette-Muller, M-L., Galvão Ferrarini, M., Vieira, C. & Gibert, P.. Chill coma recovery time of a recent invasive species *D. suzukii*, from phenotype to molecular mechanisms

2020 [*Submission*] Marin, P., Jaquet, A., Henri, H., Gibert, P. & Vieira C.. *Drosophila suzukii* oxidative stress response involves Jheh gene cluster but not transposable elements. https://doi.org/10.1101/2020.04.27.063297

2019 Marin, P., Genitoni, J., Barloy, D., Maury, S., Gibert, P., Ghalambor, C.K., Vieira, C., 2019. Biological invasion: The influence of the hidden side of the (epi)genome. Functional Ecology 34, 385–400. https://doi.org/10.1111/1365-2435.13317

2019 Lampe, N., Marin,P., Coulon, M., Micheau, P., Maigne, L., Sarramia, D., Piquemal, F., Incerti, S., Biron, D., Ghio, C., Sime-Ngando, T., Hindré, T., Breton, V., 2019. Reducing the ionizing radiation background does not significantly affect the evolution of Escherichia coli populations over 500 generations. Scientific Reports 9, 14891. https://doi.org/10.1038/s41598-019-51519-9

2016 Lampe, N., Marin,P., Castor, J., Warot, G., Incerti, S., Maigne, L., Sarramia, D., Breton, V., 2016. Background study of absorbed dose in biological experiments at the Modane Underground Laboratory. EPJ Web Conf. 124, 00006.https://doi.org/10.1051/epjconf/201612400006

2016 Lampe, N., Biron, D.G., Brown, J.M.C., Incerti, S., Marin, P., Maigne, L., Sarramia, D., Seznec, H., Breton, V., 2016. Simulating the Impact of the Natural Radiation Background on Bacterial Systems: Implications for Very Low Radiation Biological Experiments. https://doi.org/10.1371/journal.pone.0166364

Informations complémentaires

Enseignement

2016-2019 Activité complémentaire d'enseignement (200 h) — Université Claude-Bernard Lyon 1
Génétique des populations (TD/TP)
Bioinfo-biostat (TP)

Encadrement

2019 Encadrement de Prescillia Rodier en master 1 — Lyon
"Plasticité transgénérationnelle chez *Drosophila melanogaster*"

2016 Encadrement d'Anaïs Degüt en master 2 — Lyon
"Plasticité phénotypique chez *Drosophila suzukii*"

Activités scientifiques complémentaires

Mars 2018 Oral, *Résiste ! Prouve que tu existes ! Adaptation des organismes aux changements* — Association Biosphère
Vulgarisation scientifique

2016 Oral, *Nosema ceranae* and thymol interaction on bees learning ability — Paris
Symposium sur la recherche apicole en France

Remerciement

Nous arrivons avec ces lignes à l'aboutissement d'un passage de ma vie extrêmement enrichissant et que je ne pourrais oublier, dans ses plaisirs et ses difficultés. Dans les défis à relever, la sueur qui a perlée et les moments de bonheurs pris durant ces dernières années.

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Cette envolée lyrique elle t'est dédicacée.
A toujours prendre du temps pour les gens.
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Nous permettre de réussir, avec un sourire.
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prête à aider et jamais tu ne râlerais! Dans la foulée je ne vais pas oublier Nelly ou même David (le système capitaliste a encore des jours devant lui...). Merci aussi aux personnes du service informatique; Stéphane (qui m'a vu souvent, trop... dans son bureau), Bruno, Simon, Lionel et aussi Aurélie qui m'a permis de me motiver et d'arriver à dompter R! Merci aussi à Philippe W. pour son aide, ses formations et sa disponibilité!

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Merci pour cette aventure...

Table des matières

Introduction

Les activités humaines au travers d'une économie mondialisée, ont favorisé un événement reconnu comme un facteur de changement global, l'invasion biologique. De nombreuses espèces se retrouvent introduites dans des nouvelles aires géographiques, avec des conditions différentes voire inédites. Au travers de ces événements, la capacité de survivre et de continuer à envahir les aires avoisinantes pose de nombreuses questions sur la biologie de ces organismes. Ces invasions offrent un cadre d'étude pour comprendre les phénomènes d'adaptations rapides en lien avec les changements climatiques qui affectent les organismes vivants. C'est aussi une source d'étude pour comprendre le rôle de la plasticité phénotypique dans la micro-évolution des espèces ou encore, pour comprendre les mécanismes sous-jacents à la résistance aux changements d'environnements. De plus, une partie de ces invasions a des conséquences économiques et écologiques importantes. Leur étude permet de comprendre la réponse des écosystèmes lors de l'arrivée de nouvelles espèces, mais aussi de développer des moyens de lutte pour limiter leurs impacts. Le sujet de thèse a été développé pour caractériser au niveau phénotypique et moléculaire, la réponse des organismes face à différents stress abiotiques, thermiques et chimiques. Ces facteurs sont impliqués dans les changements globaux et sont déterminants dans la distribution géographique des espèces. Nous avons cherché à comprendre sur une espèce récemment invasive *Drosophila suzukii* et au travers d'un panel de populations, les différences de résistance et les mécanismes moléculaires qui pouvaient expliquer ces différences. Nous avons donc développé une approche comparative, de caractérisation phénotypique de ces résistances sur des populations d'aires envahies mais aussi de l'aire d'origine. Nous avons sélectionné par la suite des génotypes aux réponses différentes pour caractériser au niveau génétique (voir épigénétique) les différences observées au préalable. Nous avons en particulier étudié la plasticité du transcriptome mais aussi les éléments transposables dans la réponse aux stress.

1 Variations environnementales, changements globaux et invasions

1.1 Changements globaux et variations des conditions environnementales

L'écosphère, c'est à dire l'ensemble des êtres vivants et les environnements dans lesquels ils vivent en interaction ; est soumise à des variations des paramètres de ces environnements (facteurs biotiques et abiotiques) (Hutchinson, 1970 ; Huggett, 1999). On parle de changements globaux lorsque les changements environnementaux affectent l'écosphère à l'échelle de la planète (Steffen et collab., 2006 ; Oldfield et collab., 2004). Ces changements, comme les variations climatiques, sont impactés par les activités anthropiques notamment de ces derniers siècles. On parle maintenant de l'ère anthropocène (Waters et collab., 2016 ; Steffen et collab., 2006 ; Lewis et Maslin, 2015). Cette période est marquée par une accélération des changements globaux à partir du milieu du XX^{ème} siècle. Ces modifications sont diverses : réchauffement climatique, taux de dioxyde de carbone atmosphérique et océanique, usage de produits phytosanitaires, ou recrudescence des invasions biologiques (Waters et collab., 2016 ; Parmesan, 2006). L'impact des changements globaux sur l'écosphère est étudié depuis plusieurs décennies et de nombreuses revues récentes font un état de l'art détaillé des différents facteurs et de leurs conséquences (Vitousek, 1994 ; Steffen et collab., 2006 ; Sage, 2019 ; Parmesan, 2006). Un effet majeur des changements globaux, accéléré par les activités humaines, concerne la modification de la distribution des espèces et l'érosion de la biodiversité. On estime que le taux d'extinction naturelle des espèces est de deux

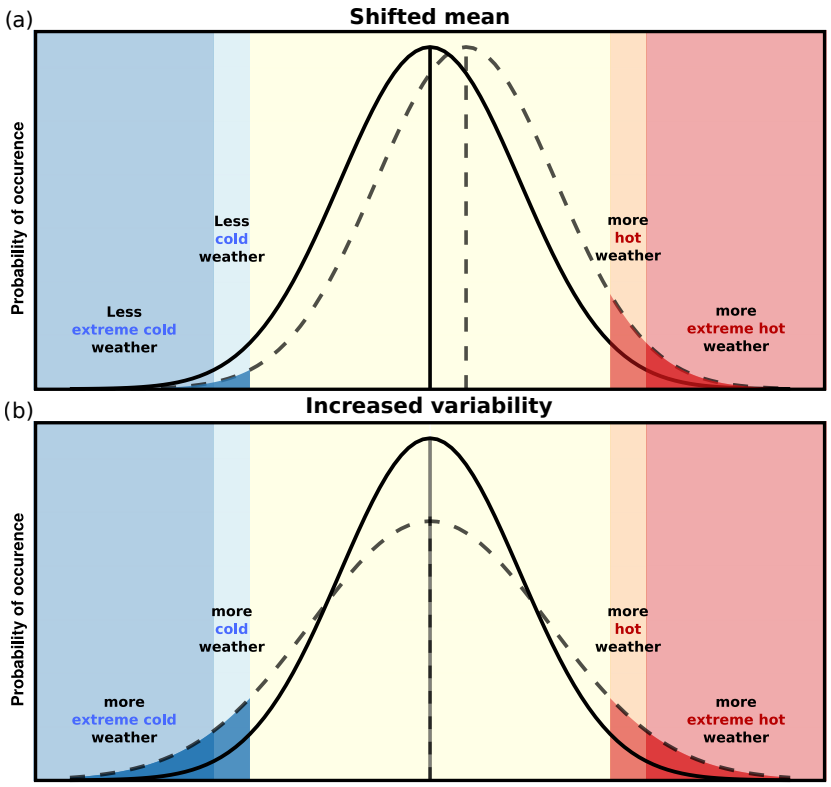

Figure 1. Schéma de l'effet des changements de température entre climat actuel et futur : **(a)** effets d'un simple déplacement de l'ensemble de la distribution vers un climat plus chaud ; **(b)** effets d'une augmentation de la variabilité de la température sans changement dans la moyenne. Source : Ummenhofer et Meehl (2017).

à trois fois plus rapide qu'attendu (Steffen et collab., 2006 ; Rockström et collab., 2009). L'Amérique du Nord aurait vu un quart des espèces d'oiseaux disparaître dans les cinquantes dernières années (Rosenberg et collab., 2019). Concernant les insectes, on estime à plus de 75% le déclin sur la biomasse des insectes volants en Allemagne (Hallmann et collab., 2017). Les facteurs majeurs de ces changements sont décrits dans de nombreux articles comme Steffen et collab. (2006), Parmesan (2006) ou Sage (2019), que nous allons utiliser par la suite pour illustrer certains concepts. Parmi ces facteurs, le changement climatique (réchauffement) est le facteur le plus étudié avec de nombreuses analyses montrant ses conséquences, sur la distribution et la phénologie des espèces et les interactions biotiques. Par exemple sur 1598 espèces étudiées, Parmesan (2006) montrent que 59% présentent des changements de distribution et de phénologie avec une précocité saisonnière de la floraison ou de l'émergence de papillons. Des événements d'asynchronie, c'est à dire de décalage dans les interactions trophiques (ex. émergence précoce de papillons par rapport à la présence de fleurs avec du nectar, conduisant à une extinction des populations) ont également été observés. Ces effets peuvent être dus, (i) soit au déplacement de la gamme de distribution thermique (température rencontrée dans l'environnement, **figure 1a**) des espèces qui doivent donc s'adapter au nouvel environnement thermique ce qui peut être bénéfique en augmentant l'aire de répartition possible de l'espèce, ou conduire à des événements de déséquilibre comme cités plus haut ; (ii) soit à l'augmentation de la fréquence et l'intensité des événements extrêmes c'est-à-dire les valeurs rarement rencontrées par un organisme (statistiquement ce sont les queues de distribution des gammes thermiques, **figure 1b**), (Schulte, 2014 ; Lynch et Gabriel, 1987 ; Gabriel et Lynch, 1992

; Chevin et Hoffmann, 2016). En effet, bien que le réchauffement climatique conduise à une réduction des périodes de froid (ex. estimation en 2070 de 10 à 90 jours selon la région, avec une température négative en Amérique du Nord, Rawlins et collab., 2016), on estime que des conditions extrêmes comme des hivers très froids ou des étés très chauds surviennent plus fréquemment avec des intensités plus fortes (Cattiaux et collab., 2010 ; Palmer et collab., 2017 ; Ummenhofer et Meehl, 2017 ; Chevin et Hoffmann, 2016). Ces conditions sont particulièrement critiques pour les espèces du fait de l'échelle de temps courte de ces changements au regard de l'évolution. Il est donc important de comprendre comment les organismes répondent aux variations de l'environnement à une courte échelle de temps, et notamment, leurs réponses au stress.

1.2 Une conséquence du changement global, les invasions biologiques

Il est maintenant admis que parmi les facteurs de changements globaux, les invasions biologiques sont un facteur important de modification de l'environnement notamment en altérant la richesse spécifique dans les aires envahies (Sage, 2019 ; Murphy et Romanuk, 2014 ; Mollot et collab., 2017 ; Bellard et collab., 2013). On parle même de la biologie de l'invasion, en tant que discipline, étudiant les espèces dites invasives (le terme français est envahissante mais nous garderons le terme emprunté de l'anglais, **figure 2**). Un consensus sur les processus d'invasion et la terminologie a été notamment proposé par Blackburn et collab. (2011). Nous définirons une espèce invasive comme :
"une espèce introduite dans un environnement dont elle était absente auparavant, qui s'établit, se reproduit et se disperse sur de nombreux sites distants du site d'introduction."

Figure 2. Illustration de différentes espèces invasives avec des conséquences, **(A)** sanitaires pour *Aedes aegypti* (moustique tigre), **(B)** inconnues pour *Physa acuta* (physe) , **(C)** écologiques pour *Trachemys scripta elegans* (tortue de Floride) ou **(D)** agronomiques pour *Bemisia tabaci* (aleurode du tabac). Source : **(A)** www.pexels.com, **(B)** Tariel et collab. (2020), **(C)** O'Keeffe (2009), **(D)** www.freestockphotos.biz.

Cette définition omet volontairement deux notions de l'invasion que l'on peut retrouver dans la littérature à savoir, (i) l'impact négatif de l'espèce invasive (économique, écologique), (ii)

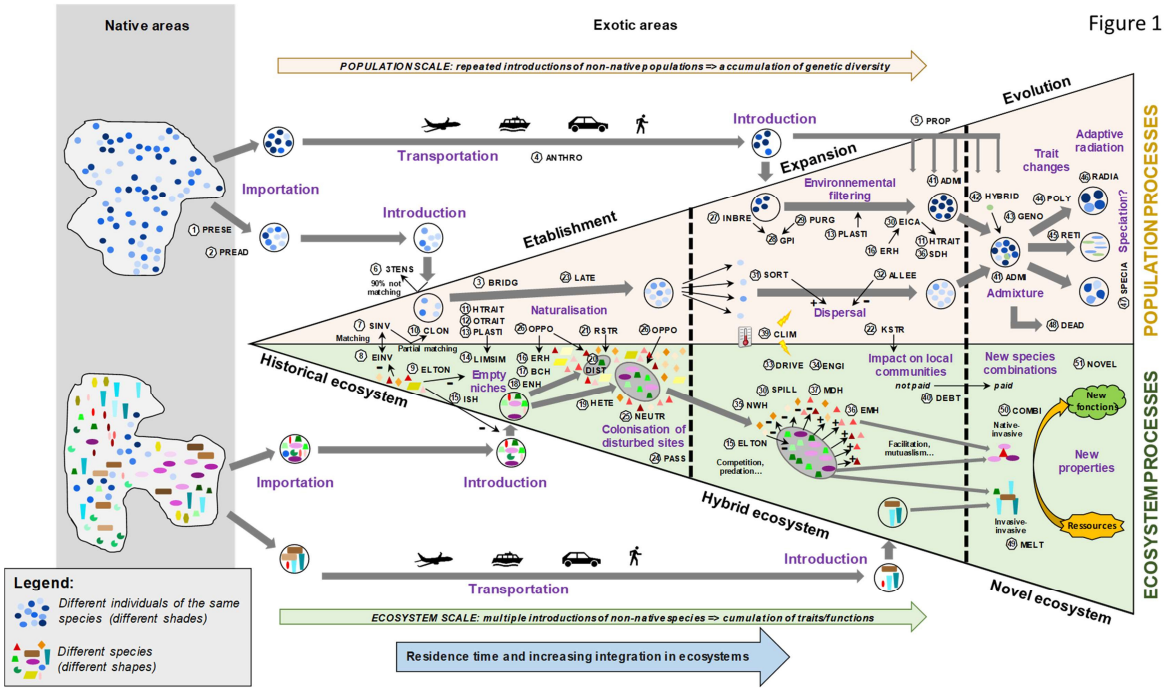

Figure 3. Illustration du processus invasif, reprenant les différentes étapes depuis le transport jusqu'à l'expansion dans de nouvelles aires. Chaque numéro correspond à un concept, mécanisme relatif à l'invasion développé par Chabrerie et collab. (2019). Source : Chabrerie et collab. (2019)

l'intervention humaine directe pour l'introduction. Considérer le statut d'invasion au regard de son impact et du rôle direct de l'humain (import volontaire) dans l'introduction, est anthropocentré et ne définit pas mieux une espèce invasive. Avant de détailler l'invasion comme facteur du changement global, il est important de définir le processus d'invasion. Des travaux récents ont permis de synthétiser les théories et hypothèses relevant du processus d'invasion avec Blackburn et collab. (2011) et Chabrerie et collab. (2019) qui vont nous servir de base pour décrire les processus en jeu. Nous pouvons résumer le processus invasif à plusieurs étapes cruciales correspondant à des barrières, c'est-à-dire des limites à franchir pour que le processus puisse être considéré comme une invasion biologique (**figure 3**) :

- **Transport** : l'étape de transport constitue une première barrière géographique, il est nécessaire que l'espèce soit transportée hors de son aire d'origine. Si dans la définition donnée plus haut, l'intervention humaine directe est omise, le fait que les activités anthropiques à la surface de la Terre accélèrent les invasions est incontestable. La mise en relation des données de trafics internationaux avec le recensement des espèces nouvellement détectées dans un territoire a permis de confirmer que les activités humaines en particulier, les échanges commerciaux internationaux sont un des facteurs, de transport et d'introduction, des nouvelles espèces dans l'environnement (Seebens et collab., 2015 ; Seebens, 2019 ; Sage, 2019 ; Chapman et collab., 2017 ; Chabrerie et collab., 2019 ; Bertelsmeier et collab., 2017). Une étude de Chapman et collab. (2017) illustre le rôle majoritaire des réseaux commerciaux comme principale source de dispersion sur 60% des espèces invasives étudiées (422 au total). De façon plus générale, les grands événements ayant marqués l'histoire humaine (guerre, globalisation...) sont associés à des pics d'invasion comme illustré par Bertelsmeier et collab. (2017), avec une étude sur 241 espèces invasives de fourmis. Par ailleurs les fac-

teurs des changements globaux peuvent interagir entre eux et modifier les flux d'invasion comme suggéré par Seebens et collab. (2015), avec une augmentation des invasions dans les pays de haute latitude du fait du réchauffement progressif.

- **Introduction** : suite au transport, l'espèce se retrouve introduite dans un nouvel écosystème. D'une part, on utilise le terme d'introduction lorsque l'espèce est cantonnée à la zone nouvellement colonisée par des mesures d'origine humaine et dont la survie n'est assurée que artificiellement par la culture. D'autre part, si l'espèce introduite se trouve libre dans l'environnement sans contrainte humaine, et qu'elle survit on ne considère pas d'étape d'introduction mais directement l'étape suivante.
- **Établissement** : une espèce est considérée comme établie dès lors qu'elle peut persister dans l'environnement sans introduction supplémentaire d'individus dans la colonie. De nombreux facteurs interviennent pour expliquer la persistance ou non de l'espèce afin de s'établir. En ciblant quelques éléments, plus détaillés dans la revue Chabrerie et collab. (2019), on perçoit que l'invasion peut être facilitée par de la présélection et de la préadaptation. La première concerne le fait qu'une espèce peut se trouver introduite au travers d'une population dont les traits sélectionnés permettent de répondre au changement d'environnement (ex. invasion par une population plus résistante au froid dans une région plus froide que l'aire d'origine). Quant à la préadaptation, elle se réfère à l'idée que les environnements d'invasion et d'origine, ont les mêmes caractéristiques. D'autres facteurs sont aussi évoqués comme la pression de propagule, c'est à dire le nombre d'introduction de l'espèce dans la nouvelle aire, qui va augmenter la probabilité d'établissement en limitant les effets négatifs associés à de petites tailles de populations par exemple. Une fois qu'une espèce est établie sur plusieurs générations elle est considérée comme naturalisée mais non invasive, en ce sens qu'elle reste limitée à la zone d'invasion initiale.
- **Expansion** : à partir du moment où une espèce établie dans une nouvelle aire, se disperse sur d'autres sites, avec un éloignement important du site initial on parle d'espèce invasive. Durant l'expansion, les espèces invasives sont confrontées au fur et à mesure que le front de migration progresse, à des variations des environnements correspondant à des étapes d'établissement successives. Le succès d'une invasion se mesure au travers de la distribution globale de l'espèce et offre l'opportunité d'étudier l'évolution de ces espèces au travers d'événements très récents, que ce soit au niveau écologique au travers de la perturbation des écosystèmes, des relations nouvelles avec les espèces invasives ; comme au niveau mécanistique pour comprendre le succès d'invasion.

1.3 Changement de l'environnement et notion de stress

Comprendre les conséquences des changements des conditions environnementales comme celles décrites en **section 1.1** ouvre le champ à l'étude des effets des conditions stressantes. L'usage du terme stress renvoie à différentes notions pouvant porter à confusion. La notion de stress est introduite notamment en 1950 par Selye comme une *"réponse aspécifique d'un organisme à toute demande"* dans une vision physiologique de l'organisme. Cependant cette notion reste trop large puisque tout changement transitoire de la physiologie pourrait être décrit comme un stress. Plus récemment Schulte (2014), a proposé un cadre unifié de la définition de stress afin de lever l'ambiguïté qui provient de l'utilisation du terme stress à la fois pour désigner la perturbation environne-

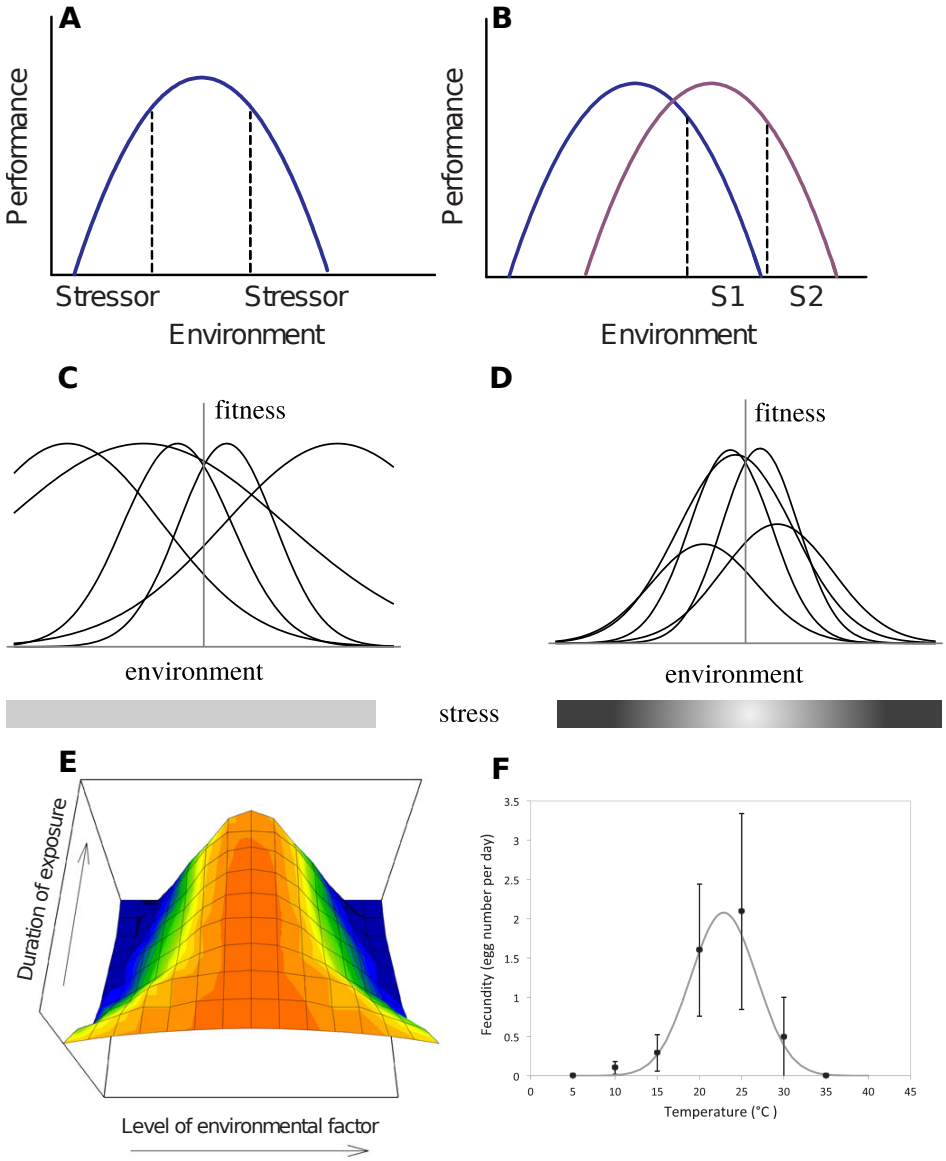

Figure 4. Exemple de courbe théorique de performances selon un gradient environnemental (A & B) supplémentée par l'échelle temporelle (E). Normes de réactions de la fitness de plusieurs génotypes en fonction de l'environnement illustrant la maladaptation (C) ou la qualité de la niche (D). Mesures empiriques de performance au travers de la fécondité chez une espèce de drosophile *D. suzukii* le long d'un gradient thermique (F). Source pour A, B, E Schulte (2014), C, D Chevin et Hoffmann (2016) et F Hamby et collab. (2016).

mentale, mais aussi la réponse à cette perturbation. Différents termes relatifs au stress coexistent dans la littérature, (i) stress, (ii) stresseur, (iii) réponse au stress ou (iv) stress environnemental (Schulte, 2014). Le stresseur se définit comme un agent biotique ou abiotique entraînant une perturbation de l'homéostasie, il implique alors une réponse physiologique ou comportementale de la part de l'organisme : la réponse au stress. Le stress est la conséquence, sur la fitness de l'organisme de l'exposition au stresseur. D'autres définitions du stress impliquent en fait la notion d'environnement *"extrême"* qui correspond à des conditions rarement rencontrées dans l'histoire évolutive d'une espèce (aussi définit comme les queues de distribution d'une gamme de tolérance le long d'un gradient d'une variable environnementale) (Chevin et Hoffmann, 2016). La réponse au stress est influencée à la fois par la nature du stresseur et de ses paramètres associés (fréquence, intensité) ainsi que l'état physiologique de l'organisme (immunité, stade de développement, comportement...) et de son histoire évolutive. La plasticité phénotypique (**section 2.2**) peut ainsi agir sur la réponse au stress en tamponnant les effets au cours de la vie d'un organisme ; tout comme les mécanismes épigénétiques (**section 3**) peuvent aussi influencer cette réponse au niveau intra- mais aussi intergénérationnelle. Enfin, sur une plus grande échelle de temps évolutif, la sélection de caractères adaptatifs peut aussi permettre aux organismes de persister dans des environnements stressants Huggett (1999). Du fait de la difficulté à estimer la fitness, on utilise la mesure de trait de personnalité en fonction d'un facteur environnemental représentée comme illustrée en **figure 4A**, **B** (**figure 4E** avec l'aspect temporel) comme proxy. Les termes de "gamme de tolérance" ou "norme de réaction" sont souvent utilisés, pour décrire les relations de dépendances (ou indépendances) des mesures phénotypiques (ou de la fitness) au gradient du facteur environnemental (Lynch et Gabriel, 1987 ; Gabriel et Lynch, 1992). Un exemple, très étudié en particulier chez les insectes, car déterminant pour la répartition géographique des espèces, correspond à la tolérance thermique comme illustrée en **figure 4F**, sur des mesures de fécondité chez des drosophiles (Klepsatel et collab., 2013 ; Hamby et collab., 2016). La description de la gamme de tolérance permet d'illustrer que les espèces présentent des limites adaptatives aux conditions environnementales. On parle de mal-adaptation quand le paysage adaptatif se décale de l'optimum, autrement dit par exemple, quand la moyenne d'un trait phénotypique est décalée par sélection directionnelle (**figure 4C**, Hoffmann et Hercus, 2000 ; Chevin et Hoffmann, 2016). On parle de qualité de l'habitat (**figure 4D**) lorsque l'environnement est stressant pour toute l'espèce, et que l'optimum de fitness est globalement plus bas que dans un autre environnement. Le stress environnemental est vu comme une force sélective importante pouvant entraîner à court terme (microévolution) des adaptations dans les populations naturelles (Hoffmann et Hercus, 2000). L'adaptation des espèces aux conditions de stress s'inscrit aussi dans la compréhension des effets des changements globaux comme décrit plus haut, qui altèrent rapidement les écosystèmes des organismes. Les populations naturelles doivent répondre à ces changements pour survivre avec un panel limité, (i) migration vers des aires géographiques plus propices, (ii) résistance aux nouvelles conditions environnementales, (iii) extinction.

1.4 Stress environnemental et réponses des espèces invasives

La dispersion à grande échelle, notion intrinsèque à la définition d'une espèce invasive (définie en **section 1.2**) ; amène ces espèces à expérimenter différentes conditions environnementales, et leurs succès impliquent que ces espèces présentent les caractéristiques phénotypiques

nécessaires à leur survie. Cette aptitude à répondre aux stress environnementaux à une échelle de temps courte fait écho à des phénomènes d'adaptations rapides qui sont très étudiés du fait de la problématique des changements globaux sur la distribution et survie des espèces. De ce fait les espèces invasives se trouvent dans la littérature comme un modèle d'étude de l'évolution et de l'adaptation des espèces en conditions stressantes ou de changements environnementaux (Whitney et Gabler, 2008 ; Prentis et collab., 2008 ; Moran et Alexander, 2014 ; Hoffmann et Sgrò, 2011 ; Hoffmann et Hercus, 2000 ; Colautti et Lau, 2015). Plusieurs aspects sont étudiés dans l'adaptation au stress chez les espèces invasives pour comprendre, (i) leur résistance (face aux variations mais aussi vis-à-vis des espèces natives), (ii) les mécanismes sous-jacents à la résistance aux stresseurs. Une riche littérature porte sur la compréhension du succès d'invasion notamment avec le rôle central que jouerait la plasticité phénotypique dans un environnement stressant, depuis H. G. Baker (1966). Cette notion de plasticité phénotypique fait l'objet d'une description particulière en **section 2.2** et **section 3**. Les environnements stressants de par leurs conséquences sur la fitness peuvent entraîner des changements rapides sur des traits en imposant une sélection directionnelle forte (Lin et collab., 2017 ; Reznick et Ghalambor, 2001 ; Hoffmann et Sgrò, 2011 ; Hoffmann et Hercus, 2000). Une définition de changement adaptatif rapide pourrait correspondre à *"un changement héritable suffisamment rapide pour que son impact écologique soit quantifiable"* (Hairston et collab., 2005). Un exemple classique provient des phénomènes de résistances aux traitements phytosanitaires sur les espèces ravageuses de cultures ou pathogènes pour l'homme, pouvant survenir en moins d'un an après usage d'un traitement (Grimmer et collab., 2015). Chez les espèces invasives, de nombreuses adaptations rapides ont été observées durant le processus d'invasion avec l'idée que certains traits seraient préférablement associés au succès invasif (Whitney et Gabler, 2008 ; Moran et Alexander, 2014 ; Dlugosch et Parker, 2008 ; Colautti et Lau, 2015 ; Card et collab., 2018). Par exemple, Moran et Alexander (2014) relèvent que des événements d'adaptation rapide, en moins de 15-20 ans ont déjà été observés permettant au moustique tigre (*Aedes albopictus*) d'ajuster sa diapause aux modifications de l'environnement ou à *Drosophila subobscura* d'adapter la taille des ailes aux conditions locales en générant un cline latitudinal. Chez les plantes, Godoy et collab. (2011) montrent par exemple que parmi quinze espèces de plantes méditerranéennes (dont 8 invasives) placées dans des conditions standards similaires, les plantes invasives présentent une meilleure résistance au stress hydrique grâce à un système photosynthétique plus efficace limitant l'effet du stresseur. Dans deux récentes méta-analyses, (Jia et collab., 2016) et (Liu et collab., 2017), comparent les performances d'espèces végétales selon différents facteurs de changements globaux, la température, le taux de dioxyde de carbone et d'azote, et le taux de précipitation. Ces deux analyses montrent que les plantes invasives semblent favorisées par ces quatre facteurs sur les différents traits mesurés (taille, fitness, vitesse de croissance…). D'autre part, parmi les différents systèmes de fixation du carbone des plantes (appelés C3, C4 ou CAM), le système C4 semble favorisé, ce qui pourrait être un facteur facilitant la compétition pour des plantes invasives présentant ce type de système par rapport aux espèces locales. Un exemple relatif à la thermotolérance vient d'une espèce marine eurytherme (gamme de tolérance thermique large) d'ascidie invasive *Diplosoma listerianum* qui présente une meilleure survie qu'une espèce locale *Distaplia occidentalis* (Zerebecki et Sorte, 2011) du fait de sa large gamme de température où elle survit, et cela semble lié au niveau d'expression de gènes de réponse au choc thermique (*Hsp*) avec un niveau plus important pour les plus thermotolérantes (Zerebecki et Sorte, 2011 ; Lock-

wood et Somero, 2011). D'autres cas de changement important d'environnement ont été observés notamment avec la tortue de Floride *Trachemys scripta elegans*. Cette espèce d'eau douce, invasive du fait de son intérêt commercial comme animal de compagnie, s'est retrouvée dans des eaux salées et présente des aptitudes à résister au stress salin impliquant des mécanismes de réponse au stress oxydant plus rapidement activés (6h après stresseur) (Ding et collab., 2019). En plus de pouvoir survivre dans un environnement différent, les mécanismes impliqués pour répondre au stress salin peuvent permettre de mieux répondre à d'autres stress comme le stress oxydant médié par des produits phytosanitaires augmentant le potentiel de résistance au stress chez cette espèce invasive. L'usage intensif et global des traitements phytosanitaires peut aussi être vu comme un facteur des changements globaux auquel les espèces invasives peuvent être confrontées (Sage, 2019). La littérature concernant l'étude d'apparition de résistance multiple et/ou rapide (moins d'un an pour certains cas) est riche d'exemples (McLaughlin et Dearden, 2019 ; Grimmer et collab., 2015 ; Dunlop et collab., 2018 ; Christie et collab., 2019 ; Campos et collab., 2014). L'espèce invasive, *Bemisia tabaci* (hémiptère, aleurode du tabac) est une bonne illustration de ces phénomènes. Cette espèce polyphage, très étudiée du fait de l'impact économique et sanitaire dans le monde est présente sur tous les continents, du Nord au Sud de l'Amérique, en Europe, Asie mais aussi en Australie (Basit, 2019 ; CABI, 2020). Elle présente des résistances à des doses importantes de traitements phytosanitaires avec plus de 630 cas de résistance répertoriés sur 60 substances différentes (`https://www.pesticideresistance.org`, Taquet et collab., 2019). Dans une étude sur l'île de la Réunion, Taquet et collab. (2019) ont étudié la réponse au stress chimique médiée par l'acétamipride et la pymétrozine sur des populations de *B. tabaci* dont une est indigène de l'île et l'autre invasive. Leur étude montre que la population autochtone est sensible aux deux traitements chimiques alors que l'espèce invasive présente une résistance pour la pymétrozine sans coût associé sur la fitness. Ces études de réponses aux stress sur les espèces invasives indiquent pour la plupart que ces dernières présentent une capacité à résister à des facteurs différents, parfois à présenter des aptitudes meilleures que les espèces natives. Cependant il n'est toujours pas possible de définir les paramètres qui permettent à ces espèces d'avoir des capacités améliorées.

2 L'invasion dans tous ses états : succès et paradoxe des espèces invasives

Nous avons précédemment présenté comment les espèces invasives étaient étudiées pour leur aptitude à répondre aux variations de l'environnement, au niveau écologique, mécanistique et évolutif avec la notion d'adaptation rapide. La description du processus d'invasion mentionne des événements pré-introductifs comme la présélection, la préadaptation ou la pression de propagule (Chabrerie et collab., 2019). D'autres hypothèses écologiques ont été suggérées afin d'expliquer le potentiel invasif comme par exemple, l'hypothèse de la niche vide ou l'hypothèse EICA (Evolution of increased competitive ability) (Plantamp, 2016). Brièvement, l'hypothèse de la niche vide postule que le succès invasif est possible du fait d'une ressource non utilisée dans la communauté envahie, permettant à l'espèce de survivre et de se reproduire. L'hypothèse EICA quant à elle suggère une réallocation des ressources de défenses vers des traits d'histoire de vie suite à la diminution de la pression de sélection des ennemis naturels absents de la zone d'invasion (prédateurs, parasites). Ces hypothèses complémentaires, seules, ne suffisent pas à expliquer le succès

des invasions, mais montrent que le processus est complexe et multifactoriel (Bossdorf et collab., 2005).

2.1 Paradoxe génétique des espèces invasives

De ce fait depuis les débuts de la biologie de l'invasion, une question persiste, comment des espèces non natives, qui ne sont pas forcément adaptées au nouvel environnement, peuvent persister, présenter des adaptations rapides, dépasser les aptitudes d'espèces locales et envahir? Ce paradoxe est d'autant plus intéressant qu'il est suggéré depuis longtemps que les événements d'introduction conduisent à une réduction de la diversité génétique pouvant mener à l'extinction sans que cela ne soit le cas pour les espèces invasives (Bock et collab., 2015 ; H. G. Baker, 1966 ; Dlugosch et Parker, 2008). Cette question a été formalisée sous le terme de paradoxe génétique des espèces invasives par Allendorf et Lundquist (2003). Dans une récente revue, Estoup et collab. (2016) montrent que ce paradoxe n'est pas aussi commun que suggéré dans la littérature et que trois conditions *sine qua non* doivent être présentes pour considérer une espèce dans ce paradoxe. Premièrement, la diversité génétique dans les populations nouvellement introduites doit être plus faible par rapport à l'aire native, suggérant des phénomènes de goulot d'étranglement (réduction importante de la population) ou d'effet fondateur (nouvelle population à partir d'un faible nombre d'individus). Deuxièmement, cette érosion de la diversité génétique ne doit pas mener à une extinction malgré les conséquences délétères induites (dépression de consanguinité, effet allee). Enfin, les populations introduites doivent présenter des adaptations aux nouvelles (différentes) conditions environnementales rencontrées. Selon ces critères, beaucoup d'espèces invasives ne semblent pas présenter de paradoxe (Wellband et collab., 2017 ; Baltazar-Soares et collab., 2017), c'est le cas pour plus de 60% d'espèces végétales et aquatiques étudiées (Roman et Darling, 2007 ; Bossdorf et collab., 2008). Plusieurs espèces invasives présentent ces trois caractéristiques du fait de biais méthodologiques pour estimer la diversité génétique, ou d'un balayage sélectif diminuant leur diversité (Estoup et collab., 2016). Mais il existe beaucoup d'espèces pour lesquelles ce paradoxe semble être confirmé. Pour ces espèces, une hypothèse pour expliquer ce paradoxe est que du fait des goulots d'étranglement rencontrés, une purge des allèles délétères peut s'opérer sur la population invasive limitant la dépression de consanguinité, c'est ce qui a été observé pour la coccinelle asiatique (*Harmonia axyrydis*) (Facon et collab., 2011). D'autres mécanismes ont été suggérés tels que l'activation des éléments transposables et/ou la mise en jeu de mécanismes épigénétiques (Vogt, 2017 ; Ullastres et collab., 2016 ; Stapley et collab., 2015 ; Richards et collab., 2012 ; Rey et collab., 2016 ; Merenciano et collab., 2016 ; Horváth et collab., 2017 ; Casacuberta et González, 2013 ; Bossdorf et collab., 2008). Ces mécanismes décrits dans un contexte invasif ont fait l'objet d'une revue publiée en 2019 présente en **section 3**.

2.2 Plasticité phénotypique en réponse au stress

Considérés auparavant comme un bruit de fond négligeable, la prise en compte des effets environnementaux dans la théorie moderne de l'évolution a permis de mieux appréhender le conflit entre expression du génotype et effet sur le phénotype (Agrawal, 2001 ; Debat et David, 2001). Le phénotype est la résultante de l'expression du génotype d'un organisme (G), des effets de l'environnement dans lequel évolue cet organisme (E) et de l'interaction de ces deux éléments (GxE). Un effet génotype-environnement, concerne la notion de plasticité phénotypique

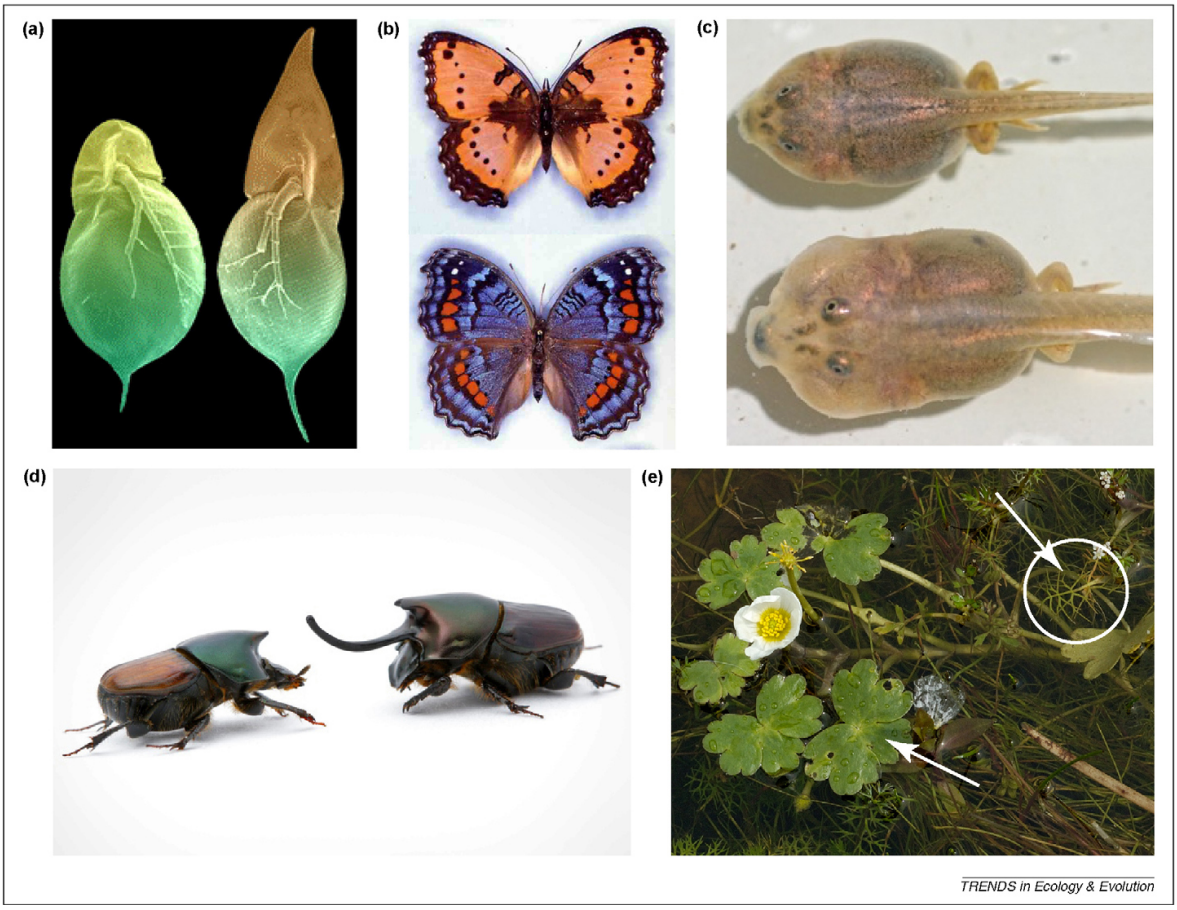

Figure 5. Illustration de la plasticité phénotypique sur différentes espèces. (**a**) *Daphnia cucullata* avec (gauche) ou sans (droite) morphe induit par le prédateur, (**b**) *Precis octavia* selon la saison sèche ou humide, (**c**) morphe omnivore et carnivore chez *Spea multiplicata*, (**d**) taille des cornes pour *Onthophagus nigriventris*, (**e**) structure foliaire large ou ramifiée sur la même plante *Ranunculus aquatilis*. Source : Pfennig et collab. (2010).

formalisée par Bradshaw (1965), en lien avec les travaux de Schmalhausen et Waddington, qui est une avancée majeure sur la compréhension de l'influence de l'environnement dans le processus d'adaptation (Pigliucci et collab., 2006 ; Debat et David, 2001). La plasticité phénotypique est définie comme *"l'aptitude d'un génotype à produire différents phénotypes en réponse à des signaux environnementaux"* (Agrawal, 2001 ; West-Eberhard, 2005 ; Pigliucci et collab., 2006 ; Reznick et Ghalambor, 2001 ; Ghalambor et collab., 2007). Une information issue de l'environnement peut être transmise à la descendance soit directement (effet maternel) soit plus indirectement (effet transgénérationnel). Elle peut s'observer à différent niveau d'un organisme (comportement, physiologie...). Un cas célèbre porte sur la réponse à la prédation d'une daphnie (*Daphnia lumholtzi*) présentant des morphes avec une tête pointue et une épine dorsale allongée induite par un signal chimique du prédateur (**figure 5**).

La plasticité phénotypique serait un des facteurs de l'invasion de cette espèce en Amérique du nord (Agrawal, 2001). Il existe différents types de plasticité selon le stade étudié (*ex.* développementale, transgénérationnelle), ou le statut adaptatif (la plasticité tend à l'optimum de la fitness, elle va dans le sens de la sélection naturelle) versus non-adaptatif (Ghalambor et collab., 2007). Un autre concept en lien avec la plasticité a été étudié par Waddington en 1953, concerne l'assimilation génétique (Waddington, 2012). Waddington a observé après induction par un stress ther-

mique, l'expression d'un nouveau variant phénotypique (via la plasticité) dans une population de *Drosophila melanogaster*. Après plusieurs générations de sélection artificielle (en appliquant le stress thermique), puis en relâchant la pression de sélection (arrêt du stress), le phénotype se maintenait dans la population. Ceci implique qu'un changement phénotypique induit initialement par l'environnement, peut se retrouver génétiquement fixé et donc que la plasticité pour ce trait soit diminuée (Pigliucci et collab., 2006). Les mécanismes moléculaires sous-jacents à la plasticité phénotypique ne sont pas tous compris, mais un cas bien décrit dans la littérature concerne la réponse aux variations thermiques chez *Drosophila melanogaster*. Du fait de le température de développement, la cuticule va se mélaniser en fonction de la diminution de la température. Cette variation est médiée par une régulation épigénétique thermosensible (Gibert et collab., 2016a). C'est l'état de la chromatine sous l'effet du signal thermique qui va être modulée au travers d'une protéine methyltransferase encodée par le gène trithorax (*trx*) qui va modifier le promoteur du gène *tan* impliqué dans la pigmentation. La plasticité phénotypique a été proposé comme un facteur clé de l'invasion et a été vite introduite dans la biologie de l'invasion ; du fait des conditions environnementales expérimentées par les espèces invasives, du paradoxe génétique et de leur succès d'invasion (H. G. Baker, 1966). Richards et collab. (2006) ont proposé trois scénarios possibles de l'effet de la plasticité sur les espèces invasives (**figure 6**). Dans le premier scénario, *« jack-of-all-trades »*, la plasticité permet aux espèces invasives d'être généralistes, c'est-à-dire que malgré les variations des conditions environnementales, leur fitness reste stable. Le scénario *« master-of-some »* prévoit au contraire, que la plasticité des traits phénotypiques induit une meilleure fitness pour les espèces invasives dans certains environnements uniquement. Finalement, le scénario *« jack-and-master »* est une combinaison des deux premiers et prévoit que l'espèce invasive présente une meilleure fitness dans tous les environnements que les espèces autochtones. Cependant dans la littérature, les mesures de plasticité des espèces invasives sont contradictoires. Certains auteurs au travers de mesures empiriques ou de méta-analyses suggèrent que les espèces invasives présentent plus de plasticité que les espèces natives (Daehler, 2003 ; Trussell et Smith, 2000 ; Sexton et collab., 2002 ; Terblanche et collab., 2010 ; Davidson et collab., 2011). Alors que d'autres résultats montrent un niveau de plasticité similaire ou moindre (Matzek, 2012 ; Godoy et collab., 2011 ; Palacio-López et Gianoli, 2011 ; Chown et collab., 2007). Il est important de noter ici que la plasticité se mesure sur des traits phénotypiques. Il est en effet possible de parler de la plasticité du trait mais en aucun cas de généraliser à l'échelle du phénotype. En effet un trait peut être plastique

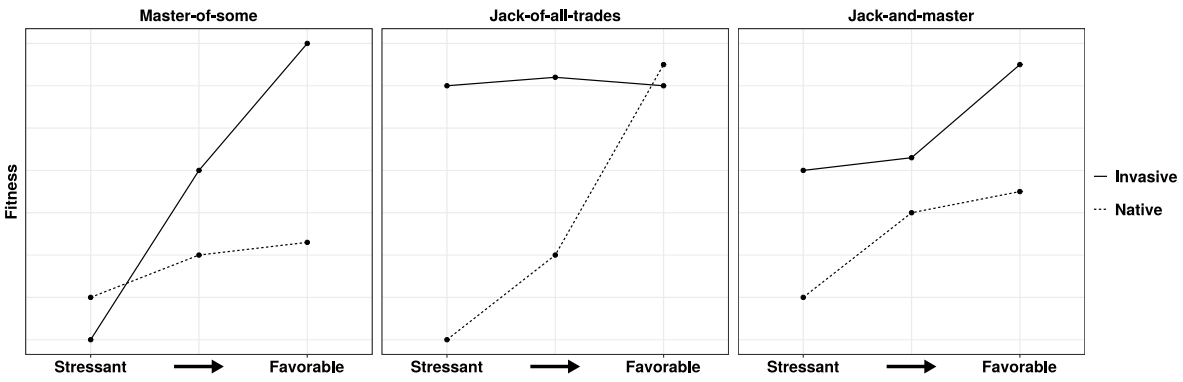

Figure 6. Illustration des trois scénarios suggérés par Richards et collab. (2006), source : repris à partir de Richards et collab. (2006).

dans certaines conditions et pas d'autres, et l'absence de mesure de plasticité d'un trait ne permet pas de conclure qu'un organisme n'est pas plastique. Lande (2015) dans une revue propose une explication sur les résultats contradictoires observés chez les espèces invasives et suggère que la plasticité est un phénomène transitoire, qui aurait plutôt lieu au début de l'invasion. Cette plasticité serait plus forte sur le front de migration qui fait face à plus de variabilité environnementale. Par ailleurs plus les aires envahies et natives sont similaires au niveau des paramètres environnementaux, moins on peut observer de plasticité chez une espèce invasive. Il y a donc une notion de dynamique de la plasticité phénotypique. D'autres facteurs sont présentés comme le coût associé à la plasticité ou les caractères labiles (changements continuels). De ce fait la plasticité phénotypique reste encore un sujet important dans la biologie de l'invasion que nous avons développé de façon plus approfondie dans la **section 3** en ajoutant la composante mécanistique de cette plasticité au travers de deux éléments, les éléments transposables et les modifications épigénétiques.

3 Réponse aux stresseurs et mécanismes moléculaires impliqués chez les espèces invasives

La capacité d'adaptation des espèces invasives dans les environnements nouvellement envahis reste encore largement mal comprise. Les conclusions, parfois divergentes sur leur capacité d'invasion ou leur capacité améliorée suggèrent que d'autres mécanismes complémentaires permettent aux espèces invasives de présenter des adaptations rapides. Ces mécanismes génétiques (ex. éléments transposables) et non-génétiques (ex. épigénétiques) ont été souvent suggérés dans la littérature, en lien avec la plasticité phénotypique. Nous avons publié une revue qui fait l'état de l'art de ces deux mécanismes en lien avec l'invasion biologique (Marin et collab., 2019). Bien que ces deux mécanismes offrent théoriquement des possibilités pour une adaptation rapide à l'environnement, les études empiriques restent très descriptives et rares dans la caractérisation de la diversité génétique versus épigénétique sans pouvoir attribuer des liens directs entre le phénotype et la diversité épigénétique observée. De même, s'il est clair que les éléments transposables dont la régulation est soumise à des processus épigénétiques, peuvent présenter un potentiel adaptatif dans des environnements stressants, en générant de la variabilité génétique ou impactant la régulation de certains gènes, aucune étude à ce jour apporte de preuve sur le rôle décisif des éléments transposables dans le succès invasif d'une espèce. Il est donc nécessaire, afin de mieux comprendre les mécanismes moléculaires sous-jacents à l'adaptation de ces espèces, de proposer des études de génomique comparative sur ces différents mécanismes.

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EPIGENETICS IN ECOLOGY AND EVOLUTION – A PRIMER

Biological invasion: The influence of the hidden side of the (epi) genome

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Abstract

1. Understanding the mechanisms underlying biological invasions and rapid adaptation to global change remains a fundamental challenge, particularly in small populations lacking in genetic variation. Two understudied mechanisms that could facilitate adaptive evolution and adaptive plasticity are the increased genetic variation due to transposable elements (TEs), and associated or independent modification of gene expression through epigenetic changes.
2. Here, we focus on the potential role of these genetic and non-genetic mechanisms for facilitating invasion success. Because novel or stressful environments are known to induce both epigenetic changes and TE activity, these mechanisms may play an underappreciated role in generating phenotypic and genetic variation for selection to act on. We review how these mechanisms operate, the evidence for how they respond to novel or stressful environments, and how these mechanisms can contribute to the success of biological invasions by facilitating adaptive evolution and phenotypic plasticity.
3. Because genetic and phenotypic variations due to TEs and epigenetic changes are often well regulated or "hidden" in the native environment, the independent and combined contribution of these mechanisms may only become important when populations colonize novel environments. A focus on the mechanisms that generate and control the expression of this variation in new environments may provide insights into biological invasions that would otherwise not be obvious.
4. Global changes and human activities impact on ecosystems and allow new opportunities for biological invasions. Invasive species succeed by adapting rapidly to new environments. The degree to which rapid responses to environmental change could be mediated by the epigenome—the regulatory system that integrates how environmental and genomic variation jointly shape phenotypic variation—requires greater attention if we want to understand the mechanisms by which populations successfully colonize and adapt to new environments.

KEYWORDS
adaptation, biological invasion, epigenetics, phenotypic plasticity, transposable elements

*Equally contributed.

1 | INTRODUCTION

In the face of current and future environmental changes, there are two possible outcomes for surviving organisms: to shift their geographical range or to adapt (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008). Dispersal to more suitable geographical areas is possible for some organisms but can be challenging for others depending on the mode of dispersal. Adapting to new environments can occur either through adaptive phenotypic plasticity or through adaptive evolution. Phenotypic plasticity is defined as the ability for a genotype to express several phenotypes according to different environmental cues and is adaptive when the environment shifts the distribution of phenotypes towards the local optimum (Ghalambor, McKay, Carroll, & Reznick, 2007). Such plastic responses represent the initial morphological, physiological or behavioural response to environmental change (Pigliucci, Murren, & Schlichting, 2006). Evolutionary responses occur across generations, and the rate at which populations can evolve is predicted to be a function of the strength of selection and the amount of standing genetic variation (e.g., Lande & Arnold, 1983). Adaptive plasticity has been proposed as one mechanism allowing for populations to colonize and persist in new environments (Davidson, Jennions, & Nicotra, 2011; Ghalambor et al., 2007; Richards, Bossdorf, Muth, Gurevitch, & Pigliucci, 2006). Yet, invasive populations also pose an interesting dilemma in that they often display evidence of rapid adaptive evolution despite lacking genetic variation; thus, invasive populations constitute good models to tackle the questions related to the mechanisms that contribute to adaptive plasticity and evolution (Simberloff & Rejmanek, 2011). A biological invasion can be defined as the success of a species to establish, develop and maintain populations outside its geographical area of origin (Theoharides & Dukes, 2007; Figure 1). The development of international trade and intercontinental transportation has accelerated the movement of non-native species to new habitats (Early et al., 2016). These non-native species can cause damage to ecological systems (e.g., Lodge, 1993), human health and economy (e.g., Pimentel et al., 2001). Biological invasions also represent "natural experiments" for evolutionary biologists, allowing investigation of evolutionary processes in real-time (Huey, Gilchrist, & Hendry, 2005).

