Phenotypic and transcriptomic responses to stress differ according to population geography in an invasive species

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Abstract

Background: Adaptation to rapid environmental changes must occur within a short time scale. In this context, studies of invasive species may provide insights 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 the differential gene expression between genotypes in response to oxidative 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. We also performed transcriptomic sequencing of selected genotypes upon and without paraquat and detected a large number of genes differentially expressed, distinguishing the genotypes 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 the invasion from the south of Asia, invasive populations of *D. suzukii* have diverged from populations in the native area regarding their genetic response to oxidative stress. This suggests that such transcriptomic changes could be involved in the rapid adaptation to local environments.

Keyword: invasive species, *D. suzukii*, oxidative stress, transposable elements, environmental changes, genotype by environment interaction.

Introduction

Rapid environmental changes, particularly related to human activity, can decisively affect living organisms, who must respond to them within a short-time scale. Understanding the mechanisms underlying these rapid responses is challenging and could help predict organism and species survival in the face of global environmental changes. The rapid adaptation of invasive species to new environments, some quite different than ancestral environments, may provide insights 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 to the success of invasive species, particularly in the case of founder populations depleted of genetic variations [3–5,7].

Genetic diversity could rapidly increase following environmental stress if there is an activation of transposable elements (TEs) or if the epigenetic control is disturbed. TEs, which are repeated sequences that can move around genomes, were discovered by B. McClintock in the 50' [8]. Depending on where TEs are inserted within the genome, they can affect the fitness of its host organism. The vast majority of new TE insertions are neutral or deleterious, and purifying selection is expected to remove them or favours their silencing [9–11]. However, some TE insertions may be advantageous and facilitate adaptation in different environments [6,12–22]. Such adaptive effects have been previously observed in response to both biotic (*e.g.,* virus infection) and abiotic (*e.g.,* oxidative stress) stress [19,20]. Moreover, stress-induced changes in the epigenetic regulation of TEs, which is often sensitive to environmental cues [14,21,23], has already been described to rapidly generate potentially advantageous changes in nearby gene regulation and facilitate rapid adaptation to environmental stress [9,13].

Here, we examined variation in the oxidative stress response of invasive and non-invasive populations of *Drosophila suzukii* with a focus on 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 [24–28]. 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 [25–28]. 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 [29,30]. 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 [31–34]. Resistance to oxidative stress has been associated with extended lifespan [34–36], 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, 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 expression. We found substantial differences among genotypes in patterns of gene expression related to oxidative stress that may explain the observed phenotypic differences and reflect population history. This work highlights the local adaptation to environmental conditions of the genotypes within a short-time scale.

Results

Among population variation for lifespan and oxidative resistance

We have measured lifespan and oxidative stress in a total of 27 isofemale lines from six geographical regions coming from the U.S.A, France and Japan. 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 [37]. 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, male and female data were pooled for 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 for Tokyo, Paris and Dayton. Populations from Watsonville and Montpellier were significantly more sensitive to paraquat treatment, with reductions in lifespan of 14.8% and 16% respectively (multiplicative effects of 0.20*0.74 and 0.20*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 in somatic tissues, 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) of gene read counts (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). CV distributions were significantly different across genotypes (paired Wilcoxon test, p-values < 0,01), which suggests significant genotype by environment interaction for transcriptomic response. The number of differentially expressed (DE) genes identified, (i) in pairwise comparisons between genotypes in control conditions, (ii) in comparisons between untreated and oxidative stress conditions for each genotype, and (iii) in pairwise comparisons between genotypes following paraquat treatment are presented in Table 2. The distribution and values of the CV were 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 higher than 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 from up-regulated genes in France) when compared to France *vs* Japan or U.S.A *vs* Japan. In the comparisons of France *vs* U.S.A, enriched terms came from down-regulated genes in the Japan genotype. 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 other two.

We detected 44 GO terms shared between the invasive genotypes (France and 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. 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 to say 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 and identified a total of 659 unique DE genes across the 3 genotypes (Fig. 4 and 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 upon oxidative stress (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 621 annotated genes out 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 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 [31]. 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 unique 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 (from $\log_2$FC = -1.03 to -10.8) and 53 upregulated (from $\log_2$FC = 1.03 to 10.48) (Fig. 6).

Among those up-regulated following paraquat treatment, we found genes related to stress response such as *Hsp* and *Cyp* genes families. The strongest up-regulated genes were a predicted gene encoding for a transcription factor A ($\log_2$fold-change = 10) and other genes in the *Hsp* gene family. Among the strongest down-regulated genes, we identified a cytochrome P450 gene that was the top down-regulated gene in all 3 genotypes and has a $\log_2$FC < -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 [38]. Out of 53 up-regulated genes, 37 had homologs in *D. melanogaster.* Enrichment analysis on this set of genes identified only one significant GO 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 [39].