The process of introduction into a new location and of spatial expansion from this point of introduction often imposes a transitory reduction in population size (e.g., Dlugosch & Parker, 2008; Peischl & Excoffier, 2015; Figure 1). Such population bottlenecks are predicted to increase inbreeding depression, increase genetic drift and decrease genetic diversity. A variety of compensatory mechanisms may act to counter the loss of genetic variation and facilitate adaptive evolution for population expansions, such as hybridization, multiple introduction events and propagule pressure, such that genetic diversity in the invasive and native area could be comparable or even greater in invaded regions (Baltazar-Soares, Paiva, Chen, Zhan, & Briski, 2017; Bock et al., 2015; Estoup et al., 2016; Facon et al., 2011; Prentis, Wilson, Dormontt, Richardson, & Lowe, 2008; Wellband, Pettitt-Wade, Fisk, & Heath, 2017). However, studies of genetic diversity and adaptive change in invasive populations rarely take into account the potential role played by transposable elements (TEs) and epigenetic changes (Oliver & Greene, 2009; Oliver, McComb, & Greene, 2013; Rey, Danchin, Mirouze, Loot, & Blanchet, 2016; Schrader et al., 2014; Stapley, Santure, & Dennis, 2015). These mechanisms can act together to rapidly increase genetic and phenotypic diversity in a population. TEs are repeated sequences present in virtually all genomes. They behave "parasitically" within the genome in that they are able to replicate and insert themselves across chromosomes. The amount of the genome made of TEs can vary from a few per cent, such as in yeast (3%; Kim, Vanguri, Boeke, Gabriel, & Voytas, 1998) up to 80% in maize (Schnable et al., 2009; Vitte, Fustier, Alix, & Tenaillon, 2014). Despite variation in their activity and impact across species, TEs have been proposed to be a relevant source of genetic variation. For example, in plants, TEs are considered as a source of genetic and epigenetic variability and thus drivers of evolution (Belyayev, 2014; Lisch, 2013; Vitte et al., 2014). Similarly, diversification and rapid evolution in angiosperms have been attributed to TE sequences (Oliver et al., 2013). In animal model systems like *Drosophila*, TEs have been described as potentially playing a role in speciation since they can be responsible for hybrid incompatibility (Kidwell & Lisch, 2001). More recently, TEs have been hypothesized to facilitate adaptation in invasive species to new environments (Schrader et al., 2014; Stapley et al., 2015). A fundamental challenge in testing this hypothesis in natural populations is linking the genetic variation generated by TEs to changes in fitness. However, the relationship between TEs and fitness is mediated by a variety of mechanisms that have evolved to find and silence deleterious TE-induced changes in gene expression. These silencing systems are largely epigenetic mechanisms, which play a role in influencing gene expression and have been proposed to independently and jointly with TEs facilitate adaptive plasticity and evolution (Lanciano & Mirouze, 2018; Rey et al., 2016; Stapley et al., 2015).

Here, we use a broad definition of epigenetics as any non-genetic molecular modification of the genome that alters gene expression. Epigenetic modifications are non-genetic changes in the sense that there is no DNA sequence change that is passed on in the germline, although there is evidence that some epigenetic modifications can sometimes be transmitted across generations (Meyer, 2018). Epigenetic mechanisms encompass three levels of well-characterized modifications: (a) DNA methylation, (b) histone modifications and (c) non-coding RNA (Allis & Jenuwein, 2016; Duncan, Gluckman, & Dearden, 2014), which can rapidly change gene expression and affect mobility of TEs. Epigenetic changes or epimutations refer to the stable modification of epigenetic marks (such as DNA methylation) on a locus that does not affect the DNA sequence. Epimutation on a locus generates an epiallele corresponding to one of two or more alleles of a given gene differing in their epigenetic properties and encoding different phenotypic characteristics. The epigenetic mechanism silencing TE insertions can be very effective, although the exact mechanism varies across species. For example, in *Drosophila*, silencing of TEs is mainly operated by histone modifications and small RNAs (Aravin, Hannon, & Brennecke, 2007), while in mammals and plants, DNA methylation is the major mechanism.

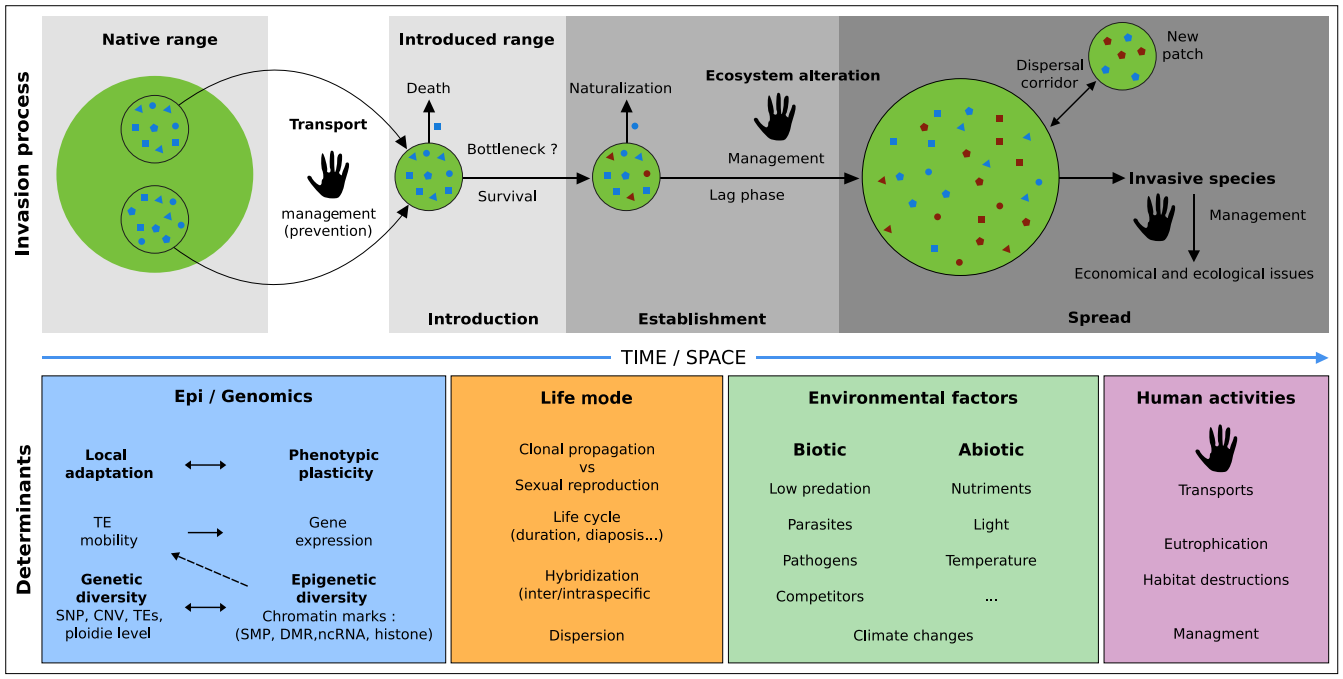

FIGURE 1 Process of invasion and its epi/genomics, life mode, environmental and human activity determinants. A species in its native area (*white frame*) is transported in a new environment (*grey frame*), often by human activities, voluntary or not. Genetic diversity (*blue shape*) is recognized as lower in introduced area. Installation of this new population can be facilitated by repeated introductions (propagule pressure). A bottleneck can take place and lead to the disappearance of the introduced species. Organisms who survive can have a different genetic (*shape*) and epigenetic (*colour*) diversity than from native area. Population becomes established and continues in time; some species can be naturalized, while others are going to continue their expansion until they become invasive. There is an uncontrolled expansion with a selection of the most performing genetic and epigenetic variants in a given environment. These invasive species can then colonize new environments and cause economic and ecological issues. During this process, a multitude of factors comes into play: Epigenetic and genetic mechanisms can modify gene expression and thus the capacity for local adaptation and phenotypic plasticity. Characteristics of organisms are also important factors to consider for the success of a biological invasion: life cycle, type of multiplication, especially clonal, possibility of hybridization (*combined shape*) and the capacities of dispersal can favour the installation of the population in a new environment. Environmental factors, biotic and abiotic, represent the sensibility of the ecosystems. Absence of predators and competitors in the introduction area, as noted in enemy release hypothesis (ERH) and its corollary evolution of increased competitive ability (EICA), as well as the presence of empty ecological niche can partially explain the success of an invasion. Besides, global climate change has been identified as a source of opportunity for new invasions. Finally, human activities are a key factor of either success or failure of an invasion. The human being acts at the origin of the process by the action of transport, voluntary or not. On the other hand, he can, with legislation and prevention, decrease or control this mechanism. He can either promote invasions by altering the ecosystems or limit their impact by controlled actions and management

Both epigenetic mechanisms and TE activity can be sensitive to the environment (Fablet & Vieira, 2011; Lanciano & Mirouze, 2018). For example, histone modifications and DNA methylation have been shown to be modified by abiotic and biotic changes to the environment (Alonso, Ramos-Cruz, & Becker, 2018; Blake & Watson, 2016; Nätt & Thorsell, 2016), and the alteration of these epigenetic mechanisms can in turn alter TE-induced changes that would otherwise not occur. Thus, the linkage between environmental change and epigenetic–TE behaviour leads to the expectation that TEs and epigenetics can contribute to an increase in the genetic and phenotypic diversity following the colonization of a new environment. The environmental induction of these mechanisms can be placed into a larger conceptual framework that considers how phenotypic plasticity can facilitate evolutionary change.

Phenotypic plasticity provides a unifying conceptual framework for incorporating the molecular mechanisms that contribute to invasion success within and across generations. Plastic responses to the environment may be reversible or non-reversible depending on the trait (Piersma & Drent, 2003; Pigliucci, 2005; West-Eberhard, 2005), and they can be adaptive, non-adaptive or neutral with respect to fitness (Ghalambor et al., 2007). We can thus view the predictable environmental induction of TEs and epigenetic changes from the same perspective used to study other environmentally induced plastic traits (Horváth, Merenciano, & González, 2017). Indeed, the epigenetic changes associated with different environmental cues provide a mechanistic understanding for the observed patterns of phenotypic plasticity, and have been hypothesized to facilitate invasions by allowing organisms to express advantageous phenotypes across a broader range of environments (Baker & Stebbins, 1965; Bradshaw, 1965; Richards, 2006; Sultan, 2001). The hypothesis that greater plasticity contributes to the success of an invasion has been supported by some

studies (e.g., Daehler, 2003; Davidson et al., 2011; Nyamukondiwa, Kleynhans, & Terblanche, 2010; Sexton, Mckay, & Sala, 2002; Trussell & Smith, 2000), but not by others (e.g., Chown, Slabber, McGeoch, Janion, & Leinaas, 2007; Godoy, Valladares, & Castro-Díez, 2011; Matzek, 2012; Palacio-López & Gianoli, 2011). Thus, while we have a compelling argument for how phenotypic plasticity and genetic variation can contribute to adaptive evolution and facilitate the invasion processes, we know far less about the contribution of TEs and epigenetic variation (Stapley et al., 2015) and specific tests of these mechanisms in natural populations remain largely unexplored (Schlichting & Wund, 2014).

In this review, we first cover the mechanistic underpinnings of how mobile TEs and epigenetic systems alter genetic variation and gene expression. We use the conceptual framework of how phenotypic plasticity or how environmentally induced changes can facilitate invasions through these mechanisms. We first review the role of TEs as drivers of genetic diversity and the epigenetic system that regulates these genomic rearrangements. We discuss the evidence that TEs generate genetic variation, and environmental conditions can release this variation and make it available to selection. We then review the epigenetic mechanisms that control gene expression, the evidence for transmission across generations, the patterns of environmental induction, and how all these processes may contribute to invasion success. We conclude that by focusing on the epigenome (i.e., the regulatory system that integrates how environmental and genomic variations jointly shape phenotypic variation) we will gain key insights into the mechanisms that contribute to invasion success.

2 | TRANSPOSABLE ELEMENTS IN INVASIVE POPULATIONS

2.1 | TEs and their regulation in the genome

Altered environmental conditions are thought to act as a source of selection that shifts genotype and phenotype frequencies towards new optima. The classical perspective is that selection acts either on standing genetic variation or on spontaneous mutations underlying the phenotypes under selection, leading to adaptive evolutionary change (Orr, 2005). However, there is an increasing appreciation that other types of genetic modifications could contribute to the genetic variation selection acts on. One such mechanism is the genetic variation generated by TEs. First discovered by McClintock (1950) in maize, TEs are defined as mobile repeated DNA sequences that can move in the genome by generating new copies of themselves and induce new mutations (Casacuberta & González, 2013; Chuong, Elde, & Feschotte, 2017; Slotkin & Martienssen, 2007). Because TEs can induce mutations in genes, alter gene regulation and disrupt recombination, they can have deleterious consequences (Slotkin & Martienssen, 2007); however, they can also generate new variation on which selection can act (Kidwell & Lisch, 2001; Stapley et al., 2015). TEs are formally classified into two families according to the transposition process. Type I, or retrotransposons, use an RNA intermediate to produce new copies of themselves. Type II DNA transposons code a transposase enzyme and transpose by a cut-and-paste mechanism (Figure 2). The majority of TE insertions in a genome are neutral or slightly deleterious, such that only small effects are expected on individual fitness. However, when they insert in locations that disrupt gene function or change gene regulation TE can have negative or (rarely) positive fitness consequences (Lanciano & Mirouze, 2018; Slotkin & Martienssen, 2007). Negative impacts of TE insertion should be under natural selection and potentially purged but could also be a source of disease as reported in human health population studies (Wang & Jordan, 2018). Positive fitness effects of TE insertions are now also reported. For example, a role for TEs in the adaptive evolution was proposed in stress response of *Drosophila melanogaster* (González, Karasov, Messer, & Petrov, 2010) and *Arabidopsis thaliana* (McCue, Nuthikattu, Reeder, & Slotkin, 2012), as well as in plant defence against fungi (Hayashi & Yoshida, 2009) or osmotic tolerance (Ito et al., 2016). Yet, to fully understand how TEs spread and are regulated throughout the genome it is important to understand the various mechanisms that have evolved to manage the consequences of these insertions.

Genomes and TEs are locked in an ongoing evolutionary arms race, where TEs act like selfish "parasites" attempting to increase their numbers, and genomes respond with a variety of mechanisms to reduce the mobility of TEs and regulate the impacts of these insertions on gene expression (Aravin et al., 2007; Slotkin & Martienssen, 2007). The regulation of TEs can occur both by transcriptional gene silencing (TGS) and by post-transcriptional gene silencing (PTGS; Castel & Martienssen, 2013; Pumplin & Voinnet, 2013; Slotkin & Martienssen, 2007), and different types of epigenetic regulation have been described in this process. These mechanisms can either use small RNAs, histone modification or DNA methylation. Small non-coding RNA from the piwi RNA (piRNA) family is implicated in TE regulation either by TGS or by PTGS. The piRNA can directly cleave transcripts of TE, avoiding the protein production and transposition, but they can also act on TE insertion and drive histone modifications which will silence the TE copies (Pedersen & Zisoulis, 2016; Sienski, Dönertas, & Brennecke, 2012; Slotkin & Martienssen, 2007; Song & Cao, 2017; Zhang, Tao, et al., 2016). These piRNA pathways are well conserved among eukaryotes (Fablet, Akkouche, Braman, & Vieira, 2014; Fablet, Salces-Ortiz, Menezes, Roy, & Vieira, 2017) and are considered a reliable immunity system contributing to the stability of the genomes (Sienski et al., 2012; Slotkin & Martienssen, 2007; Zhang, Tao, et al., 2016). At the histone level, methylation of the histone 3 lysine 9 (H3K9me3) is known to limit TE expression in *Drosophila* and mammals (Lee, 2015; Matsui et al., 2010). Some data also indicate that the histone modifications that target a specific TE insertion can spread on the region and affect the expression of nearby genes (Elgin & Reuter, 2013; Lanciano & Mirouze, 2018; Lee, 2015; Rebollo et al., 2011; Slotkin & Martienssen, 2007). DNA methylation also affects TE expression. For example, mouse embryo mutants for the DNA methyl transferase 1 (*Dnmt-1*) have higher levels of intracisternal A-particle (IAP) retrotransposon expression (Walsh, Chaillet, & Bestor,

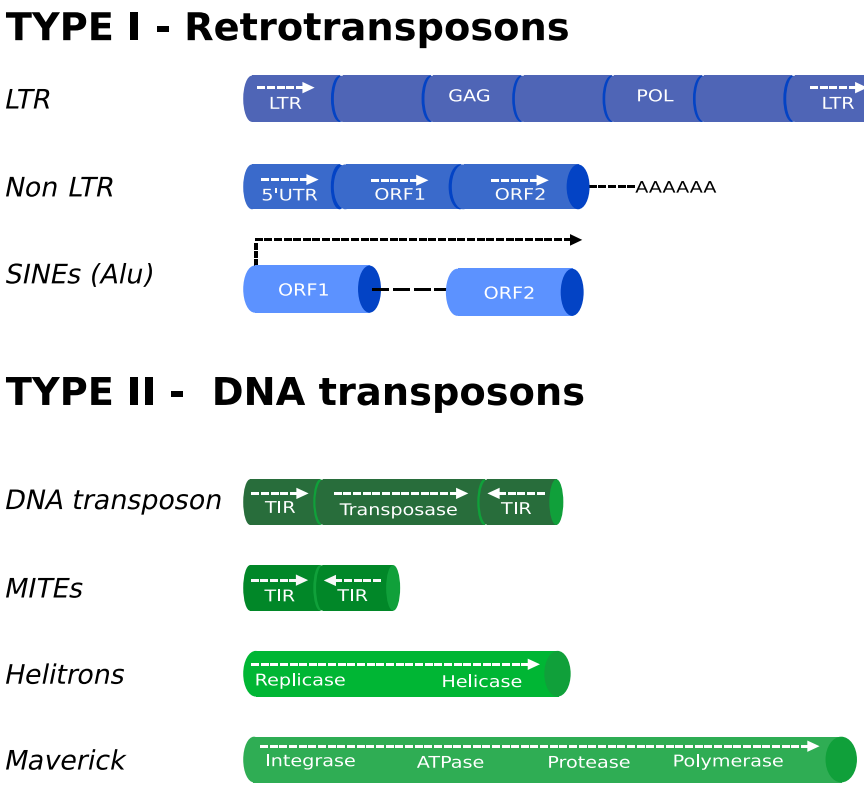

FIGURE 2 Transposable element structures. Transposable elements can be classified into types I and II according to RNA intermediate production and insertion mechanisms. Type I, called retrotransposon, use RNA intermediate via a reverse transcription step, and can be divided into two groups. Long terminal repeats (LTR) elements with direct repeats both at beginning and at end of the element. It presents POL and GAG sequences related to retroviral protein genes. Non-LTR elements also use an RNA intermediate and are characterized by a polyA tail. Short interspersed nuclear elements (SINEs) are non-autonomous transposable element (TE) that will use LINE enzymatic machinery to transpose. Type II elements, named DNA transposons, present terminal inverted repeats (TIR) at each extremity, which are recognized by transposon's transposase and allow the integration in other genome. Miniature inverted repeat transposable elements (MITEs) have no open reading frames (ORFs) and are non-autonomous TEs. Then, helitron transposons use DNA helicase mechanisms to be transposed and do not present TIR pattern. Hence, the recently discovered Maverick subgroup seems to use a self-encoded DNA polymerase and have TIRs

1998). In rice, mutation of DNMT *OsMet1b* gene reduced CG methylation and induced necrotic death in the seedlings (Yamauchi, Johzuka-Hisatomi, Terada, Nakamura, & Iida, 2014). DNA methylation can be induced by environmental conditions (see below) as shown with classic examples of TE-generated epialleles. For example, in mice, the *agouti* gene inducing coat colour modification is due to IAP retrotransposon presence, while in morning glory flowers (*Convolvulaceae*), a methylated MuLE transposon induces petal colour streaks (Slotkin & Martienssen, 2007). Thus, despite the potentially negative consequences of TEs, there are a suite of mechanisms that allow organisms to not only mitigate these impacts, but also coopt them in the process of adaptation. Yet, these mechanisms are also potentially sensitive to the environmental context organisms occur in, leading to the expectation that novel or stressful environments may induce changes that fuel evolution.

2.2 | TEs and environmental stress

Environments that fall outside the historic or normal range experienced by a population may impose stress on organisms by challenging their physiological and cellular systems and reducing fitness. How stressful environments impact the mechanisms by which organisms cope with them is thus of great interest to those interested in invasion biology because invasive populations often encounter novel environmental conditions (e.g., Stapley et al., 2015). Barbara McClintock was the first to hypothesize that the variation generated by TEs can over evolutionary time help populations survive of under stressful conditions, but limited empirical data were available to test these ideas at that time (Casacuberta & González, 2013). Specifically, novel or stressful environments have been implicated in increasing TE activity (Capy, Gasperi, Biémont, & Bazin, 2000; Lanciano & Mirouze, 2018) and disrupting the epigenetic regulation of TEs, such that previously silenced TEs become reactivated (Guerreiro, 2012; Horváth et al., 2017; Stapley et al., 2015; Vieira, Aubry, Lepetit, & Biémont, 1998). The expectation is that such loss of control over TEs will promote a rapid increase in genetic and phenotypic variation available for selection to act on, and that in some of these cases TEs will evolve to become part of the adaptive stress response (Guerreiro, 2012; Horváth et al., 2017; Stapley et al., 2015; Vieira et al., 1998). However, in a recent review of published studies,

Horváth et al. (2017) argue the links between stress and TE response are far from being a generality.

For TEs to become involved in the stress response, there needs to be a functional link between them. In rice, Naito et al. (2009) found that *mping* TE insertions had no effect on the transcriptome under control conditions, but clearly affected the expression of nearby genes under stressful cold and saline conditions. There was no indication any of these changes were adaptive or whether the TE-driven changes in gene expression were due to stress-induced disruption of the epigenetic regulatory mechanisms or some other mechanism. Using a comparative approach, González et al. (2010) identified several TE insertions that could potentially be implicated in the adaptation of *D. melanogaster* to the most southern and northern populations exhibiting clinal variation in Australia and North America. They examined both putatively neutral and adaptive insertions that had increased in these derived populations and found strong evidence for selection on a subset of the putatively adaptive insertions. When they examined the neighbouring genes to these insertions, they found they were genes previously known to be involved in adaptation to a variety of environmental factors (González et al., 2010). Other studies have also found direct links between the presence of the insertion and adaptive phenotypes related to insecticide resistance (Magwire, Bayer, Webster, Cao, & Jiggins, 2011; Merenciano, Ullastres, Cara, Barrón, & González, 2016), cold stress response, oxidative stress (Guio, Barrón, & González, 2014; Guio, Vieira, & González, 2018), xenobiotic stress (Mateo, Ullastres, & González, 2014) and resistance to sigma virus (Magwire et al., 2011). Thus, there is growing evidence that TEs can be under selection to alter patterns of expression on neighbouring genes, leading to adaptive changes in phenotypes.

2.3 | TEs in the context of phenotypic plasticity and invasion

The potential for TEs to resolve the invasion paradox of rapid evolution in response to new environments despite small population sizes and reduced genetic variation, is, in part, dependent on how TEs and their epigenetic control systems respond on ecological time-scales (Stapley, Feulner, Johnston, Santure, & Smadja, 2017). In other words, there must be a predictable increase in genetic and phenotypic variation when populations colonize new environmental conditions, and this should be caused by either the increased production of TEs or the reduced control of TEs. If the expression of TEs predictably changes in response to environmental cues, then we can study the context-dependent nature of TE activity as we would any other phenotypically plastic trait (Rey et al., 2016; Stapley et al., 2017). Thus, we can view how a change in environmental cues (e.g., a stressor) alters TE activity as a form of phenotypic plasticity, but we can also view how TEs change gene expression, providing a mechanistic explanation for patterns of phenotypic plasticity observed in fitness-related traits. In either case, the expectation is that populations likely harbour hidden genetic variation caused by TEs that is rarely revealed in their native range, because selection shapes the epigenetic control mechanisms that tightly regulate TE activity (Slotkin & Martienssen, 2007). However, in novel environments where selection has not had an opportunity to act on the epigenetic control of TEs, we expect increased misregulation of the epigenetic control system, which can lead to a "burst" of TE-related variation (Stapley et al., 2015). This burst of genetic and related phenotypic variation should fuel evolutionary responses to selection. Yet, to date no study has documented that these various mechanisms have directly contributed to the success of an invasive population, but various indirect lines of evidence suggest it is highly plausible.

The role of TEs in the success of invasive populations was reviewed by Stapley et al. (2015) where they outlined the potential ways in which TEs contribute to population expansion and adaptation, and then review the largely indirect evidence supporting this view. Here, we briefly highlight two studies where TEs have been implicated in facilitating adaptation in invasive populations. Goubert et al. (2017) studied populations of the tiger mosquito *Aedes albopictus* in their native range of Vietnam and in their invasive range throughout Europe. They used TEs as neutral markers to identify genomic regions under selection (Goubert et al., 2017). They found the majority of outlier loci had a higher frequency of insertions in the European populations, suggesting that TEs could be linked to genes that have a role in adaptation to temperate environments. Dennenmoser et al. (2017) studied copy number and TE richness in the invasive hybrid sculpin fish, *Cottus* spp., and found an increase in TE copy numbers in invasive *Cottus* populations potentially caused by hybridization. In combination with the studies described above, these studies suggest we can no longer ignore the role of TEs in the evolutionary process, although we still lack a basic understanding of what role TE-related variation plays in the early stages of population divergence when invasive populations are colonizing new environments. Future studies will need to take advantage of recently established populations or experimentally generate new populations to explicitly test if TEs and their epigenetic control play a role in biological invasions.

3 | EPIGENETIC MECHANISMS, A COMPONENT OF THE RAPID RESPONSE TO ENVIRONMENTAL CHANGES IN THE INVASION PROCESS

3.1 | Epigenetic mechanisms play a role in response to environmental change

So far we have considered epigenetic mechanisms in the context of how they control TE activity, but far more research has investigated the independent effects of epigenetic changes for their role in altering patterns of gene expression in response to both biotic and abiotic environmental variations (Amarasinghe, Clayton, & Mallon, 2014; Crisp, Ganguly, Eichten, Borevitz, & Pogson, 2016; Gómez-Díaz, Jordà, Peinado, & Rivero, 2012; Song et al., 2015; Spannhoff et al., 2011). In a diversity of organisms, ranging from invertebrate to vertebrate animals and from annual to perennial plants, environmentally induced epigenetic changes can result in adaptive responses to new and stressful environments (Becker

et al., 2011; Bräutigam et al., 2013; Conde et al., 2017; Gibert, Peronnet, & Schlötterer, 2007; Kawakatsu et al., 2016; Lafon-Placette et al., 2018; Meyer, 2015; Schmitz et al., 2011; Seymour & Becker, 2017). Some of the best examples of adaptive epigenetic effects are observed in plants. For example, following an abiotic or biotic stress plants will exhibit epigenetic changes that prime the defences against future repeated stress, resulting in a "epigenetic memory" that allows an individual to respond more quickly and effectively when the stress appears again (Lämke & Bäurle, 2017). The duration of the primed state within an individual is a key factor to survival and adaptation, and several studies have shown that the epigenetic modifications involved can be transgenerational (Mauch-Mani, Baccelli, Luna, & Flors, 2017).

The transgenerational nature of environmentally induced epigenetic variants (i.e., epimutations or epialleles) provides the critical link between plastic changes in one generation influencing the next generation. However, unlike sequence-based changes that are relatively stable and predictably transmitted regardless of environmental conditions, epigenetic variants occur at higher frequency and exhibit rapid flexibility and reversibility (Heard & Martienssen, 2014; Law & Jacobsen, 2010). For example, with respect to frequency, the rate of epimutations has been estimated at 3×10^{-4} or five orders of magnitude higher than the DNA substitution rate in *A. thaliana* (10^{-9}; see van der Graaf et al., 2015). At the same time, we also know that patterns of methylation and demethylation can rapidly shift in response to different environmental cues within and between generations, making epigenetic patterns also very flexible (Heard & Martienssen, 2014; Law & Jacobsen, 2010; Meyer, 2015). How well this epigenetic information is stored and transmitted from one generation to the next, and how effective this information is in preparing subsequent generations for responding to environmental challenges ultimately determine the importance of epigenetic variation for adaptive plasticity and evolution in response to new environments. The Arabidopsis 1001 Epigenomes project provides evidence that DNA methylation is correlated with geography and climate of origin and could be involve in local adaptation (Kawakatsu et al., 2016). Schmid et al. (2018) reported in *Arabidopsis* that epigenetic variation is subject to selection and can play a role in fast adaptive responses. However, the relative extent to which genetic and epigenetic variations contribute to plant adaptation remains to be elucidated and likely depends on the reproductive mode of the investigated species. If environments are predictable across generations, heritable epigenetic changes could provide an adaptive anticipatory response. However, if environments change between generations, a mismatch can arise between the transmitted epigenetic information and the environment of the descendants. Thus, a critical link that remains to be established is the degree to which heritable epigenetic variation underlies adaptive phenotypes.

The fundamental challenge facing researchers is to disentangle genetic and epigenetic effects on specific phenotypic traits. Establishing the genetic basis of any complex traits is already challenging given that many loci of variable effect all contribute to explain some of the phenotypic variation observed. Then, epigenetic control is an additive layer of complexity in direct interaction with genetic variation (e.g., control of TE insertion and spontaneous deamination of 5-methylcytosine into thymine) leading to a complex situation. One powerful approach that has been used to separate genetic from epigenetic effects is to create epigenetic recombinant inbred lines (epiRILs; Johannes et al., 2009; Reinders & Paszkowski, 2009) where epigenetic variants are placed on a homogeneous genetic background. This approach allows for the quantification of phenotypic variation between different lines and assumes they must be due to the epigenetic variants given the rest of the genomic background is held constant. Zhang, Fischer, Colot, and Bossdorf, (2013) used this approach in *A. thaliana* and demonstrated that epigenetic marks were responsible for phenotypic variance and for plasticity. Similar approaches reveal that for a diversity of traits epiRILs not only explain phenotypic variation, but also that transgenerational inheritance and patterns of plasticity may be purely caused by epigenetic effects (Bossdorf, Arcuri, Richards, & Pigliucci, 2010; Kooke et al., 2015; Zhang et al., 2013). For example, Cortijo et al. (2014) demonstrated that "Differentially Methylated Regions" of epiRIL lines act as epigenetic quantitative trait loci for several traits and could be stably inherited and that DNA methylation could contribute to the phenotypic plasticity of a trait, especially in stressful conditions. The same result was found in the fungus (*Neurospora crassa*) using mutants for the different epigenetic pathways (DNA methylation, histone methylation and RNA interference (Kronholm, Johannesson, & Ketola, 2016)). Another similar strategy to isolate the epigenetic basis of traits is to use clones as proposed by Conde et al. (2017), Lafon-Placette et al. (2018) and Le Gac et al. (2018) who have analysed poplar (*Populus* spp.) clones subjected to distinct environmental conditions (cold, water availability). These studies show that developmental plasticity and memory are associated with differentially expressed genes overlapped by DMRs. These genes were related to abiotic stress response and phytohormone pathways involved in complex traits such as developmental transitions during the annual cycle or drought tolerance. While such methods are not feasible in most plant and animal systems, they demonstrate that environmentally induced epigenetic modification does explain phenotypic variation and examining patterns of epigenetic changes in invasive populations is a worthwhile endeavour.

3.2 | Epigenetic landscape in invasive species

In the case of invasive species, the critical question is: What is the evidence that epigenetic mechanisms play a role in the successful colonization of new environments? To date, relatively few studies have differentiated between the role of genetic versus epigenetic variation on successful invasions (Prentis et al., 2008; Vogt, 2017). We suspect that in time evidence will accumulate that epigenetic mechanisms act as a complementary mechanism in conjunction with standing genetic variation to shape the phenotypic variation exposed to selection. Current attempts to quantify the epigenetic

contribution to population divergence are to measure genome-wide patterns of DNA cytosine methylation polymorphism using methylation-sensitive amplified fragment length polymorphism. This technique uses a number of restriction enzymes that are varyingly sensitive to cytosine DNA methylation, and cut DNA depending on the methylation status of the cytosine. Using such an approach, it is possible to compare the patterns obtained after digestion between individuals or populations to estimate genetic and epigenetic diversity (Richards, Schrey, & Pigliucci, 2012). In many studies, the experimental design consists of comparisons of epigenetic patterns between populations of the same species from several geographical areas for which the colonization history is known. While such an approach does not explicitly test the relationship between specific epigenetic changes and trait variation, it provides insight into how different environmental conditions induce genome-wide patterns of methylation. Below we briefly discuss some of the relevant plant and animal studies that have used this design and their major findings.

3.2.1 | Invasive plant examples

Given their sedentary nature, plants rely extensively on phenotypic plasticity and epigenetic mechanisms to cope with changing environments (Seymour & Becker, 2017), and there is growing evidence the patterns of methylation vary across populations occupying different environments (Foust et al., 2016; Guarino, Cicatelli, Brundu, Heinze, & Castiglione, 2015; Herrera & Bazaga, 2016; Preite et al., 2015). Thus, it is reasonable to expect that epigenetic variation may underlie invasive plant populations colonizing new environments (Richards et al., 2012). We highlight some of these examples here.

Gao, Geng, Li, Chen, and Yang (2010) investigated how epigenetic profiles change within and among natural populations of alligator weed (*Alternanthera philoxeroides*), an invasive plant in China that can grow in aquatic and terrestrial environments. This species exhibits significant changes in morphology depending on the environment it inhabits, and Gao et al. (2010) induced these differences by simulating aquatic and terrestrial environments in a common garden experiment using clones derived from natural populations occupying both environments. They found more than half of the epigenetic markers were shared under the same common garden environment independent of the population's origin; this similarity indicates that environmentally induced epigenetic reprogramming is a predictable response to environmental cues (Gao et al., 2010). Because the number of polymorphic loci was close to zero among these populations, but the epigenetic diversity was much higher (e.g., approximately a quarter of markers being polymorphic), the conclusion is that the epigenetic changes directly contribute to phenotypic changes underlying invasion success (Gao et al., 2010).

Richards et al. (2012) examined the correlation between epigenetic variation and phenotypic response for two invasive *Fallopia* species (Japanese Knotweed) in the United States. They compared individuals coming from 16 different sites in the United States and found that epigenetic differentiation was higher between populations than between both species. Moreover, they observed greater epigenetic differentiation than genetic differentiation across haplotypes. Thus, because the genetic diversity in the introduced range is less than the epigenetic diversity, it likely originated from the environmental conditions the plants experienced (Richards et al., 2012). More recently, Zhang, Parepa, Fischer, and Bossdorf (2016) have shown that epigenetic variants in Japanese knotweed are correlated with patterns of phenotypic variability of different clones, providing a link between the environment, epigenetic variation and plant phenotypes.

The specific link between methylation, gene expression and phenotypic response to the environments has recently been demonstrated by Xie et al. (2015) in A*geratina adenophora* (crofton weed). This plant originates from Mexico and was introduced to tropical regions of China before invading the north of China with differentiated cold-tolerant populations. Xie et al. (2015) studied methylation state in the C-repeat/dehydration-responsive element binding factor (CBF) pathways, which are responsible for the plant cold response via activation of cold-responsive genes. They sampled several distinct geographical populations and tested their cold tolerance. All populations were screened for expression of seven inducers of CBF pathways (RT-qPCR) and methylation state (bisulphite-PCR). In parallel, they analysed CBF inducer sequences to confirm no genetic difference in these genes or no impact at expression level. They observed a negative correlation between the methylation level of one CBF inducer, ICE1, and cold tolerance among populations. Most cold-sensitive populations were more methylated than cold-tolerant plants. The methylated state seems to be stable due to the same level in the four populations after several cold time treatments (Xie et al., 2015). These results provide some of the strongest evidence demonstrating a strong link between methylation state and rapid response to an environmental stress.

Yet, the relationship between epigenetic variation, population divergence and transgenerational inheritance across environments is not universal. Recently, Liu et al. (2018) examined genetic and epigenetic variation of native and invasive lineages of the common reed (*Phragmites australis*) in North America. They grew populations from different environments under controlled common garden conditions to test the stability of epigenetic inheritance and to partition genetic from epigenetic variation. While they found epigenetic variation to be greater than genetic variation across populations, they did not find convergence in epigenetic markings between populations occupying similar environments, suggesting the relationship between environmentally heritable induced epigenetic variations may not be stable and also dependent on the genetic background (Liu et al., 2018). In comparison with two saltmarsh species, Foust et al. (2016) found while one species showed a significant correlation between epigenetic variation and habitat, the other species did not. Both Foust et al. (2016) and Liu et al. (2018) found a correlation between the amount of genetic and epigenetic variation within a population, suggesting that separating the contribution from these sources of variation will present challenges for interpretation of these patterns.

3.2.2 | Invasive animal examples

Unlike plants that are more sedentary, many animals are mobile and experience a diversity of environments within and between generations. Epigenetic mechanisms may therefore underlie plastic responses of animals expanding their geographical ranges into new environments. Schrey et al. (2012) analysed genetic and epigenetic variation in the invasive house sparrow (*Passer domesticus*). This bird has spread all over the world and exhibits phenotypic variability in different geographical areas, despite a recent invasive period (<150 years). Schrey et al. (2012) studied epigenetic variability of populations in Nairobi (Kenya), which was colonized 50 years ago, and Tampa (Florida, USA), which was colonized over 150 years ago (Schrey et al., 2012). Comparisons of these populations reveal that the amount of within-population epigenetic variation is greater than the between-population variation and that it was associated with a poor genetic diversity. Schrey et al. (2012) suggest that epigenetic variation may have compensated for the low genetic variability and contributed to phenotypic differentiation. In another study of the same species, Liebl, Schrey, Richards, and Martin (2013) sampled seven geographically separated Kenyan populations and found a negative correlation between genetic and epigenetic diversity; higher epigenetic diversity was correlated with a decrease in heterozygosity and an increase in inbreeding (Liebl et al., 2013). The authors suggested that epigenetic variation could be a compensatory mechanism for low genetic diversity in an invading population, allowing an increase in phenotypic variability by expression of cryptic genotypes or by phenotypic plasticity. However, in a more recent comparison of Australian house sparrows, Sheldon, Schrey, Andrew, Ragsdale, and Griffith (2018) failed to detect such compensatory effects. Despite considerable phenotypic differences, Liebl et al. (2013) also failed to find a relationship between epigenetic variation and populations occupying different environments.

Ardura, Zaiko, Morán, Planes, and Garcia-Vazquez (2017) studied epigenetic variation in populations of the invasive pygmy mussel (*Xenostrobus securis*). Specifically, they compared a newly established population to existing older populations to test the hypothesis that epigenetic patterns change over time as the population undergoes sequential invasive steps. They observed a significant hypomethylation in recent invasive populations compared to older ones and suggested that hypomethylation could increase gene expression or genetic recombination, thus impacting the phenotype. The same hypomethylation was detected in one other species, *Ficopomatus enigmaticus* (Ardura et al., 2017). However, the experimental design of these studies presents potential biases due to confounding effects of different environments and the invasive status of the populations.

Huang et al. (2017) used the MSAP technique to reveal changes in DNA methylation frequency, intragroup methylation divergence and methylation differentiation after rapid environmental stress in the marine species *Ciona savignyi*. They induced variation of DNA methylation frequency and DNA methylation divergence after 1 hr of treatment, but these differences disappeared after 48 hr. These results demonstrate that DNA methylation can also be short-lived in response to environmental changes and may not persist.

Lastly, Oppold et al. (2015) and Kreß, Oppold, Kuch, Oehlmann, and Müller (2017) studied epigenetic modifications in the Asian tiger mosquito (*A. albopictus*), which has spread from South-East Asia to every continent except Antarctica and Australia. Invasive populations of this vector for many diseases have low genetic diversity (Kreß et al., 2017; Oppold et al., 2015), yet exhibit rapid adaptive responses to different environments, such as chemical compounds or resistance to cold, and showed alteration of DNA methylation levels. These results should be taken with caution since the levels of DNA methylation in insects are in general very low and some recent papers suggest an absence of significant DNA methylation in Diptera (Bewick, Vogel, Moore, & Schmitz, 2017; Provataris, Meusemann, Niehuis, Grath, & Misof, 2018).

Collectively, these publications highlight the potential role of epigenetics in the invasion process by correlating epigenetic patterns of variation with natural environmental variation, but definitive evidence that such correlations underlie invasion success remains elusive. We see several fundamental challenges that need to be overcome before we can move beyond correlation and establish causal links between environmentally induced epigenetic variation and the phenotypic changes that allow populations to expand in new environments. First, when examining genome-wide patterns of genetic and epigenetic diversity it is extremely difficult to partition out how much each of these sources of variation contributes to observed phenotypes without using experimental designs that control for genetic background. Such experiments are simply not possible for many organisms. Second, while comparing methylation patterns of invasive populations across different environments may reveal higher epigenetic diversity relative to background genetic variation, rarely is there a clear link with the phenotypic response. This challenge is not unique to epigenetic studies, as establishing the genetic basis of any complex trait is exceedingly difficult in non-model organisms. Lastly, many of the current methods such as the MSAP approach sample only a small subset of the genome, which underestimates the epi/genetic diversity and makes it even more difficult to find clear relationships with phenotypes. New genome-wide approaches are emerging to better evaluate genetic and epigenetic diversity (van Gurp et al., 2016; Pu & Zhan, 2017). Thus, while there are compelling reasons to suspect epigenetic changes to be important in the process of adapting to new environments, we still lack the necessary evidence to draw any general conclusions.

4 | CONCLUSIONS AND FUTURE DIRECTIONS

In this review, we highlight significant literature that shows potential links between TE, epigenetics and phenotypic plasticity and their role in rapid adaptation of invasive species to new environments. No broad studies have deal with these three notions concomitantly. It is clear that limited empirical data are available to test this idea, particularly because it is difficult to disentangle genetic and non-genetic variability.

Species introductions can be considered evolutionary experiments and constitute good models for investigating adaptive processes operating at short time-scales (Gibert et al., 2016). The two most commonly used approaches to investigate how invasive populations respond to new environments are as follows: (a) the synchronic approach, where comparisons of native and invasive populations are made, and (b) the diachronic approach, where the invasive population's genetic variation is monitored over time. Ideally, both approaches should be combined, but more importantly if we are to better understand the mechanisms that facilitate and constrain population expansion in new environments, we need to study populations during the initial stages of the invasion. This is the time period when population size, genetic variation and patterns of plasticity will dictate the evolutionary trajectory of the population. While detection and study of such populations are needed, the use of large-scale experiments using mesocosms and experimental introductions to simulate the invasion may ultimately be needed if we are to draw any general conclusions about what mechanisms are most important.

It has been hypothesized that invasive species or populations exhibit greater adaptive phenotypic plasticity or rapidly evolve to new environments (Ghalambor et al., 2007; Prentis et al., 2008; Richards, 2006; Figure 1). The molecular mechanisms underlying these responses have yet to be totally elucidated, but a growing body of literature shows that TEs contribute to genetic variation and epigenetic changes underlie phenotypic plasticity in gene expression. The epigenome provides a mechanistic explanation on how environmental and genomic variations are transformed into phenotypic differences. Thus, while we have reason to suspect TEs and epigenetic changes contribute to adaptive phenotypic plasticity and adaptive evolution, establishing mechanistic links between genes, the environment, gene expression and phenotypes is an exceedingly difficult task. These challenges are part of the larger debate and problem of mapping genotypes to phenotypes (Debat & David, 2001; Wagner & Altenberg, 1996). We know that the environmental context is critical to shaping how the genome is transformed into different phenotypes by altering patterns of gene expression throughout development, but by considering how the epigenome responds to different environments, we can potentially gain a better understanding of this mapping problem (Duncan et al., 2014). Our perspective here is that the potential of the epigenome to facilitate biological invasions may be hidden within the native range, where natural selection has had the opportunity to shape epigenetic control mechanisms (see also Rey et al., 2016; Stapley et al., 2017). The general hypothesis is that if new environments disrupt the epigenetic control of genetic variation, then we will observe the release of this hidden variation, which in turn will facilitate biological invasions through adaptive plasticity and or increased genetic variation. Robust tests of this hypothesis will need to capture the processes occurring during the early stages of colonization and will require comparative studies of the source and descendent populations. Such studies will also need to resolve some fundamental challenges that all epigenomic approaches face. We briefly discuss these below.

4.1 | Phenotypic plasticity versus stochastic environmental effects

Phenotypic plasticity is defined as the capacity of a genotype to produce different phenotypes in response to different environmental cues (e.g., Ghalambor et al., 2007). A defining feature of plasticity is that it represents a predictable response to the environment, such that phenotype of an individual genotype can be predicted based on the environment (Pigliucci, 2005). In contrast, environmentally induced variation that is stochastic, such as developmental noise, represents unpredictable variation (Raser & O'Shea, 2005). This distinction matters when we consider the role the epigenome plays in biological invasions. If new environments induce predictable changes in the epigenetic control of gene expression, then natural selection can shape the pattern of epigenetic change throughout the genome. The concept of predictability is central to determining whether environmentally induced variation is adaptive or non-adaptive, and how selection will act on the distribution of phenotypes (Ghalambor et al., 2015). For example, if plasticity is adaptive then it can allow populations to persist under new conditions and allow time for beneficial mutations to arise (Corl et al., 2018; Ghalambor et al., 2007; Pigliucci, 2005).

However, if new or stressful environments induce stochastic changes in patterns of methylation or histone modification, then the contribution to adaptive evolution is largely based on the probability that by chance some of the variation is beneficial. Under such a scenario, the stability of these patterns across generations is critical to the process, otherwise the patterns of variation generated in one generation will be uncorrelated with the patterns in subsequent generations. It is therefore imperative that future studies examining the role of the epigenome in biological invasions quantify how predictable environmentally induced epigenetic change is, and how heritable these changes are across generations in the presence and absence of the environmental cues.

4.2 | Linking epigenetic marks to traits and separating genetic from epigenetic variation

Identifying the genetic basis of complex traits is a fundamental and ongoing challenge in evolutionary biology, and this is particularly the case for polygenic traits subject to environmental influences. Under controlled environmental conditions, breeding experiments can be designed to partition phenotypic variation into the contributions made by genetic, epigenetic and environmental sources. However, when studying natural populations establishing links between the epigenome and specific traits is often an exceedingly difficult task. To date, most studies investigating the contribution of epigenetic mechanisms to biological invasions have simply quantified the patterns of epigenetic marks for populations occupying different environments. The assumption is that variation in epigenetic marking underlies differences in phenotypes, but such correlations are rarely tested in outbred natural populations.

Establishing the relationship between epigenetic changes and phenotypes is closely related to the general problem of separating genetic from epigenetic effects on phenotypic variation. In other words, when we observe phenotypic differences between invasive populations occupying different environments, how much of the observed variation can be directly attributed to the underlying epigenetic versus genetic diversity? In model organisms, clonal or isogenic lines can be generated that place epigenetic variation against a common genetic background, thus allowing for observed phenotypic differences to be attributed to the epigenetic effects (but see Menezes et al., 2018, for the difficulty to disentangle the two sources of variation). However, in natural populations not only does the genetic background vary across individuals and populations, epigenetic and genetic diversity can be positively correlated with each other making results even more difficult to interpret. We anticipate that future sequencing technology and advances in bioinformatic tools will continue to improve and help overcome some of these challenges, especially for non-model species (Billet, Genitoni, Bozec, Renault, & Barloy, 2018). Thus, epigenome-wide association study is an interesting approach to associate single methylation polymorphisms (used as marker) with a phenotype of interest (Lin, Barton, & Holbrook, 2016).

4.3 | Separating genetic and environmentally induced changes in TE activity

It will be important to quantify how much the role TEs play in adaptive changes in response to new environments requires a joint understanding of the epigenetic control of genetic variation. TE regulation is mediated by epigenetic marks, and the modification of their expression in new environments could reflect increased mobility or misregulation of the epigenetic control mechanisms. Distinguishing between these alternatives is important if we are interested in knowing whether new environments increase genetic variation through TE activity or simply expose existing variation that was previously hidden. Disentangling epigenetic from genetic variability is becoming easier but remains a very challenging problem, in particular if TEs are accounted for. We envision studies that do a better job of quantifying genome size, the percentage of the genome made up of TEs and the total number of small TE variations (Goubert et al., 2015; Lerat, Fablet, Modolo, Lopez-Maestre, & Vieira, 2017). This could be done by simulating genomes with different TE amounts and using software like dnaPipeTE (Goubert et al., 2015) for which no reference genome is needed. Still, the identification of insertion polymorphism in natural population which will give us indications of increased genetic variance is not an easy task, despite incredible bioinformatics developments (Villanueva-Cañas, Rech, Cara, & González, 2017). The availability of third-generation sequencing technologies will be decisive to facilitate both the incorporation of TEs in the genetic variability analyses and also the identification of specific epigenetic changes associated with TE.

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AUTHORS' CONTRIBUTIONS

C.V. and P.G. conceived the idea and initial structure of the manuscript; P.M. and J.G. wrote the first draft of the manuscript; S.M. and D.B. contributed to the epigenetics section; C.K.G. significantly reorganized and correct the manuscript. All the co-authors significantly contributed to the writing and revisions.

DATA ACCESSIBILITY

No data were collected for this manuscript.

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Objectifs de la thèse

Le succès invasif de certaines espèces questionne sur les phénomènes et mécanismes impliqués dans la résistance face à des changements environnementaux. Différents champs disciplinaires étudient ces questions. Une majorité des espèces invasives étudiées a des conséquences délétères sur les écosystèmes envahis, ou impacte directement l'espèce humaine au niveau économique ou agronomique. De ce fait, l'étude de l'invasion se fait dans une optique de lutte et diminution des conséquences de ces espèces. Dans un cadre plus académique, c'est sous un aspect évolutif que les espèces invasives intéressent, puisqu'elle représentent des événements d'adaptation rapide. Cette adaptation rapide est difficile à étudier sur des modèles biologiques plus connus comme *Drosophila melanogaster*, une espèce répartie mondialement en ayant suivie les migrations humaines il y a quelques milliers d'années (Bergland et collab., 2016). Dans un contexte de changements globaux, l'étude des espèces invasives permet de mieux comprendre les réponses des autres espèces aux changements de l'environnement. Par ailleurs, les espèces invasives offrent une opportunité *in natura* de mieux comprendre les mécanismes moléculaires sous-jacents à la réponse rapide aux stress chez une espèce. L'objectif de ce doctorat a été de décrire les réponses de différentes populations d'une espèce invasive, *Drosophila suzukii*, à deux stress environnementaux et d'initier l'étude de ces mécanismes moléculaires sous-jacents. Dans un premier temps, nous avons testé si la résistance au stress dépendait du statut d'invasion des populations ce qui suggérerait une adaptation rapide. L'analyse des mécanismes moléculaires visait à tester l'origine des variations : variabilité génétique révélée par les conditions environnementales comme les éléments transposables, variabilité non-génétique via les modifications épigénétiques. Pour ces études mécanistiques nous avons analysé le transcriptome associé aux différents phénotypes observés. Nous avons essayé de faire le lien entre changement d'expression, modifications épigénétiques, diversité en éléments transposables et gènes impliqués dans la réponse aux stresseurs. Pour déterminer s'il existait une variabilité dans les réponses aux stresseurs au sein de notre modèle biologique, nous avons comparé des populations des aires envahies et d'une aire native le Japon. L'approche expérimentale est décrite au chapitre 1 afin d'illustrer l'ensemble des méthodes mises en jeu par la suite. Nous avons tout d'abord examiné la réponse au froid (chapitre 2), souvent étudiée comme proxy de la distribution géographique des espèces ectothermes. Nous avons étudié la résistance à ce stress au niveau populationnel puis trois génotypes ont été étudiés au niveau transcriptomique, mais aussi au travers de l'expression et de la diversité des éléments transposables. Nous avons émis l'hypothèse que la résistance au froid est fonction du statut d'invasion et que les populations invasives sont plus résistantes que la population native. Une seconde étude s'est concentrée sur la résistance au stress oxydant, médiée par un herbicide, le paraquat, produit largement utilisé et répandu dans le monde (Weber et collab., 2012 ; Tsai, 2018). Ce traitement prooxydant a été choisi du fait de la littérature riche sur les effets du paraquat chez *D. melanogaster* en particulier mais aussi par les différences de traitement de par le monde. L'union européenne a interdit l'usage de ce produit en Europe en 2007, un an avant la première détection de *D. suzukii* . Cependant il reste toujours massivement utilisé au Japon et aux Etats-Unis. Nous supposions que la résistance au paraquat était plus importante aux États-Unis et au Japon, mais que du fait de la pression moins importante en Europe, les populations françaises, seraient plus sensibles. Une analyse similaire au stress thermique a été menée en comparant la survie des populations

après adjonction de paraquat, au niveau phénotypique et moléculaire (chapitre 3). Certains gènes semblent conférer une plus grande résistance au stress oxydant via l'insertion d'un élément transposable qui modifie leur l'expression (Guio et collab., 2014, 2018). Nous avons cherché à identifier si un événement similaire était présent sur *D. suzukii* en analysant finement un cluster de gènes identifiés chez *D. melanogaster* (chapitre 4).

CHAPITRE 1

Méthodes expérimentales et biologie du modèle *Drosophila suzukii*

Ce chapitre a pour vocation de présenter le modèle biologique *Drosophila suzukii* au travers de son historique d'invasion, nécessaire à la compréhension des choix méthodologiques appliqués par la suite. Il est important aussi de décrire certains aspects biologiques relatifs à sa physiologie et son cycle de développement. Afin de simplifier la lecture des chapitres suivants, nous avons dédié ce chapitre à la présentation des méthodologie expérimentales mises en œuvre dans le cadre de l'étude de la réponse au stress thermique et chimique. Nous avons détaillé l'origine des populations étudiées ainsi que leur culture en laboratoire. De plus nous avons décrits les méthodes de phénotypages utilisées ainsi que les protocoles d'analyses moléculaires concernant les études génomiques et transcriptomiques. Ces derniers ont été développé de façon succincte et plus de détails sont disponibles dans chaque chapitre.

1 Historique d'invasion

Bien que la première observation répertoriée de *D. suzukii* (Matsumura, 1931) ait été faite au Japon en 1916, son origine géographique se situe vraisemblablement en Chine. Plusieurs revues décrivent l'état de l'art sur l'invasion et la distribution de cette espèce dans le monde, avec à l'heure actuelle, une distribution mondiale allant des pays asiatiques (Chine, Corée, Japon), au continent d'Amérique (Canada, U.S.A, Amérique du Sud), jusqu'en Europe voire même en Ukraine (Asplen et collab., 2015 ; CABI, 2020 ; Gutierrez et collab., 2016 ; Lavrinienko et collab., 2017). Récemment, Fraimout et collab. (2017) par une analyse de 25 marqueurs microsatellites, sur 23 sites échantillonnés (685 individus) dans le monde, ont établi les routes d'invasion de *Drosophila suzukii* résumées sur la **figure 1.1**. En accord avec des observations antérieures et postérieures (Rota-Stabelli et collab., 2019), ils ont déterminé que l'invasion concomitante des continents américain et européen correspondait à des événements distincts. L'invasion américaine vient des îles d'Hawaï, elles-mêmes envahies en 1980 par des populations originaires du Japon et du sud de la Chine. En Europe il y aurait eu deux routes d'invasion, une directement du nord de la Chine, l'autre en provenance de la côte Est américaine. Ces deux routes distinctes se retrouvent mélangées dans certaines populations puisque des brassages génétiques ont été détectés, notamment dans les populations du nord de la France comme à Paris. Une récente modélisation de la distribution potentielle de *D. suzukii* a été faite en 2017 par dos Santos et collab. (2017), basée sur des données jusqu'en 2015 correspondant à plus de 400 sites envahis, et prévoyait que de nombreux autres pays seraient propices à l'invasion de cette espèce **figure 1.2**. Cela s'est confirmé avec sa détection en Ukraine en 2015 (Lavrinienko et collab., 2017), mais aussi au Maroc en 2019 ou en Russie (CABI, 2020). L'étendue de l'aire géographique envahie et la vitesse de cette invasion sont deux arguments sur l'usage de *D. suzukii* comme modèle biologique pour l'étude des réponses rapides aux variations de l'environnement. De plus, l'invasion parallèle sur deux continents (Europe et U.S.A) offre la possibilité de comparer et donc potentiellement d'identifier des mécanismes en réponses aux conditions environnementales nouvellement rencontrées.

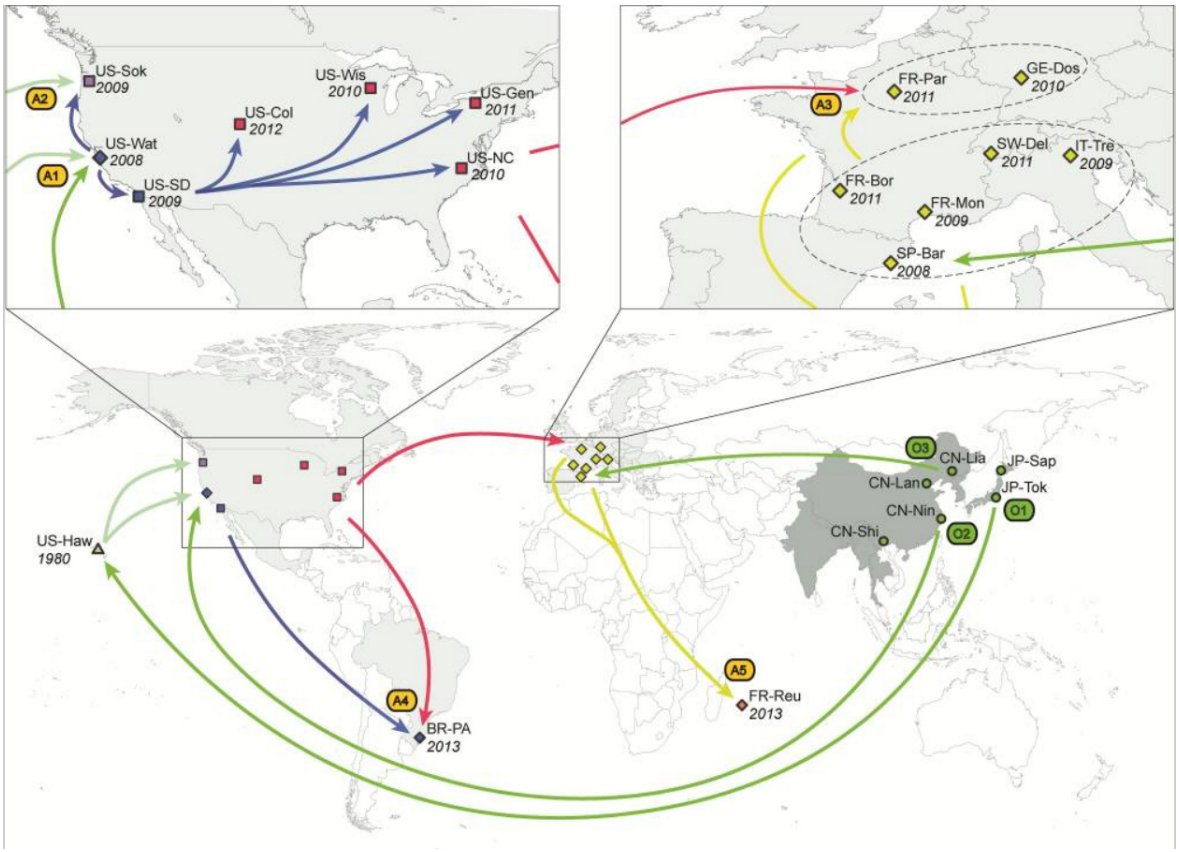


Figure 1.1. Carte du scénario d'invasion de *Drosophila suzukii* estimé par l'analyse microsatellite multilocus et les données d'observation, en utilisant une méthode de calcul bayésienne approchée (ABC). L'année d'observation de l'espèce dans le pays est indiquée en italique et la couleur grise correspond à l'aire native. Les flèches indiquent les routes d'invasion et les symboles sont les points d'échantillonnage. Source : (Fraimout et collab., 2017)

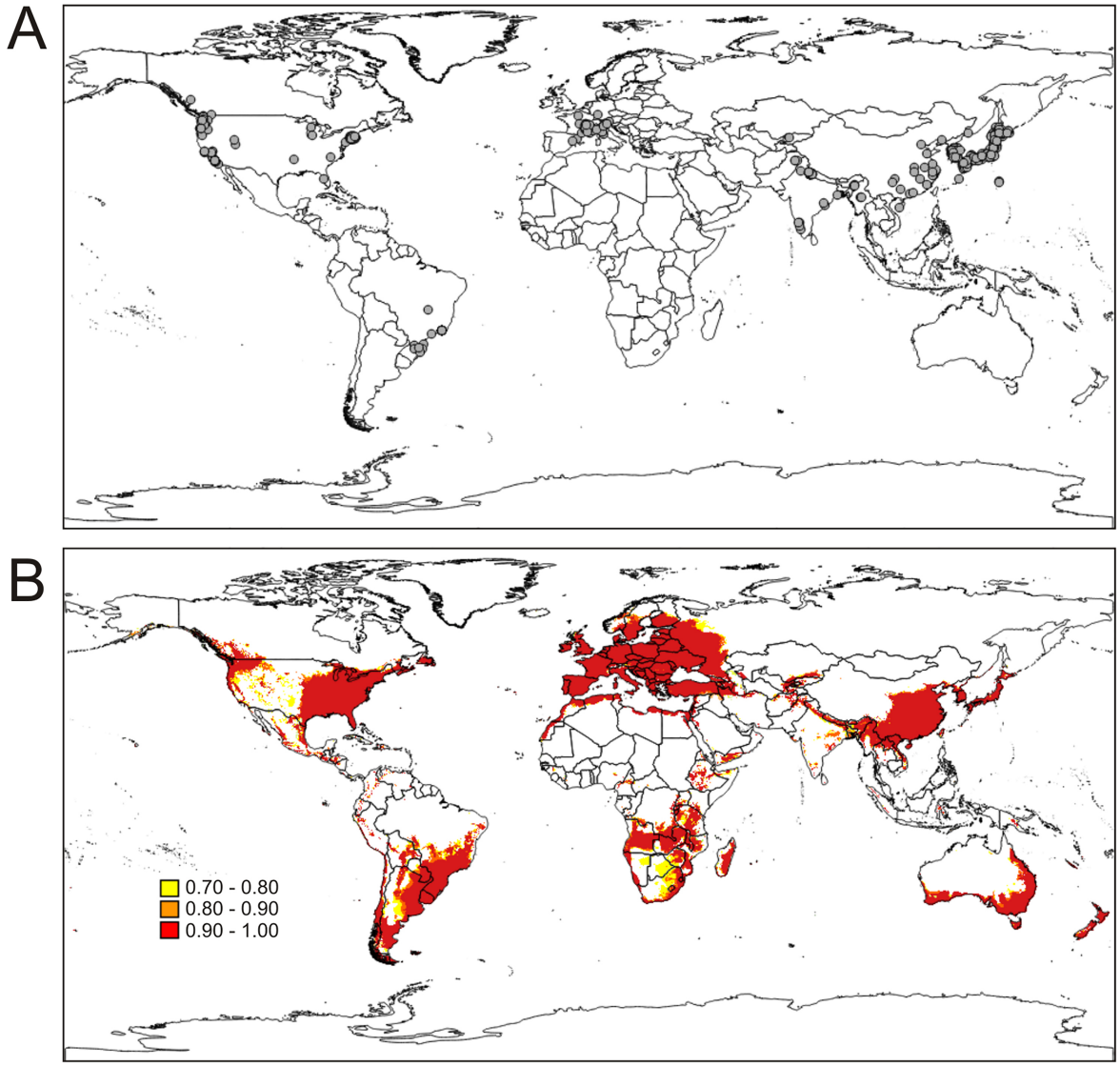


Figure 1.2. (A) carte des sites connus utilisés pour l'inférence du modèle prédictif. **(B)** modèle prédictif utilisant l'algorithme GARP (Genetic Algorithm for Ruleset Production). La légende indique l'adéquation environnementale avec *D. suzukii*. Source : (dos Santos et collab., 2017)

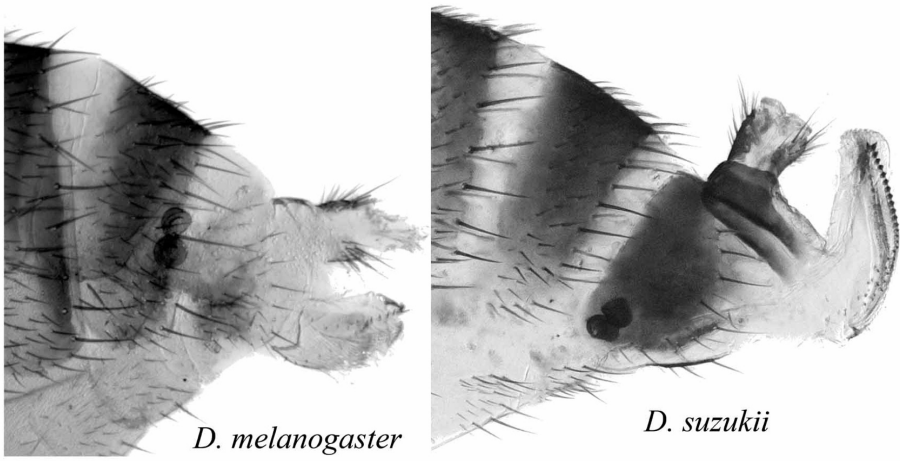
2 Biologie du modèle

La biologie de cette espèce a été étudiée de façon approfondie récemment (voir Asplen et collab., 2015 ; Dalton et collab., 2011 ; Emiljanowicz et collab., 2014 ; Hauser, 2011 ; Ryan et collab., 2016 ; Tochen et collab., 2016 ; Toxopeus et collab., 2016) et les travaux de cette dernière décennie ont été répertoriés par exemple par Hamby et collab. (2016). *Drosophila suzukii* (Matsumura, 1931), aussi connue sous le nom de mouche aux ailes tachetées (spotted wing drosophila, SWD) (*diptera, drosophilidae*) est un diptère (holométabole) rattaché au groupe phylogénétique *melanogaster* et au sous-groupe *suzukii* (**figure 1.4**) (Rota-Stabelli et collab., 2019 ; Sessegolo et collab., 2016 ; Yang et collab., 2012). Il existe un dimorphisme sexuel visible, avec d'une part des femelles de taille plus importante, d'autre part, des mâles dont les ailes comportent une tâche noire (**figure 1.3a & b**). Par ailleurs, les femelles présentent un ovipositeur sclérotinisé en dent de scie leur permettant de percer des fruits, substrat préférentiel de ponte, et d'enfouir les œufs juste sous le tégument contrairement aux autres espèces de drosophiles (**figure 1.3c & d**). Son cycle de développement varie en moyenne de 10 (27°C) à 14 (20°C) jours, avec une hygrométrie optimale vers 60-70% et en laboratoire à 25°C (Hamby et collab., 2016). La température minimale de développement est estimée à 7.2°C en condition constante et 11.6°C en régime fluctuant. La température maximale se situe à 30°C où aucune oviposition n'est observée ainsi qu'un faible taux de piégeage d'adulte en nature. Quatre phases de développement existent (ici les données sont pour une température de 25°C) : un stade œuf (24h), puis la larve (3 stades L1-L3, ~7j), en passant par une pupe (~6j), jusqu'à l'adulte. Ces paramètres sont dépendants du milieu de culture utilisé, depuis les milieux artificiels commun à *D. melanogaster* à des milieux inspirés des hôtes naturels (fruits rouges), comme les fraises, myrtilles ou framboises. Concernant la reproduction de *D. suzukii*, les femelles sont fertiles 24h après leur stade adulte, l'activité d'accouplement semble avoir son pic en début de matinée alors que la ponte s'effectue en fin de journée. La fécondité globale (nombre d'œufs total pondus pendant la vie d'une femelle) peut être de quelques œufs à près de 636 en moyenne, en condition artificielle contrôlée (Hamby et collab., 2016). On observe une fécondité moindre que chez *D. melanogaster* en condition de laboratoire. Parmi les paramètres limitant la fécondité et la ponte, un premier facteur correspond à l'élévation de température au dessus de 27°C. De plus, certains symbiotes très présents chez les arthropodes comme *Wolbachia sp.* (prévalence 52%, Weinert et collab. 2015) sont connus pour manipuler la reproduction (Cattel, 2016). Il existe une souche (*wSuz*) qui infecte naturellement *D. suzukii* mais qui semble très peu impacter la reproduction avec quelques cas de fertilité réduite (Hamby et collab., 2016). Concernant la longévité, l'espèce vie en moyenne 86 jours et jusqu'à 154 en condition contrôlée (22°C).

D. suzukii est connue pour son cycle biologique comportement une phase de diapause ovarienne, c'est à dire un arrêt de sa physiologie de la reproduction durant l'hiver (King et MacRae, 2015 ; Wallingford et Loeb, 2016 ; Wallingford et collab., 2016) avec son système reproducteur qui s'atrophie pour donner des ovaires ne contenant pas ou peu de follicules dont le développement est limité, ce qui est suivi par une absence de ponte. Par ailleurs, en suivant la diminution saisonnière des températures, la cuticule se mélanise (Dembeck et collab., 2015 ; Shearer et collab., 2016). En raison des variations colorimétriques de la cuticule, on trouve dans la littérature les notions de morphe d'été (couleur claire) et d'hiver (couleur foncée) correspondant à ces variations au cours de l'année.

(a) *D. suzukii* femelle avec un ovipositeur visible

(b) *D. suzukii* mâle avec les ailes tâchetées



(c) Illustration de l'ovipositeur sclérotinisé de *D. suzukii* (droite) comparé à *D. melanogaster* (gauche), source : Hauser (2011)

Figure 1.3. Illustration de *D. suzukii* femelle (a) et mâle (b) ainsi que l'ovipositeur sclérotinisé (c)

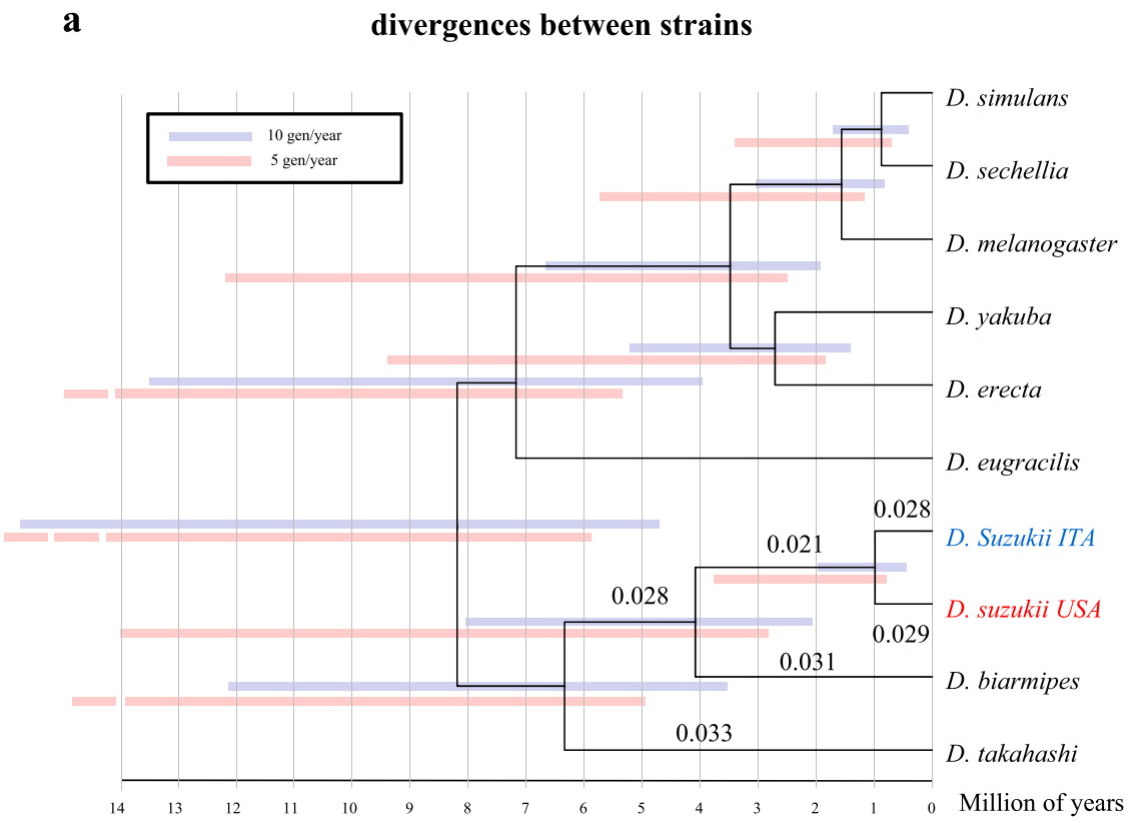


Figure 1.4. Arbre phylogénétique représentant la divergence des espèces *Drosophila sp.* Deux génomes de références (américain et européen) sont comparés pour l'espèce *D. suzukii*. Les valeurs associées aux nœuds correspondent au nombre de mutation par site et par million d'années. Source : (Rota-Stabelli et collab., 2019)

3 Méthodologie experimentale

3.1 Entretien et phénotypage des populations de *D. suzukii*

3.1.1 Echantillonnage des populations

Les expériences ont été menées en laboratoire à partir de populations échantillonnées et maintenue en laboratoire depuis 2014, aux États-Unis , France et Japon. Deux sites par pays ont été choisies pour notre projet, selon un gradient Nord-Sud ; Dayton, Watsonville sur la côte Ouest des États-Unis ; Paris, Montpellier en France et Tokyo, Sapporo au Japon (**tableau 1.1**). Des femelles sauvages ont été isolées pour générer des lignées isofemelles, c'est à dire des lignées dont tous les descendants sont issus de la même femelle. Ceci afin de pouvoir identifier dans les populations les phénotypes (donc les génotypes) particuliers. Au moins six lignées isofemelles par site d'échantillonnage (soit 36 lignées) devaient être initialement utilisées pour la suite des expérimentations. Cependant au cours du doctorat, certaines lignées se sont éteintes, nous avons donc utilisé 27 (paraquat) et 28 (froid) lignées au total.

TABLEAU 1.1 – Nomination et indications géographiques des lignées échantillonnées en France, aux U.S.A et au Japon en 2014. Les lignées isofemelles utilisées dans les expérimentations pour le stress thermique et chimique sont indiquées séparément et les lignées indiquées en gras correspondent aux lignées utilisées dans les analyses moléculaires.

Localisation	Coordonnées GPS	Lignées paraquat	Lignées froid
Sapporo (Hokkaido Japon)	43° 3' 43.545"N 141° 21' 15.754" E	S11, S20, S21, S24, S29	S11, S20, S24, S29
Tokyo (Honshu Japon)	35° 41' 22.155" N 139° 41' 30.143" E	T3, T11, T18	T10, T21
Watsonville (Californie U.S.A)	36°54'51.8"N 121°45'27.7"W	W106, W112, W113, W120, W122, W127	W106, W112, W120, W127
Dayton (Oregon U.S.A)	45° 13' 14.422" N 123° 4' 34.368" E	Sok1, Sok28, Sok58	Sok1, Sok28, Sok58, Sok76, Sok80
Paris (France)	48° 51' 23.81" N 2° 21' 7.998" E	L2, L6, L7, L21, L22,L26	L6, L7, L9, L21, L22, L26
Montpellier (France)	43° 36' 38.768" N 3° 52' 36.177" E	MT15, MT20 , MT25, MT47	MT3, MT7, MT15, MT18, MT20, MT25, MT47

3.1.2 Culture en laboratoire

Les lignées ont été conditionnées en plusieurs tubes réplicats contenant un milieu gélosé avec une feuille de papier absorbant humidifiée, à 22.5°C et 70% d'hygrométrie, pour une photopériode de 16h de jour, 8h de nuit. Les adultes émergents sont transférés dans plusieurs tubes (pour limiter une densité trop forte) jusqu'à l'âge de 3-5 jours avant d'être transférés à nouveau dans des tubes où ils pourront pondre durant trois jours. Ils sont ensuite transférés dans de nouveau tubes pour pondre pendant trois jours puis sacrifiés. Le milieu de culture utilisé correspond à celui décrit par Dalton et collab. (2011), légèrement modifié notamment en utilisant de la nipagine comme antifongique remplaçant l'acide propionique (**tableau 1.2**). Pour l'ensemble des expérimentations, nous avons utilisé des individus âgés de 4 à 7 jours pour limiter les effets de l'âge.

TABLEAU 1.2 – Recette du milieu nutritif gélifié utilisée pour la culture de *D. suzukii*, modifié d'après Dalton et collab. (2011).

Eau distillée : 1 L
Agar (Drosophila Agar Type, ref.66-103, Apex™) : 9 $g.L^{-1}$
Farine de maïs (Farine de gaudes, Moulin Giraud) : 33 $g.L^{-1}$
Ethanol 96% : 40 mL.L^{-1}
Extrait de levure (ref.75570, LYNSIDE®) : 17 $g.L^{-1}$
Sucre (sucre industriel) : 50 $g.L^{-1}$
Nipagine (Tegosept,ref.20-258, Apex™) : 4 $g.L^{-1}$
Dans 1L d'eau distillée à température ambiante, verser l'agar, la farine de maïs, l'extrait de levure et le sucre et bien mélanger.
Faites chauffer l'eau tout en mélangeant et après 10 minutes d'ébullition laisser refroidir.
Une fois la température en dessous de 53°C, ajouter la nipagine préalablement diluée dans l'alcool et remplir les tubes penchés au tiers.
Couvrir et laisser refroidir avant de stocker à 4°C pendant 1 mois.

3.1.3 Mesure de la réponse au froid

Pour étudier la réponse thermique de *D. suzukii*, nous avons mesuré le temps de réveil après un passage ponctuel au froid. La mesure de temps de réveil (chill coma recovery time) est un des proxy de la résistance à la température simple à mettre en place. Pour cela, des adultes ont été séparés selon leur sexe dans des tubes vides, puis déposés dans un cube de polystyrène contenant de la glace, lui même placé en chambre froide (4°C) avant de lancer le chronomètre. Par la suite, les mouches sont réparties dans les puits d'une plaque 54 puits, en étant toujours sur glace pour maintenir une température à 0°C. Les individus sont déposés au centre des puits sur leur partie dorsale afin de limiter les biais micro-environnementaux. Les plaques sont ensuite refermées et protégées de la lumière durant 16 heures. Puis, les plaques sont réparties sur une paillasse dans une salle à température stable (21°C) et le chronomètre est lancé. Un individu est considéré comme réveillé lorsqu'il se redresse sur ses pattes. Ne pouvant mesurer l'ensemble des individus simultanément, nous avons effectués des mesures à des dates différentes, avec différents expérimentateur.trice.s. Des essais préliminaires ont permis de constater que le temps de réveil moyen était bien inférieur à 60 minutes. Nous avons donc décidé de censurer les données dépassant ce temps de réveil. Afin de limiter les biais, les paramètres expérimentaux précédents (date, expérimentateur, censure) ont été intégrés dans les modèles statistiques. Le taux de mortalité dû au froid dans les lignées a été évalué, mais négligeable et donc non considéré (inférieur à 5%, en considérant les individus réveillés après 60 minutes). Un total de 4661 individus (28 lignées) ont été phénotypés de façon non équilibré entre les sexes et les populations. Afin d'avoir des échantillons représentatifs, nous avions fixé le nombre de mesure par condition (sexe, lignée) à 30 individus. Cependant, cinq groupes (4 mâles et 1 femelle) ont eu un nombre de mesure compris entre 16 et 26.

3.1.4 Mesure de la réponse au stress oxydant

Dans le cadre de la réponse à un environnement pro-oxydant médié par le paraquat (Dichlorure de 1,1'-Diméthyl-4,4'-bipyridinium), nous avons mesuré la survie en condition traitée et non traitée. Ceci afin d'obtenir deux informations, la résistance au paraquat mais aussi d'identi-

fier si nos populations présentaient des différences de longévité en condition contrôle. Nous avons choisi d'étudier la réponse au stade adulte. Pour cela, le paraquat (10 mM) a été supplémenté lors de la préparation du milieu de culture. Tous les trois jours, les adultes ont été transférés dans de nouveaux tubes correspondants à leur condition afin de limiter des développements bactériens ou fongiques. Cette expérimentation s'est faite selon un plan équilibré de trois réplicats par sexe pour chaque lignée (27), avec dix individus par tube. Les mesures ont été effectuées toutes les 24h jusqu'à la mort de l'ensemble des individus (3240). Cette expérience a été effectué en une seule fois par un seul expérimentateur.

3.2 Analyses moléculaires

3.2.1 Analyse génomique

L'analyse phénotypique a permis d'identifier plusieurs lignées dont les réponses sur les traits mesurés (survie et temps de réveil) étaient différentes. Nous avons choisi une lignée par pays (France, Japon et U.S.A) avec des valeurs de traits différentes. Ces 3 génotypes (S29 de Sapporo pour le Japon, W120 de Watsonville pour les U.S.A et MT47 de Montpellier pour la France) ont servi pour toutes les analyses moléculaires décrites par la suite. Nous avons séquencé ces 3 génotypes à l'aide d'une extraction au phénol-chloroforme sur 10 femelles adultes, puis nous avons envoyé les échantillons pour la préparation des librairies et le séquençage sur une plateforme (GeT-PlaGe, Génopole Toulouse/Midi-pyrénées, séquençage Illumina 150 pb paired-end).

3.2.2 Analyse transcriptomique

L'analyse transcriptomique a été effectuée simultanément pour tous les traitements (contrôle, froid et paraquat) sur des pools de 15 femelles. L'analyse transcriptomique du froid est faite en mimant les conditions expérimentales appliquées pour la mesure du temps de réveil. Nous avons laissé les individus 60 minutes pour se réveiller à 21°C, puis nous avons sacrifié et disséqué sur glace dans un tampon, afin de séparer les tissus somatiques et germinaux. Dans le cas du paraquat, nous avons laissé les adultes 24h dans un milieu avec une concentration de 20 mM avant de les disséquer. Les échantillons ont été extraits à l'aide d'un kit (RNAeasy plus mini kit) selon le protocole fourni. Nous avons appliqué un traitement supplémentaire avec de la DNAse puis les échantillons ont été stockés au congélateur à -80°C. Après contrôle de la qualité des échantillons, la construction des librairies et le séquençage ont été effectués sur une plateforme extérieure (IGBMC sequencing platform, Strasbourg, Illumina HiSeq 4000 paired-end). Au total nous avons généré 36 échantillons (2 tissus, 2 réplicats, 3 génotypes, 3 traitements). La qualité du séquençage a été controlée en utilisant FastQC (v. 0.10.1) avant nettoyage à l'aide de UrQT (v. 1.0.17) (Modolo et Lerat, 2015 ; Simon, 2010). Les données transcriptomiques ont été alignées sur un génome de référence fourni par Paris et collab. (2020) en utilisant Hisat2 puis Express pour générer des comptes (Kim et collab., 2019 ; Roberts et Pachter, 2013). L'annotation du génome de *D. suzukii* étant encore incomplète, nous avons effectué un BLASTX réciproque sur la base de données de Flybase, puis un BLASTX sur la base de données "nr" du NCBI afin de maximiser le nombre d'annotation. Sur les 16905 gènes prédit sur le génome de *D. suzukii*, nous avons pu trouver une annotation pour 53%, ce qui représente encore beaucoup de gènes sans fonction, ni annotation connu et pouvant limiter les interprétations par la suite. Nous avons mené une analyse différentielle sous

R en utilisant DESeq2 afin de mesurer, (i) l'effet des traitements sur les génotypes (contrôle versus froid ou paraquat), (ii) les différences entre génotypes (France, Japon et U.S.A, (iii) l'interaction génotype et environnement (Love et collab., 2014 ; R Core Team, 2019). Afin de limiter les faux positifs, nous avons appliqué un filtre sur le taux de faux positif (False Discovery Rate, FDR) et la valeur de taille d'effet (fold-change) en ne conservant les gènes dont l'expression présenté un score de FDR inférieur à 0.01 et un niveau d'expression au moins doublé (log_2-fold-change >1).

3.2.3 Analyse des éléments transposables

L'identification et l'analyse des éléments transposables dans le génome de référence de suzukii a été effectué par Merel *et al.* (en préparation). Une première étape a été l'identification de novo (logiciel REPET), puis les séquences consensus ont été générées et annotées. Ces séquences ont été alignée avec bwa-sw sur le génome de référence préalablement masqué en utilisant bedtools (Li et Durbin, 2010 ; Quinlan et Hall, 2010). Nous avons donc obtenu d'une part les séquences de références des ETs afin de pouvoir identifier dans les données transcriptomiques l'expression de ces éléments. D'autre part nous avons, à l'aide des données génomiques, estimé les fréquences d'insertion de ces éléments à l'aide de Popoolation-Te2 dans nos trois génotypes (Flutre et collab., 2011 ; Kofler et collab., 2016). Enfin nous avons pu utiliser les informations génomiques sur la présence des ETs dans nos 3 génomes pour identifier leur proximité avec des gènes différentiellement exprimés. L'expression des ETs a été quantifié avec le module TEcount de TEtools qui a généré des comptes pour chaque famille d'élément identifiés (Lerat et collab., 2017). Ces données ont été ajouté aux données transcriptomiques préalables pour être analysées selon le même modèle sous DESeq2, et normalisées par rapport à l'ensemble des gènes.

CHAPITRE 2

Chill coma recovery time of a recent invasive species *D. suzukii*, from phenotype to molecular mechanisms

Avant-propos

Du fait du rôle de la température dans la distribution géographique des espèces notamment chez les insectes, et de la capacité d'invasion de *Drosophila suzukii* dans des régions très diverses, du Brésil à la Pologne en passant par le Canada, nous avons cherché à savoir si durant l'invasion la réponse de *Drosophila suzukii* pouvait différer selon l'aire d'origine des populations, c'est-à-dire, les aires d'invasion et l'aire native. Avec un échantillonnage dans trois pays, comprenant une aire dite native le Japon et deux aires envahie de façon concomitante en 2008 (les États-Unis et la France), nous avons mené une analyse comparative au niveau phénotypique et moléculaire. La présence de deux invasions distinctes entre Europe et U.S.A permet d'étudier si les réponses au facteur thermique sont spécifiques ou non aux facteurs environnementaux rencontrés dans chaque aire. Nous avons donc pour cela étudié deux populations échantillonnées par continent (Sapporo et Tokyo au Japon, Watsonville et Dayton aux U.S.A, Paris et Montpellier en France), en appliquant sur un total de 28 lignées un stress ponctuel thermique (condition à 0°C pendant 16h), proxy de la résistance au froid. Nous avons analysé cette réponse au niveau populationnel (ensemble des lignées sur chaque site) afin de décrire les tendances sur les différents continents, puis nous avons choisis des lignées dont les réponses phénotypiques présentaient de fortes différences que nous avons analysé au niveau moléculaire. Nous avons pu observer que contrairement à ce qui est présent dans la littérature sur *Drosophila suzukii* mais aussi sur d'autres modèles, aucune différence de résistance n'a été observée entre sexes. Les populations de chaque aire ont en moyenne une réponse similaire, avec une plus grande résistance observée pour les populations françaises, par rapport au Japon, notamment Sapporo la plus sensible. Bien que les populations américaines ne soient pas significativement différentes, leur valeur moyenne est bien plus proche de la France que du Japon. En limitant certains biais comme la répétition expérimentale dans le temps et le nombre d'expérimentateur, nous aurions pu diminuer l'incertitude associée aux mesures et mieux estimer ces différences. L'analyse de trois génotypes venant de Sapporo, Watsonville et Montpellier illustre aussi la variabilité au niveau moléculaire. La plupart des gènes exprimés sont spécifiques à chaque génotypes avec moins de 100 gènes partagés. Par ailleurs, l'analyse des éléments transposables n'a pas révélé une explosion d'expression comme suggéré dans la littérature, avec une petite nuance pour la France qui a vu 31 familles d'éléments surexprimés contre moins de 10 dans les autres génotypes. L'analyse des insertions d'ET à proximité des gènes indique que les gènes exprimés lors du stress sont déplétés en ET, mais les gènes sensibles au traitement dont le niveau d'expression change selon le fond génétique, présentent des ETs à leur voisinage et sont des candidats pour expliquer des adaptations locales.

IN PREPARATION

Chill coma recovery time of a recent invasive species D. suzukii, from phenotype to molecular mechanisms

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Abstract

Background: The range of species distribution depends, among other things, on environmental pressures. For insects, temperature is the key factor in their geographical range. Invasive species such as *Drosophila suzukii* have a great capacity to invade new areas where environmental factors may differ from those of their native range. D. suzuki is thus a good model for studying the rapid response to environmental changes and the underlying molecular mechanisms. We analysed populations sampled from native and invasive areas to compare their responses at the phenotypic and molecular levels to cold stress. We studied the response of genes but also that of transposable elements (TE), often considered as markers of genome stability.

Results: Invasive strains were more resistant to cold than native populations and no differences were observed between sexes. During the cold stress, the transcriptome did not seem canalised, with a small number of differentially expressed genes common to all strains. France and the United States had more change in gene expression than Japan but did not have many genes that responded in the same way, despite the same phenotypic response. The expression of TEs did not show any changes as a result of cold despite the number of TE families that are expressed under control conditions. Differentially expressed genes were depleted in TE insertions. Some genes presented TE insertions with transcription factor binding sites (TFBS) that were strain specific, suggesting effects related to local adaptation.

Conclusion: *D. suzukii* cold response is population dependant with a faster recovery in invasive than native areas without sex differences. Transcriptomic study revealed higher plasticity for invasive than native genotypes with some candidate genes for local adaptation. TE expression is not modified during cold stress but some investigation on TE insertions are need.

Keywords: *Drosophila suzukii* ; invasive species ; cold stress ; chill coma recovery ; transposable elements ; environmental changes ; genotype by environment interaction

Introduction

The geographic distribution of species is determined by their ability to adapt to their environment and for ectothermic species, such as insects, the key environmental factor is temperature [1, 2]. Therefore, environmental changes of anthropogenic origin and global warming will have a strong impact on these organisms. Face to these changes, ectothermic species have limited choices, they must adapt, migrate or extinct. Invasive species are good models for studying the ability of organisms to adapt to these rapid changes [3]. Indeed, by definition, these species are introduced species, which spread through the invaded area and persist despite the new/different environment than those previously encountered [3, 4]. The fact that invasive species seem to present a reduction of the genetic diversity during invasion opens the question on how to select for adaptive variants [5–8]. Several phenomena, such as phenotypic plasticity (the ability to one genotype to express several phenotypes depending on the environmental cues) [9, 10], are expected to explain the ability of invasive species to adapt in the absence of genetic diversity [5, 7].Other mechanisms could involve cryptic genetic variations and epigenetic modifications [7, 8, 11]. Genetic variability could change rapidly fol-

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lowing environmental stress by Transposable elements (TEs) activation or epigenetic disturbance. TEs discovered by B. McClintock, are "selfish elements" which can be replicated among the genome [12]. These insertions are mainly deleterious, and purifying selection acts to remove them and silence their activity [13–15]. But, as already suggested by McClintock, TEs could have some other consequences by generating cryptic genetic diversity which could be advantageous in different environments especially related to invasive species [16–20]. Adaptive effects of the inserted TEs to both biotic and abiotic stress (*e.g.*, virus resistance or cold response) have already been shown [21, 22]. Moreover, TEs are regulated by mechanisms that are sensitive to environmental cues, such as epigenetic regulation [23–26]. We can suppose that the disruption of TEs regulation after environmental changes encountered by invasive species, could induce a burst of TEs expression [8, 27]. Furthermore, TE insertions could affect nearby genes by modifying their regulation which, in a stress context, could have various consequences and in some case increased resistance to biotic or abiotic stress [20, 28, 29]. In Drosophila species the cold hardness is a good proxy of thermal adaptation and explain geographical distribution when comparing tropical and temperate species, at the intra- and interspecies -level [1, 30]. The *Drosophila suzukii* invasive species seem to be a good model to understand rapid adaptation when acute variation, as a cold stress, occurs on short time scale because (i) we have deep knowledge on a sister species *D. melanogaster* [1, 30–34], (ii) because of the recent and concomitant (Europe and U.S.A) invasion in the last decade [35, 36]. Despite different thermal ranges between invaded (U.S.A and Europe) or native Asian area, *D. suzukii* is present in both North and South America, in Europe from the south (Spain) to the East (Poland, Ukraine) and it has also been observed in Russia [37, 38]. Two main strategies exist for insect resistance to cold stress as freeze avoidance or tolerance (reviewed in [39, 40]). Melanogaster group species are known to be chill intolerant and *D. suzukii* is not an exception [41–43]. But it's ability to invade different countries suggests some strategies not yet understood. Currently, only few studies were made at the molecular level to investigate the cold acclimation of *D. suzukii* but none of them integrate the geographical variation in cold response [44–46]. In the present manuscript, we studied populations from both a native, Japan (Sapporo & Tokyo) and invaded areas, in U.S.A (Watsonville & Dayton) and France (Paris & Montpellier). We have measured chill coma recovery time and show that the invasive populations tend to be more cold resistant than the native one. Then, we have chosen three lines with different phenotypes and genotypes (one from each country) to analyse transcriptomic profiles and identify genes responding to cold stress. Moreover, we analysed the expression of TEs during cold exposure and described how inserted elements could affect or not the expression of the nearby genes.

Results

Invasive French populations were more resistant to cold than the native

We evaluated the resistance to a cold stress by measuring CCRT on 28 monitored isofemales lines, from 6 populations in 3 countries. The mean recovery time by sex and population is presented in Table 1 and statistical analysis in Table S1 and Fig. 1. Across the populations, the range of CCRT is between 23 (Paris, France) to 45 minutes (Sapporo, Japan) (Table 1). The mean recovery time by sex and population is presented in Table 1 and statistical analysis in Table S1 and Fig. 1. We observed no differences between sex (Table 1 and value of 1.04 in Fig. 1) after cold exposure. The two native populations, Sapporo and Tokyo (Japan), had the same CCRT (p-values > 0.05), as the American (populations that were not different from Sapporo with p-value = $\sim$0.07). On the contrary, French populations presented a significantly reduced recovery time (Fig. 1) when compared to Sapporo. French flies recovered 24 to 30% more quickly respectively for Montpellier (coefficient of 0.76) and Paris (0.70, Table S1).

Gene expression was more genotype than cold dependent

We evaluate the transcriptomic responses to cold stress for three genotypes from three geographical regions (MT47 from Montpellier, France; W120 from Watsonville, U.S.A and S29 from Sapporo, Japan), hereafter referred by the country. Principal Component Analysis (PCA, Fig. 2) on RNAseq data shows that data grouped by genotype (23% of variance with axis 1). The first axis splited the 3 genotypes ordering U.S.A, France and Japan, while axis 2 separates France of the others. We can notice that for all genotypes, the cold treated samples are always above the controls. To evaluate the transcriptomic variation within each strain, between control and cold, we computed the coefficient of variation (CV) for each differentially expressed (DE) genes (Fig. S1). Japan transcriptome presents lower variation than the two other ones. Paired Wilcoxon test indicated that all two by two comparisons were significant (p-value < 0.01).

Cold stress induced a higher transcriptomic variation in the two invasive lines

Comparisons of the transcriptome between control and cold conditions revealed a total of 1073 DE genes.

Table 1: Mean (±standard deviation) of the chill coma recovery time (minutes) per population and sex.

	Sapporo (Japan)	Tokyo (Japan)	Dayton (U.S.A)	Watsonville (U.S.A)	Paris (France)	Montpellier (France)
Females	45 ±20.1	29.2 ±9.7	27.9 ±6.2	27 ±9.5	25.3 ±6.5	28 ±6.3
Males	39.8 ±10.2	30.6 ±7.8	29.9 ±3.9	29.8 ±7.1	24.8 ±5.1	29.6 ±5.3

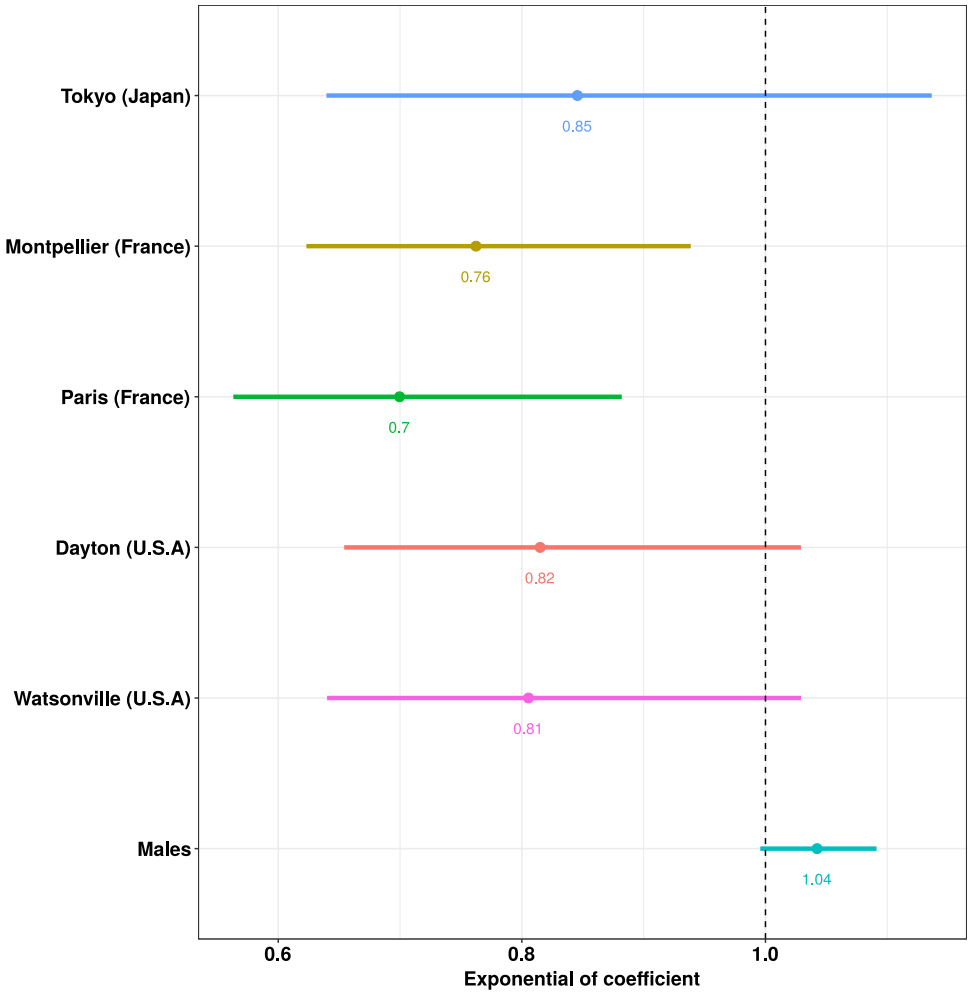

Fig. 1 Chill coma recovery time after cold exposure relative to Sapporo (Japan) population with associated confidence interval (0.95). The relative values can be interpreted as multiplicator effect compared to Sapporo reference. Values higher than 1 indicate a higher CCRT than Sapporo, while below 1, this indicate a reduced CCRT. As example, Paris (France) had a reduced CCRT (0.7) compared to Sapporo, *i.e.*, the CCRT is reduced of 30% (1-0.7). The intercept, which is the basal level of Sapporo is equal to 36.3 minutes. Male effect is the mean difference between females and males and was not significant.

The invasive genotypes from France and U.S.A presented the highest number of DE genes respectively of 544 (3.74%) and 616 (4.24%) compared to Japan with 299 (2.06%) (Table 2). Two third of genes were upregulated for both Japanese and French lines, while two-third were downregulated for the American line. A few numbers of DE genes were shared by all the lines (98) and are mainly upregulated (84/98) (Venn diagram in Fig. 3). DE genes shared between France vs Japan (88) and France vs U.S.A (89) was very similar, while Japan and U.S.A have a few numbers of shared gene (13). Gene ontology results of DE genes between

Table 2: Number of DE genes between genotypes and treatments. Pairwise comparisons between (A) untreated genotypes, (B) between treated and untreated flies within each genotype, and (C) in pairwise comparisons of cold-treated flies between different genotypes. The threshold for identifying DE genes was an adjusted p-value ≤ 0.01 and absolute log_2-fold-change ≥ 1. The proportion of DE genes is the percentage of DE genes in the expressed transcriptome (14538).

	Contrast	DE genes	Up-regulated	Down-regulated	DE rate (%)
A	France|U.S.A (control)	715	471	244	4.92
	France|Japan (control)	524	175	349	3.60
	U.S.A|Japan (control)	1023	208	815	7.04
B	Japan (cold|control)	299	199	100	2.06
	France (cold|control)	544	346	198	3.74
	U.S.A (cold|control)	616	189	427	4.24
C	France|Japan (cold)	45	16	29	0.31
	U.S.A|Japan (cold)	310	81	229	2.13
	France|U.S.A (cold)	80	68	12	0.55

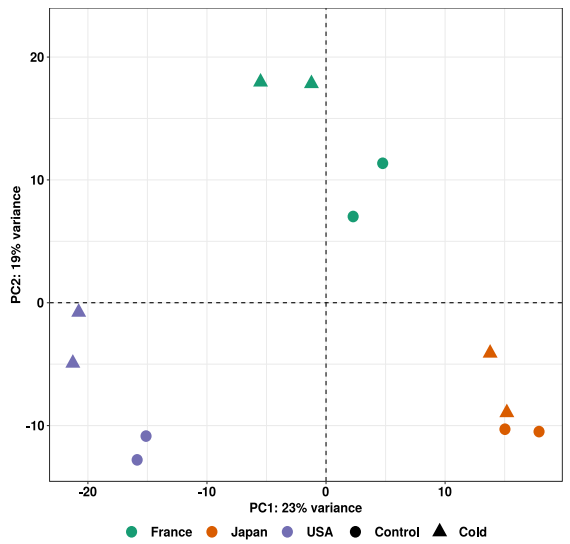

Fig. 2 PCA analysis using read counts from DESeq2. Dots correspond to the biological samples with the U.S.A in blue, Japan in red and France in green. Circles and squares correspond respectively to control and cold treatment.

cold and control showed different trends for each genotype (Fig. S2). The French genotypes (Fig. S2) responded with a major upregulation of genes, related to cold stress (abiotic, temperature stimulus), vesicle transport, multicellular process, immune system, protein folding or ageing. Similar process (stress response, immune system or response to stimulus) are present in the American genotype but also involved in circadian rhythm and rhythmic process (Fig. S2). Furthermore, only the American genotype exhibited downregulated genes related to replication, cell cycle, cell division and chromatin organisation (Fig. S2). The Japanese genotype was the one with the lowest number of enriched gene ontology terms, and they mostly showed an increase in genes related to cellular response to temperature and protein folding (Fig. S2). On the 98 common DE genes between the three genotypes, 67 were annotated had an annotated ortholog in *D. melanogaster*. Except for one gene with an unknown function, the others were regulated in the same way for all lines, *i.e.*, 14 downregulated (between -1.02 to -9.88 log_2FC) and 84 upregulated (between 1.02 to -9.65 log_2FC) (Fig. 4). Most upregulated genes were related to *Hsp* family (*Hsp70-23-68*) in a range of 3.1 to 6.1 log_2FC and also a gene encoding for a transcription factor A (8.8 to 9.6 log_2FC). Among downregulated genes, we identified the gene encoding for cytochrome-P450 (-1.7 to -9.1 log_2FC), *Smvt* gene (vitamin transporter, -1.15 to -3.1 log_2FC), *CG4563* (-1.3 to -3.9 log_2FC), *Root* (-1.1 to -2.1 log_2FC), *Ptx1* (-1.3 to -2.3 log_2FC). Gene ontology analysis revealed only one enriched term related to stress response.

Transcriptomic plasticity

Fig. 3 showed that the majority of genes induced by a cold stress were genotype dependent and a fraction of them were activated during stress response, but in a different way or magnitude depending on the genotype, which highlight the genotype by environment interaction (GEI). A total of 368 genes presented a GEI (Table 2) representing the plasticity of the transcriptome. This GEI was particularly high between USA and Japan (310 genes) by comparison with France vs Japan (45 genes) and France vs U.S.A (80 genes). It is interesting to note that the DE genes between the

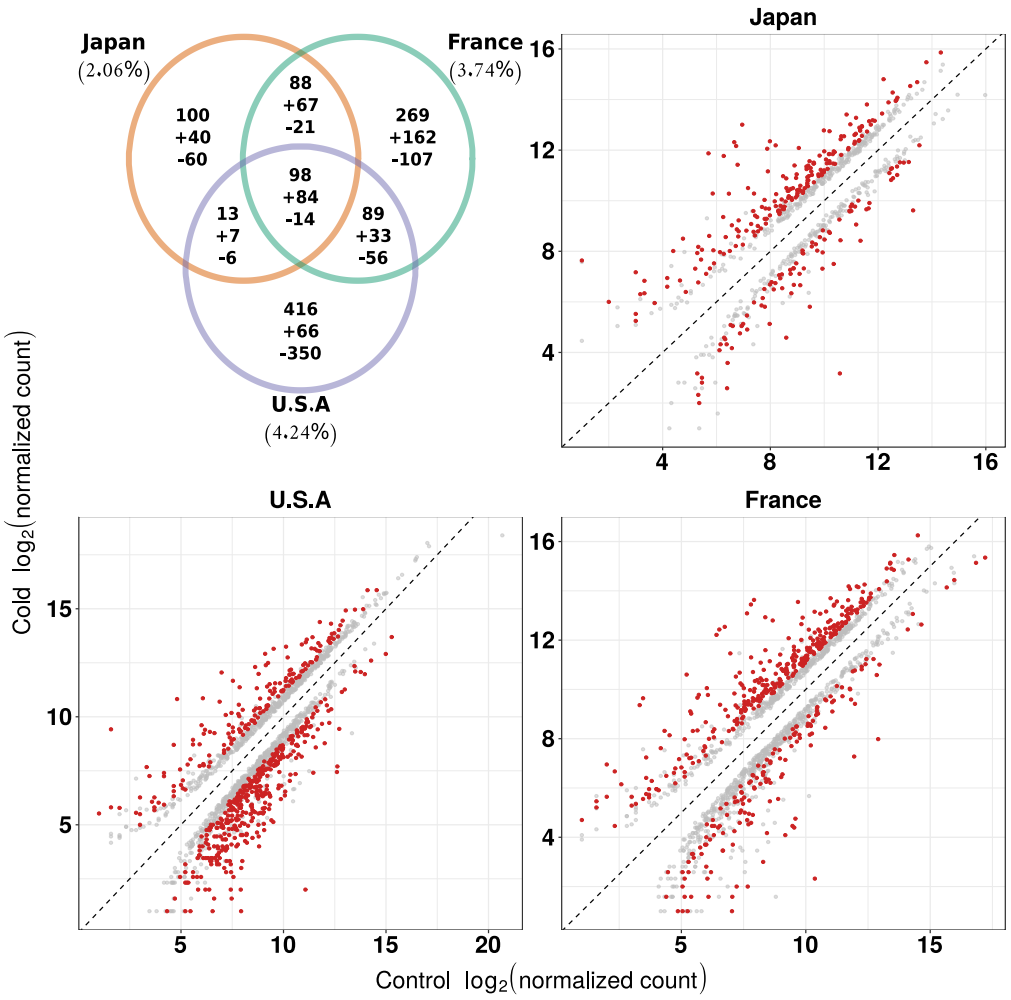

Fig. 3 Gene expression between control and cold conditions. A Venn diagram of shared and unique DE genes identified in comparisons of cold-treated and control flies within each population after cold exposure (top left). Scatter plots of log_2 normalized read count for France (top right), U.S.A and Japan (bottom panels) comparing control and treated flies. Individual genes are indicated by dots. Red colour corresponds to significant DE genes (see materials and methods).

two invasive genotypes (France vs USA) were predominantly up-regulated (85%) while for the other two contrasts, they were predominantly down-regulated (65 and 75% respectively for France vs Japan and USA vs Japan). On the total GEI DE genes, 67 were different from the two other strains, 4 between France and the others (3 unknown and 1 encoding for cytochrome-P450), 16 between Japan and the others with 4 annotated and 47 for U.S.A against the others with 26 annotated. Fig. 5 illustrates the kind of response observed for some genes. The first type of pattern is observed for *Bub1-related kinase* (*BubR1*), *cyclin dependent kinase-1* (*Cdk1*), *Nup98-96* (nucleoporin) and *Top2* (*topoisomerase 2*) (Fig. 5), corresponds to the case where Japan (in blue) was always upregulated during stress while U.S.A and France were down-regulated. The second type of pattern observed in *phosphoglycerate kinase* (*Pgk*) presented a convergence of the gene expressions of the three genotypes after cold stress, whereas they were very different in control environment. The third pattern observed in *COX5A* (*cytochrome c oxidase subunit*), *Prx2540*-1 (*Peroxiredoxin 2540*-1), *Tpi* (*Triose phosphate isomerase*), presented a situation where the gene expression was up-regulated after cold stress in U.S.A, downregulated in Japan and remained more or less stable in France. When analysing the enriched terms in genes with GEI effect, we didn't find a significant enrichment in all the contrasts concerning

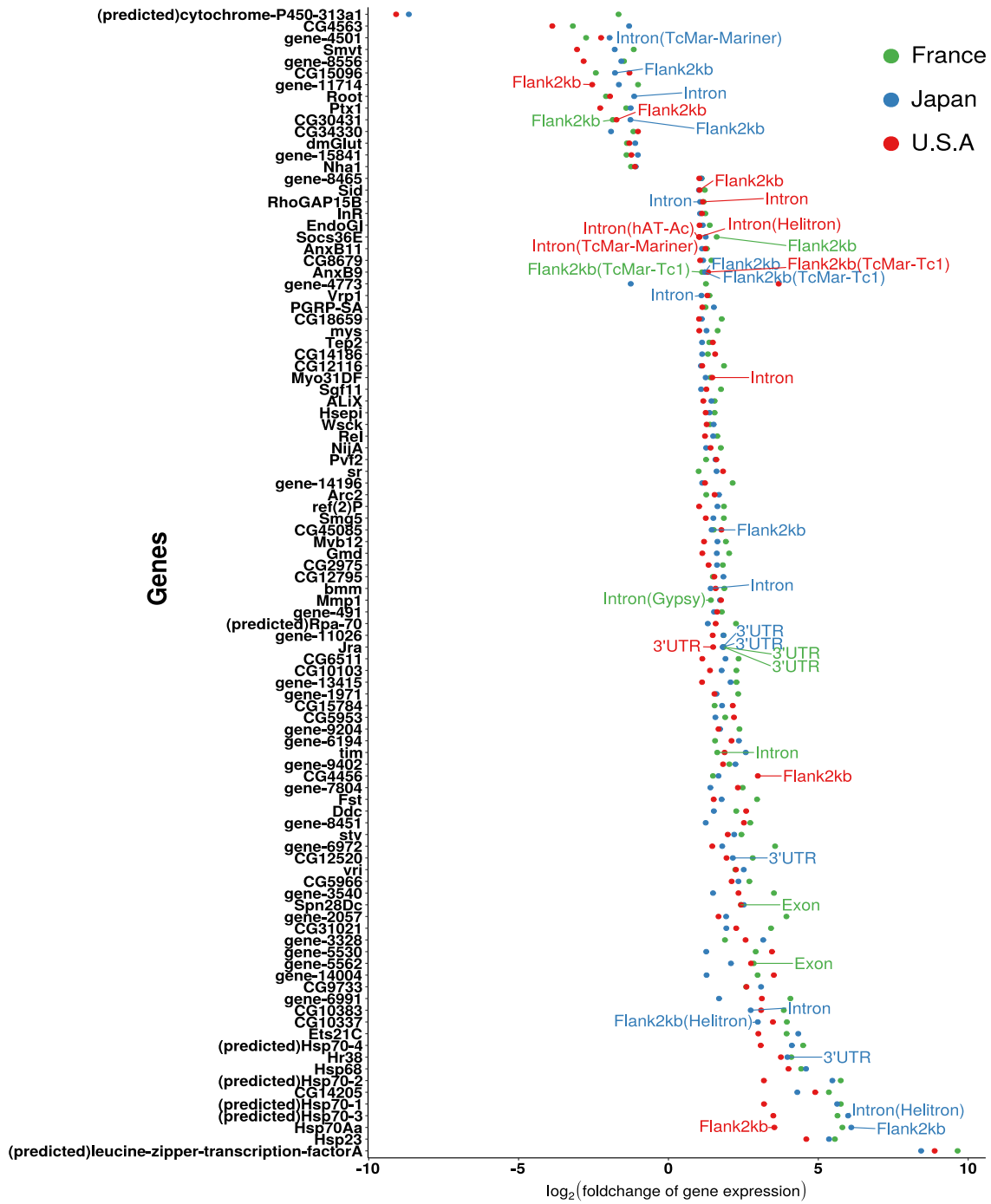

Fig. 4 DE genes (98) shared by all genotypes after cold exposure (U.S.A in red, France in green and Japan in blue). The plotted labels refer to a TE insertion (frequency ≥ 0.5) within a gene (intron, exon, 5' or 3'UTR) or within the 2kb flanking regions of a gene.

the France strain (vs Japan or U.S.A), except one term concerning catabolic process upregulated in France compared to the U.S.A. The remaining contrasts, *i.e.*, U.S.A vs Japan (Fig. S3) and especially the down-expressed genes in the American line (Fig. S3), had significant enrichment (p-value < 0.05). These terms

were mainly involved in cell cycle, DNA replication, response to DNA damages or chromatin re-organisation. Few terms are enriched in up-expressed genes in U.S.A (Fig. S3) all related to the metabolism of the ATP, carbohydrate or the metabolite precursors.

DE genes are depleted for TEs insertions in invasive genotypes

Environmental stress can induce the activation of TEs by modifying regulation mechanisms. TEs represent 33% of the *D. suzukii* genome and 1556 families were previously identified (Mérel *et al., in prep.*,[**?**]). Among these, the number of TE families DE between controlled condition and cold stress (Fig. 6, Table S2) varies from 7 in the USA (4 up-regulated), to 9 in Japan (8 up-regulated) and 31 in France (all up-regulated). The number of these families DE between genotypes after cold stress (GEI) was very low: 2 between France and Japan, 1 for the other two comparisons. We looked for TE insertions in the vicinity of DE genes (control vs cold and genes with GEI) for each of the three genotypes (see Marin *et al., in prep.*) for TE locations). Then, we tested the relation between TE insertion and gene expression states (DE or not, Table S3). We found that DE genes exhibit a lower number of TE insertions than expected int the French and the American genotypes. TEs insertions (176) near the DE genes (Table 3) were mainly in flanking (80) and intronic (85) regions. The 11 remaining insertions were detected in exons (4) with 1 in one upregulated gene in Japan and 3 in upregulated genes in France (1 annotated as *Spn28Dc*). Four TE insertions were in 3'UTR, with 1 shared by all genotypes in *Jra* (*Jun-related antigen*, encoding a transcription factor) always associated with upregulation of the gene. U.S.A genotype had 2 others TE insertions related to downregulated genes (one is *CG6834*) and Japan had also two insertions in *Hr38* and *CG12520*. From the previously 98 DE genes shared between the three genotypes (Fig. 4), 25 genes presented one or more TE insertions in the vicinity or inside the genes. From this, three were shared by all lines, namely the above mentioned *Jra*, that contained a Bel/Pao TE in 3'UTR. *CG30431* and *AnxB9* had an insertion in the 5' flanking region, that was not annotated for *CG30431* but in the case of *AnxB9* the TEs is a TcaMariner. DE genes presenting GEI were not depleted for TEs insertions except for the comparison between USA and Japan (p-value = $2.16E{-}04$, Table S3). In GEI DE genes, the distribution of the insertions was also mainly intronic (30) and in flanking regions (37) (Table 3). Two insertions in U.S.A vs Japan are in 5'UTR and 3'UTR respectively in *Rbf2* and *Caf1-180* gene (only in the Japanese genome) which presented a lower level expression in U.S.A than Japan. The Fig. 7 summarized the DE genes with GEI for which we detected TEs insertions (also Table S4). When comparing France and Japan, one gene is annotated (*MetRS-m*), with the functions related to oxidative stress response, lifespan of both fly and their photoreceptors neurons, cell proliferation in epithelial tissues [47]. One insertion in this gene is present only for France with a higher expression level than the Japan. Other genes mainly presented shared insertions with up to 5 insertions in the same gene (gene-8633). We observed an equivalent number of unique and shared TEs insertions between USA and France. France specific insertion are related to gene involved in proteolysis (*CG4053*, *CG30043* from flybase) or in oogenesis with *kelch*. Unique insertion in Japan are related to a DNA-binding transcription regulation gene (*byn*) a sodium ion transport (*ppk17*) and to *Hsp70aa*. Finally, U.S.A presented the higher number of genes presenting GEI, in contrast with Japan in which only a few genes with GEI contains TEs insertions. Insertions are mostly shared or only present in Japan. It is the only contrast in which some insertions are detected in UTR regions *e.g.* in *Caf1-180* (nucleosome assembly), *Rbf2* (transcription factor binding), *TpnC41C* (calcium ion binding) or *Nopp140* (ribosome assembly factor).

Table 3: TE insertions detected in DE genes. (A) between treated and untreated flies within each genotype, and (B) in pairwise comparisons of cold-treated flies between different genotypes. TEs were considered present only with a frequency ≥ 0.5.

	Contrast	Regulation	5'UTR	Exon	Intron	3'UTR	Flank2kb
A	France	down	0	0	8	0	11
		up	0	3	20	1	15
	Japan	down	0	0	21	0	10
		up	0	1	9	3	9
	U.S.A	down	0	0	14	2	24
		up	0	0	13	1	11
B	France|Japan	down	0	0	0	0	1
		up	0	0	4	0	2
	France|U.S.A	down	0	0	0	0	1
		up	0	0	6	0	8
	U.S.A|Japan	down	1	0	11	1	17
		up	0	0	9	0	8

Discussion

CCRT is a simple measure by which we can extrapolate climatic adaption in Drosophila at inter and intra-species levels [1, 30]. Cold resistance is associated with a clinal distribution among drosophila species but also between strains from different countries. A lower resistance to cold was also associated with a lack of genetic variation on key traits [48–50]. von Heckel et al. [49] studied the cold responses between *D. melanogaster* strains from both the ancestral (Africa: Zambia & Zimbabwe) and more recent invaded area (Europe: Sweden & Netherlands). They concluded to a higher cold resistance for European strains which they explained by a

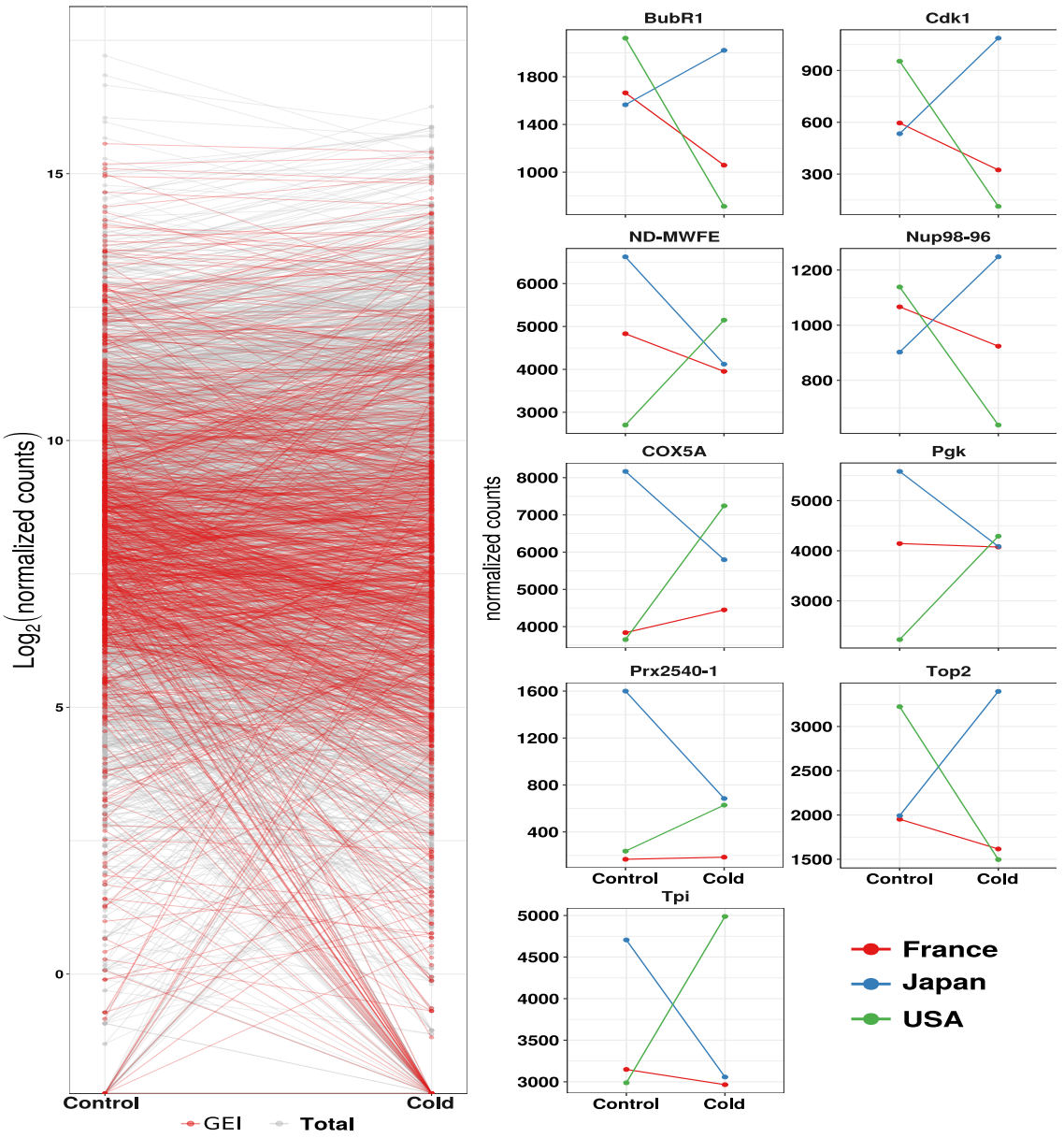

Fig. 5 Reaction norms between control and cold for DE genes using normalized log_2 read counts. (A) Reaction norms of all DE genes, with red for the GEI ones, while grey are DE genes without GEI. (B) Examples of 9 DE genes with GEI, with colours referred to the genotype (red for France, blue for Japan and green for the U.S.A)

higher canalization of the gene expression during cold. It was also hypothesised that invasive species could be more tolerant to environmental changes with, for example, a plastic response [7, 51–54]. Ayrinhac et al. [48] studied CCRT among some *D. melanogaster* populations. The value we observed in our experiences were more closed to the northern populations from his study. We have observed some differences between the populations we analysed, with Sapporo (Japan) from the ancestral location being more sensitive (30% more) than the French invasive population. American populations were not significantly different, but their

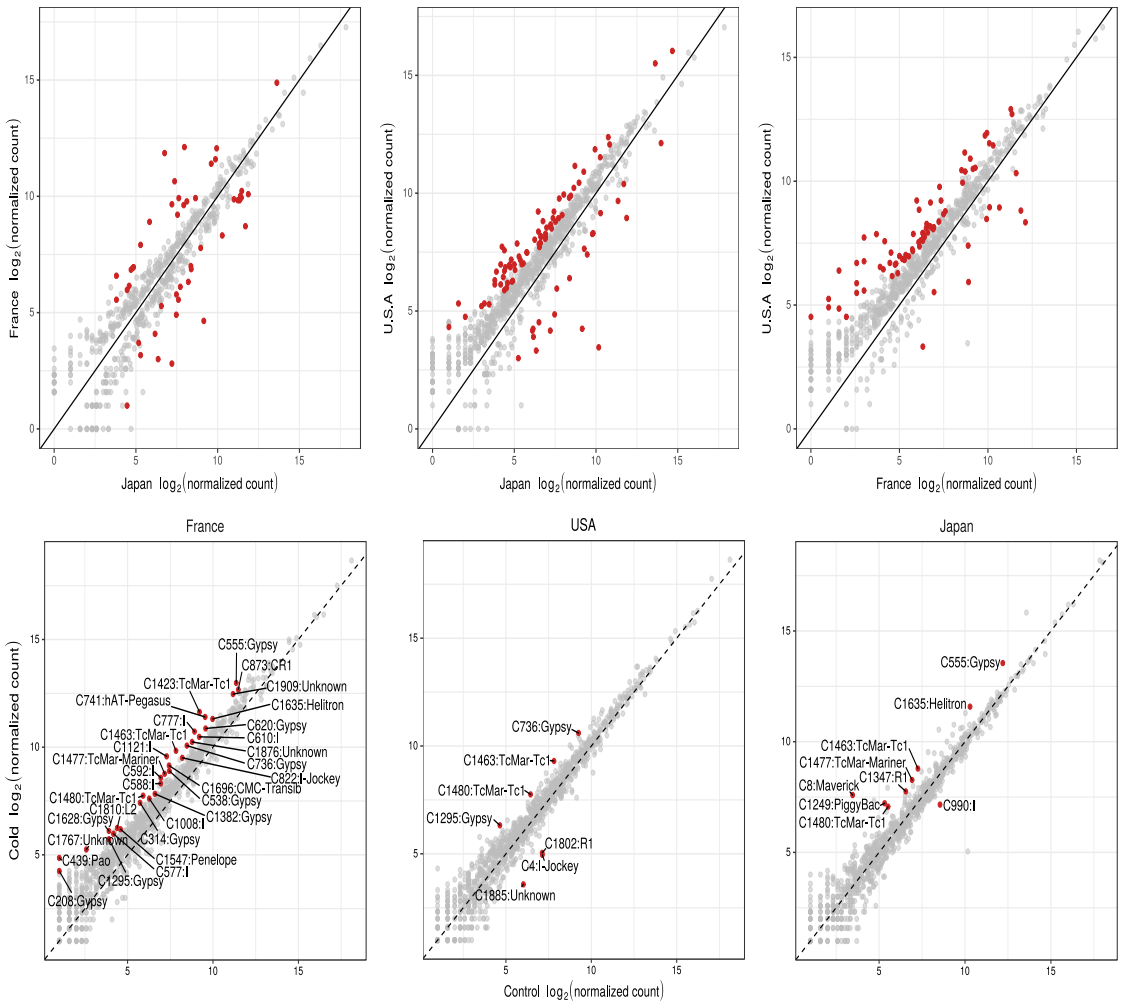

Fig. 6 TE expression between genotypes (upper panel) and between control and cold conditions for each genotype (bottom panel). Scatter plots represent the log_2-normalized read counts. Individual TE are indicated by dots. Red colour corresponds to significant DE TEs (see materials and methods).

mean value was more similar to the French population. Experimental design could partially explain difficulties to shrink estimated CCRT and others experiments on cold hardening on the invasive *D. suzukii* could help to confirm our observations. Experimental design could partially increase the variance in the measure which can explain difficulty to shrink estimated CCRT. Further experiments on cold hardening on the invasive *D. suzukii* could help to confirm our observations. *D. suzukii* are cold intolerant flies, which cannot survive to sub-zero temperature [55] despite acclimation steps, suggesting overwinter in adult stage in protected habitats. We briefly checked the average temperature in our sampled areas (data from the last 30 years, Table 4) to analyse pre-adaptation. Surprisingly, Sapporo is the only place with negative temperature during winter and the most sensitive to cold in our case. It was suggested that *D. suzukii* didn't presented pronounced cryoprotectant physiology, which could be in agreement with our results [56]. Our data suggest that phenotypic plasticity is more important in the invasive lines which allow to buffer cold stress than in native population, or a pre-selection event where only the more resistant flies to stress successfully invaded new areas. Further investigation needs to be done to characterise the phenotypic plasticity of *D. suzukii* among different populations and countries and elucidate if plasticity plays a role to cold tolerance and could explain invasive success. Sex is often associated with a difference of response during stress. We do not observe sex differences in resistance to cold. In *D. suzukii* other studies have led to contrasting re-

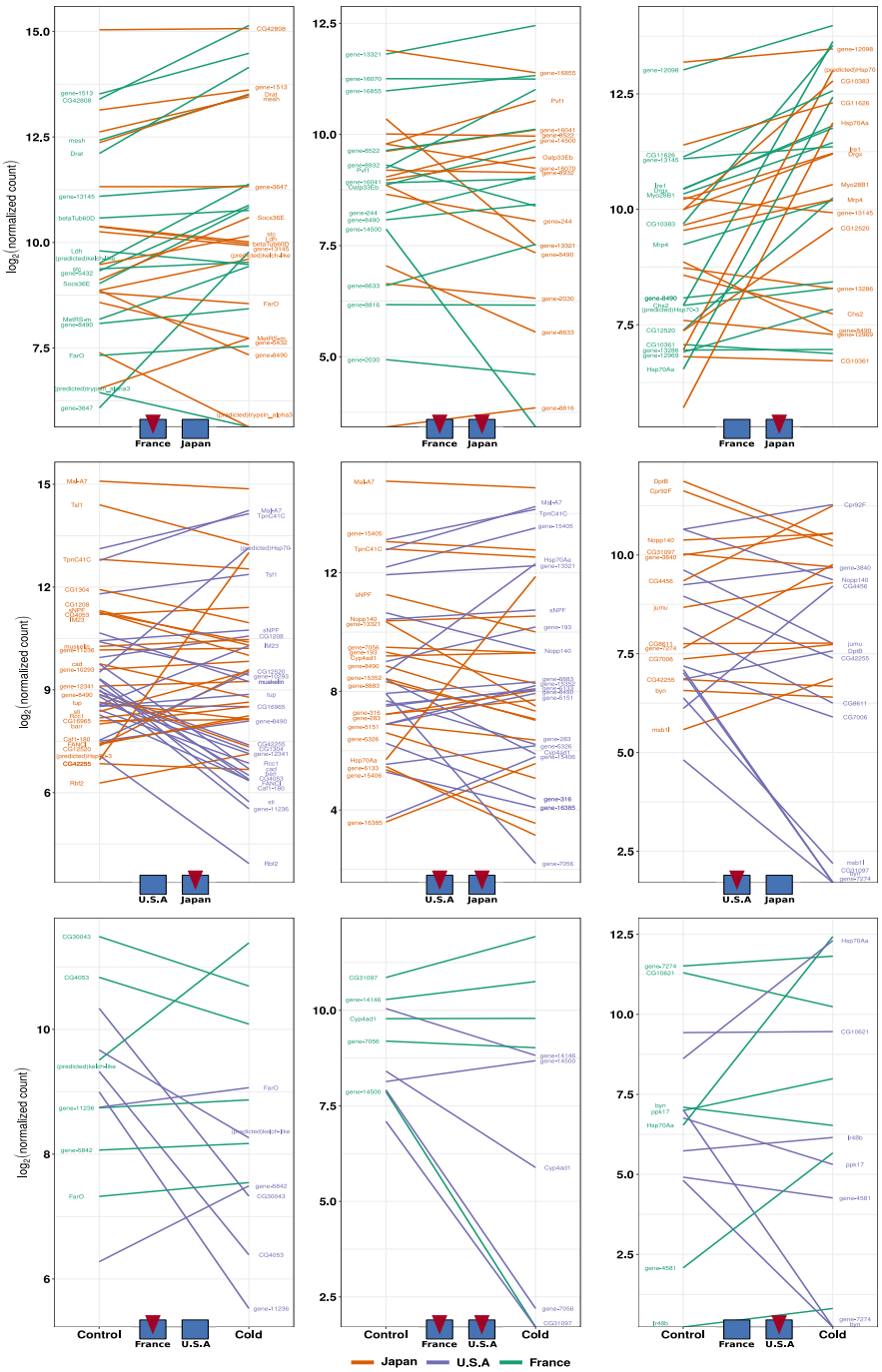

Fig. 7 DE genes in pairwise comparison between genotypes between control and cold treatment with inserted element (frequency ≥0.5). We plotted log_2 normalized counts between control and cold treatment, for each comparison (Japan versus France or U.S.A in top and middle and France versus U.S.A in bottom). Colours correspond to France (green), Japan (orange) and U.S.A (purple). The left and right plots are related to genes with insertions only present in one genotype of the comparison and middle plots are shared insertion for both genotypes.

Table 4: Thermal range in the different sampled areas (°C). Values are the most min and max daily average temperature on the year on the last 30 years (from worldweather.wmo.int except Montpellier on infoclimat.fr).

Location	Temperature (°C, min-max)
Sapporo (Japan)	-7-26.4
Tokyo (Japan)	2.5-31.1
Paris (France)	2.5-24.6
Montpellier (France)	3.2-29.5
Dayton (U.S.A)	1.8-27.3
Watsonville (U.S.A)	5.7-28.6

sults, with higher cold tolerance sometimes in males [57], sometimes in females [43, 55] and sometimes no difference [46]. Such variability of results also exists in *D. melanogaster* suggesting that cold tolerance is more dependent on the protocol used or the genotype tested than on sex [58, 59]. The following molecular analysis have been done on females only. Our results are the first to compare *D. suzukii* cold resistance for several isofemale lines, from three different continents, and especially by contrasting with ancestral location. We highlighted that the origin, more than the sex induced some variability in the cold stress response and need to be taking account when analysing resistance to this species.

Cold resistance involves a strong transcriptional plasticity

In order to identify gene expression responses to a cold stress, we choose one genotype from each continent (Watsonville U.S.A, Montpellier France and Sapporo Japan) which presented strong differences in their cold response (Watsonville: 20min, Montpellier:30min & 60min for Sapporo of CCRT). Transcriptomic analysis of cold stress experiment indicated that the amount of DE genes in Drosophila species vary in a range from 10 to 20% of the whole transcriptome [49, 60, 61]. These studies used a cut-off by adjusted p-value < 0.05. If we used the same criteria, we obtained same amount of DE genes between 10 (Japan) to 20% (U.S.A) depending of the genotype. We decided to use a more stringent cut-off in order to increase our chances of getting key gene during stress in this invasive species. Cold stress induced some mechanism to limit the injuries in Drosophila species. The literature highlighted 5 important mechanisms in cold adapted or acclimated flies related to ion and water balance regulation, resistance to oxidative stress, metabolic homeostasis, protein refolding and membrane fluidity [32, 45, 60, 62–64]. One of the first response correspond to protein refolding mediated by *Hsp* gene family [39, 40, 49, 60, 61, 63]. This genes are the first sensitive to a stress and, as previously described, we found a strong increase of their expression in all genotypes [44, 49, 60, 61, 63]. *Hsp* gene expression is often associated with a higher cold tolerance or reduced CCRT [32, 44]. However, recent analysis indicated that the *Hsp* expression is more related to repairing damage and indicate the level of damage in the flies [60, 65, 66]. In our analysis, 9 *Hsp* genes were DE with 4 *Hsp70* always upregulated in all the genotypes. Furthermore, a significant higher expression level was observed in Japan and France compared to the U.S.A and the log_2-fold-change of these genes was always ranked in the same way as the sensitivity, with the lower expression for the U.S.A, followed by the France and the Japan. This result in consistent with the literature and our data which indicates that our cold resistance measure by the CCRT is also linked to the level of *Hsp* gene. Other *Hsp* such as 23, 27, 83 were only DE in France and Japan also with a ranking by CCRT sensitivity. However, we only used 3 genotypes and we cannot generalize this relation between *Hsp* level and cold resistance, but we are in agreement with the literature. We also checked how genes related to ion and water regulation and also homeostasis could be modulated during cold stress [32, 63, 64, 67]. We identified several genes from the literature related to these mechanisms, with shared and different patterns between genotypes [32, 44, 60, 62]. We identified some genes related to glutamate transporter (*dmglut*) or sodium:proton transporter (*Nha*) which induced a cold tolerance when they are downregulated, which is the case for all the genotypes. Enriquez et al. [45] described gene expression during cold acclimation in *D. suzukii*. We didn't observe higher expression for gene related to ion transport or homeostasis, and our GO analysis is in agreement with this. Nevertheless, as Terhzaz et al. [68], we identified 2 genes encoding for diuretic neuropeptide and involved in homesostasis. Capability receptor (*CapaR*) encode a receptor for the Capa encoded neuropetide, which increase the fluidity in malpigian tube (by increasing calcium) and confers a better resistance to cold injury [68–70]. This gene was only DE in the Japanese genotype with a downregulation. Moreover, *Capa* gene is significantly upregulated in U.S.A only, despite a lower log_2-fold-change (0.8). The second gene is Diuretic Hormone 44 (*DH44*) which was significantly upregulated in invasive genotype but not in native Japanese. These results indicate that homeostasis seem to be more regulated in invasive than native genotype and could explain higher cold resistance observed by CCRT measures. Genes related to UDP-glycosyltransferases (*UGTs*) gene family involved in homeostasis and detoxification have also

been described in cold responses by an upregulation [60]. We observed that one gene (*UGT305A1*) is upregulated in France and USA, another *UGT317* is only upregulated in France while *UGT37A2* is only downregulated in U.S.A. Despite the *UGT37A2* expression pattern in the U.S.A, other UGT genes are only overexpressed in invasive than native genotype suggesting a role for cold resistance also. Other genes related to xenobiotic and oxidative stress are modulated during cold stress and seem to be implicated in cold tolerance [61, 63]. For examples, Glutathione-S-transferase gene family are overexpressed in France especially five different genes (*GstD1*, *D2*, *D5*, *D9* & *GstO3*). Other Japanese and American genotype had only one Gst gene (respectively GstD2 & GstS1) upregulated also. We could suggest that despite few differences in cold resistance between France and U.S.A, the genes involved in homeostasis, stress or detoxification seem to be more upregulated in France than in U.S.A, suggesting that French genotype has a stronger response to the cold stress. Finally, we checked for the genes involved in circadian rhythm suggested to be linked with cold acclimation (ref). Also, von Heckel et al. [49] observed that *cwo* (*clockwork orange*) is the only gene strongly DE between cold acclimated European *D. melanogaster* and an ancestral African strain [49, 65]. For the cwo gene, we observed a significant upregulation (1.7 log_2FC) only in invasive (France and U.S.A) genotypes. Despite a low log_2FC (0.8 & 0.93), this gene is also significantly different (FDR <0.01) when contrasting the French and American to the Japanese genotype. These results are in agreement with von Heckel et al. [49], where the more resistant genotypes had a higher expression level of *cwo*. Other genes related to the circadian clock (*dysc* & *pdp*) are also upregulated but log_2FC is higher than 1 only for the U.S.A. We highlighted that some differences are identified between the genotypes studied and despite the use of only one genotype per country, we observed some pattern between invasive vs native in their expression related to the cold resistance. This results above, the few numbers of shared gene compared to genotype specific expression, and the detection of GEI genes suggested that some local adaptation occurred between invasive and native genotype. Furthermore, we observed that most of the genes upregulated in France were linked to protein refolding and stress responses suggesting that difference occurred during the invasion of the U.S.A and France.

Local adaptation revealed by GEI genes

To better understand differences between genotypes, we focused our analysis on the gene fraction presenting a genotype by environment interaction (GEI). These genes are potentially the ones that can be associated to local adaptation, since each genotype produces a different response depending on the environment [71]. Local adaptation is a process by which individuals present a higher fitness in their local environment due to natural selection. A specific trait could be selected in a local population and environment as for cold tolerance in a *D. melanogaster* latitudinal gradient [1, 63, 72]. This involves the ability to deal with local temperature linked with geographical range limitation. After cold stress, the two genotypes with the highest number of DE genes (310) are Japan vs USA, while this number is the lowest (45) in Japan vs France. This can also be seen in the GO analysis where only Japan vs U.S.A contrast presented enriched terms that were related to DNA replication, cell cycle or chromatin organisation down-expressed in U.S.A compared to Japan. This suggests that the American strain would be less affected by the cold stress than the Japanese, and this GO terms are the same in U.S.A the specific analysis of down-expressed genes. The few numbers of annotated genes in the other contrasts could also explain the absence of GO enrichment. Despite the invasive status of France and U.S.A and the same response at phenotypic level, the local environment drastically influenced molecular responses between invasive strains. In a recent paper, Rota-Stabelli et al. [73] compared European and North American strains at genetic and phenotypic level [73]. They concluded that some points indicated evolutionary divergence between the two continents (*e.g.*, mutation rate, TEs copy number) and we confirmed it on the transcriptomic response during cold stress. One caveat related to genome expression analysis is related to our ability to identify biological relevant differences between conditions. We have applied a threshold (FDR < 0.01 and absolute log_2FC > 1) to identify DE genes, but its possible that less important differences would still be relevant. Genes presenting GEI are often placed in the upstream part of the regulatory networks and may be sensitive to very small differences in expression and were probably not considered in our analysis. Other examples indicated that genes with GEI are regulated by transcriptional factors, with cis or trans- regulation [74–78], and also by microRNAs, which have been identified as playing a role on the transcriptome plasticity in variable temperature environments in *D. melanogaster* [74].

Stress did not induce an increase of TEs expression

Environmental stresses have been reported to induce an increase of TE expression and eventually transposition [19, 79]. This burst of transposition could increase the genetic diversity on which selection could act. In a recent review, several examples were presented of TEs

family activation after stress, but it clearly depends on the type of stress and of the TE family [16, 80]. Also, Horváth et al. [16] suggested that under stress condition some TEs could be repressed just after the activation, indicating that stress could induce both activation and repression. Cold stress and TEs expression are not very well documented but some studies focused on plant systems. It was shown that some TEs modify nearby gene expression during cold stress in *A. thaliana*, rice or maïze for example [81–83]. In these examples, only few TE families could modify the expression up to 20% of nearby genes [83]. One example is also described in *D. melanogaster* where a *roo* insertion near a gene induced a higher cold resistance [84]. However, examples of cold resistance enhanced by TEs remain rare and are in general specific to some families [85]. We did not observe an increase of TEs expression after cold exposure in all the genotypes, even though *D. suzukii* harbors more than 30% of TEs. The French genotype presented the higher number of TEs (31 families all upregulated) compared to the others, what was considerably higher than what was found for the same genotype in response to oxidative stress. Further investigation could consider these specific families to study their link with gene regulation. This result is somehow surprising, as a much more important number of TE families are DE between genotypes in control conditions, suggesting that TE in *D. suzukii* are expressed and potentially active.

TEs inserted in GEI genes highlight candidate genes to local adaptation

If a majority of TEs insertions are removed by natural selection, some insertions could represent occasions to adaptive evolution to occur and could be retained during evolution. This has been found in several organisms especially in stressful environments [16, 19, 20, 23, 29, 64, 84]. Moreover, in a recent paper, Baduel et al. [86] described overaccumulation of TEs after whole genome duplication in *A. thaliana* and observed an enrichment of TEs insertions especially in environmental sensitive genes, which could contribute to local adaptation [86]. In another study, Li et al. [87] screened adaptive TE from 201 genome of *A. thaliana* but only 2 elements where associated with potential adaptive effect [87]. The consequence of TEs are still not well understood and we tested if the insertions could affect the gene expression under stress environment as a cold stress. We observed a depletion of TE insertions in DE genes which suggest that, in *D. suzukii*, TEs insertions are eliminated from the genome by selection, especially on genes important for cold stress response. The analysis of the TE insertions in genes that were consistently DE for all genotype show that only one gene had a fixed insertion in all genotypes. Further analysis would be necessary to conclude on the effect of the TE insertions in gene expression changes. More interesting are the genes that present a GEI, since we could hypothesis that the direction of the changes in expression could be favoured by the presence of the TE insertion. Further analysis are need to understand the fine molecular mechanism responsible for changes in gene expression for this category of genes. Moreover, difference between genotypes could be explained by genetic or epigenetic variability, inducing difference of expression by cis-regulation [7, 26, 88].

Conclusion

To our knowledge, this is the first time that a integrative analysis was made to study phenotypic responses to a cold stress on a recent invasive species followed by a transcriptomic analysis, for several cold resistant genotype from several locations. Furthermore, we investigated the potential effect of cold stress on TE expression and their regulatory consequences on nearby genes. Populations from invasive areas were more resistant than native ones. This difference was also observed at the molecular level with a different amount of DE genes and associated function. The transcriptomic study on invasive genotypes suggest a higher plasticity of the transcriptome in invasive genotype. The stress response was more genotype than environmental dependant with a lower number of shared gene after cold exposure. A significant number of genes presented an interaction genotype by environment (GEI) and further investigation for both this gene and near TEs insertion could be investigated especially by scan of genetic variant (SNP) to deeper understand fine variability between genotypes. Investigation of global pathway using all DE genes without a minimum of expression level could reveal what gene are important in the regulatory network during stress which we have not kept.

Materials and methods

Origin of *Drosophila suzukii* lines and experimental procedure

Wild living *D. suzukii* females were collected in 2014 in the native area (Japan: Sapporo and Tokyo) and two invaded areas (U.S.A: Watsonville and Dayton, France: Montpellier and Paris) (Table S5). Gravid females were isolated in culture vials to establish isofemale lines. Flies were reared on modified "Dalton" medium (Table S6) in a controlled environment: 22.5°C ±1°C, 70% ±5% RH and a 16:8 (L/D) [43]. Chill cold recovery time (CCRT) was measured after a stress of 16 hours at 0°C on a total of 28 isofemales lines (2 to 7 lines per population) with 16 to 26 flies per line (males and females). In total, CCRT was recorded for

4661 flies. Since age is known to influence CCRT in *D. melanogaster*, we controlled this factor by using 4-7 days old flies [89]. Vials containing flies of each line were first placed in an isolating polystyrene box containing ice (0°C ±0.5) and stored in a cold room at 4°C. After a few minutes, the flies went into a chill coma and we were able to place them individually in a 48 well plate. The plates were placed on the ice during the whole procedure which lasted between 5 to 10 minutes. The plates were than left in the ice during 16h, without light. After 16h, individuals were removed from cold environment to return at room temperature (22°C ±1.0). CCRT was measured by monitoring the time necessary for an adult to stand on its legs (see [89]). Preliminary essays indicated that *D. suzukii* wake-up occurred before 60 minutes, then, we decided to censor after 1-hour time limit. The experiments have been performed in different dates and by several man experimenters. These two parameters were included into the statistical model.

CCRT analysis

CCRT analysis was made in two steps on R software (3.6.0, [90]). First, we fitted several distributions (normal, Weibull and logistic) on log transformed times, using fitdistcens function from the fitdistrplus package (v.1.0-14, [91]). We used this approach to take into account right censored data (after 60 minutes of recording). Normal distribution was selected after graphical comparison with others and by the log-likelihood values of models. The distribution was summarized for each sample (every line, sex, date of recording and experimenter) by mean and standard deviation. Then a linear mixed model was fitted to those means using lmer function from lme4 (v.1.1-21, [92]) and p-values were estimated using lmerTest (v.3.1-0, [93]) with population as a fixed factor and lines, dates and experimenters as the random factors. We previously added the sex in interaction with the population, but the model selection indicated a fewer contribution for this term, so we added as only an additive factor, to simplify the analysis. The model coefficients after exponential transformation were reported with their confidence intervals (0.95) in Table S1 and in Fig. 1. Those effects can be interpreted as multiplicative effect on the mean recovery time compared to the reference chosen here as the group of Sapporo (Japan) population (*e.g.*, Montpellier (France) has a value of 0.77 which indicate a recovery time of 0.77 compared to the Sapporo reference centered 1, or also a reduced of time of 1-0.77 = 0.23 or 23%). Normality and homoscedasticity of residuals and normality of random effects were confirmed graphically after logarithmic transformation of means recovery times.

RNA extraction and sequencing

We selected three isofemale lines from the three continents to perform transcriptomic analysis (S29, W120 and MT47 respectively from Sapporo (Japan), Watsonville (U.S.A) and Montpellier (France)). Fifteen 4-7 days old females per replicate were exposed as previously described for the CCRT experimentation (16h to 0°C). Then, flies returned to 22°C ±1.0 during 1h before to be dissected (to keep only the somatic tissue) on ice with phosphate buffer saline solution, then frozen in liquid nitrogen and stored at -80°C. We produced 2 biological replicates per treatment and line (*i.e.*, 12 samples). We used the RNAeasy Plus Mini Kit (Qiagen) to extract total RNA from carcasses following the protocol provided by manufacturer. Samples were treated with DNAse (ref AM2224, AMbion™) according to manufacturer instructions and stored at -80°C. RNA amount and quality was checked using Qubit™ (Thermo Fisher Scientific) and 2100 Bioanalyser instrument (Agilent). RNA libraries and sequencing were performed by the IGBMC sequencing platform (Strasbourg, France), a member of the 'France Génomique' consortium (ANR-10-INBS-0009). Libraries were constructed using the TruSeq® Stranded mRNA Library Prep Kit following manufacturer's recommendations. The libraries were sequenced on Illumina High HiSeq 4000 with paired-end 100 base pair long reads.

Transcriptome analysis

Between 69.13 to 99.69 million of pair-end reads were generated on the 12 libraries, quality was assessed using FastQC (v. 0.10.1, [94]), a trimming step was made with UrQT (v. 1.0.17, minimum phred score of 20, [95]) and quality was confirmed with FastQC. RNA data were mapped on reference genome of *D. suzukii* produced by Prud'hommes laboratory [96] using HISAT2 (v. 2-2.1.0, [97]) and read counts on genes were computed with eXpress [98]). A reciprocal BLASTN (2.2.26, [99]) was made between *D. suzukii* genes and FlyBase, a database of Drosophila genes and genomes (archive data: $FB2018_06$). Another BLASTX was made for no matched genes with the previous BLASTN, on the nr database from NCBI. Matched hits for BLASTX was tagged with the term "(predicted)". On the 16905 predicted gene from the genome, 8428 matched with Flybase database and others 478 on the nr database (52.7% of total genes). Differential expression analysis was made using DESeq2 package (v. 1.24.0, [100]) on R (v. 3.6.0). Expression levels were analysed to measure the differences, between genotypes, mediated by the environment (control and cold), and the genotype environment interaction (GEI). The lfcShrink function was used to estimate log_2-fold-change and identify differentially expressed (DE) genes using the ashr R package [101].

DE genes were those with an FDR-adjusted p-value below 0.01 and absolute log_2-fold-change > 1. The coefficient of variation (CV, standard deviation/mean) on normalized counts was computed for each genotype, between control and cold. Cold experiment was made in same time of another studied stress (paraquat), previously described (Marin *et al.*, *in prep.*). The analysis of the differences between genotypes in untreated condition had already been described in this previous paper, so the informations are available in Marin *et al., in prep.*. Transcriptomic experiment was made in same time for both cold and oxidative stress. This last was previously described in another publication (Marin *et al., in prep.*). We briefly summarized results from untreated condition, but more details are available in Marin et al., (*in prep.*). In control environment, we observed variability between the genotypes (3.6 to 7.0% of the transcriptome DE) with 70% of the DE genes under 2 log_2-fold-log_2change (log_2FC). The gene ontology analysis was made to investigate the genotype differences related to the local adaptation where genotypes evolved. The analysis suggested a similarity between invasive genotypes (France and U.S.A) with fewer number of enriched terms. Contrary to observed in the invasive genotypes, the Japanese is different from the others with some enriched GO terms. These terms were mainly related to translation, protein metabolic process, ribosome biogenesis, response to hyperoxia and immune response (antibacterial related), downregulated in invasive strains (Marin *et al., in prep.*).

Transposable element (TE) identification and expression analysis

The locations of genomic TEs insertions in the three genotypes (S29, W120 and MT47) were previously identified (Marin *et al.*, *in prep.*). Briefly, using a custom TE library (Mérel *et al., in prep.*) obtained from the reference genome [96], we identified the TE insertions with Popoolation-Te2 [102]. From the output of PopoolTE2 we retain fixed insertions when insertion frequency was higher than 0.8 and absent when insertion frequency was inferior to 0.5. Using the gene annotation of the reference *D. suzukii* genome we have identified TEs insertions present inside genes (exon, intron, 5' and 3' UTR) and ±2kb flanking regions (see Marin *et al., in prep.*).

TE expression analysis

TE expression was quantified using the TEcount module from the TEtools software [103]. Briefly, TEcount sums reads aligned against copies of each TE family annotated from the reference genome creating an output table of expression arranged by TE family [96]. Differential expression of TEs between cold-treated and control flies for each isofemale line was computed using a merged file with the RNA counts for genes and TE families, and following normalization using DESeq2.

Gene ontology analysis

We performed a GO enrichment analysis directly on the geneontology.org website, using homologs in *D. melanogaster* to discover over or underrepresented gene functions from the lists of DE genes [104]. P-values were calculated using a Fisher test for enriched GO terms and adjusted with the Benjamini-Hochberg correction for multi-testing [105]. GO terms with FDR $\leq$0.05 were defined as significantly enriched. The GO terms were reduced to representative non-redundant terms using the REVIGO tool and manual curation [106].

Competing interests

The authors declare that they have no competing interests.

Author's contributions

P. M. produced data, conceived and wrote the manuscript draft. C. V. & P. G. designed experiments, edited the manuscript. J. P. trimmed NGS data and help in bioinformatic analysis. A. J. calibrated experimental design and produced data. M.L. D.M. reorganized, corrected statistical analysis and revised manuscript. M. G.F. produced gene ontology analysis. V. M. produced all related TEs informations (genome annotations, frequency insertions).

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List of supplementary tables and figures

Additional Fig. S1 — Distribution of coefficient of variation (%) for DE genes in the genotypes (France, Japan and U.S.A).
Additional Fig. S2 — Gene ontology enrichment analysis for all genotypes after cold exposure.
Additional Fig. S3 — Gene ontology enrichment analysis for genotype pairwise comparison after cold exposure.
Additional Table S1 — Summary of linear mixed model for chill coma recovery time.
Additional Table S2 — Number of TE family differentially expressed between genotypes and treatments.
Additional Table S3 — Contingency table of DE gene ($DE^{+/-}$) and inserted elements ($TE^{+/-}$).
Additional Table S4 — Genes with significant GEI effect and detected TEs insertions.
Additional Table S5 — Origin of isofemale lines of *D. suzukii*
Additional Table S6 — Recipe of diet medium modified

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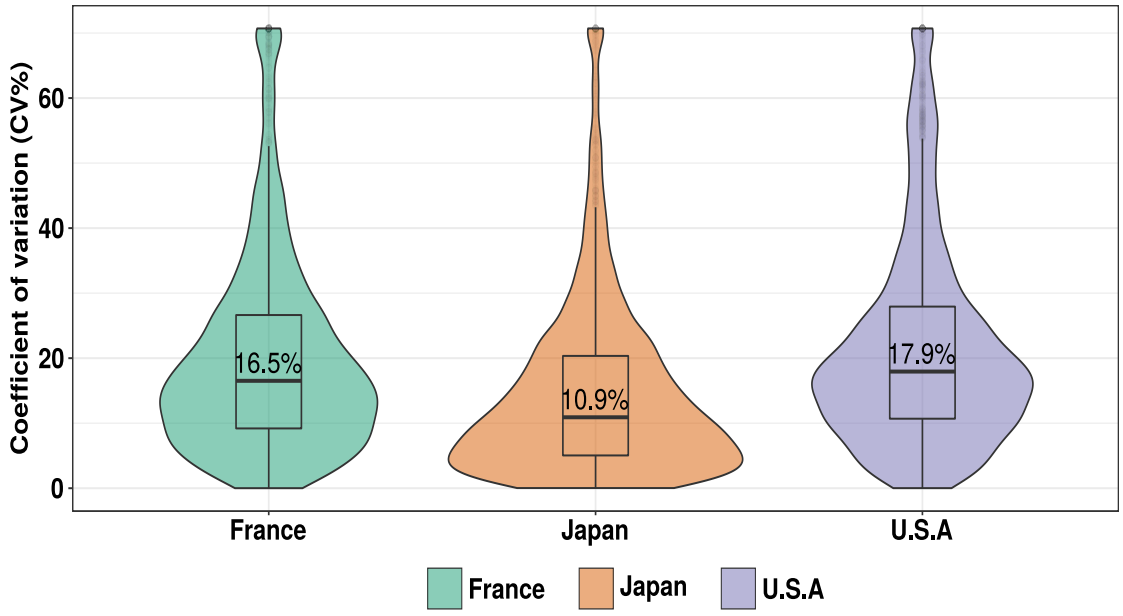

Fig. S1 Distribution of coefficient of variation (%) for DE genes in the genotypes (France, Japan and U.S.A). Coefficient of variations were calculated using the standard deviation and mean on control and cold values. Central values correspond to the median. Paired Wilcoxon test are significant in all comparisons (p.value ¡ 0.05).

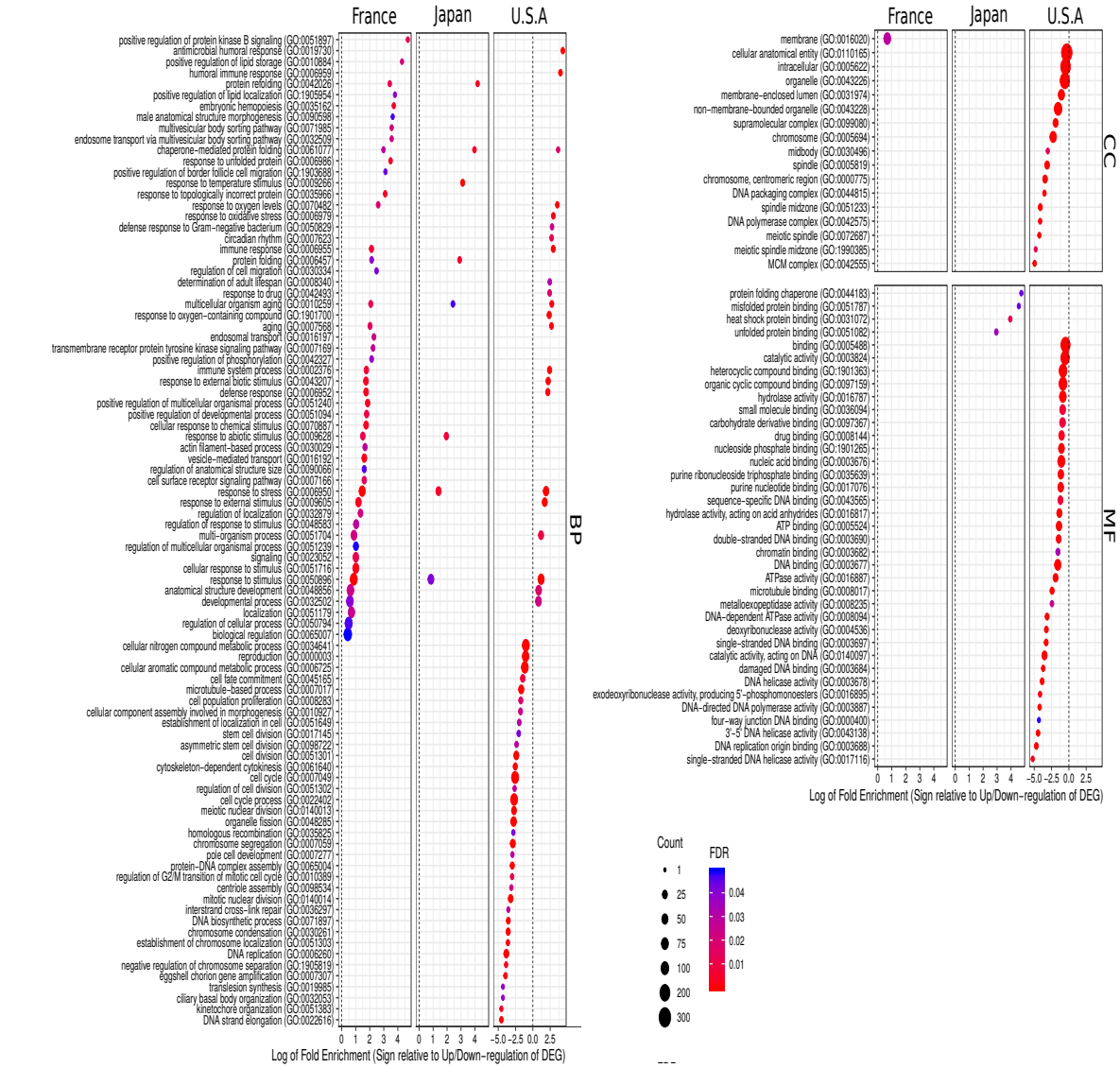

Fig. S2 **Gene ontology enrichment analysis for all genotypes after cold exposure. BP: Biological Process; CC: Cellular Components; MF: Molecular Function.** The size of dots corresponds to the number of genes in each category and the colour to the FDR (only terms with p.value ¡ 0.05 are presented). Gene ratio (x axis) correspond to the number of genes from our data compared to genes within a GO term. Down-regulated in a genotype are symbolized with negative gene ratio and a positive gene ratio for up-regulated genes.

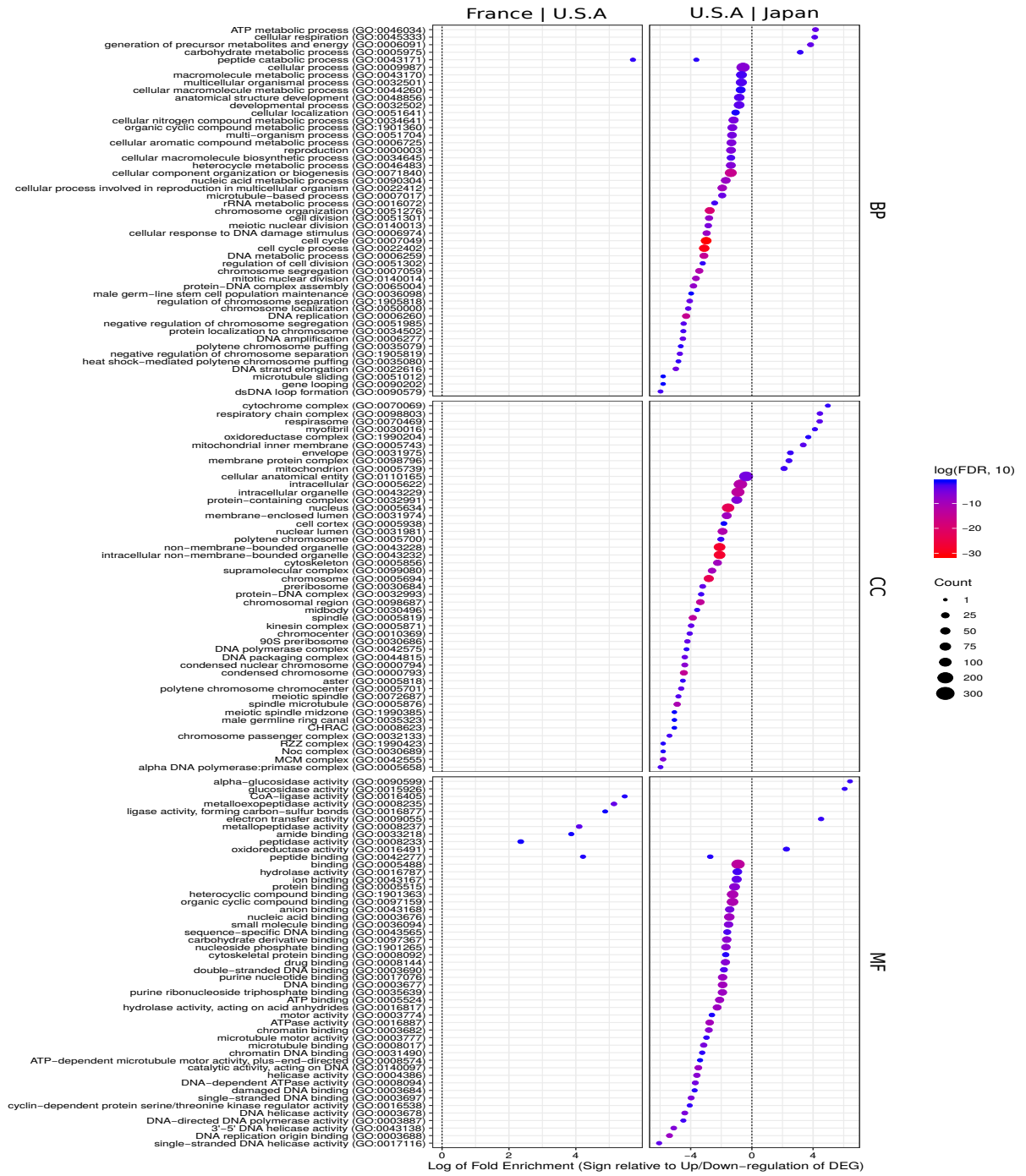

Fig. S3 Gene ontology enrichment analysis for genotype pairwise comparison after cold exposure. BP: Biological Process; CC: Cellular Components; MF: Molecular Function. The size of dots corresponds to the number of genes in each category and the colour to the FDR (only terms with p.value ¡ 0.05 are presented). Gene ratio (x axis) correspond to the number of genes from our data compared to genes within a GO term. Down-regulated in a genotype are symbolized with negative gene ratio and a positive gene ratio for up-regulated genes.

Table S1: **Summary of linear mixed model for chill coma recovery time.** Model is centered on the Females and Sapporo (Japan) city and data were previously log transformed.

Factor	Coefficient	exp(coef)	exp(CI2,5)	exp(CI97,5)	Std.error	df	Tvalue	Pr(>|t|)
Males	0.0414875	1.0423602	0.9957236	1.0911883	0.0230610	76.11	1.799031	0.0759779
Tokyo (Japan)	-0.1677253	0.8455861	0.6395516	1.1365070	0.1397431	24.26	-1.200240	0.2416361
Dayton (U.S.A)	-0.2045594	0.8150063	0.6541613	1.0291084	0.1105659	26.11	-1.850114	0.0756477
Watsonville (U.S.A)	-0.2163620	0.8054437	0.6400758	1.0291578	0.1150136	24.21	-1.881186	0.0720243
Paris (France)	-0.3572115	0.6996245	0.5631185	0.8820250	0.1087840	27.06	-3.283676	0.0028306
Montpellier (France)	-0.2713542	0.7623464	0.6231118	0.9386228	0.1000671	23.28	-2.711723	0.0123646

Table S2: **Number of TE family differentially expressed between genotypes and treatments.** Pairwise comparisons between (A) untreated genotypes, (B) between treated and untreated flies within each genotype, and (C) in pairwise comparisons of cold-treated flies between different genotypes. The threshold for identifying DE TEs was an adjusted p.value ≤ 0.01 and absolute log_2-fold-change ≥ 1. The proportion of DE TEs is the percentage of DE TEs in the expressed transcriptome (1556).

Contrast	DE TEs	Up-regulated	Down-regulated	DE rate (%)
France|U.S.A (control)	78	10	68	5.01
France|Japan (control)	48	22	26	3.08
U.S.A|Japan (control)	92	70	22	5.91
Japan (cold|control)	9	8	1	0.58
France (cold|control)	31	31	0	1.99
U.S.A (cold|control)	7	4	3	0.45
France|Japan (cold)	2	2	0	0.13
U.S.A|Japan (cold)	1	0	1	0.06
France|U.S.A (cold)	1	1	0	0.06

Table S3: **Contingency table of DE gene ($DE^{+/-}$) and inserted elements ($TE^{+/-}$).** Observed and expected insertion numbers are indicated for (A) genes in each genotype after cold exposure or (B) in pairwise comparison between genotypes after cold exposure. P-value were adjusted using the Benjamini-Hochberg method. TEs were considered present only with a frequency ≥ 0.8.

	Contrast	DE	obs(TE-)	obs(TE+)	est(TE-)	est(TE+)	padj
A	France	DE-	13982	2379	14025	2336	1.52e-06
		DE+	509	35	466	78	
	Japan	DE-	14012	2594	14024	2582	9.44e-02
		DE+	265	34	253	46	
	U.S.A	DE-	13877	2412	13941	2348	3.34e-12
		DE+	591	25	527	89	
B	France|Japan	DE-	13763	3097	13766	3094	3.84e-01
		DE+	40	5	37	8	
	France|U.S.A	DE-	13907	2918	13915	2910	7.92e-02
		DE+	74	6	66	14	
	U.S.A|Japan	DE-	13577	3018	13605	2990	2.16e-04
		DE+	282	28	254	56	

Table S4: **Genes with significant GEI effect and detected TEs insertions.** Foldchange corresponds to the difference in pairwise comparison (contrast) between genotypes after cold exposure with associated adjusted p-value. TE information are related to the location of the insertion in the genome, the family and sub-family when available and the genotype were the insertion frequency is ≥ 0.5.

gene symbol	log2FoldChange	Padj	Contrast	Location	TEs family	TEs sub-family	Genotype insertion
gene-13321	3.296537	0.0000173	FrancevsJapan	Intron	NA	NA	France|Japan
gene-14500	-22.766859	0.0000000	FrancevsJapan	Flank2kb	NA	NA	France|Japan
gene-244	1.082941	0.0015759	FrancevsJapan	Intron	BEL-14_-DEl-I	Pao	France|Japan
gene-3647	3.338811	0.0003358	FrancevsJapan	Intron	NA	NA	France
MetRS-m	1.253194	0.0062150	FrancevsJapan	Flank2kb	NA	NA	France
gene-8490	1.568408	0.0000131	FrancevsJapan	Intron	Mariner-3_Dro	TcMar-Tc1	Japan
gene-8490	1.568408	0.0000131	FrancevsJapan	Flank2kb	NA	NA	France|Japan
gene-8490	1.568408	0.0000131	FrancevsJapan	Intron	Copia-2_-DYa-I	Copia	France
gene-8633	1.351592	0.0073052	FrancevsJapan	Exon	NA	NA	France|Japan
gene-8633	1.351592	0.0073052	FrancevsJapan	Flank2kb	Gypsy-33_-DEl-I	Gypsy	France|Japan
gene-8633	1.351592	0.0073052	FrancevsJapan	Intron	Gypsy-2_-DKi-I	Gypsy	France|Japan
gene-8633	1.351592	0.0073052	FrancevsJapan	Intron	NA	NA	France|Japan
gene-8633	1.351592	0.0073052	FrancevsJapan	Intron	Jockey-1_-DEu	I-Jockey	France|Japan
gene-11236	3.056016	0.0000105	FrancevsUSA	Intron	NA	NA	France
byn	1.949532	0.0074285	FrancevsUSA	Flank2kb	NA	NA	U.S.A
(predicted)kelch-like	2.945773	0.0000001	FrancevsUSA	Flank2kb	NA	NA	France
gene-14146	1.234126	0.0015794	FrancevsUSA	Intron	NA	NA	France|U.S.A
gene-14500	-22.728827	0.0000000	FrancevsUSA	Flank2kb	NA	NA	France|U.S.A
CG30043	2.076761	0.0000002	FrancevsUSA	Flank2kb	NA	NA	France
CG31097	10.012267	0.0000231	FrancevsUSA	Intron	NA	NA	France|U.S.A
CG4053	1.614953	0.0019973	FrancevsUSA	Flank2kb	NA	NA	France
gene-4581	1.953724	0.0062221	FrancevsUSA	Flank2kb	NA	NA	U.S.A
ppk17	1.531587	0.0062670	FrancevsUSA	Intron	NA	NA	U.S.A
gene-7056	4.791224	0.0000065	FrancevsUSA	Flank2kb	Helitron-N2_DBi	Helitron	France|U.S.A
gene-7274	2.087427	0.0099339	FrancevsUSA	Intron	NA	NA	U.S.A
gene-7274	2.087427	0.0099339	FrancevsUSA	Intron	NA	NA	U.S.A
Cyp4ad1	2.249800	0.0000291	FrancevsUSA	Flank2kb	NA	NA	France|U.S.A
Hsp70Aa	1.322001	0.0075020	FrancevsUSA	Flank2kb	NA	NA	U.S.A
gene-10293	-1.003783	0.0003048	USAvsJapan	Intron	NA	NA	Japan
jumu	-1.542164	0.0072462	USAvsJapan	Flank2kb	Harbinger-2_DRh	PIF-Harbinger	U.S.A
gene-11236	-3.203387	0.0000163	USAvsJapan	Flank2kb	Mariner-1_DF	TcMar-Mariner	Japan
gene-12341	-1.240123	0.0015052	USAvsJapan	Intron	Copia-2_-DYa-I	Copia	Japan
Nopp140	-1.008612	0.0054519	USAvsJapan	3'UTR	NA	NA	U.S.A
Nopp140	-1.008612	0.0054519	USAvsJapan	Flank2kb	PARISa_-DSu	TcMar-Tc1	U.S.A|Japan
CG42255	-1.079953	0.0014640	USAvsJapan	Flank2kb	NA	NA	Japan
CG42255	-1.079953	0.0014640	USAvsJapan	Intron	NA	NA	U.S.A
byn	-4.226951	0.0016181	USAvsJapan	Flank2kb	NA	NA	U.S.A

gene-13321	2.814820	0.0000543	USAvsJapan	Intron	NA	NA	U.S.A|Japan
Rcc1	-1.236033	0.0087513	USAvsJapan	Intron	NA	NA	Japan
sti	-4.299961	0.0000002	USAvsJapan	Intron	Helitron-1_DF	Helitron	Japan
gene-15352	2.210957	0.0005771	USAvsJapan	Flank2kb	Mariner-4_DAn	TcMar-Tc1	U.S.A|Japan
gene-15405	1.056440	0.0077937	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-15405	1.056440	0.0077937	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-15405	1.056440	0.0077937	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
gene-15405	1.056440	0.0077937	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-15405	1.056440	0.0077937	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-15405	1.056440	0.0077937	USAvsJapan	Intron	Copia-2_-DYa-I	Copia	U.S.A|Japan
gene-15405	1.056440	0.0077937	USAvsJapan	Intron	CR1-5_-DRh	CR1	U.S.A|Japan
gene-15406	2.600362	0.0035826	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-15406	2.600362	0.0035826	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
gene-15406	2.600362	0.0035826	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-15406	2.600362	0.0035826	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-15406	2.600362	0.0035826	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-15406	2.600362	0.0035826	USAvsJapan	Intron	NA	NA	U.S.A|Japan
Rbf2	-3.273245	0.0004509	USAvsJapan	5'UTR	hAT-6_-DRh	hAT-Ac	Japan
gene-16385	-1.842476	0.0083080	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
CG1208	1.376369	0.0001014	USAvsJapan	Intron	NA	NA	Japan
gene-193	1.907095	0.0000885	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
gene-283	-1.402809	0.0078160	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
gene-283	-1.402809	0.0078160	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-283	-1.402809	0.0078160	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-283	-1.402809	0.0078160	USAvsJapan	Intron	R1-2_DEu	R1	U.S.A|Japan
gene-316	-1.720290	0.0018201	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
gene-316	-1.720290	0.0018201	USAvsJapan	Intron	NA	NA	U.S.A|Japan
TpnC41C	1.124468	0.0000487	USAvsJapan	5'UTR	NA	NA	U.S.A|Japan
TpnC41C	1.124468	0.0000487	USAvsJapan	Intron	Helitron-2N1_DVir	Helitron	U.S.A|Japan
TpnC41C	1.124468	0.0000487	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
TpnC41C	1.124468	0.0000487	USAvsJapan	Intron	NA	NA	U.S.A|Japan
TpnC41C	1.124468	0.0000487	USAvsJapan	Intron	NA	NA	U.S.A|Japan
TpnC41C	1.124468	0.0000487	USAvsJapan	Intron	Gypsy-5_-DRh-LTR	Gypsy	Japan
TpnC41C	1.124468	0.0000487	USAvsJapan	Intron	Gypsy-9_-DEu-LTR	Gypsy	U.S.A|Japan
CG31097	-6.437234	0.0003101	USAvsJapan	Intron	NA	NA	U.S.A
Cpr92F	1.301102	0.0066037	USAvsJapan	Intron	Mariner-1_DF	TcMar-Mariner	U.S.A
CG4053	-1.524466	0.0019066	USAvsJapan	Flank2kb	NA	NA	Japan
CG7006	-1.078352	0.0076418	USAvsJapan	Intron	NA	NA	U.S.A
gene-5151	1.052500	0.0073796	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-5151	1.052500	0.0073796	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-5151	1.052500	0.0073796	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
gene-5151	1.052500	0.0073796	USAvsJapan	Intron	Jockey-5_-DAn	I-Jockey	U.S.A|Japan
gene-5326	1.802625	0.0002679	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-5326	1.802625	0.0002679	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-6133	1.823502	0.0054519	USAvsJapan	Intron	NA	NA	U.S.A|Japan
gene-6133	1.823502	0.0054519	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
gene-6133	1.823502	0.0054519	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan

gene-6133	1.823502	0.0054519	USAvsJapan	Intron	BEL-3_-DBi-I	Pao	U.S.A|Japan
msb1l	-3.341862	0.0028642	USAvsJapan	Intron	NA	NA	U.S.A
cad	-1.638992	0.0005969	USAvsJapan	Flank2kb	NA	NA	Japan
barr	-2.637136	0.0003015	USAvsJapan	Intron	Copia_LTR	Copia	Japan
gene-7056	-4.775971	0.0000061	USAvsJapan	Flank2kb	Helitron-N2_DBi	Helitron	U.S.A|Japan
sNPF	1.171204	0.0020012	USAvsJapan	Intron	BEL-21_-DTa-I	Pao	U.S.A|Japan
sNPF	1.171204	0.0020012	USAvsJapan	Intron	NA	NA	Japan
gene-7274	-7.252668	0.0004675	USAvsJapan	Intron	NA	NA	U.S.A
gene-7274	-7.252668	0.0004675	USAvsJapan	Intron	NA	NA	U.S.A
IM23	1.020780	0.0072976	USAvsJapan	Intron	NA	NA	Japan
FANCI	-2.136577	0.0011812	USAvsJapan	Flank2kb	NA	NA	Japan
Cyp4ad1	-2.388876	0.0000128	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
muskelin	-1.161202	0.0033218	USAvsJapan	Flank2kb	NA	NA	Japan
Mal-A7	1.075391	0.0087614	USAvsJapan	Intron	NA	NA	U.S.A|Japan
Mal-A7	1.075391	0.0087614	USAvsJapan	Intron	I-5_DBi	I	Japan
gene-8490	1.939201	0.0000001	USAvsJapan	Intron	Mariner-3_Dro	TcMar-Tc1	Japan
gene-8490	1.939201	0.0000001	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
gene-8883	1.420790	0.0000398	USAvsJapan	Flank2kb	Harbinger-2_DRh	PIF-Harbinger	U.S.A|Japan
gene-8883	1.420790	0.0000398	USAvsJapan	Intron	NA	NA	U.S.A|Japan
Hsp70Aa	-1.950249	0.0009190	USAvsJapan	Flank2kb	NA	NA	U.S.A|Japan
(predicted)Hsp70-3	2.006042	0.0003101	USAvsJapan	Intron	Helitron-N2_DBi	Helitron	Japan
Caf1-180	-1.924261	0.0070919	USAvsJapan	3'UTR	Mariner-16_DRh	TcMar-Tc1	Japan
CG8611	-1.432928	0.0015313	USAvsJapan	Flank2kb	NA	NA	U.S.A

Table S5: **Origin of isofemale lines of *D. suzukii* sampled in 3 countries (Japan, U.S.A and France) with their location and invasive status.**

Location	Coordinates	Status	Lines
Sapporo. Hokkaido. Japan	43° 3' 43.545"N 141° 21' 15.754" E	Native	S11, S20, S24, S29
Tokyo. Honshu. Japan	35° 41' 22.155" N 139° 41' 30.143" E	Native	T20, T21
Watsonville. California. U.S.A	36°54'51.8"N 121°45'27.7"W	Invasive	W106, W112, W120, W127
Dayton. Oregon. U.S.A	45° 13' 14.422" N 123° 4' 34.368" E	Invasive	Sok1, Sok28, Sok58, Sok76, Sok80
Paris. France	48° 51' 23.81" N 2° 21' 7.998" E	Invasive	L6, L7, L9, L21, L22, L26
Montpellier. France	43° 36' 38.768" N 3° 52' 36.177" E	Invasive	MT3, MT7, MT15, MT18, MT20, MT25, MT47

Table S6: Recipe of diet medium modified from Dalton et al. [43]

Distilled water: 1 L
Agar (Drosophila Agar Type, ref.66-103, Apex™): 9 $g.L^{-1}$
Cornmeal (Farine de gaudes, Moulin Giraud): 33 $g.L^{-1}$
Ethanol 96%: 40 $ml.L^{-1}$
Yeast (ref.75570, LYNSIDE®): 17 $g.L^{-1}$
Sugar (supermarket sugar) 50 $g.L^{-1}$
Nipagin (Tegosept,ref.20-258, Apex™): 4 $g.L^{-1}$
Bring to boil agar, cornmeal, yeast extract and sugar in distilled water. Then wait out of the fire about 10 minutes until the mixture cooled to 53°C before adding diluted nipagin in 96% ethanol. Medium is then poured in vials and cooled at room temperature before to be stored at 4°C.

CHAPITRE 3

Differences in phenotypic and transcriptomic responses to stress is related to population geography in an invasive species

Avant-propos

Les traitements phytosanitaires font parti des changement globaux et sont largement utilisés dans le monde. De ce fait, beaucoup d'organismes sont soumis à ces stress chimiques. Un des traitements les plus utilisés comme herbicide est le paraquat. Son effet pro-oxydant est bien connu chez *D. melanogaster* en particulier. Son usage est réglementé en Europe avec une interdiction depuis 2007, soit un an avant la première détection de *D. suzukii* en Europe. Cependant son usage est toujours légal aux États-Unis et au Japon. En appliquant une approche similaire à l'étude de la réponse thermique au chapitre 2, nous avons tout d'abord caractérisé la diversité phénotypique entre les six populations en étudiant la survie en condition contrôle et traitée au paraquat. En analysant l'espérance de vie en condition contrôle des populations, nous avons observé qu'à l'exception d'une population américaine (Watsonville), les populations invasives ont une longévité accrue par rapport aux populations japonaises. De façon surprenante, le stress oxydant a révélé que les populations invasives venant de Montpellier et Watsonville était plus sensibles que celle de Sapporo au Japon. Nous avons donc un effet génotype par environnement important. Au niveau moléculaire, les populations américaines et françaises, présentent un nombre de gènes important et majoritairement surexprimés avec toujours peu de gènes en commun. De même que pour le froid, le stress oxydant ne semble pas influer sur l'expression des ETs chez *D. suzukii*. Nous avons identifié des gènes dont l'expression est dépendante du génotype et de l'environnement avec des insertions uniques à un génotype particulier. Par ailleurs une analyse complémentaire en site de reconnaissance pour des facteurs de transcriptions (séquences reconnues par des protéines pour modifier l'expression des gènes) a révélé que plusieurs séquences d'ETs à proximité de gènes présentaient des sites de reconnaissance pour des régulation au stress oxydant.

IN PREPARATION TO GENOME BIOLOGY

Differences in phenotypic and transcriptomic responses to stress is related to population geography in an invasive species

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Abstract

Background: Adaptation to rapid environmental change must occur over short time scales. Studies of invasive species may provide insight into the underlying mechanisms of rapid adaptation as these species have repeatedly encountered and successfully adapted to novel environmental conditions. Here we investigated how invasive and non-invasive populations of *D. suzukii* deal with an oxidative stress at both the phenotypic and molecular level. We also investigated the impact of transposable element insertions on gene expression differences, observed between the populations and in response to stress.

Results: Invasive populations lived longer in the untreated condition than non-invasive Japanese populations. As expected, lifespan was greatly reduced following exposure to paraquat, but this reduction varied among genotypes (a genotype by environment interaction, GEI) with invasive genotypes appearing more affected by exposure than non-invasive genotypes. In our analysis of gene expression, we detected a large number of expression differences distinguishing the populations in the untreated environment. While a small core set of genes were differentially expressed by all genotypes following paraquat exposure, much of the response of each population was unique. Interestingly, we identified a set of genes presenting genotype by environment interaction (GEI). Many of these differences may reflect signatures of history of past adaptation. Transposable elements (TEs) were not activated after oxidative stress and differentially expressed (DE) genes were significantly depleted of TEs.

Conclusion: In the decade since invasion from the south of Asia, invasive populations of *D. suzukii* have diverged from populations in the native area indicating rapid adaptation to local environment.

Keywords: *Drosophila suzukii* ; invasive species ; oxidative stress ; transposable elements ; environmental changes ; genotype by environment interaction

Introduction

Rapid environmental change, particularly related to human activity, can decisively affect living organisms, who must respond to them in a short time. Understanding the mechanisms underlying these rapid responses is challenging and could help predict organism and species survival in the face of global environmental change. The rapid adaptation of invasive species to new environments, some quite different than ancestral environments, may provide insight into such mechanisms [1, 2] including hormonal regulation of suites of traits, or epigenetic gene regulation [3–6]. Phenotypic plasticity, *i.e.*, the ability of a genotype to express different phenotypes in different environments, is a possible explanation of invasive species success, particularly in the case of founder populations depleted of genetic variation [4–6]. Genetic diversity could rapidly increase following environmental stress if there is an activation of transposable elements (TEs) or if epigenetic control is disturbed. TEs, which are repeated sequences that can move around genomes, were discovered by B. McClintock in the 50' [7]. Depending on where TEs insert in the genome, they can affect the fitness of the organism. The vast majority of new TE insertions are neutral or deleterious, and purifying selection is expected to remove them or favor their silencing [8–10]. However, some TE insertions may be advantageous and

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facilitate adaptation in different environments [3, 11–20]. Such adaptive effects have been previously observed in response to both biotic (*e.g.*, virus infection) and abiotic (*e.g.*, oxidative stress) stress [13, 21]. Moreover, stress-induced changes in the epigenetic regulation of TEs, which is often sensitive to environmental cues [12, 18, 22], could rapidly generate potentially advantageous changes in nearby gene regulation and facilitate rapid adaptation to environmental stress [10, 19]. Here, we examined variation in the stress response of invasive and non-invasive populations of *Drosophila suzukii* with an analysis of molecular mechanisms potentially underlying the observed phenotypic differences. *D. suzukii* is an Asian species of the melanogaster group that invaded North America and Europe in 2008 [23–27]. Outside of Asia, *D. suzukii* is now found in both North and South America, and throughout most of Europe, from southern Spain easterly into Poland, Ukraine and Russia [23–26]. As *D. suzukii* has spread throughout the world, it has encountered and successfully colonised many different, potentially stressful environments. Paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride) is one of the most widely used herbicide in the world [28, 29]. Exposure to paraquat leads to the production of ROS (reactive oxygen species) and has often been used in the lab as a proxy to study oxidative stress [30–33]. Resistance to oxidative stress has been associated with extended lifespan [30, 34, 35], a trait possibly under selection during invasion of a new area. Furthermore, paraquat has been banned since 2007 in Europe but is still used in the U.S.A and Japan. In this study, we compared field-sampled *D. suzukii* genotypes collected in their native area of Japan with genotypes collected in invaded areas in the U.S.A and France. For each genotype, we measured lifespan in both the presence and absence of paraquat exposure, where we identified an effect of genotype and a genotype-by-environment interaction effect (GEI). We went further by examining the transcriptomic response of single genotypes from each location along with analysis of TE expressions. We found substantial differences among genotypes in patterns of gene expression related to oxidative stress that may underly our observed phenotypic differences and reflect population history. This work highlights the local adaptation to environmental conditions of the genotypes in a short time scale.

Results

Among population variation for lifespan and oxidative resistance

As expected, oxidative stress had a strong negative effect on survival, with an average decrease in lifespan of 80% when paraquat was present in the medium (multiplicative coefficient of 0.20, Fig. 1). Median lifespans of flies are presented in Table 1 for each population, sex and treatment and statistical analysis of survival is presented in Fig. 1 and Table S1. Sex differences in lifespan and changes in lifespan in response to stress are present in some species. However, we did not find a main effect of sex or any significant interactions with sex in our preliminary statistical model (see materials and methods). Therefore, sex was removed from subsequent analysis. In the untreated condition, flies from the two Japanese populations had the shortest lifespan and were not significantly different. For flies sampled in the United States, those from Watsonville had a median lifespan very similar to the Japanese populations and were not different from the reference Sapporo population (Fig. 1, value = 1.01, corresponding to about 1% greater lifespan than the reference Sapporo population). However, flies from Dayton lived the longest (value=1.44, a 44% relative increase). The two populations collected in France lived on average 25-28% longer than flies in the Sapporo population (1.25 and 1.28 for Paris and Montpellier, respectively). The decline in lifespan following paraquat treatment was variable among populations (genotype by environment interaction). Compared to the Sapporo reference population, there were non-significant reductions in resistance of 7 and 9% in populations from Tokyo (multiplicative effect of 0.93) and Paris (0.91) respectively. Genotypes sampled in Dayton (1.13) lived 13% longer than those from the reference Sapporo population, although this difference was not significant. Populations from Watsonville and Montpellier were significantly more sensitive to paraquat treatment, with reductions in lifespan of 26% and 20% respectively (multiplicative effects of 0.74 and 0.80 in Fig. 1). We observed a low but significant correlation among genotypes for lifespan across the two environments (r=0.28, p-value = $3.3e^{-4}$, Fig. S1).

Transcriptomic variability among genotypes

We quantified gene expression of three genotypes, one from each geographical sampling location (Montpellier (MT47): France, Watsonville (W120): U.S.A & Sapporo (S29): Japan), hereafter referred by the country where flies were sampled. We choose these three genotypes because of their difference in lifespan. A Principal Component Analysis (PCA) (Fig. 2) clearly showed genotype-specific clustering, independent of the treatment. To evaluate variation in the transcriptomic response of each genotype to paraquat treatment, we computed the coefficient of variation (CV) for each differentially expressed (DE) genes between control and treated flies (Fig. S2). Paired Wilcoxon tests comparing expression in the two treatment groups for DE genes between genotypes were

Table 1: **Median lifespan (days) by sex, treatment, and population with bracketed 0.25 & 0.75 quantiles.** Values were calculated from estimated median (see materials and methods) at population level (line effects estimation as random effect was negligible (SD = 0.12) from the linear mixed model).

	Sapporo (Japan)	Tokyo (Japan)	Montpellier (France)	Paris (France)	Dayton (U.S.A)	Watsonville (U.S.A)
Females control	32.0 (26.0-39.8)	32.0 (29.6-41.0)	40.5 (35.3-46.3)	41.0 (34.5-49.3)	51.4 (39.7-54.6)	34.8 (27.4-42.4)
Females paraquat	6.8 (5.0-9.1)	5.3 (5.0-7.7)	5.5 (5.2-6.3)	7.1 (5.4-8.2)	9.1 (8.7-9.8)	5.1 (3.8-6.5)
Males control	32.6 (24.65-37.85)	33.2 (27.32-44.43)	39.4 (36.69-44.41)	39.9 (36.48-48.3)	40.4 (39.46-46.96)	33.9 (26.51-38.92)
Males paraquat	6.1 (4.85-7.57)	7.0 (5.89-8.2)	7.5 (6.66-8.08)	7.6 (6.81-9.02)	12.0 (10.78-12.91)	4.4 (3.16-5.17)

all significant (p-value < 0.01). The number of differentially expressed (DE) genes identified, (i) in pairwise comparisons between genotypes in control conditions, (ii) in comparisons between untreated and oxidative stressed conditions for each genotype, and (iii) in pairwise comparisons between genotypes following paraquat treatment are presented in Table 2. We found that the distribution and values of the CV are in agreement with the distribution of DE genes shown in the Table 2, suggesting that the difference in DE gene proportions between the genotypes are due to biological variation and not a bias of statistical power.

Genotypic variation in gene expression in untreated flies

Pairwise comparisons of gene expression of untreated flies between the three genotypes revealed 715 DE genes between France and U.S.A (4.92% of the total transcriptome), 524 between France and Japan (3.6%), and 1023 between U.S.A and Japan (7.04%) (Table 2 and Fig. S3). Most of these DE genes (70%) had an absolute log_2-fold-change below 2 (Fig. S3) and only 60 had an absolute log_2-fold-change > 5. To further examine these DE genes, we performed a Gene Ontology analysis (Fig. 3). The rationale was to identify transcriptomic differences possibly related to adaptation of the different genotypes to their respective environments. In the comparison of France vs U.S.A, there were fewer enriched terms (all of them up-regulated in France) compared to the number of enriched terms from comparisons between France and Japan or U.S.A. In comparisons with France and U.S.A, enriched terms were always down-regulated in Japan. These results suggest a greater similarity between the two invasive genotypes, France and U.S.A. The greater enrichment of GO terms in comparisons between Japan and either the U.S.A or France suggests this population is extremely different than the two. We detected 44 GO terms shared between the invasive genotypes (France, U.S.A) in comparison with the non-invasive Japan genotype. These terms were mainly related to translation, protein metabolic process, ribosome biogenesis, response to hyperoxia, and immune response (antibacterial related). All of these terms were down-regulated in the invasive genotypes (U.S.A or France) when compared to the non-invasive Japan genotype. Enriched terms present only in the comparison U.S.A vs Japan were also similar for enrichment with the aforementioned terms. We also detected other functional terms in molecular function (MF) that seemed to be specifically down-regulated in the U.S.A genotype (so they appear in both U.S.A vs Japan and France vs U.S.A results): carbohydrate transport and energy metabolism. It is plausible that these functions are compromised in the U.S.A genotype. Taken together, these enrichment analyses suggest transcriptomic differences in translation, protein metabolic process, ribosome biogenesis, response to hyperoxia, and immune response (antibacterial related), which have been down-regulated in invasive genotypes compared to the non-invasive Japanese genotype.

Oxidative stress induces genes upregulation in invasive genotypes

We compared changes in gene expression between flies in control and oxidative conditions (Fig. 4) and identified a total of 659 DE genes across the 3 genotypes (Table 2). The Japan genotype had the fewest DE genes (122 genes, representing 1.10% of the transcriptome) in response to paraquat treatment, followed by the U.S.A (281 genes, 2.46%) and France (531 genes, 4.51%). Of all DE genes, most were upregulated (435/659). When comparing DE genes among genotypes, we observed that fewer genes were shared between Japan and the other two genotypes (Fig. 4), with respectively 4 and 23 genes uniquely shared with U.S.A and France. The comparison between France and U.S.A showed that a greater number of DE genes were uniquely shared (114) between these two genotypes. A gene ontology enrichment analysis for each genotype was performed with the 621 annotated genes of the 659 DE genes. We were able to detect enriched terms for down-regulated genes in the Japan genotype and for up-regulated genes in the U.S.A and France genotypes. These observations are in accordance with the fact that, a functional major up-regulation of genes in response to paraquat was only observed in invasive

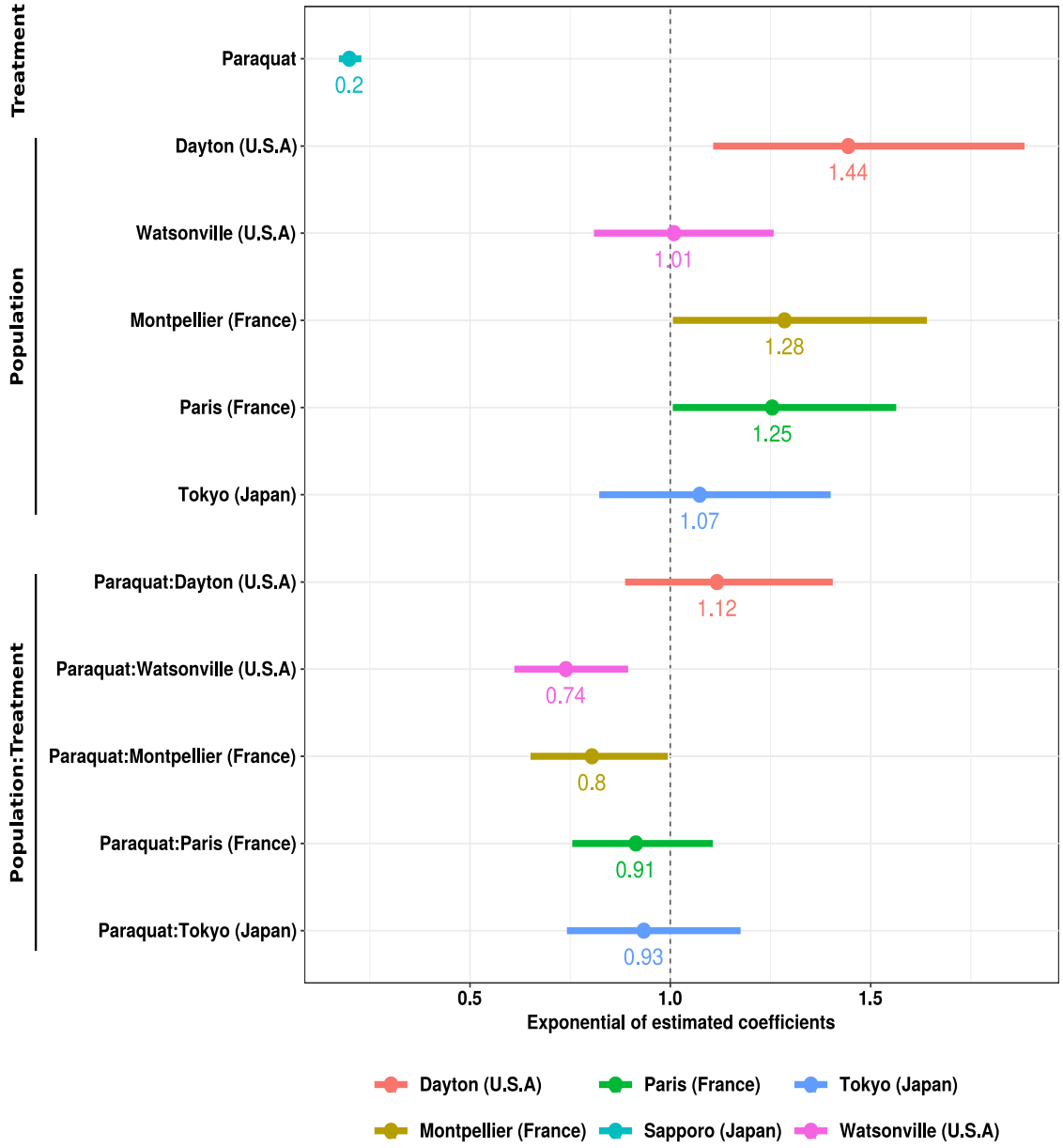

Fig. 1 Lifespans under control and paraquat-treated conditions relative to the Japan Sapporo population with confidence intervals (0.95). The relative values are within each treatment group and can be interpreted as a multiplicator effect compared to the reference level (Sapporo population, female and control condition). The intercept, which is the basal level of the Sapporo females in untreated condition, is equal to 31.96 days. Paraquat correspond to the mean effect of the treatment on Sapporo. Mean values of the populations correspond to the effect of the population on the lifespan compared to Sapporo in untreated condition and the last are the interaction term after paraquat exposure. As examples, the effect of paraquat reduced the lifespan of Sapporo to 20% (0.2) of the initial value in untreated condition. Values higher than 1 indicate an increase in the lifespan compared to Sapporo, while below 1 this indicate a higher sensitivity (*e.g.*, Paraquat:Watsonville correspond to the interaction term and indicates that after paraquat exposure, Watsonville remains more sensitive than Sapporo with a difference of 26% (1-0.74)).

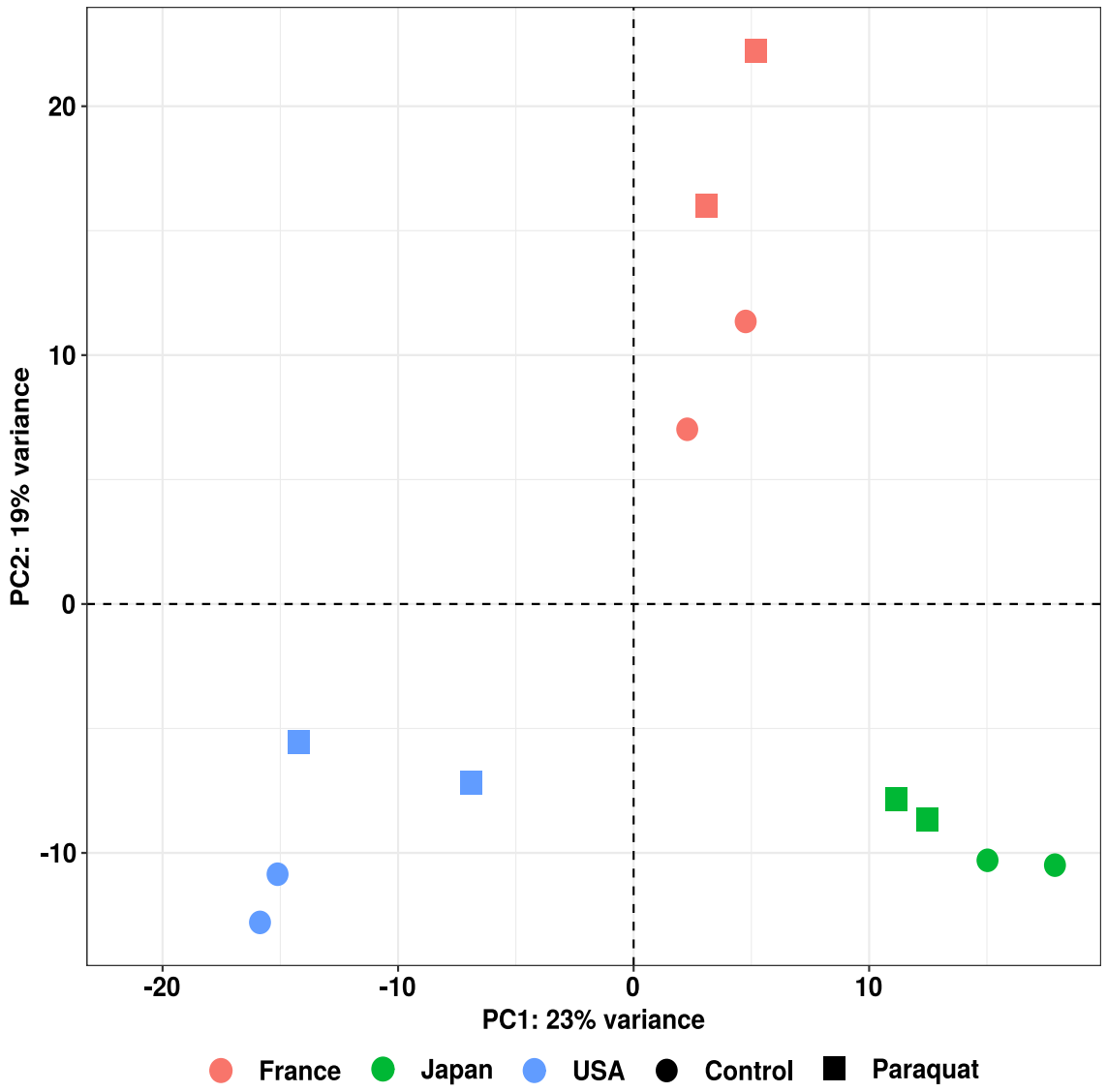

Fig. 2 PCA analysis using read counts from DESeq2. Dots correspond to the biological samples with the U.S.A in blue, Japan in red and France in green. Circles and squares correspond respectively to control and paraquat treatment.

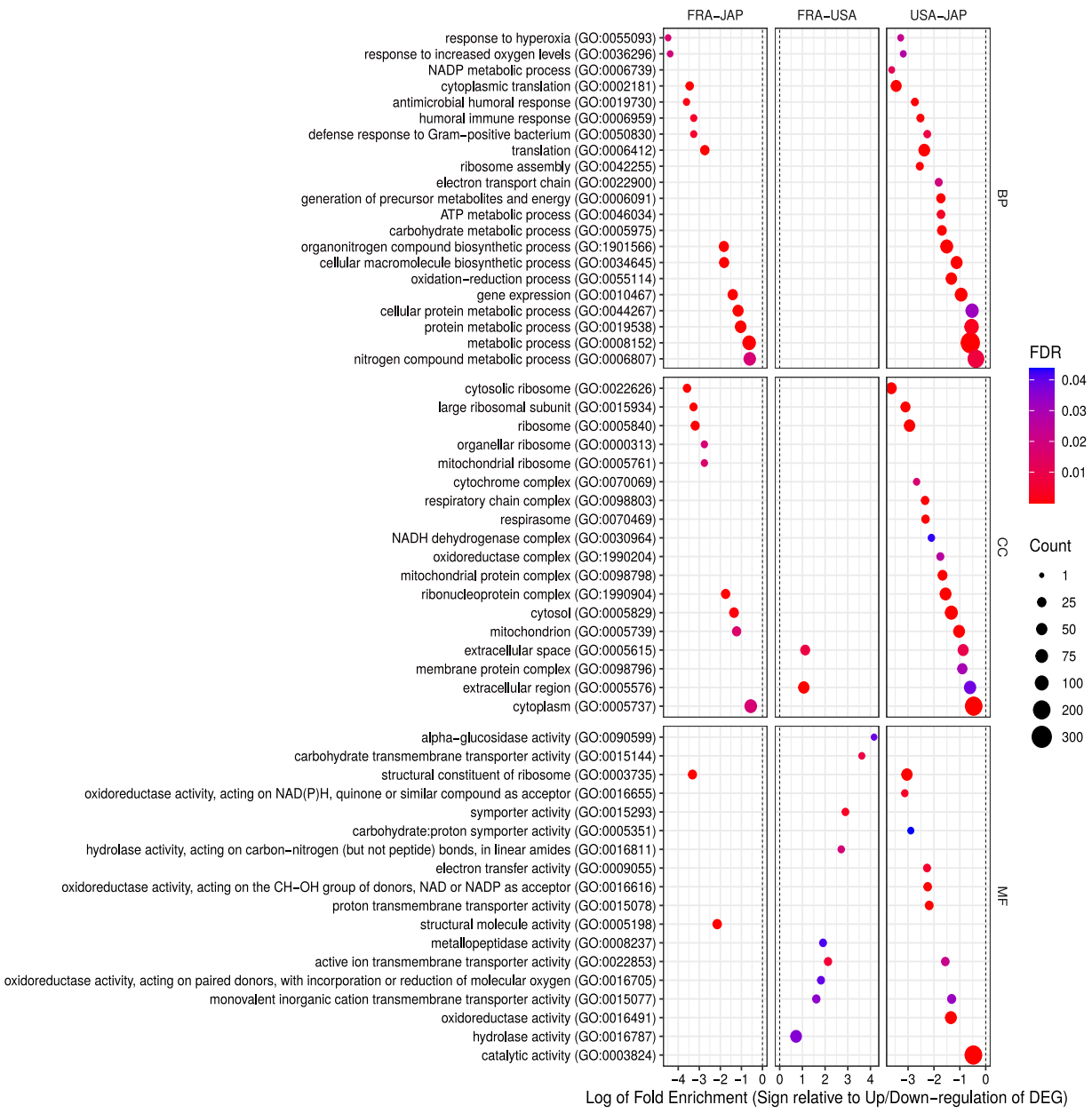

Fig. 3 Gene ontology enrichment analysis for all genotypes in control condition. BP: Biological Process; CC: Cellular Components; MF: Molecular Function. The size of dots corresponds to the number of genes in each category and the colour to the FDR. Gene ratio correspond to the number of genes from our data compared to genes within a GO term. Down-regulated genes from pairwise comparisons (*e.g.*, France-Japan) are symbolized with negative gene ratio and a positive gene ratio for up-regulated genes.

Table 2: **Number of DE genes between genotypes and treatments.** Pairwise comparisons between (A) untreated genotypes, (B) between treated and untreated flies within each genotype, and (C) in pairwise comparisons of paraquat-treated flies between different genotypes. The threshold for identifying DE genes was an adjusted p.value ≤ 0.01 and absolute log_2-fold-change ≥ 1. The proportion of DE genes is the percentage of DE genes in the expressed transcriptome (14538).

	Contrast	DE genes	Up-regulated	Down-regulated	DE proportion (%)
A	France|Japan control	524	175	349	3.6
	France|U.S.A control	715	471	244	4.92
	U.S.A|Japan control	1023	208	815	7.04
B	Japan (paraquat|control)	122	74	48	0.84
	France (paraquat|control)	531	354	177	3.65
	U.S.A (paraquat|control)	281	214	67	1.93
C	France|Japan paraquat	138	105	33	0.95
	France|U.S.A paraquat	65	19	46	0.45
	U.S.A|Japan paraquat	62	57	5	0.43

genotypes. When comparing the GO terms enriched in up-regulated genes from invasive genotypes (Fig. 5), terms such as ligase activity, oxidation-reduction, ATP binding, drug binding and ion binding were common to France and U.S.A. As observed in related species, paraquat can indeed cause DNA damage via oxidative stress [33]. The French genotype had a greater number of specific enriched terms, mostly related to DNA repair (including aforementioned ligase activity and telomere maintenance, among others), protein translation, protein refolding and mitochondrion. The U.S.A genotype had other enriched terms related to carbohydrate metabolism, detoxification, and response to metal ion. There were no enriched GO terms among up-regulated genes in the Japan genotype. Enriched terms for down-regulated genes in the Japan genotype were mainly related to immune response (to bacteria), response to increased oxygen levels (hyperoxia) and peptidase activity (Fig. 5). Overall, it appeared that while paraquat induced increased expression for genes related to oxidation-reduction, detoxification, drug/metal binding, DNA repair and protein refolding in invasive genotypes, it reduced the expression of important genes for the antioxidant response in the non-invasive genotype.

DE genes common to the three genotypes were mostly upregulated with oxidative stress

From a total of 659 DE genes between control and paraquat exposure, 67 were shared by all genotypes. This set of core genes were regulated in the same way for the three genotypes: 14 down-regulated (between -1.03 to -10.8 log_2FC) and 53 upregulated (between 1.03 to 10.48 log_2FC) (Fig. 6). Among those up-regulated following paraquat treatment, we found genes related to stress response such as *Hsp* and *Cyp* genes family. The most up-regulated genes were a predicted gene encoding for a transcription factor A (log_2-fold-change of 10) and genes in the *Hsp* gene family. Among the most down-regulated genes, we identified a cytochrome P450 gene that was the most down-regulated gene in all 3 genotypes and with a log_2FC $<$ -10 in the Japanese genotype. We performed a GO enrichment analysis for the set of 67 genes common to the three genotypes. For down-regulated genes, only 9 of the 14 genes had a homolog in *D. melanogaster*. Enriched terms were associated with peptidoglycan metabolic process and negative regulation of NK cell differentiation involved in the immune response. However, all enriched terms were related to two genes: PGRP-SC1a and PGRP-SC1b. PGRPs (Peptidoglycan recognition proteins) are important in recognizing and degrading bacterial peptidoglycan, although PGRP-SC1b has not shown antibacterial activity and may instead be a scavenger protein. Out of 53 up-regulated genes, 37 had homologs in *D. melanogaster*. Enrichment analysis of this set of genes identified only one significant gene ontology term: ligase activity (which is related to DNA repair). Four of the five genes within this GO term were tRNA-ligases, which may play a role in protecting cells against oxidative damage following their translocation into the nucleus.

The stress response is variable among genotypes

We identified a total of 213 genes with a significant GEI, which represent the set of genes with expression differentially modulated by oxidative stress according to genotype (Fig. 7A). When comparing differences in the response of invasive genotypes to the noninvasive Japan genotype, we found 62 differentially modulated genes with the U.S.A genotype and 138 with

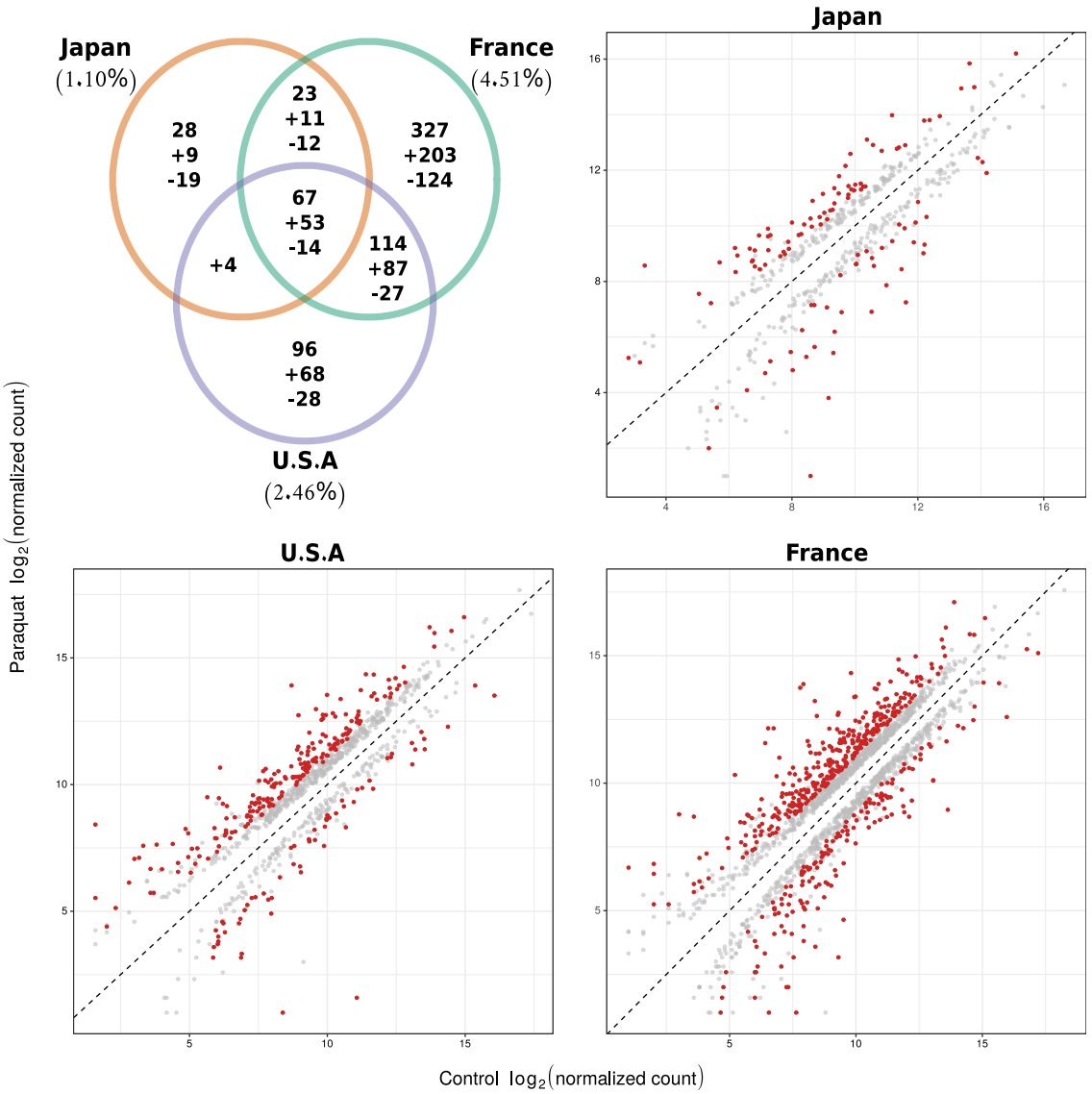

Fig. 4 Gene expression between control and paraquat conditions. A Venn diagram of shared and unique DE genes identified in comparisons of paraquat-treated and control flies within each population after paraquat exposure (top left). Scatter plots of log_2 normalized read count for Japan (top right), U.S.A and France (bottom panels) comparing control and treated flies. Individual genes are indicated by dots. Red colour corresponds to significant DE genes (see materials and methods).

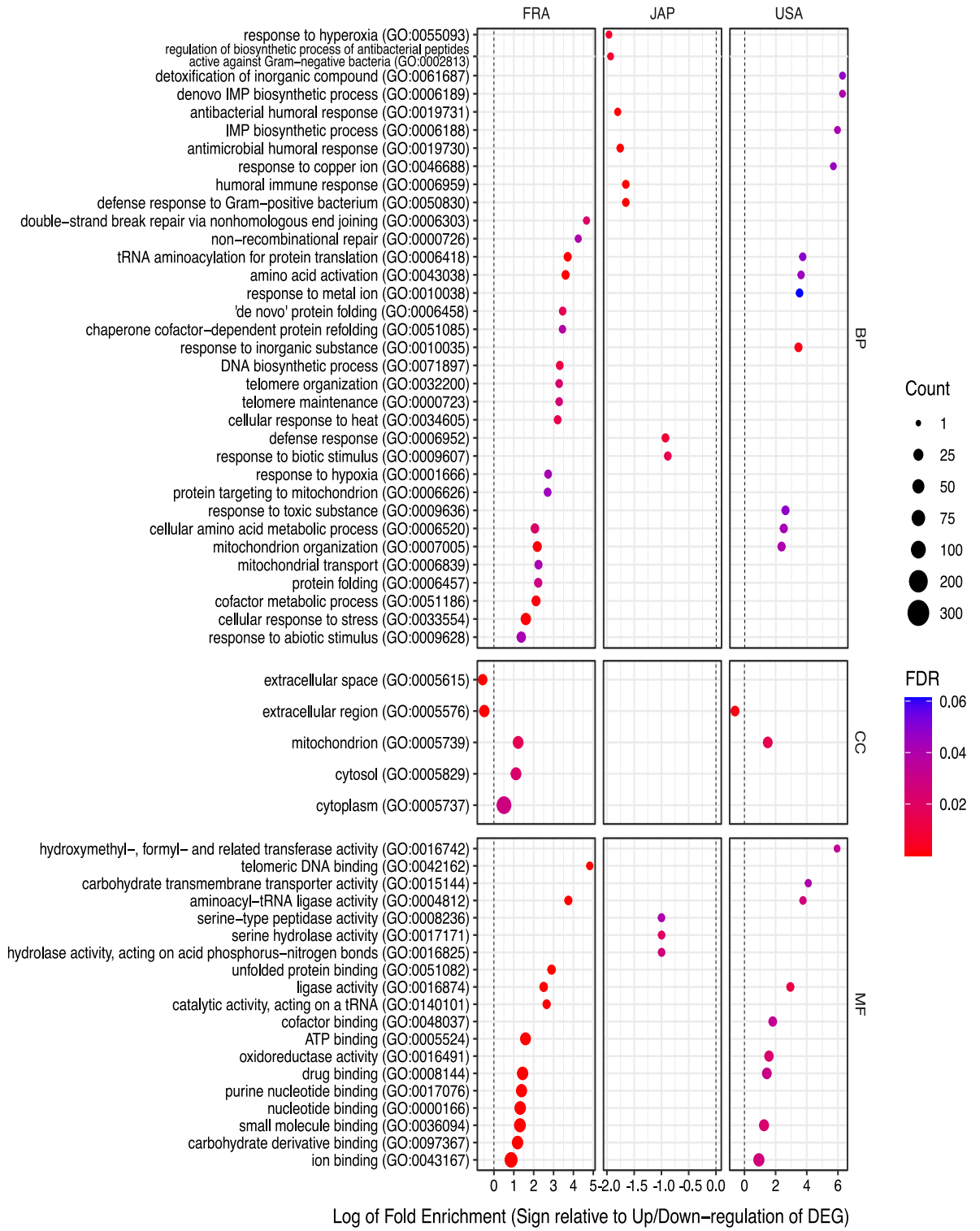

Fig. 5 Gene Ontology analysis for up- and down-regulated genes induced upon treatment with paraquat. Up- and downregulated DE gene lists from the three pairwise comparisons (Paraquat vs Control) were used in this analysis in order to detect enriched functions. For this we used: 243 genes up-regulated and 105 genes downregulated in the French strain, 134 genes up-regulated and 42 genes down-regulated in the USA strain and 31 genes down-regulated from the Japanese strain. No functional enrichment with up-regulated genes from the Japanese genotype was detected.

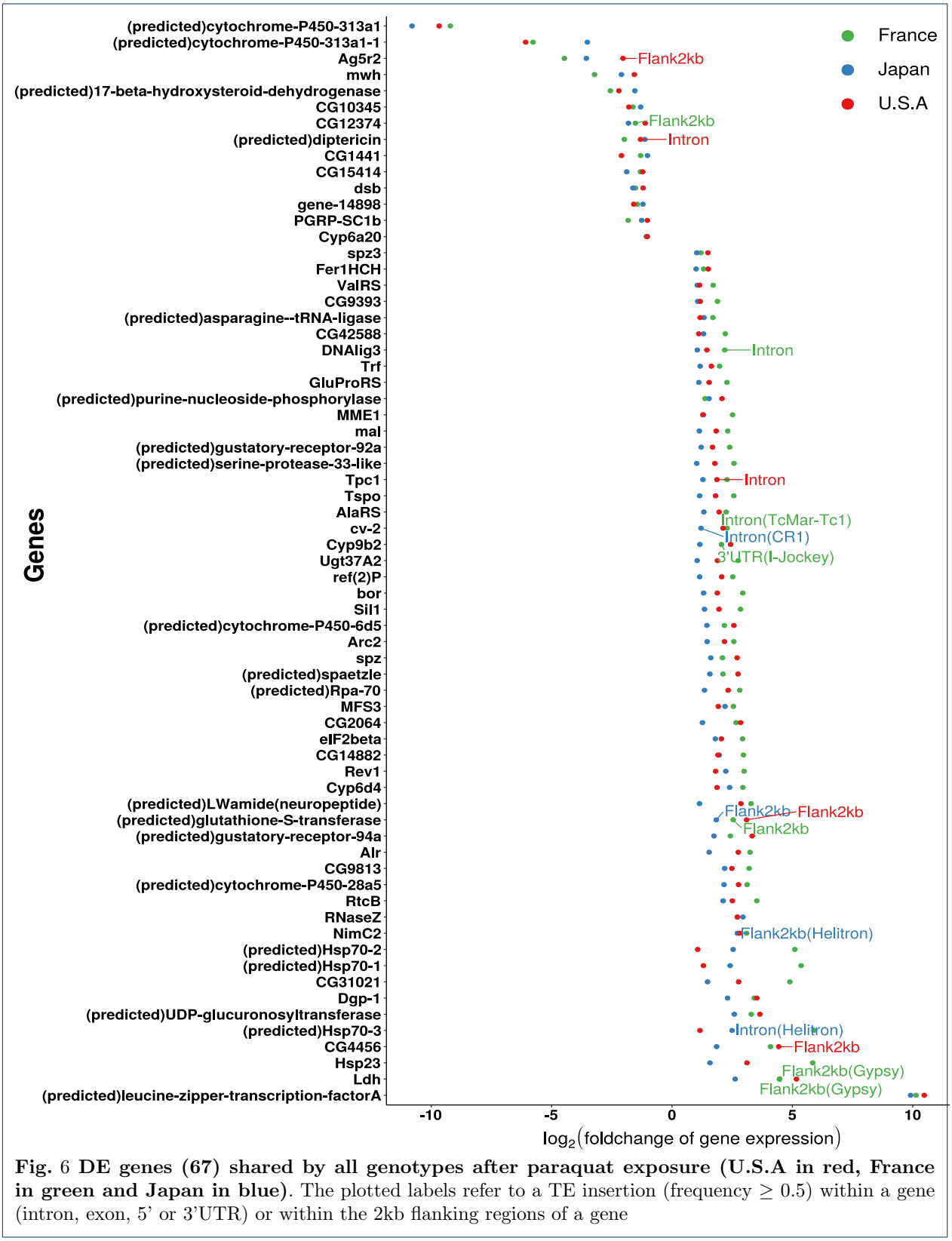

Fig. 6 DE genes (67) shared by all genotypes after paraquat exposure (U.S.A in red, France in green and Japan in blue). The plotted labels refer to a TE insertion (frequency ≥ 0.5) within a gene (intron, exon, 5' or 3'UTR) or within the 2kb flanking regions of a gene

the France genotype (Table 2). Most of these differences were due to greater up-regulation in the invasive genotypes (57/62 and 105/138). We identified 52 genes where the GEI was driven by a differential response in only one genotype compared to the other two. This included 22 genes differentially modulated in the France genotype compared to Japan and U.S.A, 14 in Japan compare to France and U.S.A, and 16 for U.S.A against others. We have presented some examples of these genes (Fig. 7B and Table S2), selected for the greatest log_2-fold-change and illustrating cases in which the magnitude of the response to paraquat differed among genotypes. For example, *dysc* and *FarO* were down-regulated in France and upregulated in U.S.A and Japan. The *Hsp* genes *Hsp68* and *Hsp70Aa* were strongly up-regulated following paraquat treatment in France, with a log_2FC ≥ 2, compared to the much-reduced changes in expression in Japan or U.S.A. In the USA genotype, *Mec2* was strongly down-regulated compared to the increased expression following treatment in the other genotypes. Oxidative stress appeared to upregulate *CCHa2*, *RpL40* and *Tsf1* only for the Japanese genotype. These examples highlight the potential effect of genotype-specific responses to oxidative stress.

TE expression is not sensitive to oxidative stress

Environmental changes can affect the expression of TEs by lifting epigenetic repressive regulation mechanisms. In control condition, differentially expressed TEs (DETEs) identified in pairwise comparisons between genotypes represented from 3.06 to 5.91% of total number of TE families annotated in the *D. suzukii* genome (Table S3). The U.S.A genotype exhibited a greater level of expression of TEs compared to the French or Japanese genotypes, with almost 70 TE families up-regulated in U.S.A genotype in comparisons with either France or Japan. By contrast, a similar number of both up- and down-regulated DETES were identified in the comparison between France and Japan (Fig. 8). It should be noted that TE expression levels were very low, and that reads corresponding to TEs did not exceed 3 to 7% of the total transcriptome. After paraquat exposure, very few TE families changed in their expression level (Table S3). In total, only 12 TE families were differentially expressed (Fig. 8). Six TE families in France and three in Japan were up-regulated. In the U.S.A genotype, differential expression of five TE families was observed, with three showing up-regulation and the remainder down-regulated. Among the DETEs, all classes of TE families were represented. We observed a differential expression in two of the genotypes in a Copia cluster and a Tc1 mariner cluster, which could suggest specific activation of these TE families in the presence of oxidative stress.

DE genes during oxidative stress are not enriched in TE insertions

TEs represent 33% of the *D. suzukii* genome and can potentially interfere with gene expression during stress [35]. We looked for TE insertions, for each of the three genotypes, in the vicinity of genes differentially expressed in response to oxidative stress, including all DE genes from comparisons control vs paraquat and genes with a significant GEI. The distribution of TEs in the three genomes was not different (Chi-square test = 0.67, Table S4). We then tested the dependence of TE insertions and gene expression states (DE or not) after paraquat exposure (Table S5). Chi-square tests for the three genotypes showed that DE genes had fewer than expected TE insertions (p-value < 0.05). We then focused on 115 TE insertions, the majority of which were in introns (57) or in ±2kb flanking regions (50) around DE genes (Table 3). Of the remaining 8 TEs, 7 were associated with up regulated genes (*JMJD4* (5'UTR), *Act42A* (exon), *Cyp9b2* (3'UTR), *CG8728* (3'UTR), *Cyp6a22* (3'UTR), *CG6834* (3'UTR), and one non annotated gene (exon). One insertion was associated with a down-regulated gene, *CG4409* (exon).

Table 3: **Insertion location of TEs in genes differentially expressed.**

		5'UTR	Exon	Intron	3'UTR	Flank 2kb
France	Down	0	1	17	0	8
	Up	0	2	18	2	19
U.S.A	Down	0	0	6	0	5
	Up	1	0	12	2	10
Japan	Down	0	0	2	0	3
	Up	0	0	2	0	5

Core DE genes are not enriched with TE insertions

In agreement with a depletion of TE in DE genes, of the 67 core DE genes that responded to paraquat treatment in similar ways across the three genotypes (Fig. 6), we found only 11 genes with one or more TE insertions. Among these 11 genes, only one (a gene predicted to encode a glutathione transferase) had a shared element present at the same position in all three genotypes (helitron family 1kb upstream the gene).

Distribution of TEs among GEI genes

For DE genes showing evidence of a GEI, TEs presence was generally not associated with the status of the gene for the DE, except for genes with a GEI between U.S.A and France or Japan (p-value = 0.0123, Table S5). A GEI interaction indicates that the magnitude or direction of changes in expression following treatment could differ depending on the genotype. Fig. 9 summarizes detected TE insertions in GEI DE

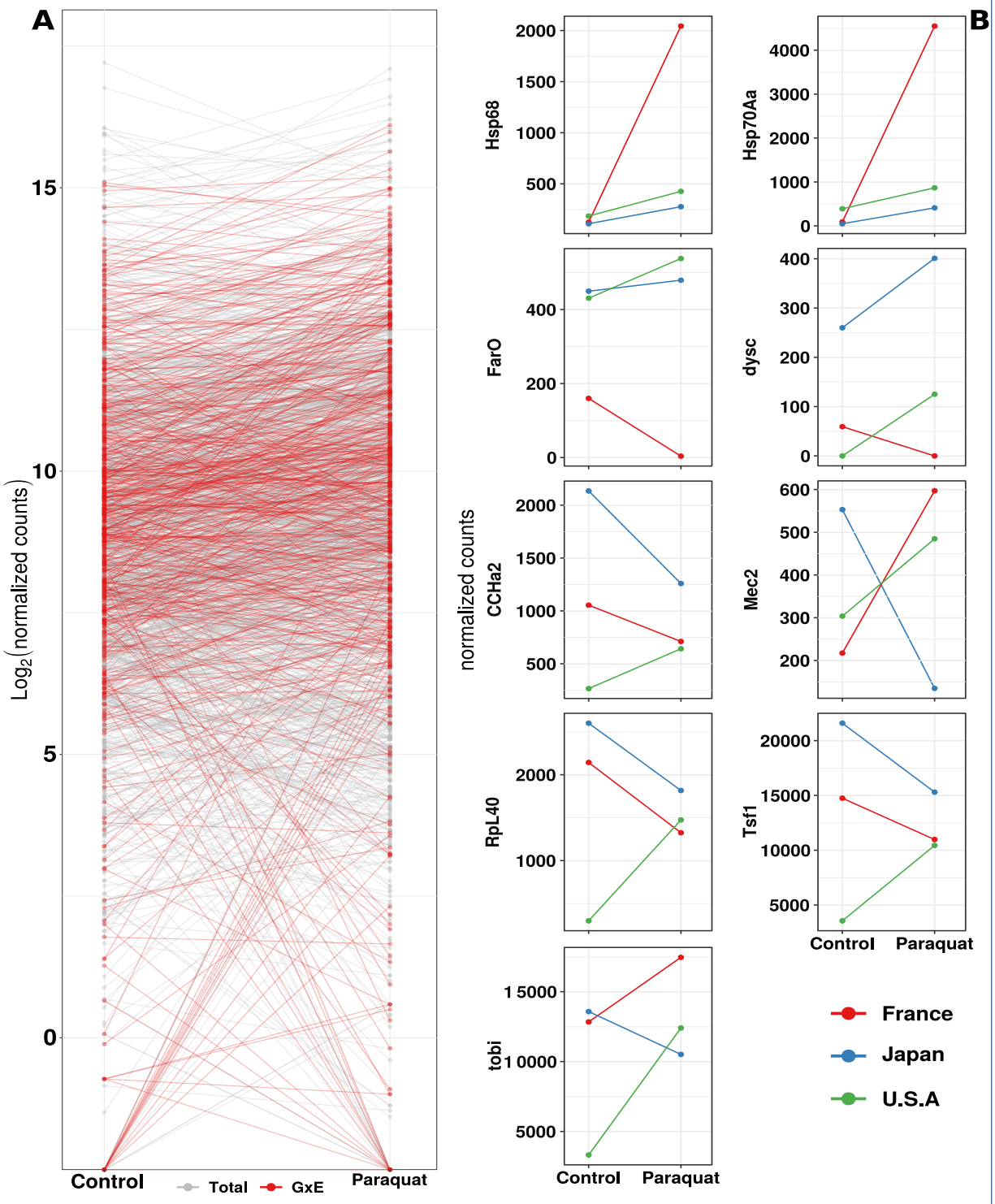

Fig. 7 Reaction norms between control and paraquat for DE genes using log_2 normalized read counts. (A) Reaction norms of all DE genes, with red for the GEI ones, while grey are DE genes without GEI. (B) Examples of 9 DE genes with GEI, with colours referred to the genotype (red for France, blue for Japan and green for the U.S.A)

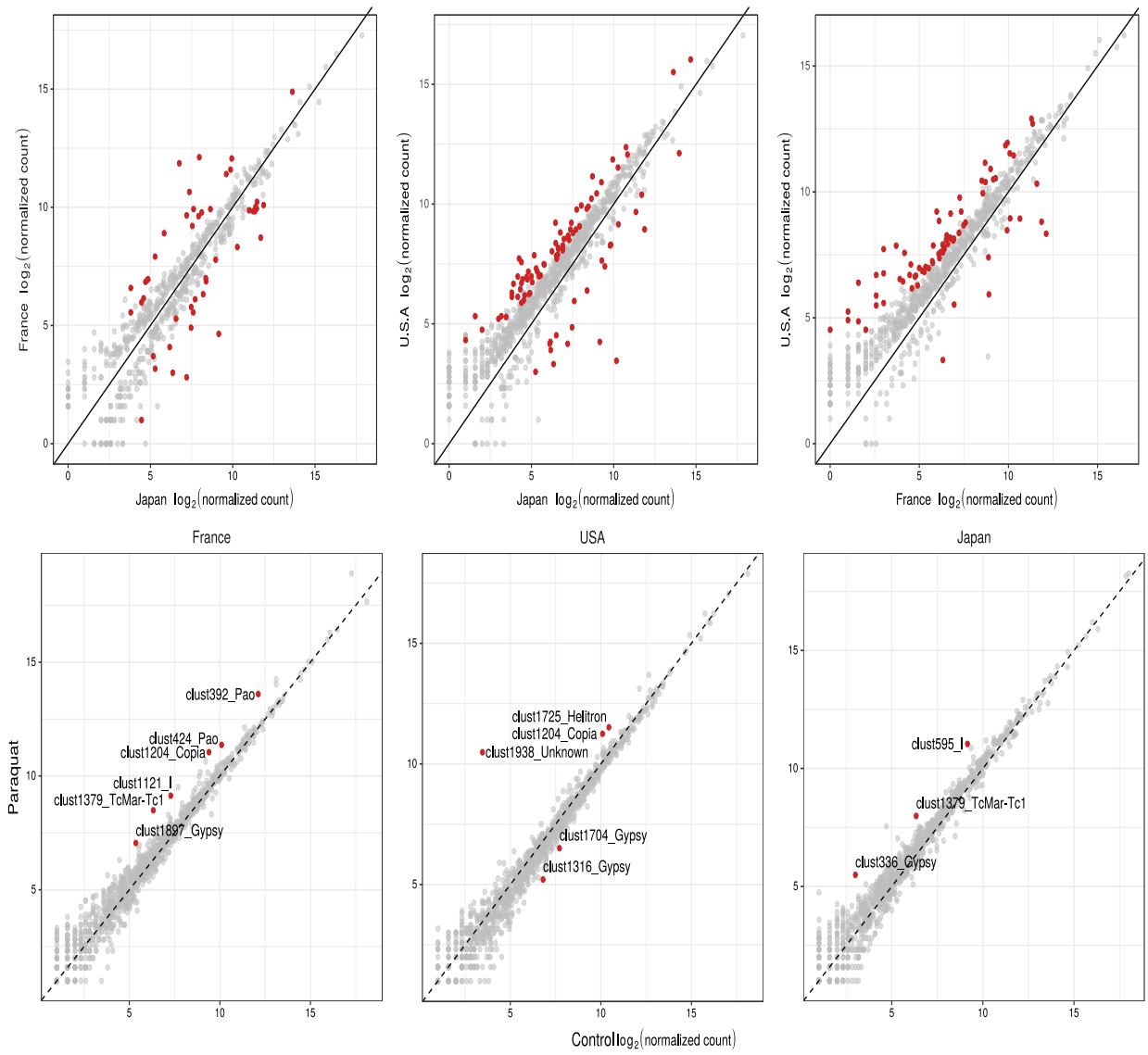

Fig. 8 TE expression between genotypes (upper panel) and between control and paraquat conditions for each genotype (bottom panel). Scatter plots represent the log_2-normalized read counts. Individual TE are indicated by dots. Red colour corresponds to significant DE TEs (see materials and methods).

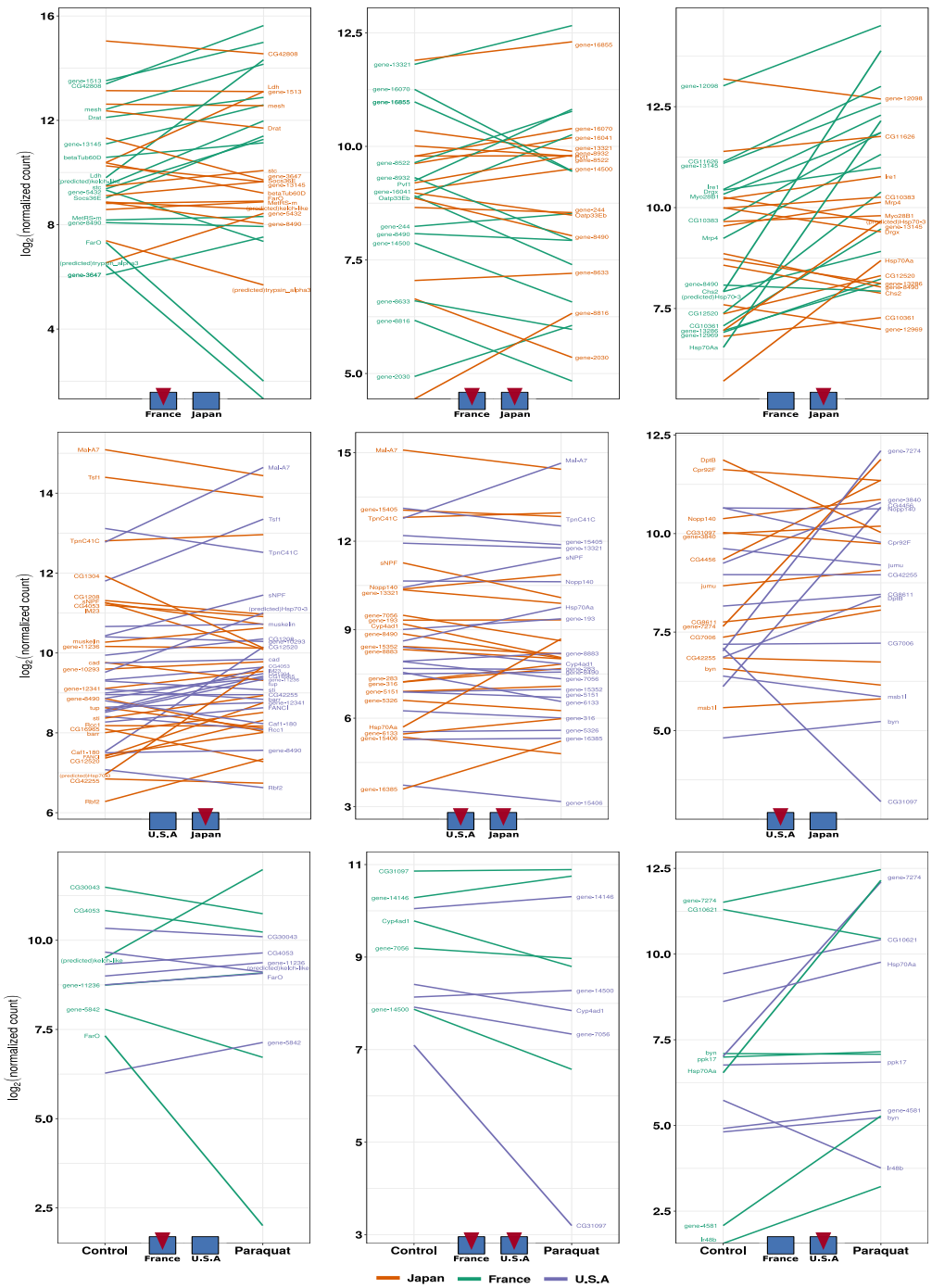

Fig. 9 DE genes in pairwise comparison between genotypes between control and paraquat treatment with inserted element (frequency ≥0.5). We plotted log_2 normalized counts between control and paraquat treatment, for each comparison (Japan versus France or U.S.A in top and middle and France versus U.S.A in bottom). Colours correspond to France (green), Japan (orange) and U.S.A (purple). The left and right plots are related to genes with insertions only present in one genotype of the comparison and middle plots are shared insertion for both genotypes.

genes for the different genotypes (also Table S6). 37 genes had at least one insertion between France and Japan, 10 in comparison between U.S.A and Japan and 6 between France and U.S.A. No insertion was fixed in all genotypes. Three genes were differentially expressed between France and the others, *FarO* (Fatty acyl-CoA reductase), a predicted *kelch* gene (which plays an essential role in oogenesis, where it is required for cytoskeletal organization), and *Hsp70-Aa* (a protein involved in response to heat shock and hypoxia). *Kelch* and *FarO* both had a TE insertion in France, with, respectively, a greater and lesser expression compared to other genotypes. In the case of *Hsp70-Aa*, this gene always showed greater expression in the France genotype compared Japan and the USA which both contained an insertion absent in the French genotype. Another example is the gene *CG12520*, which has an inserted element in the 3'UTR only in Japan and is less expressed than other genotypes. To understand if TE insertions close to GEI genes harbour TFBS that could be implicated in gene expression modifications, we screened 3 transcription factor binding sites (TFBS) from the antioxidant responses elements group (ARE) [36]. We first analyzed enrichment in TFBS for all TE copies identified in the GEI genes (Fig. S4A). Of 196 candidate sequences, 36 had at least 1 TFBS, most of them related to a CnC element. Of those with putative TFBS, they were related to Pao and Gypsy TE families. When specifically examining insertions in the genes with GEI (Fig. S3B), we found only 4 hits, 3 in unannotated genes and one in the annotated gene stc, identified in the comparison between France and Japan. Interestingly, stc encodes a NFX1 family transcription factor implicated in modulating adult lifespan and aging phenomena, where a trade-off with the oxidative stress response is known [30]. Furthermore, the TE insertion and expression difference was only present in the French genotype. In GEI analyses of the U.S.A versus Japan, only one hit was significant, but shared for the 2 genotypes.

Discussion

D. suzukii genotypes vary in lifespan and response to oxidative stress

Previous studies founded a positive association between stress resistance and extended lifespan or aging in *D. melanogaster* [30, 37, 38]. In this species, the ROS (reactive oxygen species) defences are mediated by both immune and antioxidant response pathways. A similar association may be expected in *D. suzukii*, a species from the melanogaster group that diverged 8 Mya. However, until now, no extensive study had been performed using *D. suzukii* wild-type genotypes. We observed a significant positive correlation between lifespan in standard conditions and under oxidative stress. However, not all fly genotypes responded to oxidative stress in same way, resulting in a significant genotype-by-environment interaction (Fig. 1 and Table 1), a result also observed in *D. melanogaster* [32]. For example, Japanese populations had the lowest lifespan in the untreated condition but were more resistant to oxidative stress than genotypes from Watsonville or Montpellier (France). This GEI suggests possible local adaptation of the different populations to paraquat, perhaps associated with differences in herbicide use in the three countries. Paraquat is one of the most used herbicides in the world and is widely used in Japan and U.S.A, but forbidden in Europe since 2007 [28, 29]. The presence of *D. suzukii* in Europe has been reported since 2008, and flies are therefore unlikely to have encountered paraquat in the field since their arrival [24, 27, 39]. This could have resulted in relaxed selective pressure for oxidative stress resistance and explain why the French Montpellier population was more sensitive than Japanese and American lines (except for Watsonville). The Paris population was not significantly different than the Japanese Sapporo population and we suggest that an admixture event that occurred in the North of France with flies from U.S.A could explain the difference between the two French populations [27]. The difference between the two American populations are strange but molecular analysis revealed that a copper detoxification pathway, discussed below, could be involved. Our results demonstrate the importance of considering different populations in such studies and should probably be confirmed by a larger sampling.

Basal gene expression is different between invasive and native genotypes

erformed a transcriptomic analysis on genotypes from each of the three sampled locations in order to possibly identify molecular processes underlying variation in the oxidative stress response. We first evaluated DE genes between the three genotypes in untreated conditions (control). In *D. melanogaster*, genotypic differences accounted for 7.3% of DE genes showing microenvironment plasticity among as set of 16 DGRP lines reared under carefully controlled standard conditions [40]. This result is in agreement with our results, where almost 7% of the transcriptome was differentially expressed among genotypes. Most of these DE genes correspond to biological processes such as metabolism or protein production and may possibly reflect genotype-specific differences related to local adaptation. In general, the level of expression for DE genes in invasive genotypes (U.S.A and France) was lower than in the native genotype from Japan, suggesting this genotype

has by default a higher level of transcription. Among down-regulated genes, we found significant GO term enrichment in invasive genotypes related to translation, protein metabolic process, ribosome biogenesis, response to hyperoxia and immune response.

Relationship between oxidative stress response, phenotype and gene expression

Exposure to paraquat affected the expression of up to 5% of the transcriptome (703 DE genes between control and paraquat) with a majority of DE genes being up-regulated. Similar changes in gene expression have been observed in *D. melanogaster*, with 608 to 111 DE genes identified after exposure to 5mM or 15mM of paraquat [41]. In response to oxidative stress following exposure to hydrogen peroxide, 1639 DE genes were identified [41, 42]. Interestingly, the proportion of the transcriptome affected by oxidative stress differed between native and invasive genotypes. The Japanese genotype appeared highly stable, with fewer DE genes in response to paraquat than either invasive genotype (Fig. 4). Furthermore, the number of DE genes uniquely affected by paraquat exposure was much lower in the Japanese genotype (28) than either the U.S.A (96) or France (327) genotypes. It should be pointed out that the two invasive lines used in the transcriptomic analysis, Watsonville (USA) and Montpellier (France), were the lines most affected by paraquat exposure in our phenotypic analysis (Fig. 1), suggesting that stress sensitivity could be linked to greater transcriptional deregulation. The French genotype had by far the greatest number of DE genes (almost twice as many as the American genotype). Consistent with the hypothesis of transcriptional deregulation, this result could reflect a lack of adaptation to paraquat, which has been banned as an herbicide in Europe since 2007, just prior to the arrival of *D. suzukii*. A core set of 67 DE genes were shared by all genotypes in their response to paraquat. This core set of common DE genes likely corresponded to those directly implicated in the oxidative stress response. In agreement with this idea, we found that among these genes, some are known to be generally activated under stress, such as *Hsp* or genes in the cytochrome gene family [43–47]. At the transcriptomic level, the Japanese genotype appeared very different from the other two. First, as discussed above, the transcriptional response to paraquat involved a much smaller portion of the genome and there were fewer DE genes unique to this genotype. Second, under standard, control conditions, this genotype had the highest level of expression. At the phenotypic level, the Japanese genotype had the lowest lifespan under standardized control conditions but was then, one of the genotypes most resistant to oxidative stress. Together these results suggested that the Japanese genotype harbored some constitutive defenses to oxidative stress. In the absence of oxidative stress, the expression of constitutive defense may come at a cost of reduce lifespan but would result in greater resistance when flies encounter paraquat. In the case of the French and American genotypes, many up-regulated genes are directly related to the oxidative stress response (GO enriched for oxidation-reduction, immune response and ion binding), which could indicated they are experiencing a greater amount of oxidative damage and explain their lower lifespan under stress conditions. A surprising result comes from the GO analysis of DE genes in response to paraquat exposure in the U.S.A genotype. We identified an enrichment in terms related to copper detoxification that was absent in the other genotypes. Previous research has demonstrated a trade-off between copper tolerance and sensitivity to paraquat [48, 49]. Thus, the greater sensitivity of the Watsonville genotype to paraquat exposure could reflect previous exposure and adaptation to copper in the environment. In support of this hypothesis, information from the California Pesticide Information Portal (https://calpip.cdpr.ca.gov/main.cfm) indicates a sizeable use of copper-based agricultural products, especially fungicides, with 525 Kg reportedly used in 2017. Portal, data from 2017, calpip.cdpr.ca.gov).

Genotype-specific transcriptional responses to paraquat exposure

To better understand genotype-specific responses to paraquat exposure, we focused on genes presenting a genotype-by-environment interaction (GEI). If differences in the transcriptional response to paraquat exposure reflect adaptive changes evolved in response to local environmental conditions then analysis of genes with GEI may provide insight into the mechanisms of local adaptation [50]. Genes with GEI have often been identified in studies of oxidative stress responses (see e.g., Jordan et al., 2012) [51]. Genetic variation in transcriptomic plasticity could contribute to rapid adaptation to novel environments during the invasive process, possibly due to variation in both cis and trans regulatory sequences [44, 52–56]. We found evidence of GEI for the transcriptional response to paraquat in only a small part of the transcriptome. Most DE genes with evidence of GEI showed a greater change in the level of expression in invasive genotypes versus the native one. Due to the large number of genes that remain unannotated in the *D. suzukii* genome, a complete scenario of the genome-wide transcriptional response to oxidative stress is difficult to achieve. This may be

particularly problematic when attempting to understand the functional relevance of genotype-specific responses. However, our results confirmed that for parts of the genome, the transcriptional response to oxidative stress varies among genotypes, and that some of these differences may reflect population history. Interestingly, the French genotype showed massive up-regulation of some Hsp's compared to the USA and Japan genotypes (Fig. 7). These genes are known to be highly responsive to temperature [44] and also to oxidative stress (see review [47]). In general, gene ontology analysis revealed an enrichment in GO terms related to oxidative stress (oxygen level, hyperoxia, hypoxia, stress) for up-regulated genes in invasive genotypes relative to the native one. These GO terms were also enriched for up-regulated genes with evidence of GEI in the comparison between France and the U.S.A. One caveat of the genome-wide expression analysis is our statistical power to identify biologically relevant differences in expression levels. We have applied a threshold (FDR < 0.01 and absolute log_2FC > 1) to identify DE genes, but it is possible that genes with more subtle changes in expression are important. Indeed, genes showing evidence of GEI are often found in upstream parts of regulatory networks, where even very small differences in expression could have pronounced phenotypic consequences. Also, genes with GEI are often associated with genetic variation in cis or trans- regulatory sequences, and a further investigation would be necessary to identify such factors [44, 52, 53, 57, 58].

TE insertions are depleted near oxidative stress sensitive genes

TEs have been described as stress sensitive and their activation by stress-responsive elements (SREs) in promoting regions could generate a burst of transposition and facilitate adaptation by increasing the genetic diversity upon which selection could act [19, 59]. A recent review cited several examples of TE family activation following stress, which may depend on the type of stress and the TE family [15, 60]. Horváth et al. [15] also suggested that under stressful conditions, some TEs could be repressed just after their activation, indicating that stress could induce both activation and repression. TE transcription is a prerequisite to TE activity [15]. Our analysis of the TE transcriptome after stress induction showed that in *D. suzukii* very few TEs are activated, with a maximum of 6 TE families unregulated following exposure to paraquat in the French genotype. This result is somewhat surprising, as a greater number of TE families are DE between genotypes in control conditions, suggesting that TEs in *D. suzukii* are capable of being expressed and potentially active. Most TE insertions are neutral or slightly deleterious, but some may be beneficial and implicated in adaptation [18, 19, 59, 60]. The impact of TE insertions is often through their effect on gene expression, likely due to the addition of regulatory sequences, present in the TE, that can modulate how genes are expressed, particularly during stress [13]. While TEs have been implicated as playing an important role in the success of invasive species, by generating genetic diversity and thus compensating for bottleneck effects after introduction, no empirical data exists in support of this hypothesis [3, 6, 16, 59]. As 33% of the *D. suzukii* genome is composed of TEs, we tested the hypothesis that TEs could modulate gene expression by the addition of regulatory regions. We found that the distribution of TEs did not differ among the three genomes, and, as observed in other Drosophila species, a majority of the insertions were in intergenic and intronic regions [61]. However, when we specifically analyzed DE genes, we observed a depletion of TE insertions, suggesting that TE insertions in stress response genes may be limited by strong purifying selection. This paucity of TE insertions was also observed for DE genes that were shared by the three genotypes. Only one gene, encoding for a glutathions-transferase, had a shared element in flanking region. Finally, we tested for the presence of TE insertions in genes presenting GEI. We detected more insertions in genes with GEI than in other DE genes, suggesting that this category of genes may be more accepting of TE insertions. Several insertions were found in 3', 5'UTR and could have regulatory consequence on those genes. While it is possible that the presence of TE insertions could affect gene expression in a manner consistent with the genotype-specific responses we observed, further analyses are needed to understand the molecular mechanisms responsible for changes in gene expression for this category of genes.

Conclusion

Our results showed a difference in paraquat resistance between native and invasive populations of *D. suzukii*, that is not homogeneous between populations on the same continent. The differences observed between the two French populations could be explained by differential admixture subsequent to colonization in these two regions of France. In the United States, possible local adaptation to copper in the environment in Watsonville, as revealed by the molecular analysis, may explain the difference in resistance to paraquat. Further research is required to test these hypotheses and to better understand population differences in paraquat resistance. At the molecular level, the level of gene expression is more strongly genotype-dependent than stress-dependent. Finally, we showed that contrary to

expectations, oxidative stress does not induce significant activation of TEs and that there is a depletion of TE insertions under stressful conditions for the three genotypes of *D. suzukii* studied. Together our results highlighted the importance of not focusing on a single genotype in phenotypic or transcriptomic analyses of stress responses, that phenotypic and molecular approaches may complement each other for better understanding of these traits, and that it is important to test assumptions of the still neglected compartment of the role of TEs in adaptive evolution.

Materials and methods

Drosophila suzukii lines rearing conditions and phenotyping

D. suzukii genotypes were sampled in 2014 in the native area (Japan: Sapporo and Tokyo) and two invaded areas (U.S.A: Watsonville and Dayton and France: Montpellier and Paris) (Table S7). To establish isofemale lines, a single gravid female was placed in a culture vial, and the line maintained thereafter with a low larval density in vials containing modified "Dalton" medium (Table S8) in a controlled environment: 22.5°C ±1°C, 70 % ±5% RH and a 16:8 (L/D) [62]. We used paraquat (methyl viologen dichloride hydrate, ref. 75365-73-0, Sigma-Aldrich®) to mimic oxidative stress. Paraquat (10mM) was added to the cooling medium, before pouring into vials. Control vials were made at the same time but without adding paraquat. In the experiment, ten 4-7-day old flies were placed in experimental vials and transferred to new vials every 3 to 4 days to limit microbial development. Both males and females were tested and kept in separate vials. Survival was monitored by visual inspection every 24h. There were three replicate vials for each combination of the 27 isofemale lines (Table S7), sex, and paraquat treatment, for a total of 324 vials.

Survival data analysis

The analysis of survival data was performed in two steps on R software (v.3.6.0, [63]). First, for each replicate (10 survival times), we used the fitdistcens function from the fitdistrplus package (v.1.0-14, [64]) to determine which of several distribution models (Weibull, lognormal and gamma) were most appropriate for analysis of our right censored data (33 flies) data. The Weibull distribution was chosen after graphical comparison with others, also confirmed using loglikelihood ratio of the models. The distribution for each replicate was summarized using the median. Second, a linear mixed model was fit to the log transformed medians using the lmer function of lme4 (v.1.1-21, [65]), and p-values were estimated using lmerTest (v.3.1-0, [66]) with treatment, sex and population (the 6 sampled cities) entered as fixed factors and isofemale line as a random factor). The main effect of sex and interactions with both treatment and population were removed after AIC comparison from the final model for analysis. The interaction between population and treatment (GEI effect) was kept in the model. Model coefficients are reported with their confidence intervals (0.95) in Table S9 and after exponential transformation on Fig. 1. These effects can be interpreted as multiplicative effect on the median lifespan compared to a reference, here chosen as the non-exposed group from Sapporo. So, for example, with the untreated Sapporo flies centered on 1, an effect of 0.2 for paraquat-treated Sapporo flies means they have 20% of the survival time of Sapporo flies without paraquat. Normality and homoscedasticity of residuals and normality of random effects were confirmed graphically after logarithmic transformation of median survival times. We also examined the correlation across the isofemale lines between log-transformed survival times in control and paraquat-treated conditions using a Pearson test in R (Fig. S1).

DNA extraction and sequencing

We sequenced one isofemale line per country: S29, W120 and MT47 respectively from Sapporo (Japan), Watsonville (U.S.A) and Montpellier (France). DNA was extracted using phenol chloroform extraction from a pool of 10 adult females. Libraries and sequencing were performed by the platform GeT-PlaGe, Génopole Toulouse / Midi-pyrénées (France), using Illumina (150 bp) TruSeq Nano pair end. We obtained between 33,362,864 and 72,022,388 reads per library. Sequences were cleaned using Trimmomatic with default parameters.

RNA extraction and sequencing

We used the same three isofemale lines (S29, W120 and MT47) for our analysis of gene expression. For each of two biological replicates, fifteen 4-7 days old females were exposed for 24h to medium supplemented with paraquat (20mM) or without paraquat (*i.e.*, a total of 12 samples). Flies were dissected on ice in a phosphate buffer saline solution, and the somatic tissue then frozen in liquid nitrogen and stored at -80°C. We used the RNAeasy Plus Mini Kit (Qiagen) to extract total RNA from carcasses following the protocol provided by manufacturer. Samples were treated with DNAse (ref AM2224, AMbionTM) according to manufacturer instructions and stored at -80°C. RNA amount and quality was checked using QubitTM (Thermo Fisher Scientific) and the 2100 Bioanalyser instrument (Agilent). RNA libraries and sequencing were

performed on the GenomEast platform, a member of the 'France Génomique' consortium (ANR-10-INBS-0009). Libraries were constructed using the TruSeq® Stranded mRNA Library Prep Kit following manufacturer's recommendations. The libraries were sequenced on Illumina High HiSeq 4000 with paired-end 100 base pair long reads.

Transcriptome analysis

Between 62.76 to 120.122 million pair-end reads were generated from the 12 libraries. Quality was assessed using FastQC (v. 0.10.1), a trimming step implemented with UrQT (v. 1.0.17, minimum phred score of 20), and quality was reconfirmed again using FastQC [67, 68]. RNA data were mapped on the *D. suzukii* reference genome using HISAT2 (v. 2-2.1.0) and read counts for genes were computed with eXpress [69–71]. We performed a reciprocal BLASTN (2.2.26) between the *D. suzukii* genes and the *Drosophila melanogaster* database (FlyBase, dm6 version) (archive data: $FB2018_06$) in order to identify orthologues [72]. Another BLASTX was performed against the NCBI nr database, using predicted genes in *D. suzukii* for which no orthologues were detected in *D. melanogaster*. Matched hits from this BLASTX were tagged with the term "(predicted)". Of the 16905 annotated genes in the *D. suzukii* genome, 8428 matched with a Flybase gene and 478 others on the nr database (52.7% of total genes). Differential expression analysis was made using DESeq2 package (v. 1.24.0) on R (v. 3.6.0) [73]. We built a model estimating the effects of genotype (France, U.S.A and Japan), the environment (control and paraquat), and the genotype-by-environment interaction (GEI effect). The lfcShrink function was used to estimate log_2-fold-change and identify differentially expressed (DE) genes using the ashr R package [74]. DE genes were those with an FDR-adjusted p-value below 0.01 and absolute log_2-fold-change > 1. The coefficient of variation (CV, standard deviation/mean) on normalized counts was computed for each genotype, between control and paraquat.

Transposable element (TE) identification

The reference genome was masked using a custom TE library (Mérel et al., in prep). The Python script create-reads-for-te-sequences.py was used to generate reads corresponding to the TE library using the following parameters : —read-length 125, –max-error-rate 0, –boost 10) [71, 75]. The reads were then mapped to the reference genome using bwa bwasw (v0.7.17) [76]. Aligned bases were masked using bedtools, bamtobed, and bedtools maskfasta (v2.20.0) [77]. This process of read generation and mapping was repeated 200 times. Note that sequences smaller than 500 bp were removed from the TE library. Forward and reverse reads were mapped separately to a fasta file containing the masked reference genome and the TE library. The mapping was done using bwa bwasw. For each line, the resulting single-end read alignments files were merged using PoPoolationTE2 se2pe (v1.10.04) [75]. PoPoolationTE2 pipeline was used to estimate TE frequencies in each sample. The following options were used in the analysis: –map-quality 15 (ppileup module), –mode joint, –signature-window minimumSampleMedian, min-valley minimumSampleMedian, –min-count 2 (identify signature module), –max-otherte-count 2, –max-structvar-count 2 (filterSignatures module), –min-distance -200, –max-distance 300 (pairupSignatures module). In the PoPoolationTE2, hierarchy file was a file allowing multiple slightly diverged sequences to be assigned to one family, and all sequences with cross mapping reads were regrouped in the same family. The cross mapping was investigated by generating TE reads using create-reads-for-te-sequences.py (–read-length 125, –max-error-rate 0, –boost 50) and mapping the reads to the TE library using bwa bwasw. The software was run using the S29, W120 and MT47 DNAseq data. Using the gene annotation of the reference *D. suzukii* genome we identified TEs insertions present in genes (exon, intron, 5' and 3' UTR) and ±2kb flanking regions. We tested the dependence of TE insertions with the state of the genes (DE or not) using a Chi-square test. We considered as absent, TEs with insertion frequency < 0.2 and present when > 0.8. Intermediate frequencies were removed to limit bias. For studies of TE insertions and expression of DE genes, we considered a potential effect of an insertion when frequency > 0.5. TE expression analysis TE expression was quantified using the TEcount module from the TEtools software [78]. Briefly, TEcount sums reads aligned against copies of each TE family annotated from the reference genome creating an output table of expression arranged by TE family [71]. Differential expression of TEs between paraquat-treated and control flies for each isofemale line was computed using a merged file with the RNA counts for genes and TE families, and following normalization using DEseq2.

TFBS screening

TE sequences inserted in flanking regions located ±2kb from differentially expressed genes were screened for transcription factor binding sites (TFBS). We selected three TFBS (CNC, HSF and DL) related to antioxidant response element family (ARE) from the literature [36]. TFBS were screened in R (v. 3.6.0) using the JASPAR2018 database R library (v.1.1.1) and TFBSTools R library (v.1.22.0) [79, 80]. PFM matrices were extracted (CnC:MA0530.1, HSF:MA0486.2,

DL:MA0022.1) before a PWM conversion with the pseudocount value set to 0.8. The minimum score value for the screening was fixed at 0.95 to minimize false positives due to small TFBS sequence sizes. P-values were adjusted with the Benjamini-Hochberg correction for multi-testing [81].

Gene ontology analysis

We performed a GO enrichment analysis directly on the geneontology.org website, using homologs in *D. melanogaster* to discover over or underrepresented gene functions from the lists of DE genes [82]. P-values were calculated using a Fisher test for enriched GO terms and adjusted with the Benjamini-Hochberg correction for multi-testing [81]. GO terms with FDR $\leq$0.05 were defined as significantly enriched. The GO terms were reduced to representative non-redundant terms using the REVIGO tool and manual curation [83].

Competing interests

The authors declare that they have no competing interests.

Author's contributions

P. M. produced data, conceived and wrote the manuscript draft. C. V. & P. G. designed experiments, edited the manuscript. J. P. trimmed NGS data and help in bioinformatic analysis. A. J. calibrated experimental design and produced data. M.L. D.M. reorganized, corrected statistical analysis and revised manuscript. M. F. helped on transcriptomic analysis and manuscript correction. M. G.F. produced gene ontology analysis. V. M. produced all related TEs informations (genome annotations, frequency insertions).

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List of supplementary tables and figures

Additional Fig. S1 — Correlation between median lifespan under or not paraquat treatment for every lines and sex
Additional Fig. S2 — Distribution of the coefficient of variation (%) of DE genes after exposure to paraquat
Additional Fig. S3 — Scatter plots and histograms of differentially expressed genes in pairwise comparisons between populations under control conditions
Additional Fig. S4 — Transcription factor binding analysis in TEs and toward DE genes
Additional Table S1 — Table of the linear mixed model with estimated coefficient and associated statistic.
Additional Table S2 — Table of some differentially expressed genes under control condition (p-adjusted $\leq$0.01 and log_2FC $>$ 1).
Additional Table S3 — Number of DE TEs between control and oxidative (paraquat) condition for each genotype and between the different genotypes for both conditions.
Additional Table S4 — Observed genomic distribution of TE insertions in Japan, U.S.A, France.
Additional Table S5 — Contingency table (observed and expected) of DE genes and TE insertions detected toward 2kb for the three genotypes.
Additional Table S6 — GEI DE genes with inserted element
Additional Table S7 — Geographical location of isofemale lines.
Additional Table S8 — Recipe of diet medium modified

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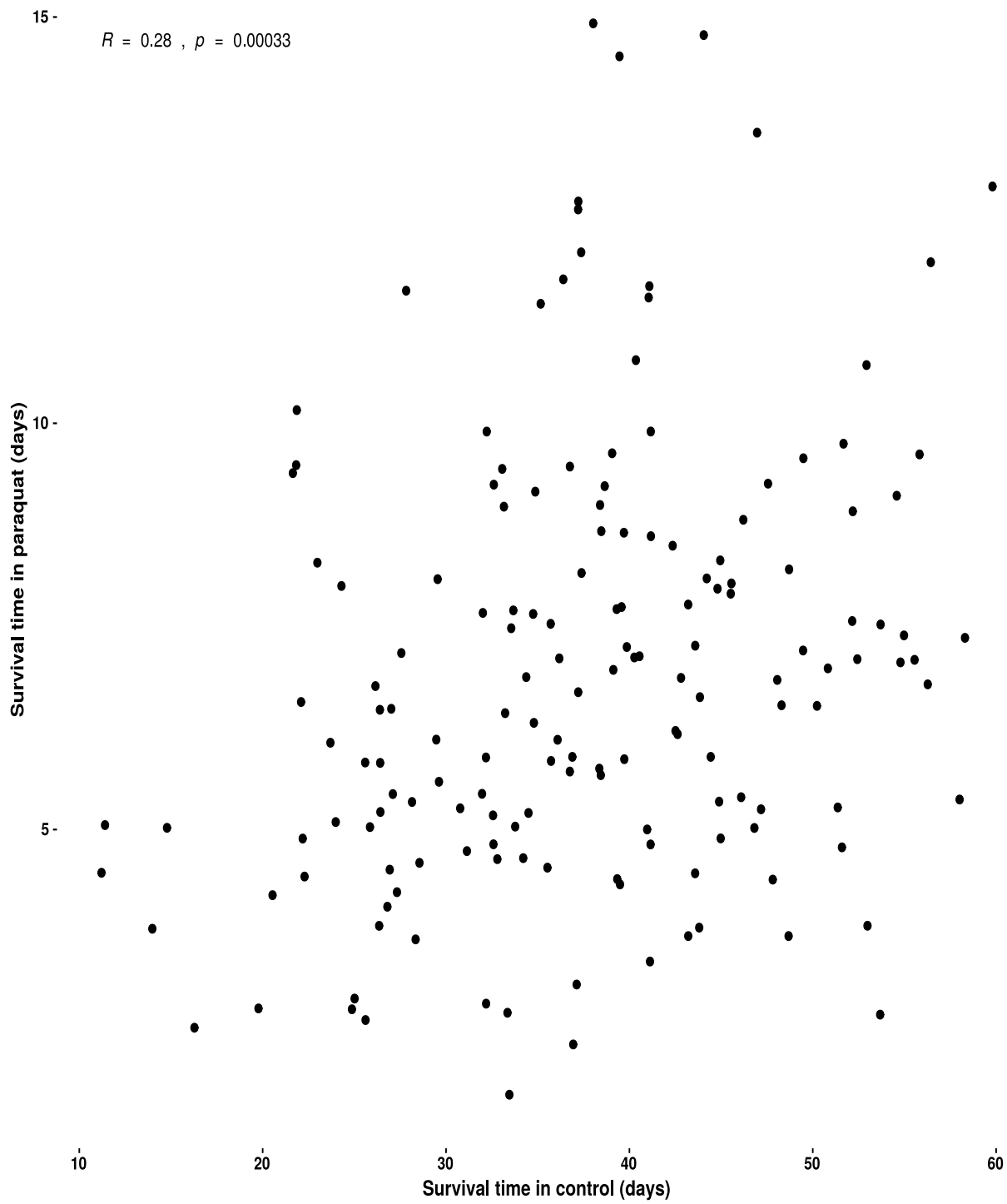

Fig. S1 **Correlation between median lifespan under or not paraquat treatment for every line.** Correlation test was made using Pearson method. Each dot represent the median for both males and female per isofemale lines and replicates.

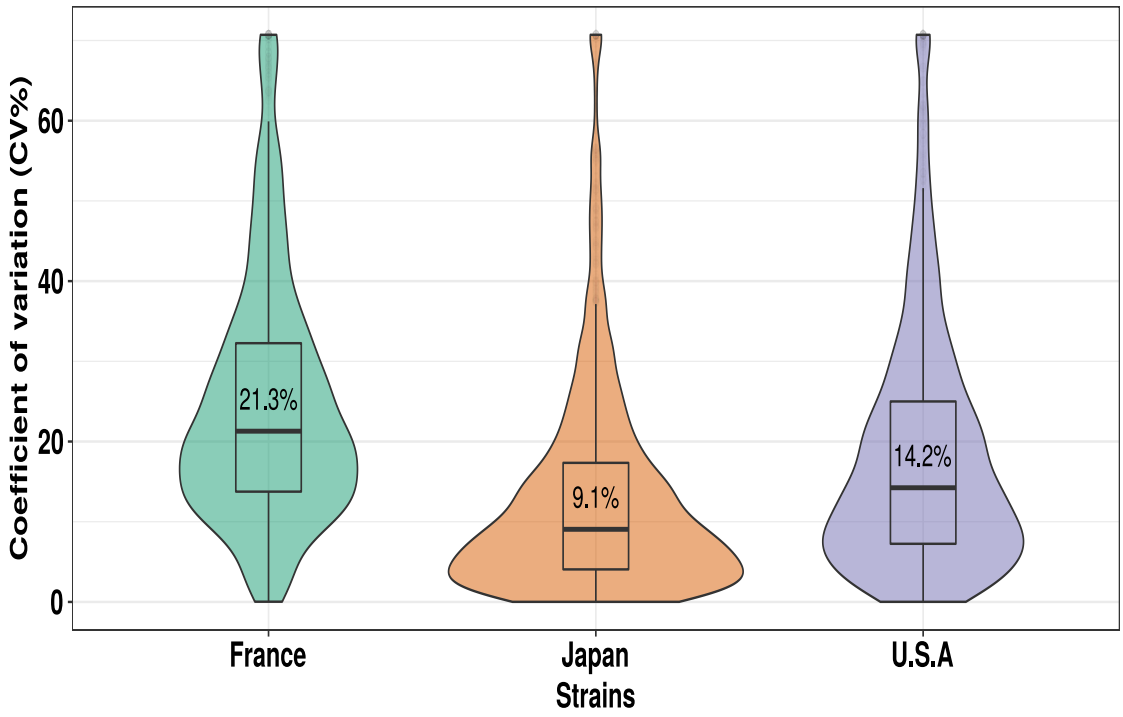

Fig. S2 **Distribution of the coefficient of variation (%) of DE genes after exposure to paraquat.** Coefficients of variation was calculated using the standard deviation and mean counts in control and paraquat treated flies. Central values correspond to the median coefficient of variation. Pairwise comparisons of medians were done using a paired Wilcoxon test and all comparison were significants (p-value ¡ 0.01).

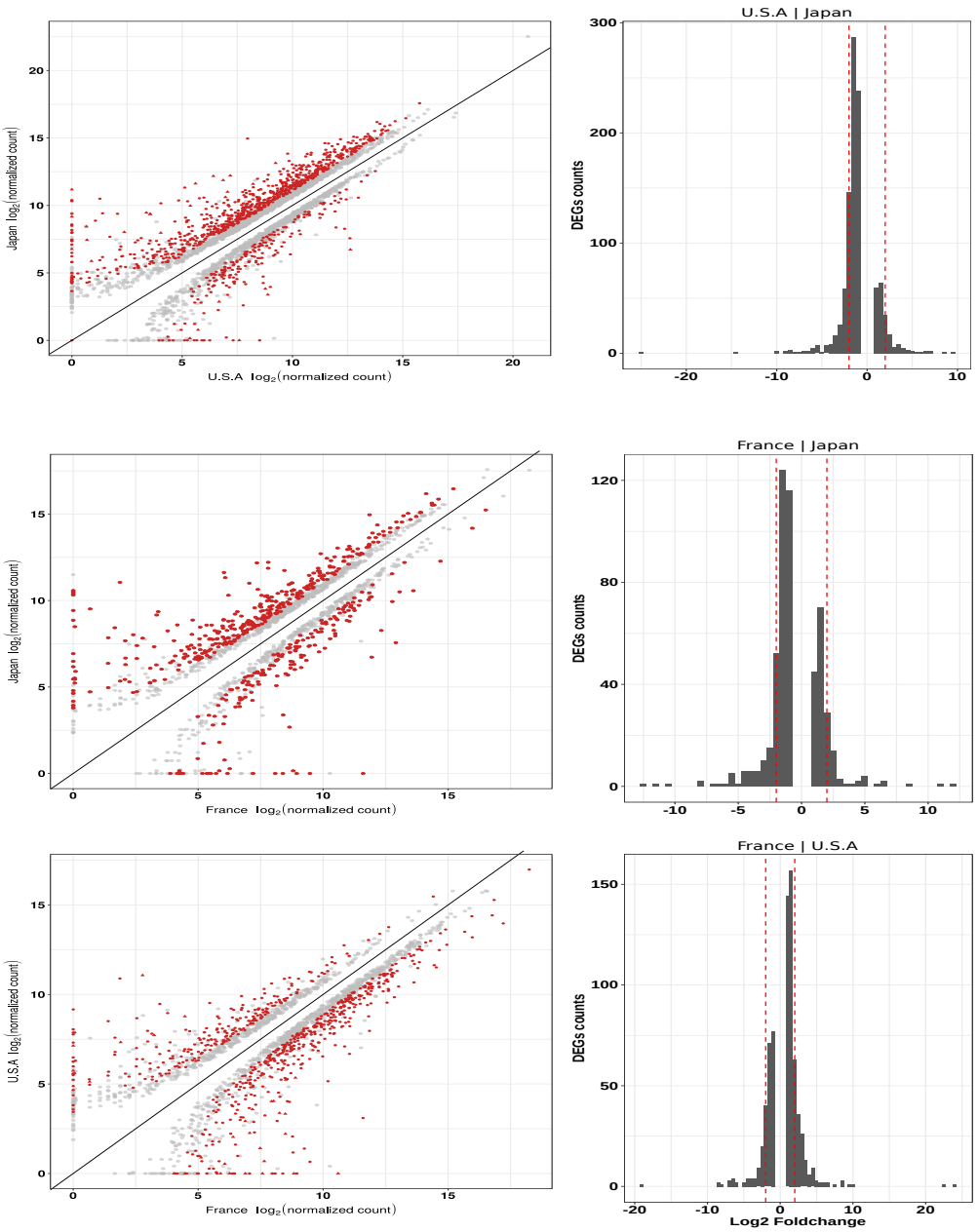

Fig. S3 **Differentially expressed genes in untreated condition.** Scatter plots (left) of significant differentially expressed genes in pairwise comparisons between populations under control conditions using log_2 of normalized counts. Histograms (right) of log_2-fold-changes for DE genes in pairwise comparisons between populations under control conditions. Red lines correspond to threshold of log_2-fold-change = 2.

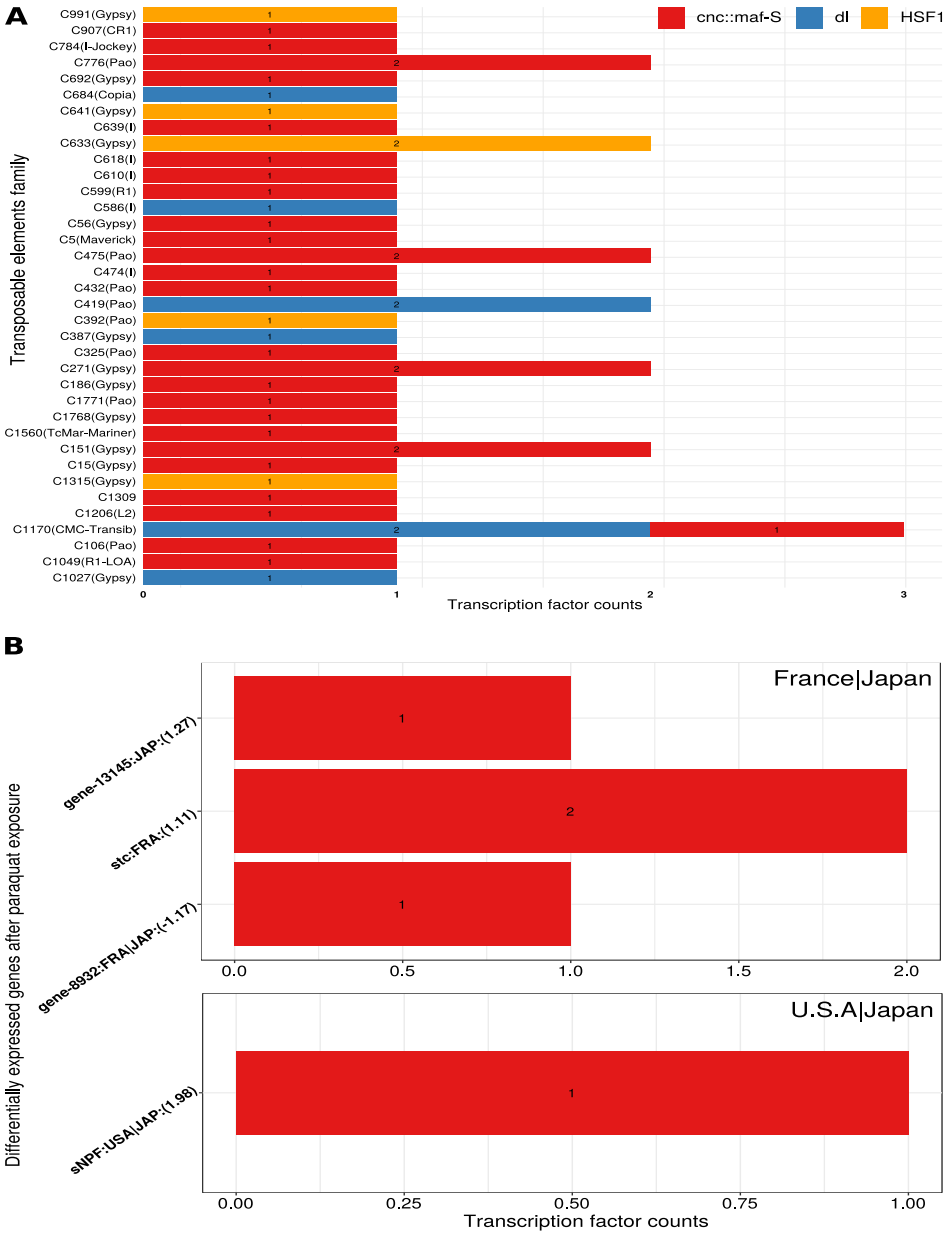

Fig. S4 **Transcription factor binding analysis in TEs and toward DE genes**. (A) Transcription factor binding site numbers in candidate TEs. (B) DE genes identified from comparisons between populations after paraquat treatment (a GEI). Colours correspond to the 3 screened TFBS, CnC (red), dl (blue), and HSF1 (orange). Gene names are labelled on the y-axis, followed by the genotype where insertion is detected (FRA for France, JAP for Japan, USA for U.S.A and possible combination for shared), and the log_2-fold-change of expression in contrasted genotypes.

Table S1: **Table of the linear mixed model with estimated coefficient and associated statistic.** Model is centered on the Sapporo population reference, in untreated condition. Data were previously log transformed for normality. Isofemale line was included as a random effect and we used exponential of coefficient value as multiplicative effect to interprate.

Factor	Estimate	exp(estimate)	CI(2.5)	CI(97.5)	Std. Error	df	t value	Pr(>|t|)
Intercept)	3.444	31.322	26.614	36.862	0.081	41.46	42.456	8.50e-36
Paraquat	-1.615	0.199	0.173	0.229	0.072	297.00	-22.537	2.92e-66
Tokyo (Japan)	0.071	1.073	0.823	1.400	0.132	41.46	0.533	5.97e-01
Dayton (U.S.A)	0.367	1.444	1.107	1.884	0.132	41.46	2.774	8.27e-03
Watsonville (U.S.A)	0.009	1.009	0.809	1.258	0.110	41.46	0.081	9.36e-01
Paris (France)	0.227	1.254	1.006	1.564	0.110	41.46	2.063	4.55e-02
Montpellier (France)	0.251	1.285	1.006	1.640	0.122	41.46	2.060	4.57e-02
Paraquat:Tokyo (Japan)	-0.069	0.934	0.742	1.175	0.117	297.00	-0.586	5.58e-01
Paraquat:Dayton (U.S.A)	0.110	1.116	0.887	1.405	0.117	297.00	0.941	3.47e-01
Paraquat:Watsonville (U.S.A)	-0.302	0.739	0.611	0.895	0.097	297.00	-3.114	2.03e-03
Paraquat:Paris (France)	-0.090	0.914	0.755	1.106	0.097	297.00	-0.927	3.54e-01
Paraquat:Montpellier (France)	-0.218	0.804	0.651	0.994	0.108	297.00	-2.026	4.37e-02

Table S2: **Table of some differentially expressed genes (p-adjusted $\leq$0.01 and log_2FC $>$ 1).** These genes are exampled in Fig. 7 in the genotype environment interaction (GEI) with flybase information available.

Gene ID	Information	Biological process	Molecular function
dysc	dyschronic (dysc) encodes a protein that regulates the localization of the calcium-activated potassium channel encoded by slo. The product of dysc impacts circadian locomotor patterns, synaptic morphology, active zone structure, and both spontaneous and evoked neurotransmitter release.	regulation of synaptic growth at neuromuscular junction, rhabdomere development, locomotor rhythm, muscle cell cellular homeostasis, positive regulation of ion transmembrane transporter activity, negative regulation of neuromuscular synaptic transmission, photoreceptor cell axon guidance, sensory perception of sound	
FarO	-	long-chain fatty-acyl-CoA metabolic process, negative regulation of cell growth, positive regulation of lipid storage	fatty-acyl-CoA reductase (alcohol-forming) activity, long-chain-fatty-acyl-CoA reductase activity
Hsp68	Heat shock protein 68 (Hsp68) encodes a protein involved in lifespan determination and response to heat shock and starvation.	protein refolding, cellular response to heat, chaperone cofactor-dependent protein refolding, response to starvation, response to unfolded protein, protein folding, determination of adult lifespan	protein folding chaperon, heat shock protein binding, ATP binding, ATPase activity, coupled, unfolded protein binding, misfolded protein binding, ATPase activity, unfolded protein binding
Hsp70Aa	Heat-shock-protein-70Aa (Hsp70Aa) encodes a protein involved in response to heat shock and hypoxia.	chaperone cofactor-dependent protein refolding, response to hypoxia, response to heat, cellular response to unfolded protein, protein refolding, cellular response to heat, response to unfolded protein, vesicle-mediated transport, heat shock-mediated polytene chromosome puffing	protein folding chaperone, heat shock protein binding, ATPase activity, unfolded protein binding, ATP binding, misfolded protein binding, ATPase activity, coupled
Mec2	-	nephrocyte filtration	protein binding, inferred from physical interaction with sns
CCHa2	Insufficient genetic data for FlyBase to solicit a summary.	neuropeptide signaling pathway	neuropeptide hormone activity
Tsf1	Transferrin 1 (Tsf1) encodes an iron binding protein induced during the immune response. Iron sequestration is a classical host defense mechanism to combat bacterial infection. [Date last reviewed: 2019-03-14]	olfactory behavior, response to fungus	-
tobi	-	carbohydrate metabolic process, glycoside catabolic	hydrolase activity, hydrolyzing O-glycosyl compounds
RpL40	Insufficient genetic data for FlyBase to solicit a summary.	Translation, protein ubiquitination, cytoplasmic translation, ubiquitin-dependent protein catabolic process, translation , protein ubiquitination, cellular protein modification process, modification-dependent protein catabolic process	structural constituent of ribosome, protein tag, protein tag, ubiquitin protein ligase binding, structural constituent of ribosome

Table S3: **Number of DE TEs between control and oxidative (paraquat) condition for each genotype and between the different genotypes for both conditions.** DE TE threshold made with adjusted p.value $\leq$0.01 and absolute log_2-fold-change ≥ 1. The rate corresponds to number of DE TE on total TE families (2030).

Carcasses	DE TEs	Up-regulated	Down-regulated	DE rate (%)
France|Japan control	48	22	26	3.08
France|U.S.A control	78	10	68	5.01
U.S.A|Japan control	92	70	22	5.91
Japan (paraquat|control)	3	3	0	0.19
France (paraquat|control)	6	6	0	0.39
U.S.A (paraquat|control)	5	3	2	0.32
France|Japan paraquat	1	1	0	0.06
France|U.S.A paraquat	2	2	0	0.13
U.S.A|Japan paraquat	0	0	0	0.00

Table S4: **Observed genomic distribution of TE insertions in Japan, U.S.A, France.**

	intergenic	±2kb flanking	5'UTR	3'UTR	intron	Exon
France	17142	2179	66	76	2469	115
U.S.A	19210	2399	69	78	2582	124
Japan	18924	2354	73	87	2687	133

Table S5: Contingency table (observed and expected) of DE genes and TE insertions detected toward 2kb for the three genotypes. P-value associated correspond to the Pearson chi-square test result. The three first rows correspond to DE genes in every genotypes after paraquat exposure and last 3 rows to GEI genes in every contrasted genotypes.

Contrast	DE	obs(TE-)	obs(TE+)	est(TE-)	est(TE+)	padj
France	DE-	13994	2380	14036	2338	1.52e-06
	DE+	497	34	455	76	
Japan	DE-	14162	2621	14174	2609	1.23e-02
	DE+	115	7	103	19	
U.S.A	DE-	14203	2421	14228	2396	2.16e-04
	DE+	265	16	240	41	
France|Japan	DE-	13689	3078	13690	3077	9.34e-01
	DE+	114	24	113	25	
France|U.S.A	DE-	13918	2922	13927	2913	1.23e-02
	DE+	63	2	54	11	
U.S.A|Japan	DE-	13799	3044	13808	3035	1.23e-02
	DE+	60	2	51	11	

Table S6: GEI DE genes with inserted element, unknown gene in Flybase was reported with name "gene" followed by a number. Contrast column correspond to the lines tested for GEI. Type is the structure where is inserted the element with the position of right and inserted line correspond to the line where is detected the element.

Gene symbol	log_2FoldChange	Contrast	Type	Insertion position	Inserted line
CG10383	1.753125	France | Japan	intron	11431663	Japan
CG12520	1.892906	France | Japan	3'UTR	16312823	Japan
Mrp4	1.354537	France | Japan	intron	3059393	Japan
FarO	-4.923014	France | Japan	intron	7509567	France
Ire1	1.168464	France | Japan	exon.part	1288004	Japan
CG11626	1.328294	France | Japan	flank2kb	1760496	Japan
(predicted)Hsp70-3	3.007337	France | Japan	intron	2816351	Japan
Drgx	1.027039	France | Japan	intron	23636644	Japan
Drgx	1.027039	France | Japan	flank2kb	23660595	U.S.A
mesh	1.497837	France | Japan	intron	1007389	France
Myo28B1	1.175073	France | Japan	5'UTR	7819926	Japan
Mocs1	1.115113	France | Japan	intron	18050475	U.S.A
(predicted)kelch-like	2.399565	France | Japan	flank2kb	14580423	France
stc	1.106598	France | Japan	flank2kb	23035304	France
CG42808	2.321616	France | Japan	intron	6914954	France
Pvf1	1.262606	France | Japan	flank2kb	924620	Japan
Pvf1	1.262606	France | Japan	5'UTR	928977	Japan
Pvf1	1.262606	France | Japan	flank2kb	924620	France
Pvf1	1.262606	France | Japan	5'UTR	928977	France
Pvf1	1.262606	France | Japan	flank2kb	924620	U.S.A
Pvf1	1.262606	France | Japan	5'UTR	928977	U.S.A
JMJD4	1.152672	France | Japan	5'UTR	12034085	U.S.A
Ldh	1.414352	France | Japan	flank2kb	2962505	France
Ldh	1.414352	France | Japan	flank2kb	2962902	France
gene-8816	-6.929561	France | Japan	exon.part	826521	Japan
gene-8816	-6.929561	France | Japan	flank2kb	828928	Japan
gene-8816	-6.929561	France | Japan	exon.part	826521	France
gene-8816	-6.929561	France | Japan	flank2kb	828928	France
gene-8816	-6.929561	France | Japan	flank2kb	828928	U.S.A
CG4456	1.689628	France | Japan	flank2kb	7586469	U.S.A
Oatp33Eb	1.420563	France | Japan	flank2kb	2565015	Japan
Oatp33Eb	1.420563	France | Japan	flank2kb	2565015	France
Oatp33Eb	1.420563	France | Japan	flank2kb	2565015	U.S.A
Oatp33Eb	1.420563	France | Japan	flank2kb	2566617	U.S.A
gene-16041	-1.556090	France | Japan	intron	51130	Japan
gene-16041	-1.556090	France | Japan	intron	53804	Japan
gene-16041	-1.556090	France | Japan	intron	51130	France
gene-16041	-1.556090	France | Japan	intron	53804	France
gene-16041	-1.556090	France | Japan	intron	51130	U.S.A
gene-16041	-1.556090	France | Japan	intron	53804	U.S.A
Hsp70Aa	1.956436	France | Japan	flank2kb	2824573	Japan
Hsp70Aa	1.956436	France | Japan	flank2kb	2824573	U.S.A
gene-742	1.419717	France | Japan	flank2kb	2573404	U.S.A
CG3513	2.132744	France | Japan	flank2kb	23660595	U.S.A
Socs36E	1.322709	France | Japan	flank2kb	12039658	France
Socs36E	1.322709	France | Japan	intron	12042814	U.S.A
Socs36E	1.322709	France | Japan	intron	12046161	U.S.A

Socs36E	1.322709	France | Japan	intron	12046748	U.S.A
gene-8522	1.051557	France | Japan	exon.part	8449067	Japan
gene-8522	1.051557	France | Japan	exon.part	8449067	France
gene-8522	1.051557	France | Japan	exon.part	8449067	U.S.A
Drat	1.079845	France | JapanF	intron	2761492	France
gene-12098	1.386302	France | Japan	intron	6814225	Japan
lncRNA:CR45936	-1.419969	France | Japan	intron	16923423	U.S.A
gene-2030	1.575219	France | Japan	intron	5579171	Japan
gene-2030	1.575219	France | Japan	intron	5579171	France
gene-2030	1.575219	France | Japan	intron	5579171	U.S.A
gene-3647	1.941648	France | Japan	intron	2104897	France
gene-3647	1.941648	France | Japan	intron	2099877	U.S.A
gene-1513	1.070880	France | Japan	intron	145053	France
(predicted)trypsin_alpha3	-2.153081	France | Japan	intron	6743283	France
gene-13286	1.164912	France | Japan	3'UTR	16312823	Japan
gene-13145	1.267960	France | Japan	intron	16350711	Japan
gene-13145	1.267960	France | Japan	intron	16351595	France
Chs2	1.075446	France | Japan	flank2kb	22441443	Japan
Chs2	1.075446	France | Japan	flank2kb	22441443	U.S.A
gene-12969	1.158750	France | Japan	flank2kb	18466778	Japan
gene-8932	-1.170218	France | Japan	intron	5844	Japan
gene-8932	-1.170218	France | Japan	intron	5844	France
gene-8932	-1.170218	France | Japan	intron	5844	U.S.A
gene-5432	-1.652789	France | Japan	intron	9876320	France
betaTub60D	1.037268	France | Japan	3'UTR	12121904	France
gene-16855	-1.081314	France | Japan	flank2kb	439861	Japan
gene-16855	-1.081314	France | Japan	flank2kb	440304	Japan
gene-16855	-1.081314	France | Japan	flank2kb	439861	France
gene-16855	-1.081314	France | Japan	flank2kb	440304	France
gene-16855	-1.081314	France | Japan	flank2kb	439861	U.S.A
gene-16855	-1.081314	France | Japan	flank2kb	440304	U.S.A
gene-16070	-1.190533	France | Japan	intron	45349	Japan
gene-16070	-1.190533	France | Japan	intron	53781	Japan
gene-16070	-1.190533	France | Japan	intron	56597	Japan
gene-16070	-1.190533	France | Japan	intron	92529	Japan
gene-16070	-1.190533	France | Japan	intron	97953	Japan
gene-16070	-1.190533	France | Japan	intron	99403	Japan
gene-16070	-1.190533	France | Japan	intron	118960	Japan
gene-16070	-1.190533	France | Japan	intron	45349	France
gene-16070	-1.190533	France | Japan	intron	53781	France
gene-16070	-1.190533	France | Japan	intron	56597	France
gene-16070	-1.190533	France | Japan	intron	92529	France
gene-16070	-1.190533	France | Japan	intron	97953	France
gene-16070	-1.190533	France | Japan	intron	99403	France
gene-16070	-1.190533	France | Japan	intron	118960	France
gene-16070	-1.190533	France | Japan	intron	45349	U.S.A
gene-16070	-1.190533	France | Japan	intron	53781	U.S.A
gene-16070	-1.190533	France | Japan	intron	56597	U.S.A
gene-16070	-1.190533	France | Japan	intron	92529	U.S.A
gene-16070	-1.190533	France | Japan	intron	97953	U.S.A
gene-16070	-1.190533	France | Japan	intron	99403	U.S.A
gene-16070	-1.190533	France | Japan	intron	118960	U.S.A
CG33282	1.002304	France | Japan	intron	19704856	U.S.A
CG10361	1.045731	France | Japan	flank2kb	18922002	Japan

(predicted)Hsp70-3	4.281151	France | U.S.A	intron	2816351	Japan
Hsp70Aa	4.230943	France | U.S.A	flank2kb	2824573	Japan
Hsp70Aa	4.230943	France | U.S.A	flank2kb	2824573	U.S.A
FarO	-5.293578	France | U.S.A	intron	7509567	France
(predicted)kelch-like	2.863579	France | U.S.A	flank2kb	14580423	France
CG10621	-1.446417	France | U.S.A	3'UTR	11134738	U.S.A
gene-5842	-1.775330	France | U.S.A	intron	9902605	Japan
gene-5842	-1.775330	France | U.S.A	intron	9902872	Japan
gene-5842	-1.775330	France | U.S.A	intron	9905803	France
CG16965	-1.049352	France | U.S.A	flank2kb	3227015	Japan
gene-9109	6.739623	France | U.S.A	intron	2816351	Japan
Ir48b	2.818729	France | U.S.A	intron	10573980	Japan
Ir48b	2.818729	France | U.S.A	intron	10573980	U.S.A
Tsf1	-1.099044	France | U.S.A	intron	1957700	Japan
Ldh	2.339272	U.S.A | Japan	flank2kb	2962505	France
Ldh	2.339272	U.S.A | Japan	flank2kb	2962902	France
CG12520	1.480822	U.S.A | Japan	3'UTR	16312823	Japan
sNPF	1.983076	U.S.A | Japan	intron	14521385	Japan
sNPF	1.983076	U.S.A | Japan	intron	14523939	Japan
sNPF	1.983076	U.S.A | Japan	intron	14521385	France
sNPF	1.983076	U.S.A | Japan	intron	14521385	U.S.A
CG4456	2.260613	U.S.A | Japan	flank2kb	7586469	U.S.A
Mal-A1	2.109920	U.S.A | Japan	flank2kb	5265200	France
CG16965	1.430053	U.S.A | Japan	flank2kb	3227015	Japan
Mal-A7	2.224882	U.S.A | Japan	intron	5210868	Japan
Mal-A7	2.224882	U.S.A | Japan	intron	5211873	Japan
Mal-A7	2.224882	U.S.A | Japan	intron	5211873	France
Mal-A7	2.224882	U.S.A | Japan	intron	5211873	U.S.A
gene-3840	1.106626	U.S.A | Japan	intron	1916531	U.S.A
tup	1.030389	U.S.A | Japan	intron	11241399	Japan
DptB	2.623785	U.S.A | Japan	intron	4000182	U.S.A
CG4372	1.269428	U.S.A | Japan	flank2kb	1467799	France
Ance-2	1.004916	U.S.A | Japan	intron	5677285	France
CG1304	1.656842	U.S.A | Japan	flank2kb	2387338	Japan
Mal-A3	1.215228	U.S.A | Japan	flank2kb	5257029	France
Tsf1	1.261203	U.S.A | Japan	intron	1957700	Jap

Table S7: **Geographical location of isofemale lines.** *D. suzukii* flies were sampled in 3 countries (Japan, U.S.A and France) with their location and invasive status. Line name is indicated with bold type for the line use in molecular analysis.

Location	Coordinates	Status	Lines
Sapporo (Hokkaido, Japan)	43° 3' 43.545"N 141° 21' 15.754" E	Native	S11, S20, S21, S24, **S29**
Tokyo (Honshu, Japan)	35° 41' 22.155" N 139° 41' 30.143" E	Native	T3, T11, T18
Watsonville (California, U.S.A)	36°54'51.8"N 121°45'27.7"W	Invasive	W106, W112, W113, **W120**, W122, W127
Dayton (Oregon, U.S.A)	45° 13' 14.422" N 123° 4' 34.368" E	Invasive	Sok1, Sok28, Sok58
Paris (France)	48° 51' 23.81" N 2° 21' 7.998" E	Invasive	L2, L6, L7, L21, L22, L26
Montpellier (France)	43° 36' 38.768" N 3° 52' 36.177" E	Invasive	MT15, MT20, MT25, **MT47**

Table S8: Recipe of diet medium modified from Dalton et al. [62]

Distilled water: 1 L

Agar (Drosophila Agar Type, ref.66-103, Apex™): 9 $g.L^{-1}$

Cornmeal (Farine de gaudes, Moulin Giraud): 33 $g.L^{-1}$

Ethanol 96%: 40 $ml.L^{-1}$

Yeast (ref.75570, LYNSIDE®): 17 $g.L^{-1}$

Sugar (supermarket sugar) 50 $g.L^{-1}$

Nipagin (Tegosept,ref.20-258, Apex™): 4 $g.L^{-1}$

Bring to boil agar, cornmeal, yeast extract and sugar in distilled water. Then wait out of the fire about 10 minutes until the mixture cooled to 53°C before adding diluted nipagin in 96% ethanol. Medium is then poured in vials and cooled at room temperature before to be stored at 4°C.

CHAPITRE 4

Drosophila suzukii oxidative stress response involves *Jheh* gene cluster but not transposable elements

Avant-propos

Il a été récemment observé chez *D. melanogaster*, qu'une insertion adaptative *Bari-Jheh* proche d'un groupe de gènes *Jheh* (*Juvenile Hormone Epoxy Hydrolase*), modifiait l'expression des gènes lors d'un stress oxydant (Guio et collab., 2014). Nous avons voulu savoir si un tel événement d'insertion adaptative, pouvait avoir eu lieu chez *D. suzukii*, du fait de l'usage intensif de ce produit dans le monde et donc dans les aires où *D. suzukii* est présente. Pour cela nous avons décidé de nous focaliser sur le même cluster de gènes *Jheh* (*Jheh* 1, *Jheh* 2 et *Jheh* 3), en caractérisant une lignée isofemelle de chaque point d'échantillonnage (S29 pour Sapporo, T18 pour Tokyo, Sok28 pour Dayton, W120 pour Watsonville, MT47 pour Montpellier et L7 pour Paris). Nous avons étudié la survie des six lignées, puis le niveau d'expression des gènes *Jheh* par RT-qPCR ainsi que la diversité génétique de la région génique par la détection d'éléments transposable et de séquences de facteurs de transcriptions. En effet dans le cas de *Bari-Jheh*, l'insertion présentait un motif ARE (antioxidant response element), reconnu par des facteurs de transcriptions modifiant alors l'expression des gènes en aval lors d'un stress oxydant. Nos résultats ont montré que l'espérance de vie en condition non traitée suit la tendance générale observée au niveau de la population avec une plus faible longévité pour les populations japonaises au regard de l'espérance des populations française ou de Dayton au U.S.A. Cependant l'induction d'un stress oxydant via le paraquat montre que les japonaises ne sont pas plus sensibles que les autres lignées, au contraire, la France pour les mâles et pour les femelles (à l'exception de Montpellier) est plus sensible que les autres populations. L'interdiction en 2007 du paraquat en Europe est une hypothèse pour expliquer ces différences. Au niveau de l'expression des gènes nous avons observé que chez les mâles, le traitement ne modifie que très peu l'expression alors que les femelles en particulier pour *Jheh-1* et *Jheh-2* ont des changements. Trois réponses se dessinent, Sapporo est étrangement la seule lignée dont tous les gènes sont sous-exprimés après ajout de paraquat. Les lignées américaines ont un niveau stable dans tous les cas alors que les lignées françaises ont une surexpression des gènes *Jheh-1* et *Jheh-2* mais pas *Jheh-3* . Nous n'avons pas pu mettre en lien la diversité génétique observée au travers des éléments et des séquences de facteurs de transcriptions avec les réponses phénotypique et moléculaires.

READY TO SUBMIT

Drosophila suzukii oxidative stress response involves *Jheh* gene cluster but not transposable elements

Pierre Marin[1], Angelo Jaquet[1], Hélène Henri[1], Patricia Gibert[1] & Cristia Vieira-Heddi[1]

ABSTRACT

The study of the mechanisms involved in adaptation remains a timely issue, particularly in the context of global changes. To better understand these mechanisms of rapid adaptation, invasive species are a good model because they are subjected to new and/or different environmental factors. Using different lines of different geographical origin of the invasive pest *Drosophila suzukii*, we characterized the phenotypic response to oxidative stress. Subsequently, we tested the involvement of the *Jheh* gene cluster in this response and the possible role of transposable elements. We show that the resistance to oxidative stress of the lines appears to be related to their invasive status and we confirm the role of the *Jheh* gene cluster in this response. We have not identified any transposable elements in this gene region that could influence the expression of the gene.

KEYWORDS: invasive species, *Drosophila suzukii*, oxidative stress, rapid adaptation, transposable elements, Juvenile Hormone Epoxy Hydrolase

Introduction

The rapid spread of invasive species in a huge spectrum of environments relies on multiple factors, from genetics to phenotypic plasticity, probably including fine molecular mechanism such as hormonal production or epigenetic gene regulation (Marin *et al.*, 2019 ; Stapley *et al.*, 2015 ; Beldade *et al.*, 2011). Phenotypic plasticity, *i.e.*, the ability of a genotype to express different phenotypes in different environments (Ghalambor *et al.*, 2015) has been proposed as one of the most promising explanations for invasive success, particularly in the case of founder population depleted of genetic variation (Marin *et al.*, 2019 ; Estoup *et al.*, 2016). Among deleterious environments that can be encountered by invasive species, oxidative stress caused by phytosanitary products is one of them. The invasive pest, *Drosophila suzukii*, is a good model to investigate the adaptive process during invasion (Gibert *et al.*, 2016). This species which belong to the group of the fruit fly *D. melanogaster* , originally comes from Asia and was detected simultaneously both in North America (U.S.A) and in Europe in 2008. North America was invaded by native Japan populations derived from Hawaii. In Europe, several introductions were detected from U.S.A and from China (Fraimout *et al.*, 2017). Currently, *D. suzukii* is present in both North and South America, in Europe from the south (Spain) to the East (Poland, Ukraine) and it has also been observed in Russia (Lavrinienko *et al.*, 2017 ; CABI, 2020). Characterization of the phenotypic and molecular responses of *D. suzukii* to changing environmental conditions may provide information to the mechanisms involved in the ability of invasive species to cope with environmental variation. Paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride) is one of the most widely used herbicide in the world leading to the production of ROS (reactive oxygen species) (Tsai, 2018). Oxidative stress due to the use of paraquat in the field has also been used in the laboratory as a good proxy for studying stress resistance (Rzezniczak *et al.*, 2011 ; Bus and Gibson, 1984). Paraquat was banned since 2007 in Europe but is still used in many other regions like in U.S.A or Japan. Paraquat exposition is known to induce a reduction in the lifespan associated with changes in gene expression (Vermeulen *et al.*, 2005 ; Liguori *et al.*, 2018 ; Finkel and Holbrook, 2000). One of the candidate genes involved in paraquat resistance is the cluster of *Jheh* (*Juvenile hormone epoxy hydrolase*) genes, which are not only involved in the lifespan but also in response to the oxidative environment (Guio *et al.*, 2014 ; Flatt and Kawecki, 2007). Moreover in *D. melanogaster* , an insertion of a transposable element (TE) *Bari-Jheh*, near the cluster of the *Jheh* genes has been described as driving an increase of resistance in presence of paraquat (Guio *et al.*, 2014). Using several strains of *D. suzukii*, we measured responses to oxidative stress at the phenotypic and molecular level. We made the hypothesis that different genetic backgrounds from native and invasive populations will have different responses to oxidative stress and that the *Jheh* cluster may be involved on it. Due to the over-representation of TEs in the genome of *D. suzukii* (33% of the repeated elements,Sessegolo *et al.*, 2016), compared to other *Drosophila* species, we looked for the presence of TEs in this region in the different lines. We monitored lifespan after paraquat exposure and measured the expression of three genes of *Jheh* cluster *Jheh-1* , *Jheh-2* and *Jheh-3* in six isofemale lines, four from the invasive regions, North America (Watsonville and Dayton) and France (Paris and Montpellier) and two from the native area, Japan (Sapporo and Tokyo). We evaluated the genetic diversity within and between lines by sequencing introns of the *Jheh* genes, searched for TEs and for transcription factor binding sites (TFBS). Our results suggest a strong effect of the genotype on the resistance to stress and changes in *Jheh* expression levels, with no link with TEs.

Materials and methods

Drosophila suzukii lines and rearing conditions

D. suzukii lines were sampled in 2014 from one native country (Japan: Sapporo and Tokyo) and 2 invaded areas (U.S.A: Watsonville and Dayton and France: Montpellier and Paris). Field-inseminated females were isolated to establish half-sib families called isofemale lines commonly used to investigate *Drosophila*

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natural populations (David *et al.*, 2005). Flies were reared in modified medium (drosophila agar type, ref.66-103, Apex™, 9 $g.L^{-1}$; cornmeal 33 $g.L^{-1}$; yeast, dried yeast, ref.75570, LYNSIDE®17 $g.L^{-1}$; industrial sugar 50 $g.L^{-1}$; nipagin, Tegosept, ref.20-258, Apex™4 $g.L^{-1}$; 96% ethanol 40 $ml.L^{-1}$; distilled water 1 L) from Dalton *et al.* (2011), in a humidified, temperature-controlled incubator at 22.5°C, 70% of relative humidity and a 16:8 LD cycle. The recipe of the modified medium was to bring to boil agar, cornmeal, yeast extract and sugar in distilled water. Then wait out of the fire about 10 minutes until the mixture cooled to 53°C before adding diluted nipagin in 96% ethanol. Medium is then poured in vials and cooled at room temperature before to be stored at 4°C. All the experiments were made with 4 to 7 days old flies.

Oxidative stress resistance experiments

We used paraquat (methyl viologen dichloride hydrate, ref. 75365-73-0, Sigma-Aldrich®) to mimic oxidative stress. Oxidative stress was assessed by adding paraquat directly in the medium (10 mM) before the cooling step and below 53°C. The control experiment was made with the same medium but without paraquat. We used one isofemale line per locality (total of six) named Montpellier (France), Paris (France), Sapporo (Japan), Tokyo (Japan), Dayton (U.S.A.) and Watsonville (U.S.A.). We made three replicates per line and per sex, with ten flies per replicate. Survival was monitored every 24h. Flies were transferred into new vials every three to four days to limit microbial development.

RT-qPCR analysis of *Jheh* genes

We quantified the expression of the three *Jheh* genes (*Jheh-1* , *Jheh-2* and *Jheh-3*) by RT-qPCR after induction of oxidative stress and in control condition. Adult males and females were exposed during 24h to medium culture with 20 mM of paraquat. We made three replicates per sex and treatment and used four flies per replicate. After 24h the flies were immediately dissected in PBS 1X solution (Gibco Thermo-Fisher) in order to extract carcasses for both sexes and eliminate germline tissues. RNA extraction was made using 96-well Direct-zol™RNA Kits by Zymo according to the manual and RNA was treated with DNAse. cDNA were obtained from 0.5 µg of RNA using SuperScriptTM IV VILOTM Master Mix (Invitrogen). Negative control was made with cDNA without RT. cDNA were stored at -80°C before qPCR quantification. Gene expression was quantified by quantitative PCR and we used *Rp49* to the normalization step. Primers were designed using the *D. suzukii* referenced genome (Table S1, Chiu *et al.*, 2013). Their efficiency was between 91.1% to 97.2% (*RP49*: 91.6%, *Jheh-1* : 97.2%, *Jheh-2* : 95.2%, *Jheh-3* : 91.1%). 2 µl of sample were supplemented with 5 µL of Sybr-Green mix 2X, 0.3 µl of each primer and 2.4 µl of free water. We made technical duplicates for each sample. The PCR program was the follow: 95°C for 10 minutes, 15 seconds at 95°C, 10 seconds at 60°C and 72°C for 40 cycles.

Genetic diversity of isofemale lines

We sequenced intronic regions of *Jheh* gene cluster of the six lines used in this study. DNA was extracted from 10 females per line with EZ-10 Biobasic 96 well DNA extraction (EZ-10 96 well plate genomic, ref. BS437, Biobasic) following the manufacturer instructions. Primers were designed to flank the intronic regions for the three *Jheh* genes (Table S1) and Phusion DNA Polymerase (2 U/µL) was used to amplify sequences. The same PCR program was used for all the couple of primers: 98°C for 10 minutes, and 30 seconds at 98°C, 1 minute at 56°C and 20 seconds at 72°C for 40 cycles and 1 minute to 72°C. Sequence of both strands were made directly from the PCR product by BIOFIDAL sequencing company. Sequences were manually curated with CLC Main Workbench 8 software (Qiagen) before alignment (Muscle alignment) to generate haplotypes by line for each intron. MEGA X software was used to calculate pairwise comparison and nucleotide diversity using p-method option (Table S2, Table S3) (Kumar *et al.*, 2018).

Detection of Transposable elements and transcription factor binding sites

We sequenced the intergenic regions of the *Jheh* gene cluster, plus the 5' and 3' regions of the cluster (Table S1). DNA was extracted from one female per population as described above. Classical PCR method was used with the following program, 10 minutes at 95°C, 30 seconds at 95°C, 30 seconds at 63°C, 3 minutes at 72°C and 15 minutes at 72°C for 25 cycles for the region before *Jheh-1* and between *Jheh-1* and *Jheh-2* , 35 cycles for the region between *Jheh-2* and *Jheh-3* and 30 cycles after *Jheh-3* . We identified TEs in the intergenic regions by a blast against a homemade data base of the TE sequences from the *D. suzukii* reference genome (Paris *et al.*, 2020 ; Mérel *et al.*, *in prep*). For TFBS (Table 1), we used conSite website to screen all TFBS from insect

Table 1. Transcription factor analysed with the PWM matrix ID from JASPAR2018

Trancription factors	PWM ID	Species	Origin
HSF (heat shock factor)	MA0486.2	*Homo sapiens*	
HIF1 (hypoxia inducible factor)	MA0259.1	*Homo sapiens*	
DL (Dorsal)	MA0022.1	*D. melanogaster*	
MTF1 (Metal response element-binding Transcription Factor-1)	PB0044.1	*Mus musculus*	
DEAF1 (Deformed epidermal autoregulatory factor-1)	MA0185.1	*D. melanogaster*	From Villanueva-Cañas *et al.*, 2019
CAD (caudal)	MA0216.2	*D. melanogaster*	
NUB (nubbin)	MA0197.2	*D. melanogaster*	
XBP1 (X box binding protein-1)	MA0844.1	*Homo sapiens*	
CnC (cap-n-collar)	MA0530.1	*D. melanogaster*	
Br(var4)			
(broad complex 4)	MA0013.1	*D. melanogaster*	
Hb (hunchbak)	MA0049.1	*D. melanogaster*	
Ubx (Ultrabithorax)	MA0094.2	*D. melanogaster*	From conSite website Sandelin *et al.*, 2004
Cf2 (Chorion factor 2)	MA0015.1	*D. melanogaster*	
Snail(sna)	MA0086.2	*D. melanogaster*	

Mainly matrix come from *D. melanogaster* model, but several as HSF, HIF1 and XBP1 come from human, while MTF1 come from mice.

in our sequences (Sandelin *et al.*, 2004). To complete our analysis, we used the TFBS obtained from Villanueva-Cañas *et al.* (2019) and we extracted PFM (position frequency matrix) of the 14 TFBS from the JASPAR2018 database (v.1.1.1) (Parcy *et al.*, 2017). Then, we used TFBSTools (v.1.22.0) package from R software (v. 3.6.0) to convert in PWM (position weight matrix), and then search on the 6 lines and the reference genome of *D. suzukii* (Tan and Lenhard, 2016 ; R Core Team, 2019 ; Paris *et al.*, 2020).

Statistical analysis

Survival monitoring

Survival data were analyzed using a linear mixed model with lmer function from lme4 provided on R (v. 3.6.0) after a log transformation, confirmation of normality and homoscedasticity (Bates *et al.*, 2015). This model was chosen after log-likelihood comparison between models (linear model with raw or log transformed data, survival model with a Weibull distribution). We analyzed sexes separately to limit interaction terms, and focused on the effect of the treatment, the lines and their interaction. Biological replicates were added as random effect and we plotted exponential of the values and associated confidence interval on the Fig. S1. Those effects can be interpreted as multiplicative effect on the mean lifespan compared to the reference chosen here as the non-exposed group from Sapporo (e.g. the Sapporo reference is centered on 1 and the effect of paraquat 0.18 involves a survival time under paraquat for Sapporo of 0.18 or 18% of the survival time without paraquat).

qPCR analysis

RT-qPCR raw data were analyzed using R and EasyqpcR library (1.21.0) based on $\delta\delta Ct$ method for the quantification and normalization with *RP49* (Sylvain, 2012). Data were analyzed separately for the three genes (*Jheh-1 -2* and *-3*) and sex using a linear model (ANOVA2, Table S4) after log transformation to validate homoscedasticity and normality. Pairwise comparisons were made using a Tuckey test.

Results

D. suzukii wild type lines have significant differences in lifespan

To investigate the influence of the genotypes from different geographical origins on the lifespan, we compared the invasive and native *D. suzukii* lines in control condition (Fig. 1A, Fig. S1 & Table 2). The lifespan ranges from 31 to 55 days for females and from 25 to 45 days for males. For females we observed a strong genotype effect related to geographic location: the genotypes that lived the longest were those of Dayton and Paris (about 1.88-1.96 times more than Sapporo, Fig. S1). Sapporo, Tokyo, and Watsonville were not significantly different and with the lowest lifespan. For males, the four invasive genotypes from Europe and U.S.A had a higher lifespan than Sapporo. Tokyo was similar to Sapporo.

As expected, exposure to paraquat reduced life span on average from 82 to 77% for females and males (Fig. 1A, Fig. S1). The two lines with the best paraquat resistance in absolute value were still Dayton and Paris in both sexes (Fig. 1A and Table 2). We then wanted to have an estimate of paraquat sensitivity (*i.e.*, the slope difference Fig.1 B) taking into account the 'natural' longevity of each line by estimating the value of the interaction coefficients (*i.e.*, the slope difference compared to Sapporo) in Table 2 and statistically tested in Fig. S1. Again, the effect was not similar between genotypes and sexes. For females, Paris was the line presenting significantly the highest sensitivity (-0.87, Table 2) with a reduction of 28% of the life span compared to Sapporo (Fig. S1). For males, the reduction in life span was significantly the highest for Montpellier and Watsonville (-0.84 and -0.83) with a reduction from 34 and 32% by comparison with Sapporo. These results reveal a strong genotype-by-environment interaction in the response to oxidative stress and also a sex effect. It is interesting to note that despite the shorter life span of Japanese genotypes, and in particular of Sapporo in the absence of treatment, these genotypes were the most resistant to paraquat exposure, as shown by the lowest ratio of paraquat lifetime to control lifetime (Table 2).

Table 2. Mean (±SD) of survival time (days) for *D. suzukii* males and female's lines, in control and paraquat condition.

Lines	Females		Males	
	Control	Paraquat	Control	Paraquat
Sapporo (Japan)	31 ±13.3	5.4 ±2.3	25.4 ±5.9	6.2 ±2.1
Tokyo (Japan)	27.1 ±9.1	5.1 ±1.9	29.2 ±11.5	7.7 ±4.9
Dayton (U.S.A)	53 ±8.4	9.5 ±3	42.8 ±16.3	11 ±4.1
Watsonville (U.S.A)	34.9 ±13.8	6.6 ±2.3	34.5 ±11.2	5.5 ±2.1
Paris (France)	55.4 ±9.4	7.2 ±1.8	41.9 ±14.8	8.1 ±2.8
Montpellier (France)	37.9 ±8.4	5.7 ±2.2	44.7 ±12.2	7.5 ±3.8

Jheh genes expression changes with the paraquat treatment

To investigate the effect of paraquat-mediated oxidative stress on the gene expression level, we focused on *Jheh* gene cluster described as potentially involved in stress response in insects and mammals (Oesch *et al.*, 2000 ; Guio *et al.*, 2014). We quantified the level of expression of the *Jheh* genes (*Jheh-1* , *Jheh-2* and *Jheh-3*) in adult males and females flies for the six genotypes described above (Fig. 2). We observed strong differences between males and females. For males, gene expression was not significantly different between control and paraquat treatment for the six genotypes and for *Jheh-1* , -2, but a global downregulation after paraquat exposure in *Jheh-3* without difference between genotypes (Table S4). In females, the effect of paraquat was different according to the gene and the genotype (Table S4). For *Jheh-1* and *Jheh-2* , oxidative stress resulted in a significant increase of gene expression for the two French genotypes and the Tokyo genotype. On the contrary, the Sapporo genotype exhibited a significant reduction of *Jheh-1* expression in presence of paraquat. For *Jheh-3* , we observed a downregulation of the gene expression only for the Sapporo genotype.

Low Genetic diversity of lines in *Jheh* cluster

To assess the levels of neutral genetic diversity within and between lines, we sequenced intronic regions for *Jheh* genes for each genotype (Fig. 3). As expected, the within-line polymorphism was very

Table 3. Within pi diversity for the six genotypes of D. suzukii among the seven intronics regions sequenced.

Lines	Jheh-1.1	Jheh-1.2	Jheh-1.3	Jheh-2.1	Jheh-2.2	Jheh-2.3	Jheh-3.2
Paris	0.0000	0.0000	0.0000	0.0460	0.0000	0.0000	0.0000
Montpellier	0.0000	0.0169	0.0000	0.0502	0.0081	0.0000	0.0339
Sapporo	0.0000	0.0000	0.0396	0.0546	0.0000	0.0000	0.0000
Tokyo	0.0000	0.0000	0.0000	0.0324	0.0000	0.0144	0.0113
Dayton	0.0000	0.0000	0.0000	0.0449	0.0000	0.0096	0.0000
Watsonville	0.0792	0.0000	0.0198	0.0466	0.0000	0.0000	0.0000
Mean pi diversity	0.0070	0.0080	0.0177	0.0390	0.0214	0.0333	0.0209

The first intron of Jheh-3 was not successfully sequenced and was omitted. Mean diversity per intron was calculated using the most common sequence of the six genotypes.

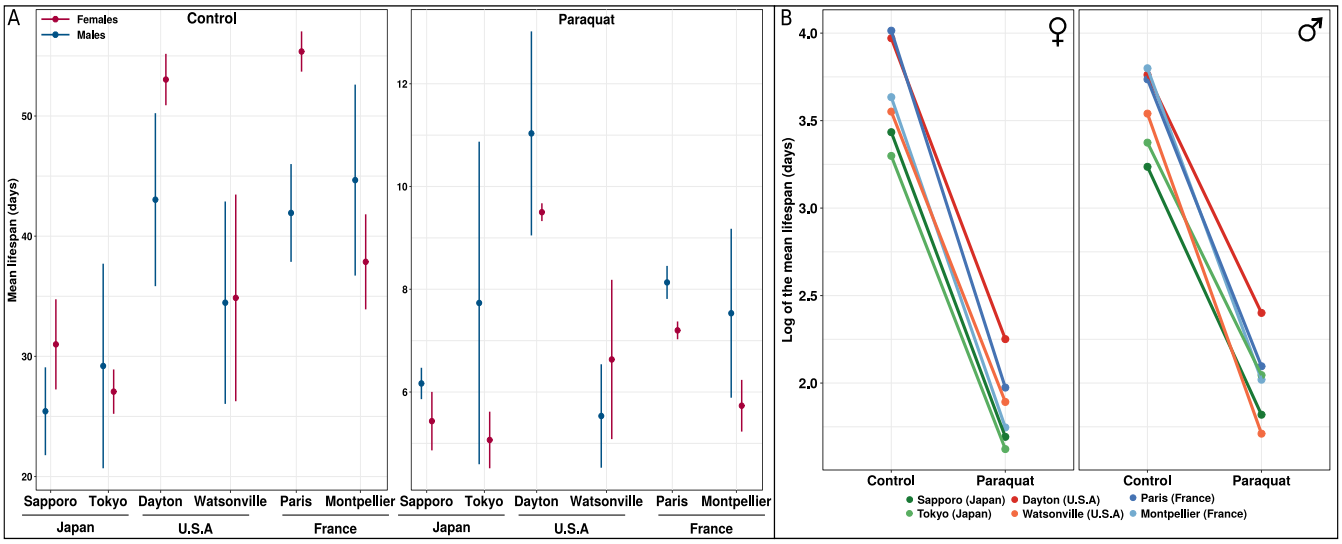

Fig. 1. Mean (±SD) of survival time (days) of *D. suzukii* genotypes (A) and reaction norm (log of the mean) (A). (**A**) mean values under control and paraquat condition for females (dark pink) and males (dark blue) with associated SD. (**B**) reaction norm between treatments (control and paraquat) for both females and males for the 6 genotypes using log transformed mean value. The slope differences between the curves highlight the difference of sensitivity among genotypes.

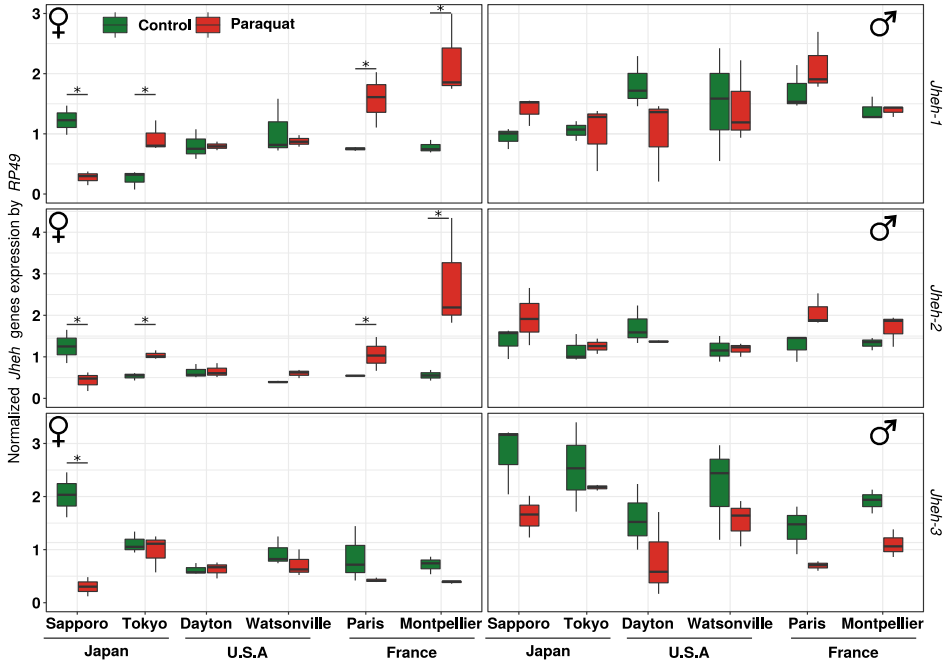

Fig. 2. *Jheh* gene expressions (*Jheh-1, -2, -3*) after normalization by *Rp49* in control (green) and paraquat (red) condition for females on the left and males on the right.

low (Table 3, Fig. S2), with the exception of Watsonville with 0.0792 for the first intron of *Jheh-1* . The number of haplotypes was also low (Table S2). The first intron of *Jheh-2* presents the highest levels of diversity, contrasting with the other introns. These correspond to residual polymorphism that is still maintained in the lines despite the laboratory rearing. Depending on the intronic regions we found between two to four haplotypes per genotype (Table S2). We computed the diversity between genotypes (global intronic nucleotide diversity π) using the most common haplotype for each intron, showing that on average these values are very small, with the highest value for *Jheh-2* .1 as mentioned above (Table S3).

Jheh harbour transcription factor binding sites (TFBS)

Transcription factor binding site (TFBS) are cis regulatory sequences that are recognized by transcription factors and modify gene expression. Several TFBS are known to be involved

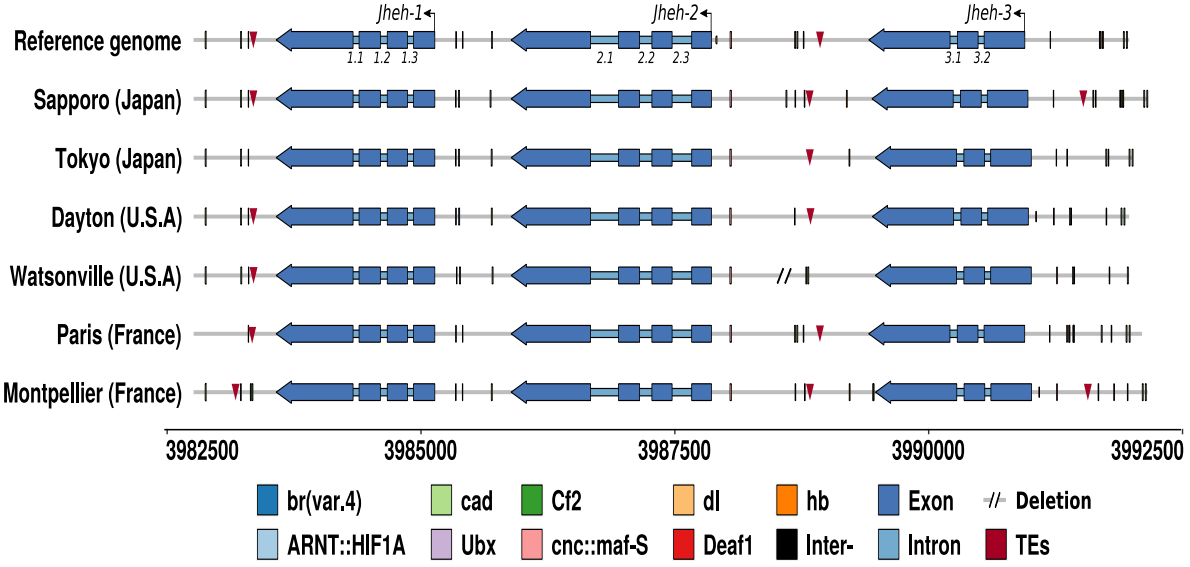

Fig. 3. Blue squares are exon and blue rows represent intronic regions. Red triangles are the detected TEs in intergenic regions and TFBS are indicated by vertical rectangles. Scale is indicated below the figure in base pair.

during oxidative response. We detected 9 of the 14 previous identified TFBS in the intergenic regions: HIF1A, br, cad, Cf2, Deaf1, CnC, dl, hb and Ubx (Fig. 3 & Fig. 4, Table S5). Comparison of the number of TFBS between genotypes (Fig. 4) revealed several differences but not clear link with the changes in expression observed for the *Jheh* genes. For example, the Sapporo genotype which consistently showed a decrease in the expression of all three genes, did not appear to have a different specific TFBS. The two French genotypes which exhibited systematically an increase of expression after paraquat treatment appeared to have an increased number of putative TFBS. For example, the two French genotypes showed two Deaf1 motives when compared to the other genotypes upstream of TSS of the *Jheh-1* gene. In the case of the *Jheh-2* gene, the French genotypes presented a significant number of TFBS, with for example six TFBS for the Montpellier genotype (2Ubx, 2hb, CnC and cad). In this region, no genotype showed the same pattern of TFBS and it was similar for *Jheh-3* .

Transposable elements do not affect *Jheh* gene expression

The presence of TE in the vicinity or within the *Jheh* cluster could impact the gene expression during oxidative stress because they could bring Antioxidant Response Element for transcription factors or by modifying chromatin state (Guio *et al.*, 2014 ; Guio and González, 2015). Surprisingly, and even if *D. suzukii* harbors more than 30% of TEs, no full insertion was observed in the *Jheh* cluster indicating that we are probably in regions of high recombination. However, we did identify small pieces of TE that are quite conserved between the genotypes but no TFBS were detected in these sequences (Fig. 3, Table 4 & S5). No obvious link seems to exist between gene expression and the presence of TE in the *Jheh* cluster.

Discussion

A growing body of literature suggests that responses to oxidative stress in Drosophila may be mediated by insertions of TEs, that in some cases could affect gene expression or the chromatin structure (Guio *et al.*, 2014). In *D. melanogaster* , the *Jheh* gene cluster has been shown to be involved in the response to paraquat treatment and associated with local adaptation. Guio *et al.* (2014) compared *D. melanogaster* genotypes with and without *Bari-Jheh* TE insertion, and showed that, (i) TE insertion had a cost in the absence of stress, (ii) TE insertion confer increased survival in the presence of oxidative stress, (iii) TE insertion provides antioxidant response elements (AREs) that contribute to altered gene expression (Guio *et al.*, 2014 ; Guio and González, 2015). In this study, we analyzed the expression of the *Jheh* gene cluster in several genotypes of *D. suzukii* to test whether the *Jheh* gene cluster is involved in the oxidative stress response and whether TEs could also be associated with alterations in gene expression. We first measured the life span of flies without treatment. We showed that flies of the Japanese genotypes exhibited the shortest lifespan in both males and females. Surprisingly, these lines showed an increased resistance to oxidative stress. The French lines were more sensitive to paraquat than the American ones, although notable differences were observed between lines from the same continent. The negative association we observed between longevity and paraquat resistance had not been observed in previous work with *D. melanogaster* , in which the opposite association was observed (Liguori *et al.*, 2018 ; Finkel and Holbrook, 2000). It could be argued that the use of paraquat in Europe has been banned since 2007, which could lead to a loosening of selection on genes related to paraquat resistance, as observed in other organisms (Shaw, 2000 ; Campos *et al.*, 2014). We then measured the expression of the *Jheh* genes previously reported to be involved in the oxidative response. Consistent with the literature of *D. melanogaster* , we found sex-specific responses to oxidative stress (Weber *et al.*, 2012 ; Guio *et al.*, 2014). For *Jheh-1* and *Jheh-2* , we observed a significant effect of genotype and treatment, but only for females, contrary to what was reported in *D. melanogaster* . For *Jheh-3* , treatment and genotype effect were significant for

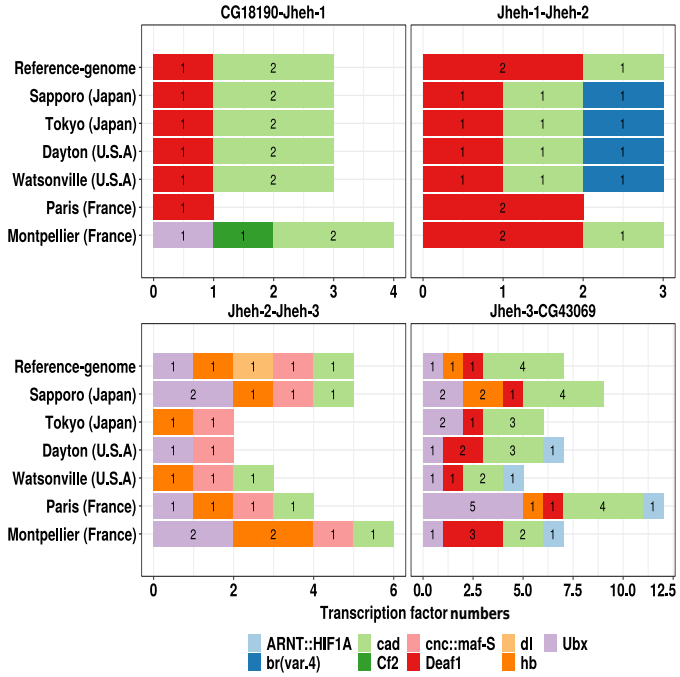

Fig. 4. On the 14 screened sequences, 8 had at least one hit. Intergenic regions are upstream Jheh-1(*CG18190-Jheh-1*), between *Jheh-1* and *-2* (*Jheh-1-Jheh-2*), between *Jheh-2* and *-3* (*Jheh-2-Jheh-3*) after downstream Jheh-3 (Jheh-3-CG43069).

both males and females. These differences in gene expression could not be associated with the presence of TEs insertions, since only partial sequences were present in the intergenic regions. The presence of various TFBS could contribute to the observed differences. We also quantified the polymorphism in our lines, which could be associated with differences in gene expression. We did not observe total homozygosity in the lines but genetic diversity was much lower than what is observed in natural populations of *D. melanogaster* . Lack *et al.* (2016) studied populations from several continents and measured values of nucleotide diversity of up to 0.401 within the population. For inbred DGRP (Drosophila Genetic Reference Panel) lines, the mean intronic diversity was 0.0076 ±0.008, which is close to the values we observed (MacKay *et al.*, 2012). It is therefore unlikely that the residual polymorphism in the *Jheh* gene region can explain the differences in gene expression. The striking result in our analysis is the similar pattern of changes in the expression of *Jheh-1* and *Jheh-2* in females of European genotypes, with an increase in expression, which was associated with lower resistance to oxidative stress, since these are the most sensitive genotypes. On the contrary, the Sapporo genotype systematically showed a reduction in the expression levels of the three genes, which could also be associated with increased resistance in the presence of paraquat, but this was not observed for the Tokyo genotype.

Conclusion

In conclusion, our work shows for the first time how various genotypes of *D. suzukii* respond to oxidative stress and suggests that populations found in invaded areas are more sensitive than Japanese populations, specially the French ones. We have also confirmed that the *Jheh* gene cluster is involved in the response to oxidative stress also in *D. suzukii*, independently of the presence of TE in intergenic regions. This work also suggests that the genetic background and probably trans regulatory sequences are involved in gene expression and stress response. Further phenotypic and genomic studies on natural populations are needed to better understand the success of invasive species such as *D. suzukii*.

Table 4. Size differences (bp) between the six genotypes and the reference genome

		expected size (reference genome)	Sapporo (Japan)	Tokyo (Japan)	Dayton (U.S.A)	Watsonville (U.S.A)	Paris (France)	Montpellier (France)
CG18190-Jheh-1	Size	809	0	-2	0	1	0	12
	TE insertion	82	82	abs	82	82	82	abs
Jheh-1 -Jheh-2	Size	756	-17	-6	-2	2	1	7
	TE insertion	abs	abs	abs	abs	abs	abs	abs
Jheh-2 -Jheh-3	Size	1541	31	-64	35	-297	-1	66
	TE insertion	41	48	49	91	abs	41	49
Jheh-3 -CG43069	Size	1021	171	-8	58	-55	29	122
	TE insertion	abs	58	abs	abs	abs	abs	36

TE insertions are indicated by their size or abs if they are absent.

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Competing interests

The authors declare that they have no competing interests.

Contribution

P. M. produced data, conceived and wrote the manuscript draft. C. V. & P.G. designed experiments, edited the manuscript. A. J. calibrated experimental design. H. H. helped to RT-qPCR method and analysis.

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Supplementary

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Additional tables and figures

Additional Fig. S1 — Model representation using exponential of the coefficient and confidence interval (95%)

Additional Fig. S2 — π genetic diversity

Additional Table S1 — Designed primer using Primer3 plus

Additional Table S2 — Genetic structure of *D. suzukii* genotypes with the number of haplotypes

Additional Table S3 — Pairwise genetic distance

Additional Table S4 — Summaries of the ANOVA 2

Additional Table S5 — Table of TEs & TFBS detected in the 6 genotype and the reference genome

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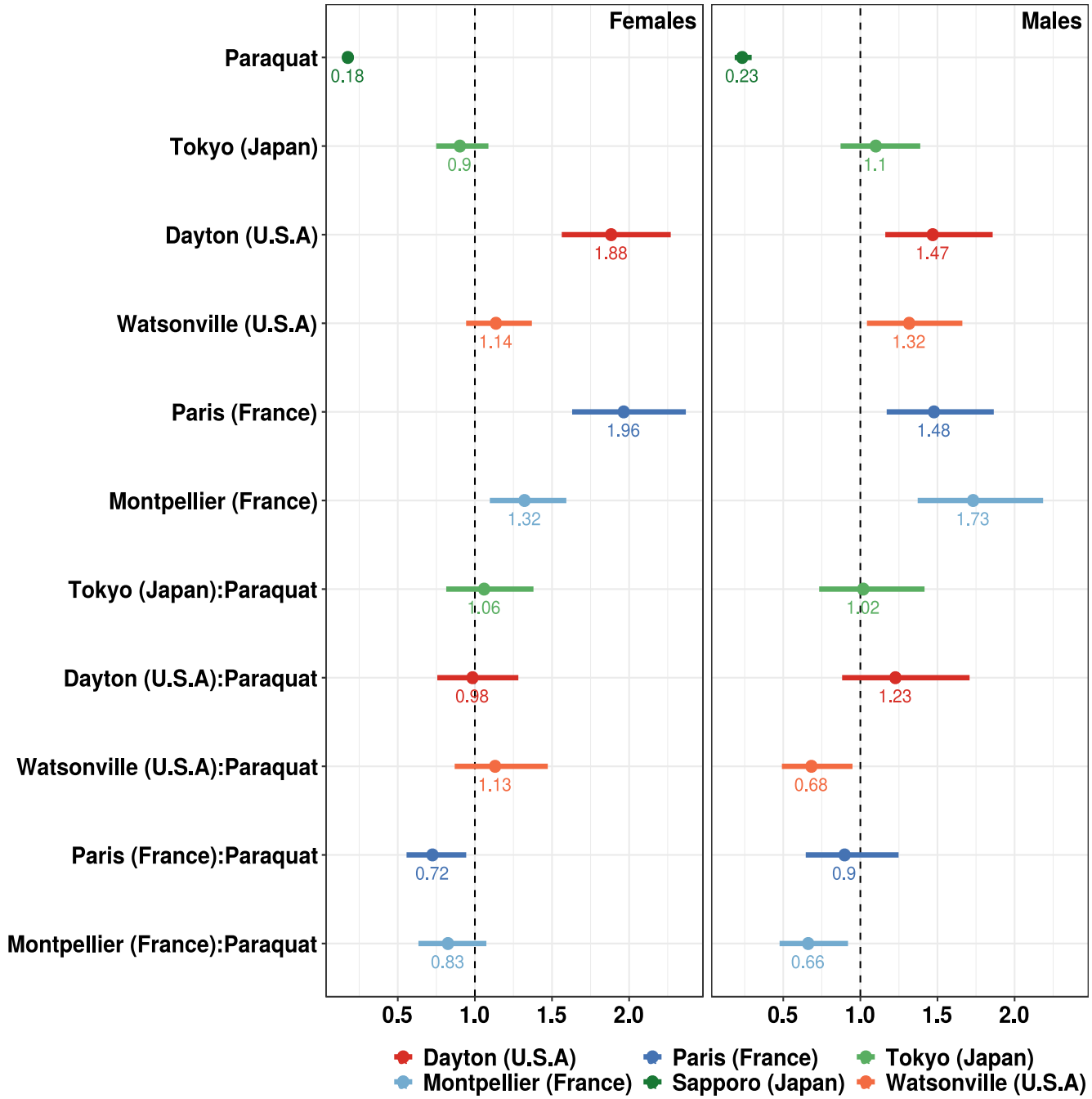

Fig. S1. The values were exponentially transformed to be interpreted as multiplicator effect vertical rows correspond to the reference. p-values associated are above 0.05 when confidence interval crosses the vertical row.

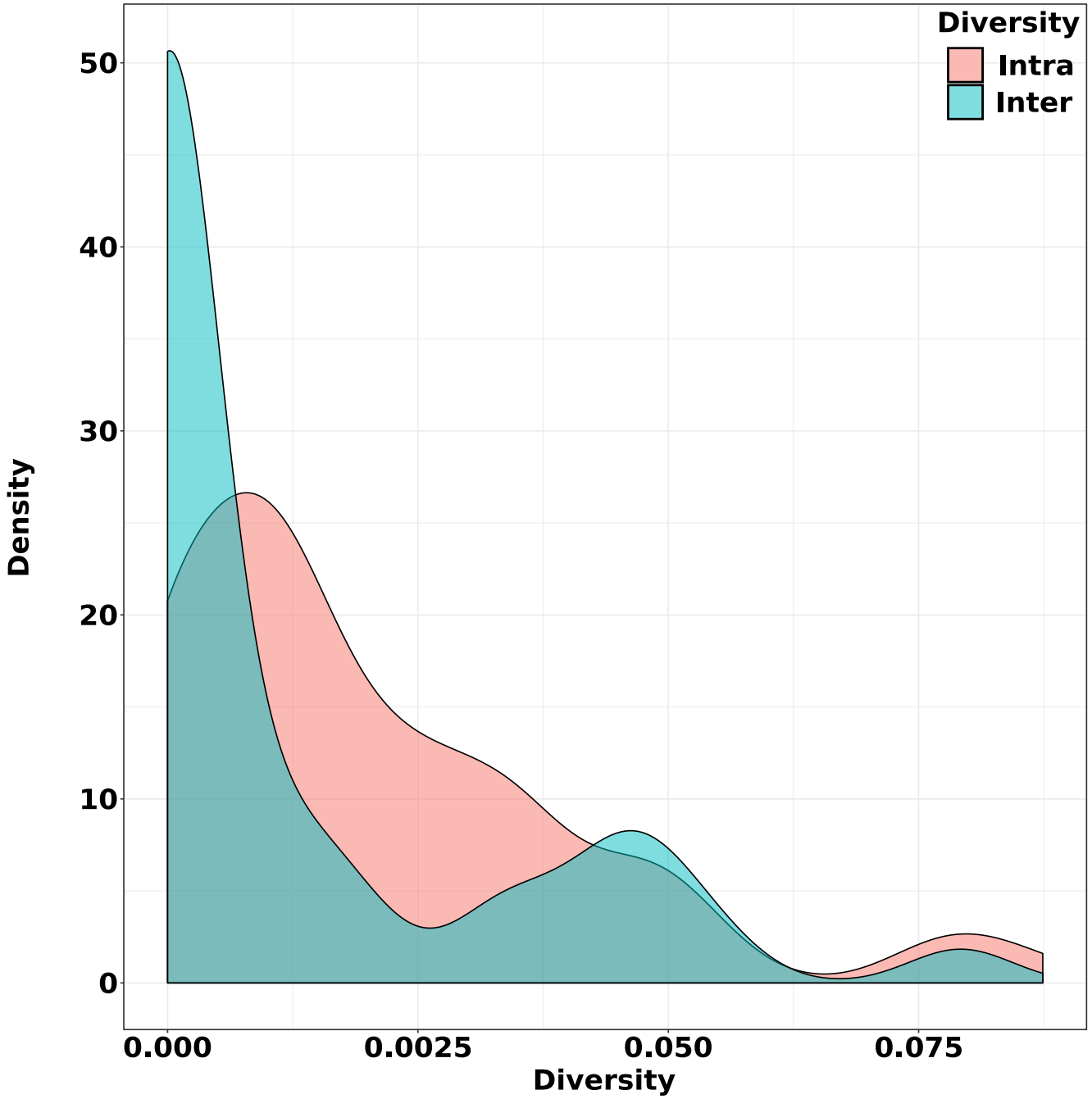

Fig. S2. Pi diversity is indicated on the x axis and y axis correspond to a smoothed representation of counts.

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Table S1. Designed primer using Primer3 plus.

Name	Sequence
Jheh-1	Forward: GAGCAACCTGGACAAGAACAAC Reverse: TATCCCAAGCGCTGCATAAG
Jheh-2	Forward: AGAAGCTGGACCACTACCAAAC Reverse: AGAACCTTCTTGGGCTTCTGG
Jheh-3	Forward: AGTACGCTTTTGAGGTCGTG Reverse: AGACGCAGCATCAAGTTTCG
Rp49	Forward: CCGCTTCAAGGGACAGTATC Reverse: GACGATCTCCTTGCGCTTCT
DS10_00005800-Jheh-1	Forward: GTGTCCCTGGACCATGTTGT Reverse: GGAGGACACTTTGCGGCTAT
Jheh-1-Jheh-2	Forward: GCCAATGGCCAGTACACAGA Reverse: GCCCCAGAAGCTGTACGATG
Jheh-2-Jheh-3	Forward: GCAAAGTGAGCATGATTTGGC Reverse: CAACCCTGTGAACCGAGCTA
DS10_0005804-Jheh-3	Forward: GCAATTAGCTCCCACTCGGT Reverse: CGTGACACTGCAGTTTATGGC
Jheh-1_intr1	Forward: GAGCGGATCCTAGACCCTTC Reverse: GCTGGTCGGAGGTAAGTTGT
Jheh-1_intr2	Forward: AAGAAAGTGCATGCGTAGCC Reverse: TGGCAGTTCAACCACTTCAC
Jheh-1_intr3	Forward: ATTGAGGCGGCTCTTTAGGT Reverse: CGGAGGTGATAAACAACAAACTT
Jheh-2_intr1	Forward: GAGGCCTGGAATTGGAAAAT Reverse: TCTCGAGGGAATAAGAGGTTCA
Jheh-2_intr2	Forward: CGGCTTGGCATGAATAAAGT Reverse: ACGGAGATCCAGGGGTAAGT
Jheh-2_intr3	Forward: CCTCAATTACCTGTGGGGTAAA Reverse: CCCGAGGTAAGCTATGTTTCA
Jheh-3_intr2	Forward: GCCTTCTCGTGAACGTAGTGA Reverse: CAAGCAGTACACGACCGAGA

Table S2. Sequence length used to calculate mean diversity per population per intron. Mean diversity was computed as the mean between the most common haplotype from the 6 lines.

Lines	# of haplotypes	# of flies	Size of the alignment	Mean diversity
		Jheh-1 intron 1		
Paris (France)	1	6	97pb	0.0070
Montpellier (France)	1	10		
Sapporo Japan)	1	6		
Tokyo (Japan)	1	10		
Dayton (U.S.A)	1	10		
Watsonville (U.S.A)	2	7-2		
		Jheh-1 intron 2		
	N haplotype	N	Alignment	Mean diversity
Paris (France)	1	10	119pb	0.0080
Montpellier (France)	2	4-3		
Sapporo Japan)	1	10		
Tokyo (Japan)	1	10		
Dayton (U.S.A)	1	10		
Watsonville (U.S.A)	1	10		
		Jheh-1 intron 3		
	N haplotype	N	Alignment	Mean diversity
Paris (France)	1	10	133pb	0.0177
Montpellier (France)	1	10		
Sapporo Japan)	2	7-2		
Tokyo (Japan)	1	10		
Dayton (U.S.A)	1	10		
Watsonville (U.S.A)	2	5-5		
		Jheh-2 intron 1		
	N haplotype	N	Alignment	Mean diversity
Paris (France)	2	4-2	250pb	0.0390
Montpellier (France)	2	5-2		
Sapporo Japan)	2	4-3		
Tokyo (Japan)	4	2-2-1-1		
Dayton (U.S.A)	3	04/02/01		
Watsonville (U.S.A)	2	5-4		
		Jheh-2 intron 2		
	N haplotype	N	Alignment	Mean diversity
Paris (France)	1	8	165pb	0.0214
Montpellier (France)	3	04/03/02		
Sapporo Japan)	1	10		
Tokyo (Japan)	1	8		
Dayton (U.S.A)	1	9		
Watsonville (U.S.A)	1	10		
		Jheh-2 intron 3		
	N haplotype	N	Alignment	Mean diversity
Paris (France)	1	8	211pb	0.0333
Montpellier (France)	1	8		
Sapporo Japan)	1	10		
Tokyo (Japan)	2	5-4		
Dayton (U.S.A)	2	4-2		
Watsonville (U.S.A)	1	10		
		Jheh-3 intron 2		
	N haplotype	N	Alignment	Mean diversity
Paris (France)	1	10	118pb	0.0209
Montpellier (France)	2	7-3		
Sapporo Japan)	1	9		
Tokyo (Japan)	3	07/02/01		
Dayton (U.S.A)	1	9		
Watsonville (U.S.A)	1	10		

Journal of Experimental Biology (2019) 00, jebxxxxxx. doi:10.1242/jeb.xxxxxx

Table S3. Bold values represent genetic diversity between haplotype of a lineage.

Jheh-1.1	Paris	Montpellier	Sapporo	Tokyo	Dayton	Watsonville
Paris	0.0000					
Montpellier	0.0104	0.0000				
Sapporo	0.0104	0.0000	0.0000			
Tokyo	0.0208	0.0104	0.0104	0.0000		
Dayton	0.0105	0.0000	0.0000	0.0105	0.0000	
Watsonville	0.0104	0.0000	0.0000	0.0104	0.0000	0.0792
Jheh-1.2	Paris	Montpellier	Sapporo	Tokyo	Dayton	Watsonville
Paris	0.0000					
Montpellier	0.0084	0.0169				
Sapporo	0.0000	0.0084	0.0000			
Tokyo	0.0000	0.0084	0.0000	0.0000		
Dayton	0.0000	0.0084	0.0000	0.0000	0.0000	
Watsonville	0.0000	0.0084	0.0000	0.0000	0.0000	0.0000
Jheh-1.3	Paris	Montpellier	Sapporo	Tokyo	Dayton	Watsonville
Paris	0.0000					
Montpellier	0.0079	0.0000				
Sapporo	0.0157	0.0236	0.0396			
Tokyo	0.0079	0.0000	0.0236	0.0000		
Dayton	0.0238	0.0159	0.0379	0.0159	0.0000	
Watsonville	0.0236	0.0157	0.0226	0.0157	0.0152	0.0198
Jheh-2.1	Paris	Montpellier	Sapporo	Tokyo	Dayton	Watsonville
Paris	0.0460					
Montpellier	0.0502	0.0502				
Sapporo	0.0546	0.0462	0.0546			
Tokyo	0.0502	0.0167	0.0378	0.0324		
Dayton	0.0254	0.0466	0.0511	0.0466	0.0449	
Watsonville	0.0502	0.0084	0.0462	0.0084	0.0466	0.0466
Jheh-2.2	Paris	Montpellier	Sapporo	Tokyo	Dayton	Watsonville
Paris	0.0000					
Montpellier	0.0121	0.0081				
Sapporo	0.0303	0.0303	0.0000			
Tokyo	0.0061	0.0061	0.0242	0.0000		
Dayton	0.0364	0.0364	0.0242	0.0303	0.0000	
Watsonville	0.0000	0.0121	0.0303	0.0061	0.0364	0.0000
Jheh-2.3	Paris	Montpellier	Sapporo	Tokyo	Dayton	Watsonville
Paris	0.0000					
Montpellier	0.0240	0.0000				
Sapporo	0.0144	0.0096	0.0000			
Tokyo	0.0144	0.0096	0.0000	0.0144		
Dayton	0.0144	0.0096	0.0000	0.0000	0.0096	
Watsonville	0.0825	0.0874	0.0777	0.0777	0.0777	0.0000
Jheh-3.2	Paris	Montpellier	Sapporo	Tokyo	Dayton	Watsonville
Paris	0.0000					
Montpellier	0.0339	0.0339				
Sapporo	0.0254	0.0085	0.0000			
Tokyo	0.0339	0.0169	0.0085	0.0113		
Dayton	0.0000	0.0339	0.0254	0.0339	0.0000	
Watsonville	0.0339	0.0169	0.0085	0.0000	0.0339	0.0000

Table S4. Summary of the ANOVA 2 by gene and sex.

Gene	Sex	Estimate	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Jheh-1	Females	Treatment	1	0.7578741	0.7578741	5.4488993	0.0286769
		Genotype	5	5.7726966	1.1545393	8.3008099	0.0001334
		Treatment:Genotype	5	7.6349146	1.5269829	10.9785737	0.0000172
		Residuals	23	3.1990137	0.1390876	NA	NA
	Males	Treatment	1	0.0375774	0.0375774	0.1612375	0.6915729
		Genotype	5	1.5806168	0.3161234	1.3564255	0.2754043
		Treatment:Genotype	5	1.4780750	0.2956150	1.2684280	0.3096968
		Residuals	24	5.5933488	0.2330562	NA	NA
Jheh-2	Females	Treatment	1	1.3696002	1.3696002	13.3644443	0.0013171
		Genotype	5	2.7850846	0.5570169	5.4353244	0.0019155
		Treatment:Genotype	5	5.2867104	1.0573421	10.3174554	0.0000276
		Residuals	23	2.3570606	0.1024809	NA	NA
	Males	Treatment	1	0.2389486	0.2389486	4.2568387	0.0500705
		Genotype	5	0.6101519	0.1220304	2.1739557	0.0908517
		Treatment:Genotype	5	0.4690600	0.0938120	1.6712490	0.1799009
		Residuals	24	1.3471889	0.0561329	NA	NA
Jheh-3	Females	Treatment	1	2.8083330	2.8083330	21.6508485	0.0001105
		Genotype	5	1.8020725	0.3604145	2.7786163	0.0417902
		Treatment:Genotype	5	3.4117019	0.6823404	5.2605045	0.0022994
		Residuals	23	2.9833315	0.1297101	NA	NA
	Males	Treatment	1	2.5825122	2.5825122	13.5076975	0.0011917
		Genotype	5	4.5987363	0.9197473	4.8106909	0.0034625
		Treatment:Genotype	5	0.6865256	0.1373051	0.7181674	0.6161005
		Residuals	24	4.5885164	0.1911882	NA	NA

Table S5.: Detected transcription factor binding sites (TFBS) and TEs among the six genotypes and the reference genome of *D. suzukii*. We screened TFBS in intergenic regions before, between and after the *Jheh* genes. Transcription factor (TF) and transposable éléments (TE) names were indicated with their detected positions (bp).

Genotype	Gene	subgene	Element	Start	end
Dayton (U.S.A)	CG18190-Jheh-1	RLX-incomp_Blc1935_Dsuz-L-B2033-Map1_reversed	TE	3983310	3983388
Montpellier (France)	CG18190-Jheh-1	RXX_Blc1636_Dsuz-B-R3220-Map20_reversed	TE	3983164	3983184
Paris (France)	CG18190-Jheh-1	RLX-incomp_Blc1935_Dsuz-L-B2033-Map1_reversed	TE	3983310	3983388
Reference genome	CG18190-Jheh-1	RLX-incomp_Blc1935_Dsuz-L-B2033-Map1_reversed	TE	3983310	3983388
Sapporo (Japan)	CG18190-Jheh-1	RLX-incomp_Blc1935_Dsuz-L-B2033-Map1_reversed	TE	3983310	3983388
Tokyo (Japan)	CG18190-Jheh-1	RXX-LARD_Blc2842_Dsuz-L-B3109-Map1_reversed	TE	3983339	3983380
Watsonville (U.S.A)	CG18190-Jheh-1	RLX-incomp_Blc1935_Dsuz-L-B2033-Map1_reversed	TE	3983312	3983390
Dayton (U.S.A)	Jheh-2-Jheh-3	RXX-LARD_Blc2479_Dsuz-L-B2652-Map1	TE	3988791	3988881
Montpellier (France)	Jheh-2-Jheh-3	RXX-LARD_Blc2479_Dsuz-L-B2652-Map1	TE	3988807	3988855
Paris (France)	Jheh-2-Jheh-3	RLX-incomp-chim_Blc427_Dsuz-L-B425-Map1	TE	3988909	3988949
Reference genome	Jheh-2-Jheh-3	RLX-incomp-chim_Blc427_Dsuz-L-B425-Map1	TE	3988910	3988950
Sapporo (Japan)	Jheh-2-Jheh-3	RXX-LARD_Blc2479_Dsuz-L-B2652-Map1	TE	3988804	3988850
Tokyo (Japan)	Jheh-2-Jheh-3	RXX-LARD_Blc2479_Dsuz-L-B2652-Map1	TE	3988805	3988853
Dayton (U.S.A)	Jheh-3-CG43069	RXX-LARD_Blc5020_Dsuz-L-B5102-Map1	TE	3991489	3991526
Montpellier (France)	Jheh-3-CG43069	RXX-LARD_Blc4946_Dsuz-L-B5036-Map1	TE	3991470	3991507
Reference genome	Jheh-3-CG43069	RXX-LARD-chim_Blc2440_Dsuz-L-B2608-Map1	TE	3991670	3991701
Sapporo (Japan)	Jheh-3-CG43069	RYX-incomp_Blc4021_Dsuz-L-B4274-Map1	TE	3991449	3991507
Tokyo (Japan)	Jheh-3-CG43069	DHX-incomp_Blc652_Dsuz-B-R2897-Map20	TE	3991666	3991691
Watsonville (U.S.A)	Jheh-3-CG43069	RXX_Blc2359_Dsuz-B-R8146-Map6_reversed	TE	3991575	3991608
Paris (France)	Jheh-3-CG43069	RXX-LARD_Blc434_Dsuz-L-B429-Map1	TE	3991559	3991592
Dayton (U.S.A)	CG18190-Jheh-1	cad	TF	3983223	3983233
Dayton (U.S.A)	CG18190-Jheh-1	cad	TF	3982874	3982884
Dayton (U.S.A)	CG18190-Jheh-1	Deaf1	TF	3983298	3983303
Montpellier (France)	CG18190-Jheh-1	cad	TF	3983335	3983345
Montpellier (France)	CG18190-Jheh-1	cad	TF	3982874	3982884
Montpellier (France)	CG18190-Jheh-1	Ubx	TF	3983223	3983230
Montpellier (France)	CG18190-Jheh-1	Cf2	TF	3983319	3983328
Paris (France)	CG18190-Jheh-1	Deaf1	TF	3983298	3983303
Reference genome	CG18190-Jheh-1	cad	TF	3983223	3983233
Reference genome	CG18190-Jheh-1	cad	TF	3982874	3982884
Reference genome	CG18190-Jheh-1	Deaf1	TF	3983298	3983303
Sapporo (Japan)	CG18190-Jheh-1	cad	TF	3983223	3983233
Sapporo (Japan)	CG18190-Jheh-1	cad	TF	3982874	3982884
Sapporo (Japan)	CG18190-Jheh-1	Deaf1	TF	3983298	3983303
Tokyo (Japan)	CG18190-Jheh-1	cad	TF	3983223	3983233
Tokyo (Japan)	CG18190-Jheh-1	cad	TF	3982874	3982884
Tokyo (Japan)	CG18190-Jheh-1	Deaf1	TF	3983298	3983303
Watsonville (U.S.A)	CG18190-Jheh-1	cad	TF	3983225	3983235
Watsonville (U.S.A)	CG18190-Jheh-1	cad	TF	3982874	3982884
Watsonville (U.S.A)	CG18190-Jheh-1	Deaf1	TF	3983300	3983305
Dayton (U.S.A)	Jheh-1-Jheh-2	cad	TF	3985692	3985702
Dayton (U.S.A)	Jheh-1-Jheh-2	Deaf1	TF	3985342	3985347
Dayton (U.S.A)	Jheh-1-Jheh-2	br(var.4)	TF	3985371	3985381
Montpellier (France)	Jheh-1-Jheh-2	cad	TF	3985692	3985702
Montpellier (France)	Jheh-1-Jheh-2	Deaf1	TF	3985341	3985346
Montpellier (France)	Jheh-1-Jheh-2	Deaf1	TF	3985407	3985412
Paris (France)	Jheh-1-Jheh-2	Deaf1	TF	3985342	3985347
Paris (France)	Jheh-1-Jheh-2	Deaf1	TF	3985408	3985413
Reference genome	Jheh-1-Jheh-2	cad	TF	3985694	3985704
Reference genome	Jheh-1-Jheh-2	Deaf1	TF	3985343	3985348
Reference genome	Jheh-1-Jheh-2	Deaf1	TF	3985409	3985414
Sapporo (Japan)	Jheh-1-Jheh-2	cad	TF	3985680	3985690
Sapporo (Japan)	Jheh-1-Jheh-2	Deaf1	TF	3985341	3985346
Sapporo (Japan)	Jheh-1-Jheh-2	br(var.4)	TF	3985370	3985380
Tokyo (Japan)	Jheh-1-Jheh-2	cad	TF	3985691	3985701
Tokyo (Japan)	Jheh-1-Jheh-2	Deaf1	TF	3985341	3985346

Tokyo (Japan)	Jheh-1-Jheh-2	br(var.4)	TF	3985370	3985380
Watsonville (U.S.A)	Jheh-1-Jheh-2	cad	TF	3985700	3985710
Watsonville (U.S.A)	Jheh-1-Jheh-2	Deaf1	TF	3985350	3985355
Watsonville (U.S.A)	Jheh-1-Jheh-2	br(var.4)	TF	3985379	3985389
Dayton (U.S.A)	Jheh-2-Jheh-3	cnc::maf-S	TF	3988041	3988055
Dayton (U.S.A)	Jheh-2-Jheh-3	Ubx	TF	3988678	3988685
Montpellier (France)	Jheh-2-Jheh-3	cad	TF	3989450	3989460
Montpellier (France)	Jheh-2-Jheh-3	cnc::maf-S	TF	3988043	3988057
Montpellier (France)	Jheh-2-Jheh-3	hb	TF	3989217	3989226
Montpellier (France)	Jheh-2-Jheh-3	hb	TF	3989452	3989461
Montpellier (France)	Jheh-2-Jheh-3	Ubx	TF	3988684	3988691
Montpellier (France)	Jheh-2-Jheh-3	Ubx	TF	3988777	3988784
Paris (France)	Jheh-2-Jheh-3	cad	TF	3988701	3988711
Paris (France)	Jheh-2-Jheh-3	cnc::maf-S	TF	3988043	3988057
Paris (France)	Jheh-2-Jheh-3	hb	TF	3988679	3988688
Paris (France)	Jheh-2-Jheh-3	Ubx	TF	3988764	3988771
Reference genome	Jheh-2-Jheh-3	dl	TF	3987904	3987915
Reference genome	Jheh-2-Jheh-3	cad	TF	3988702	3988712
Reference genome	Jheh-2-Jheh-3	cnc::maf-S	TF	3988044	3988058
Reference genome	Jheh-2-Jheh-3	hb	TF	3988680	3988689
Reference genome	Jheh-2-Jheh-3	Ubx	TF	3988765	3988772
Sapporo (Japan)	Jheh-2-Jheh-3	cad	TF	3988594	3988604
Sapporo (Japan)	Jheh-2-Jheh-3	cnc::maf-S	TF	3988043	3988057
Sapporo (Japan)	Jheh-2-Jheh-3	hb	TF	3989189	3989198
Sapporo (Japan)	Jheh-2-Jheh-3	Ubx	TF	3988683	3988690
Sapporo (Japan)	Jheh-2-Jheh-3	Ubx	TF	3988774	3988781
Tokyo (Japan)	Jheh-2-Jheh-3	cnc::maf-S	TF	3988042	3988056
Tokyo (Japan)	Jheh-2-Jheh-3	hb	TF	3989215	3989224
Watsonville (U.S.A)	Jheh-2-Jheh-3	cad	TF	3988809	3988819
Watsonville (U.S.A)	Jheh-2-Jheh-3	cnc::maf-S	TF	3988042	3988056
Watsonville (U.S.A)	Jheh-2-Jheh-3	hb	TF	3988790	3988799
Dayton (U.S.A)	Jheh-3-CG43069	ARNT::HIF1A	TF	3991698	3991705
Dayton (U.S.A)	Jheh-3-CG43069	cad	TF	3991337	3991347
Dayton (U.S.A)	Jheh-3-CG43069	cad	TF	3991874	3991884
Dayton (U.S.A)	Jheh-3-CG43069	cad	TF	3991845	3991855
Dayton (U.S.A)	Jheh-3-CG43069	Deaf1	TF	3991182	3991187
Dayton (U.S.A)	Jheh-3-CG43069	Deaf1	TF	3991006	3991011
Dayton (U.S.A)	Jheh-3-CG43069	Ubx	TF	3991351	3991358
Montpellier (France)	Jheh-3-CG43069	ARNT::HIF1A	TF	3991877	3991884
Montpellier (France)	Jheh-3-CG43069	cad	TF	3992054	3992064
Montpellier (France)	Jheh-3-CG43069	cad	TF	3992024	3992034
Montpellier (France)	Jheh-3-CG43069	Deaf1	TF	3991182	3991187
Montpellier (France)	Jheh-3-CG43069	Deaf1	TF	3991744	3991749
Montpellier (France)	Jheh-3-CG43069	Deaf1	TF	3991006	3991011
Montpellier (France)	Jheh-3-CG43069	Ubx	TF	3991589	3991596
Reference genome	Jheh-3-CG43069	cad	TF	3991933	3991943
Reference genome	Jheh-3-CG43069	cad	TF	3991674	3991684
Reference genome	Jheh-3-CG43069	cad	TF	3991696	3991706
Reference genome	Jheh-3-CG43069	cad	TF	3991903	3991913
Reference genome	Jheh-3-CG43069	Deaf1	TF	3991182	3991187
Reference genome	Jheh-3-CG43069	hb	TF	3991695	3991704
Reference genome	Jheh-3-CG43069	Ubx	TF	3991667	3991674
Sapporo (Japan)	Jheh-3-CG43069	cad	TF	3992103	3992113
Sapporo (Japan)	Jheh-3-CG43069	cad	TF	3991844	3991854
Sapporo (Japan)	Jheh-3-CG43069	cad	TF	3991866	3991876
Sapporo (Japan)	Jheh-3-CG43069	cad	TF	3992073	3992083
Sapporo (Japan)	Jheh-3-CG43069	Deaf1	TF	3991182	3991187
Sapporo (Japan)	Jheh-3-CG43069	hb	TF	3991572	3991581
Sapporo (Japan)	Jheh-3-CG43069	hb	TF	3991865	3991874
Sapporo (Japan)	Jheh-3-CG43069	Ubx	TF	3991597	3991604
Sapporo (Japan)	Jheh-3-CG43069	Ubx	TF	3991837	3991844

Tokyo (Japan)	Jheh-3-CG43069	cad	TF	3991924	3991934
Tokyo (Japan)	Jheh-3-CG43069	cad	TF	3991665	3991675
Tokyo (Japan)	Jheh-3-CG43069	cad	TF	3991894	3991904
Tokyo (Japan)	Jheh-3-CG43069	Deaf1	TF	3991176	3991181
Tokyo (Japan)	Jheh-3-CG43069	Ubx	TF	3991688	3991695
Tokyo (Japan)	Jheh-3-CG43069	Ubx	TF	3991281	3991288
Watsonville (U.S.A)	Jheh-3-CG43069	ARNT::HIF1A	TF	3991698	3991705
Watsonville (U.S.A)	Jheh-3-CG43069	cad	TF	3991337	3991347
Watsonville (U.S.A)	Jheh-3-CG43069	cad	TF	3991877	3991887
Watsonville (U.S.A)	Jheh-3-CG43069	Deaf1	TF	3991182	3991187
Watsonville (U.S.A)	Jheh-3-CG43069	Ubx	TF	3991351	3991358
Paris (France)	Jheh-3-CG43069	ARNT::HIF1A	TF	3991784	3991791
Paris (France)	Jheh-3-CG43069	cad	TF	3991961	3991971
Paris (France)	Jheh-3-CG43069	cad	TF	3991368	3991378
Paris (France)	Jheh-3-CG43069	cad	TF	3991686	3991696
Paris (France)	Jheh-3-CG43069	cad	TF	3991931	3991941
Paris (France)	Jheh-3-CG43069	Deaf1	TF	3991177	3991182
Paris (France)	Jheh-3-CG43069	hb	TF	3991367	3991376
Paris (France)	Jheh-3-CG43069	Ubx	TF	3991408	3991415
Paris (France)	Jheh-3-CG43069	Ubx	TF	3991412	3991419
Paris (France)	Jheh-3-CG43069	Ubx	TF	3991346	3991353
Paris (France)	Jheh-3-CG43069	Ubx	TF	3991410	3991417
Paris (France)	Jheh-3-CG43069	Ubx	TF	3991414	3991421

Discussion générale et conclusion

Discussion

Avec l'accélération des changements globaux, le nombre d'introduction d'espèces n'a cessé d'augmenter au cours des dernières décennies (Seebens et collab., 2017). Malgré les conséquences, souvent décrites comme délétères sur le mode de vie humain, ces événements offrent des cadres d'études des mécanismes de l'adaptation. En effet, comme développé en introduction, plusieurs hypothèses et processus peuvent expliquer le succès d'invasion de nouvelles aires par des espèces. Doit-on considérer que les espèces invasives sont des espèces avec des propriétés particulières? Ou au contraire, est-ce simplement l'ensemble d'événements stochastiques qui permettent à une espèce d'envahir? Ce dernier cas impliquerait que toutes les espèces seraient potentiellement invasives et que les conditions environnementales déterminent l'invasion. Une façon de comprendre le potentiel invasif des espèces consisterait à comparer des différences dans le succès invasif de plusieurs populations d'une même espèce. Cependant identifier des cas d'échec d'invasion semble difficile (Wellband et collab., 2017). Il ne faut donc pas oublier que nous concentrons notre attention seulement sur des espèces pour lesquelles un succès invasif est confirmé ce qui ne représente pas l'ensemble des événements d'invasion possible. Par ailleurs, plusieurs exemples de mécanismes moléculaires ont été identifiés permettant de répondre à des variations de l'environnement dans des conditions stressantes. Il est donc nécessaire de compléter nos connaissances sur ces mécanismes moléculaires. Nous pouvons citer les mécanismes épigénétiques, en lien avec la plasticité phénotypique par exemple (Zhang et collab., 2013 ; Kooke et collab., 2015). Différents cas illustrent comment l'épigénome peut être modifié par les signaux environnementaux (Gibert et collab., 2007 ; Dubin et collab., 2015). De plus les éléments transposables ont été décrits comme pouvant avoir des effets adaptatifs dans le cadre de résistance à des stress biotiques ou abiotiques (Merenciano et collab., 2016 ; Marin et collab., 2019 ; Horváth et collab., 2017 ; Guio et collab., 2014). Sur ce dernier point, il est nécessaire de rappeler que les éléments transposables sont par définition des éléments qui se répliquent via la machinerie d'un hôte indépendemment des effets sur ce dernier. De ce fait les événements adaptatifs via les insertions de TEs que ce soit par l'ajout de nouvelle fonction ou le changement dans la régulation des gènes restent des événements rares. La difficulté de définir les conditions de succès et les mécanismes sous-jacents aux invasions, provient peut-être, du fait qu'elles mettent en jeu différents processus qui peuvent différer entre espèces et donc que plusieurs combinaisons peuvent être impliquées. Dans ce cas il n'est pas déraisonnable de vouloir mieux caractériser ces mécanismes moléculaires. La littérature du XXI^{ème} siècle est d'ailleurs de plus en plus axée sur ces mécanismes. Cependant Marin et collab. (2019) soulignent que ces études sont très corrélatives voire spéculatives sur le rôle des mécanismes épigénétiques ou des éléments transposables. Les études épigénétiques sont limitées à la comparaison des variabilités génétiques et épigénétiques, et à l'observation des changements selon le profil d'invasion mais ne peuvent en aucun cas conclure une relation de causalité. Il existe

peu de cas dans la nature où on décrit un lien direct entre l'invasion (et la résistance à un stress particulier) et un état épigénétique particulier (Xie et collab., 2015). Ceci peut s'expliquer par les méthodes encore difficiles à mettre en place pour étudier l'épigénome dans un cadre écologique, c'est pourquoi il est encore nécessaire de s'intéresser à des cas maîtrisés en laboratoire. Nous avons donc inscrit nos travaux de thèse dans l'idée de mieux comprendre les mécanismes moléculaires en jeu au cours d'une invasion biologique en identifiant les variations au sein d'une espèce invasive entre populations selon l'environnement et l'état d'invasion. Dans cette optique, nous avons considéré une espèce invasive récente, *Drosophila suzukii,* avec deux invasions concomitantes mais distinctes et cela afin de pouvoir comparer si une similitude dans les processus existait. Nous avons cherché à identifier des phénotypes de résistance et sensibilité que nous pourrions expliquer au niveau moléculaire. Par la suite nous avons décrit les réponses au stress chez *D. suzukii* au travers de ses gènes mais aussi au niveau des éléments transposables. Un autre aspect moléculaire que nous avons voulu étudier concernait les réponses au niveau épigénétique. Nous voulions voir quelle part ces modifications pouvaient avoir dans le changement d'expression des gènes face à un environnement stressant. Cet aspect avait été aussi envisagé dans une approche transgénérationnelle (détaillée par la suite en perspective) que nous avons simplifiée par une étude sur les modifications dans les tissus germinaux sur une seule génération. Nous allons proposer une synthèse des résultats obtenus durant ce doctorat en déclinant les observations phénotypiques d'une part et d'autre part les variations observées au niveau du transcriptome. Nous développerons aussi un volet concernant les éléments transposables et dont les résultats que nous avons obtenus sont loin des attentes développées dans la littérature. Enfin nous finirons cette conclusion sur un volet de perspectives pour proposer des idées dans la continuité des travaux effectués.

1 Caractérisation phénotypique de la résistance au stress thermique et chimique chez *D. suzukii*

1.1 Le sexe ne modifie pas la réponse au stress

Chez les drosophiles comme chez de nombreux organismes, il est souvent décrit que la réponse aux stress est dépendante du sexe (Jensen et collab., 2007 ; Enriquez et collab., 2016). Cependant dans la littérature, bien que l'effet sexe soit souvent observé, il n'est pas toujours en faveur (plus grande résistance) d'un sexe particulier. Dans le cas du froid par exemple, la littérature sur *D. melanogaster* est tantôt en faveur des mâles (Kelty et Lee, 2001 ; Jensen et collab., 2007), tantôt des femelles (David et collab., 1998) et c'est aussi le cas des études avec *Drosophila suzukii* (Shearer et collab., 2016 ; Plantamp et collab., 2016 ; Enriquez et Colinet, 2017). Nous avons pris en compte ce paramètre-là dans nos études expérimentales en mesurant la variabilité due au sexe. Dans les deux cas étudiés, l'effet du stress par choc thermique (0°C) ou au travers d'un environnement pro-oxydant, les effets du sexe sont négligeables. Pour être plus précis, lors de nos analyses initiales, nous avions inclus l'effet du sexe et nous avions observé quelques effets mineurs. Par exemple, pour le stress thermique dans deux populations (Watsonville et Montpellier) les mâles se réveillent en moyenne quelques minutes plus tard que les femelles. De même lors du stress au paraquat certaines lignées présentaient des différences selon le sexe qui étaient gommées lors de l'analyse à une échelle populationnelle. Ceci semble indiquer que la variabilité dans la réponse est

majoritairement dépendante du génotype plutôt que du sexe dans notre cas. Une autre hypothèse dans le cas du froid pourrait être liée au trait étudié, qui n'aurait pas permis de discriminer des différences à un changement de température brusque et non létal.

1.2 Variabilité phénotypique géographique

Nous avons observé que l'origine géographique des populations étudiées modifiait significativement la valeur des traits mesurés. Par exemple la longévité, c'est à dire l'espérance de vie en condition contrôle des lignées des populations invasives de France et des U.S.A est bien plus grande que celle des populations natives (de 25 à 44% supérieure). En valeur médiane, les populations japonaises vivent ~32 jours quand les populations invasives vivent entre 40 et 50 jours. Cependant, une population invasive (Watsonville, U.S.A) est une exception puisqu'elle n'est pas différente des populations japonaises. Nous détaillerons le cas de cette population dans la partie suivante. L'espérance de vie de *D. suzukii* est similaire à celle de *D. melanogaster* (Hamby et collab., 2016 ; Flatt et Kawecki, 2007 ; Emiljanowicz et collab., 2014). Par contre, la littérature sur *D. suzukii* montre une variabilité importante dans les valeurs de ce trait. Shearer et collab. (2016) dans une étude sur la plasticité à la température, observent des valeurs maximales (sexes et morphes confondus) inférieures à 30 jours à 20°C et 28°C, ce qui est au même niveau que notre population la moins longévive en valeur médiane. Leur étude portait sur une population provenant de l'état de l'Oregon à environ 160 km d'une de nos populations la plus longévive Dayton. Dans une autre étude par Grumiaux et collab. (2019), en régime constant à 25°C sur une population provenant de France, les valeurs médianes sont similaires aux nôtres avec entre 40 et 50 jours d'espérance de vie. Dans les données recensées par Hamby et collab. (2016), l'espérance de vie varie grandement selon l'étude avec des facteurs influant comme la température, l'hygrométrie, la nutrition. Cependant elles restent comparables à celles connues chez *D. melanogaster* avec une espérance autour de 60 jours. Plusieurs études ont suggéré que l'espérance de vie dépend de la capacité à résister au stress oxydant, qui est un facteur de vieillissement (Tullet et collab., 2017 ; Morrow et collab., 2016 ; Liguori et collab., 2018 ; Lee et collab., 2009 ; Landis et collab., 2004 ; Hajjar et collab., 2018 ; Finkel et Holbrook, 2000 ; Deepashree et collab., 2019). Nous avons observé que la sensibilité au paraquat était spécifique à chaque population et que la corrélation entre stress oxydant et longévité n'était pas forte ($r = 0,28$). Comme mentionné dans l'introduction, Richards et al. (2006) proposent différents scénarios décrivant comment la plasticité phénotypique adaptative peut contribuer au succès de l'invasion. Dans le cas d'un stress oxydant, conduisant inéluctablement à une mortalité accrue, la conséquence de la plasticité d'un (ou plusieurs) trait (adaptatif) serait de maintenir la fitness et donc de limiter la mortalité due au stress. Autrement dit, la sélection naturelle devrait favoriser les génotypes ayant une plasticité phénotypique de traits physiologiques sous-jacents leur permettant de garder leur fitness stable même lors d'un stress. Dans l'hypothèse d'un rôle adaptatif de la plasticité phénotypique dans l'invasion, les populations invasives devraient donc avoir une fitness plus stable que les populations natives. Dans notre étude, le proxy utilisé pour mesurer la fitness est la longévité, et on peut donc estimer sa stabilité par la pente de la droite entre les deux environnements. On observe que cette pente est la plus forte pour Watsonville (U.S.A) et Montpellier (France) alors que Paris (France) et Dayton (USA) ont des pentes similaires aux japonaises. Une hypothèse pour expliquer ce résultat pourrait être liée à des pressions de sélection très fortes aux USA et au Japon liées à l'utilisation du paraquat contrairement à l'Europe

où cette molécule est interdite. Cependant cela n'explique pas les différences observées entre les deux populations françaises qui pourraient être liées aux routes d'invasion différentes pour ces deux populations. En effet, Fraimout et collab. (2017), montrent que des événements de brassage génétique ont eu lieu entre les États-Unis et le nord de la France uniquement. Dans le cadre de la réponse au froid, nous avons aussi observé que les populations invasives avaient globalement une tendance à une meilleure résistance au stress thermique que les natives. Cette tendance était claire pour les populations françaises, beaucoup moins pour les populations américaines. Il est important de noter que contrairement à l'expérience sur la survie au paraquat, la mesure de la réponse thermique a été plus difficile pour plusieurs raisons. Tout d'abord, il a été nécessaire de réaliser cette expérience sur plusieurs périodes, avec plusieurs expérimentateur.trice.s et bien que ces paramètres aient été pris en compte, leurs effets sur la variabilité n'est pas négligeable. De plus, du fait de problèmes d'élevage, pour certaines populations, peu de lignées ont pu être phénotypées, par exemple seulement deux lignées (avec peu d'individus) pour Tokyo dont l'intervalle de confiance englobe tous les autres. En modifiant les conditions de mesure ou le trait mesuré, il aurait été possible de limiter certains biais (nombre d'expérimentateurs, mesures espacées dans le temps) (Hoffmann et collab., 2003). Cependant la difficulté d'élevage de ce modèle biologique en laboratoire reste un élément majeur dans la variabilité des réponses mesurées. Il a été montré chez *D. melanogaster* que la mesure du temps de réveil est un proxy de la résistance au froid et donc en partie de la distribution des espèces (Ayrinhac et collab., 2004). Dans notre étude, il semblerait que les différences observées reflètent plus le caractère invasif ou non des populations que les variations climatiques. En effet, nous observons que les populations invasives ont une tendance à mieux résister que les populations natives. Par ailleurs quand on regarde les valeurs moyennes de température dans les régions échantillonnées, Sapporo est la plus sensible alors que les valeurs saisonnières sont les plus froides avec des valeurs négatives en hiver, ce qui n'est pas le cas pour les autres sites. La résistance thermique de *D. suzukii* au froid a été étudié par de nombreux auteurs, et il apparaît que *D. suzukii* est une espèce comme *D. melanogaster* sensible au froid et qui ne peut survivre longtemps à des températures en dessous de 0°C (Jakobs et collab., 2015). Il semble que la résistance au froid n'est pas simplement liée à la gamme de température rencontrée dans l'environnement et que d'autres facteurs peuvent expliquer les différences observées dans nos données. Pour résumer, nous observons que les populations invasives ont tendance à vivre plus longtemps en condition de laboratoire et à mieux résister au stress thermique. Cependant la réponse au paraquat suggère que d'autres facteurs sont impliqués dans la réponse au stress du fait des effets génotype par environnement observés. Est-il alors possible de relier les différences au niveau transcriptomique avec les phénotypes observés? C'était l'objectif de la seconde partie de ce doctorat : caractériser les changements moléculaires en lien avec le stress environnemental avec l'idée d'apporter des réponses sur nos observations phénotypiques. Cela sur plusieurs aspects, changement transcriptomique, variabilité des éléments transposables et un volet que nous discuterons à la fin, l'épigénétique.

2 Caractérisation du transcriptome de *D. suzukii* en condition stressante

Afin de pouvoir plus clairement faire le lien entre nos observations phénotypiques et nos analyses moléculaires, il aurait été idéal de pouvoir conduire une analyse moléculaire sur toutes les lignées phénotypées. Cependant pour des questions économiques et logistiques (taille du jeu de données à analyser et coût de séquençage) nous avons restreint notre analyse. Néanmoins une étude à petite échelle permet d'abord de mettre en évidence des différences moléculaires, qui peuvent être confirmées par des analyses complémentaires, comme des analyses qPCR voire le séquençage de quelque lignées supplémentaires. Nous avons décidé d'étudier trois lignées isofemelles provenant des trois aires géographiques, Japon (Sapporo, S29), France (Montpellier, MT47) et U.S.A (Watsonville, W120).

2.1 Empreinte géographique sur le profil transcriptomique

Nous avons observé qu'au niveau moléculaire, le facteur génotype prévaut sur le facteur environnemental. Autrement dit, l'expression des gènes est dépendante du génotype plus que de l'effet du traitement et ce peu importe le traitement étudié. Selon nos filtres, le nombre de gènes qui répondent au stress varient entre 100-500 (paraquat) et 300-600 (froid). Les gènes exprimés indépendamment des génotypes représentent entre 70 et 100 gènes ce qui montre encore que la réponse est plutôt spécifique à chaque génotype. Parmi ces gènes, on retrouve systématiquement les gènes codants pour des heat shock protein (*Hsp*) fortement exprimés mais aussi des facteurs de transcription. Dans le cas du paraquat, quasiment tous les gènes ont été annotés et nous retrouvons des gènes impliqués dans les processus de détoxification contre les xénobiotiques, qui sont aussi source de résistance Mishra et collab. (2018) ; Chen et collab. (2016). Dans le cas du froid, nous retrouvons des gènes sensibles au froid comme *frost*, mais tous les gènes ne sont pas annotés ce qui limite l'identification de gènes candidats. En effet environ la moitié du génome de *D. suzukii* a pu être annoté à l'aide d'orthologues chez *D. melanogaster* en majorité. Ces gènes communs sont donc ceux impliqués dans les premières réponses au stress de manière générale. On peut noter que systématiquement, le génotype de Sapporo par rapport aux autres, présente un nombre de gènes différentiellement exprimés deux fois moins importants et qui ne semble pas associé à un biais expérimental dans la quantification. De ce fait la majorité des gènes exprimés chez Sapporo sont partagés par les autres génotypes. Le transcriptome de ce génotype natif semble donc plus canalisé que celui des deux génotypes invasifs, ce qui serait un facteur facilitant la réponse au stress dans les environnements envahis. Il serait nécessaire de confirmer cela sur d'autres génotypes des différentes aires. Du fait des variabilités importantes entre génotypes, nous pouvons supposer aussi que des processus d'adaptation locale ont eu lieu car de nombreuses différences existent entre les génotypes invasifs étudiés. En ce sens nous avons identifié qu'une part du transcriptome présentait une expression génotype et environnement (traitement) dépendante, c'est à dire que des gènes sensibles au stress ne sont pas exprimés dans le même sens ou amplitude selon le génotype étudié. Ces gènes constituent aussi une piste pour des événements d'adaptation locale sur un temps court. Quand on regarde ces gènes dont la proportion peut représenter entre 1 et 2% du transcriptome au paraquat ou au froid on retrouve des gènes comme *Hsp70* dont l'expression pour Montpellier est entre deux (froid) et quatre (paraquat) fois plus forte que l'ex-

pression des autres génotypes. Ces gènes sont cependant peu annotés ce qui limite encore l'identification de gènes particuliers. Mishra et collab. (2018), ont étudié la réponse à des insecticides au niveau transcriptomique chez deux populations de *D. suzukii*. Ils ont par ailleurs analysé les variants nucléotidiques et les insertions délétions dans les gènes différentiellement exprimés entre leurs populations. Une telle analyse sur nos données permettrait de déterminer si la variabilité de ces gènes particuliers peut s'expliquer par de la variabilité génétique cryptique. Par ailleurs, nous avons choisi un filtre basé sur l'amplitude de l'expression en considérant négligeable tout gène dont l'expression ne variait pas d'un facteur deux. Cependant les gènes génotype et environnement dépendants sont décrits comme des gènes impliqués en amont dans les voies de régulation et des réponses aux stress et, pour avoir un effet il n'est pas forcément nécessaire d'avoir des fortes variations de niveau d'expression. Par ailleurs, si l'on considère que des changements phénotypiques sont apportés par un ensemble de petits changements d'expression plus que par des effets forts de quelques gènes, une analyse plus large, avec une approche réseau permettrait de mieux caractériser le transcriptome de *D. suzukii*.

2.2 Quelles conséquences sur les éléments transposables ?

Chez *D. suzukii*, le contenu en éléments transposables représente ~33% du génome, un des plus importants du groupe *melanogaster* (Sessegolo et collab., 2016). Ceux ci peut représenter une source de variabilité génétique favorisant l'invasion notamment pour des populations rencontrant des réductions de diversité génétique lors de l'invasion. Nous avons donc développé un pan d'analyse dédié aux éléments transposables, afin d'étudier l'effet du stress environnemental sur l'expression des éléments transposables, puis si leur présence pouvait avoir un impact sur la régulation des gènes avoisinants.

2.3 Absence d'explosion de l'expression des ETs

Dans un premier temps nous nous sommes intéressés à l'expression des ETs chez *D. suzukii* en condition de stress. La littérature, suggère que les signaux environnementaux entraînent des modifications dans la régulation des gènes, notamment en condition de stress avec des changements épigénétiques (Sahu et collab., 2013 ; Rey et collab., 2016 ; Nestler, 2016 ; Mirouze et Paszkowski, 2011). Comme les mécanismes de régulation des ETs sont aussi épigénétiques, leur expression est dépendante des conditions environnementales rencontrées. Par ailleurs plusieurs auteurs suggèrent que les ETs seraient un facteur facilitant l'invasion par la génération de variant génétique pré- post-adaptatif (Stapley et collab., 2015 ; Schrader et collab., 2014 ; Rey et collab., 2016). On parle notamment d'explosion de l'expression des ETs suite à un stress, générant de la variabilité qui peut être source de sélection (Laudencia-Chingcuanco et Fowler, 2012 ; Belyayev, 2014). Pour faire cette analyse nous avons identifié les ETs de notre espèce et nous avons analysé l'expression de ces familles dans les différentes conditions (contrôle, froid et paraquat) sur nos 3 génotypes. Contrairement à ce qui est suggéré, nous n'avons observé aucune explosion dans l'expression suite aux stress appliqués. Sur plusieurs centaines de familles d'éléments identifiés dans nos génotypes, moins de 10 éléments ont été exprimés dans chaque condition. Une exception concerne Montpellier suite au stress thermique. 31 familles d'éléments toutes surexprimées ont été détectées ce qui n'était pas le cas lors du traitement au paraquat, indiquant une sensibilité différente des éléments selon le traitement appliqué. Ce cas mériterait d'être approfondi pour

caractériser les familles et identifier les positions de ces éléments. L'expression des éléments transposables chez *D. suzukii*, n'est pas modifiée par des stress environnementaux comme le froid ou le paraquat, ce qui ne valide pas l'hypothèse de l'explosion des éléments chez une espèce invasive quel que soit son statut (invasive et native). Bien que le niveau d'expression des ETs soit faible, nous pouvons dire que les ETs dans le génome ne sont pas inactifs car nous avons observé un nombre d'ETs différentiellement exprimés plus important en condition contrôle que après traitement entre les génotypes. Il serait intéressant de pouvoir étudier si les mécanismes de régulations des éléments ont été affectés par les stress pour comprendre le faible nombre d'éléments exprimés. Par ailleurs, une autre conséquence des ETs dans le génome vient de leur proximité avec des gènes et donc de leur possibilité d'influencer leur régulation. Plusieurs études montrent que dans certains cas particuliers, des insertions d'éléments peuvent être adaptatives, comme c'est le cas avec *Bari-Jheh* un élément transposable qui augmente l'expression des gènes *Jheh* dans un contexte de stress oxydant chez *D. melanogaster* (Guio et collab., 2014).

2.4 Conséquences des ETs sur la régulation de l'expression de gènes voisins

Pour comprendre l'implication des insertions sur la régulation des gènes de *D. suzukii*, nous avons cherché toutes les insertions à l'intérieure des gènes et dans les 2 kb de région flanquante, en amont et en aval. Nous avons observé que la distribution des insertions entre nos trois génotypes n'étaient pas différentes au niveau des composantes des gènes (intron, exon...), mais que les gènes différentiellement exprimés suite au stress, étaient systématiquement moins riches en insertions. Ces gènes sont importants pour la réponse au stress et ont peut supposer que la sélection dessus est forte et va donc limiter le maintien d'insertions non adaptatives proches de ces gènes. Cependant quelques centaines de gènes différentiellement exprimés présentent des insertions plutôt intergéniques et introniques mais parfois quelques cas dans des régions 5' ou 3'UTR. Ces insertions pourraient au travers de modifications épigénétiques changer l'expression de ces gènes dans des conditions stressantes. Nous avons en particulier identifié que beaucoup de gènes avec une interaction génotype par environnement avaient des insertions à proximité. Nous n'avons pas pu confirmer les effets de ces insertions pour le moment, mais nous fournissons une source de candidats à étudier qui est une première étape. Il serait nécessaire pour compléter cette analyse des éléments, d'étudier d'autres génotypes pour confirmer.

3 Le cas particulier de *Jheh* dans la réponse au stress oxydant

Dans notre étude sur l'effet du stress oxydant chez *D. suzukii*, nous avons développé une expérimentation présentée en chapitre 4, qui s'intéressait particulièrement à un groupe de gène, *Jheh*. Chez *D. melanogaster*, certaines populations présentent une insertion en amont de ces gènes qui induit une surexpression des gènes en condition de stress oxydant (Guio et collab., 2014). Nous avons donc cherché à comprendre si les gènes *Jheh* étaient également impliqués dans la réponse au stress oxydant chez *D. suzukii* et si leur expression était dépendante du fond génétique des différentes lignées utilisées. Nous avons utilisé six lignées, une par aire échantillonnée et analysé la réponse phénotypique et moléculaire chez les mâles et les femelles. Au niveau phénotypique, ces six lignées ont des réponses proches de celles observées au niveau populationnel. Au niveau moléculaire, nous avons pu observer que le paraquat modifie l'expression des gènes *Jheh* de façon

sexe et génotype dépendant. En effet chez les mâles, nous n'avons pas observé d'effet du traitement sur l'expression des gènes, contrairement aux femelles. Cependant dans le cas des femelles, nous observons trois types de réponses selon l'aire géographique. Sur les lignées américaines, le traitement n'a pas modifié l'expression des gènes de *Jheh*. En France, l'expression de *Jheh-1* et *Jheh-2* est augmentée suite au traitement pour Montpellier, et seulement sur *Jheh-1* pour Paris. Au Japon, les deux lignées sont différentes avec Tokyo qui voit une expression (de faible intensité) plus grande sur *Jheh-1* et *Jheh-2* après stress. Sapporo a un profil d'expression totalement opposé aux autres puisque pour tous les gènes, le niveau d'expression en condition contrôle est plus élevé alors qu'il est fortement sous-exprimé après ajout du paraquat. Pour essayer de comprendre les différences d'expression chez les femelles de nos six lignées, nous avons analysé la diversité génétique autour du groupe de gène ainsi que la présence d'éléments transposables associée à des sites de reconnaissance de facteurs de transcriptions. Nous n'avons pas détecté d'éléments insérés, du moins complet, mais seulement des fragments de quelque dizaines de paires de bases. Ces fragments n'étaient pas associés à des séquences de facteurs de transcriptions supplémentaires. L'analyse de ces derniers a indiqué que la plupart des sites CnC sont partagés, et les quelques sites variables n'expliquent pas les variations d'expression des gènes observées. Il semble que les gènes *Jheh* soient sensibles au stress oxydant particulièrement chez les femelles, mais aucun événement d'insertion adaptative n'a été détecté, l'expression des gènes *Jheh* semble donc marginale dans la réponse au stress oxydant.

Perspectives et conclusion

Au travers de ce doctorat nous avons essayé pour la première fois sur un modèle invasif, de caractériser des populations sur plusieurs aires géographiques au niveau phénotypique mais aussi moléculaire, en étudiant les réponses à deux stress abiotiques. Nous avons conduit une approche intégrative bien que limitée par le nombre de lignée que nous avons analysé au niveau moléculaire. Nous avons aussi intégré dans notre approche l'étude des éléments transposables qui sont chez les espèces invasives encore peu étudiés. Les données générées, en particulier moléculaires, soulèvent des questions que nous n'avons pas pu développer dans ce doctorat. Ces données pourront, entre autres, être utilisées pour cartographier les voies métaboliques et les cascades de signalisation en réponse au stress, étudier des groupes de gènes spécifiques dans un contexte de stress, identifier des gènes candidats pour l'adaptation locale ou la résistance au stress. Par ailleurs, nous avions initialement envisagé d'étudier les conséquences trans-générationnelles du stress environnemental causée par la température ou le paraquat. Du fait des difficultés techniques générant un retard dans le travail de thèse, mais aussi par la logistique qu'impliquerait une expérimentation trans-générationnelle, nous avons abandonné cet axe de travail. Si l'invasion de *D. suzukii* dans le monde est importante, en laboratoire le maintien de lignées isofemelles est plus difficile. L'utilisation de milieu standard développé sur *D. melanogaster* n'est pas optimale, la culture en grand volume (cage à populations) est contrainte du fait du nombre de lignées à maintenir (Iacovone et collab., 2015). Malgré cela, pour étudier les conséquences transgénérationnelles qui pourraient exister, nous avons choisi de caractériser, suite à un stress, les conséquences sur les tissus germinaux chez les femelles. Nous avons effectué cela sur les mêmes trois génotypes étudiés au préalable, pour tenter de comprendre si des effets sur la descendance pouvaient opérer. Cette analyse a porté sur le transcriptome mais aussi l'épigénome. En effet dans notre projet nous avions envisagé l'approche transgénérationnelle au travers de variabilité non génétique expliquant en partie la transmission des informations à la descendance. Les mécanismes épigénétiques sont connus pour être impliqués dans la réponse au stress dans beaucoup d'espèces étudiées surtout chez les plantes, et ce aussi au niveau transgénérationnel (Wibowo et collab., 2016 ; Sahu et collab., 2013 ; Rey et collab., 2016 ; Mirouze et Paszkowski, 2011 ; Kinoshita et Seki, 2014). C'est aussi un mécanisme étudié dans le cadre de la plasticité phénotypique (Zhang et collab., 2013 ; Schlichting et Wund, 2014 ; Kooke et collab., 2015 ; Beldade et collab., 2011). Chez les espèces invasives beaucoup d'études sont faites sur l'épigénome des espèces mais restent encore trop spéculatives (Zhang et collab., 2016 ; Xie et collab., 2015 ; Schrey et collab., 2012 ; Richards et collab., 2012 ; Pu et Zhan, 2017 ; Marin et collab., 2019 ; Liebl et collab., 2013 ; Gillies et collab., 2016 ; Gao et collab., 2010). Les modifications épigénétiques sont diverses. Elles impliquent trois niveaux, (i) les modifications sur l'ADN comme la méthylation des cytosines, (ii) les marques sur les histones et (iii) les modifications post-traductionnelles comme certaines marques sur des protéines ou la régulation

par des ARN non codants (Duncan et collab., 2014 ; Allis et Jenuwein, 2016). Nous avions choisi d'étudier la modification des histones car elle est impliquée dans la régulation des ETs et sensible aux signaux environnementaux (Rey et collab., 2016 ; Guio et collab., 2018 ; Gibert et collab., 2016b). Deux marques ont été sélectionnées, $H_3K_4me_3$ (histone 3 lysine 4 triméthylée) et $H_3K_9me_3$ (histone 3 lysine 9 triméthylée), connues respectivement pour leur effet activateur et répresseur sur les gènes ainsi que sur les ETs pour $H_3K_9me_3$. Actuellement nous avons analysé une partie des données transcriptomiques sur les ovaires, mais l'analyse des données épigénétiques n'est pas terminée. Les résultats préliminaires montrent que l'expression des gènes reste génotype plus qu'environnement dépendante. La variabilité entre génotypes en condition contrôle est bien bien plus importante qu'après traitement. Entre 300 et 1700 gènes sont différents entre génotypes, quand après un stress, au maximum 100 gènes sont différentiellement exprimés. Un gène est partagé par tous les génotypes après le stress au paraquat et 12 après le froid. Contrairement aux expérimentations sur carcasses, plusieurs gènes ont un sens d'expression antagoniste entre populations, ce qui n'a jamais été observé dans nos précédentes analyses. Concernant l'expression des éléments transposables, quasi aucune expression après stress n'a été détectée (5 éléments au mieux pour Sapporo). Jusqu'à 60 familles sont différentiellement exprimées en condition contrôle quand on compares les génotypes. Cela suggère aussi que le stress n'affecte pas l'expression des éléments dans les ovaires. L'analyse épigénétique nous permettrait aussi de voir si les ETs sont réprimés aussi bien en condition contrôle que traitée par des marques d'histone ce qui pourrait expliquer l'absence d'expression au travers d'une machinerie de régulation qui n'a pas été impactée par les stress. Pour terminer, l'approche trans-générationnelle, reste pour moi un volet à développer en particulier s'il est appuyé par des analyses transcriptomiques couplées à des données épigénétiques, et à la mesure de traits au cours des générations. Ceci aurait offert une cartographie moléculaire et une identification des réponses au stress à plus long terme encore jamais effectuées sur des espèces invasives. Selon son organisation ce volet aurait permis aussi, de suivre au cours du temps la variabilité transcriptomique associée au paysage épigénétique, voire d'effectuer une expérimentation similaire à Waddington pour étudier la canalisation génétique.

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