The stress response is variable across genotypes

We identified a total of 213 unique genes with a significant GEI, which represent the set of genes with expression differentially modulated by oxidative stress according to genotype (Fig. 7). When comparing differences in the response of invasive genotypes to the non-invasive Japan genotype, we found 62 differentially modulated genes with the U.S.A genotype and 138 with the France genotype (Table 2). Most of these differences were due to greater up-regulation DE genes 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. 7 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 $\log_2 FC \geq 2$, 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 [17]. In our experiments, TE expression levels were very low, and reads corresponding to TEs did not exceed 3.8 to 7.1% of the total transcriptome. In control condition, differentially expressed TEs (DETEs) identified in pairwise comparisons between genotypes represented from 3.08 (48 families) to 5.91% (92) of total number of TE families annotated in the *D. suzukii* genome (Table S3). The U.S.A genotype exhibited a greater level of DE 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. Moreover, a similar number of both up- and down-regulated DETES were identified in the comparison between France and Japan (Fig. 8).

After paraquat exposure, very few TE families changed in their expression levels (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 upon 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 [40]. To test for an enrichment or depletion of TE insertions around DE genes, we first test if the distribution of TEs in the three genomes was not significantly 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 in genes and the 2kb flanking region (p-value < 0.05).

We then focused on all the 115 TE insertions present in the DE genes, the majority of which were in introns (57) or in ±2kb flanking regions (50) around DE genes (Table S6). 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)*.

Shared DE genes are not enriched with TE insertions

In agreement with a depletion of TE in DE genes, of the 67 shared DE genes that responded to paraquat treatment in similar ways across the three genotypes (Fig. 6), we founded 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

A GEI interaction indicates that the magnitude or direction of changes in expression following treatment could differ depending on the genotype. We found a total of 53 genes with at least one TE insertion.

The DE genes showing evidence of a GEI, present the same distribution of TE insertion as all genes, except for genes with a GEI between France and U.S.A (p-value = 0.016, Table S5) in which they are less frequent and no insertion was shared in all genotypes. Also, the TE insertions were associated with high or low level of expression (Fig. 9 summarizes detected TE insertions in GEI DE genes for the different genotypes, also Table S7). For example, three genes were differentially expressed between France and the others genotypes ; *FarO (Fatty acyl-CoA reductase)*, *kelch* (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. *Hsp70-Aa* had an insertion in Japan and U.S.A and showed lower expression than in France. Another example is the gene *CG12520*, which has a TE insertion in the 3'UTR in Japan and a lower expression.

TEs may impact neighbouring gene expression due to the transcription factor binding sites (TFBSs) they harbour. We investigate whether this was the case in our data set and focused on three TFBSs of the antioxidant responses elements group (ARE) [41]. We first analysed enrichment in TFBS for all TE families identified in the GEI genes (Fig. S4A). Of 196 TE families, 36 had at least one TFBS, most of them related to a CnC (Cap'n'collar) element. The TE sequences with putative TFBS were related to Pao and Gypsy families. We founded 4 GEI genes with TFBS linked to a TE insertion (Fig. S4B). Only one was annotated as *stc* gene *(shuttle craft)*, which encodes a NFX1 family transcription factor implicated in modulating adult lifespan and aging [42].

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* [34,43,44]. In this species, the ROS defences are mediated by both immune and antioxidant response pathways. A similar association may be expected in *D. suzukii,* a species which diverged from the melanogaster group ~8 Mya. However, until now, no extensive study had been performed using *D. suzukii* wild-type genotypes. Here, 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 the same way, resulting in a significant genotype-by-environment interaction (Fig. 1 and Table 1), in accordance to what was reported 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 (U.S.A) 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 [29,30]. 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 [24,27,45]. This could have resulted in a 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, on the other hand, was not significantly different than the Japanese Sapporo population; 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 [24]. The difference between the two American populations seemed odd at first; however, the transcriptomic analysis revealed that a copper detoxification pathway specific to the Watsonville population (as discussed below) could be involved. Even though we should confirm these findings with a larger sampling, our results demonstrate the importance of considering different populations in such studies.

Basal gene expression is different between invasive and native genotypes

We performed a transcriptomic analysis on genotypes from each of the three sampled locations in order to identify molecular processes underlying variation in the oxidative stress response. We first identified DE genes between the three genotypes in untreated conditions (control).

In *D. melanogaster*, genotypic differences accounted for 7.3% of DE genes showing micro-environment plasticity among as set of 16 DGRP (Drosophila Genetic Reference Panel) lines reared under carefully controlled standard conditions [46]. This result is in agreement with our results, in which almost 7% of the transcriptome was differentially expressed across genotypes. Most of these DE genes correspond to biological processes such as metabolism or protein synthesis 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 for the DE genes. Among down-regulated genes, we found significant enriched GO terms 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 1111 DE genes identified after exposure to 5mM or 15mM of paraquat [47]. In response to oxidative stress following exposure to hydrogen peroxide, 1639 DE genes were identified [47,48].

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 both invasive genotypes (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, prior to the arrival of *D. suzukii*.

A set of 67 DE genes were shared by all genotypes in their response to paraquat. This set of common DE genes likely corresponded to those directly implicated in stress response. In agreement with this idea, we founded genes such as *Hsp* or genes of the cytochrome gene family [49–54].

At the transcriptomic level, the Japanese genotype appeared dissimilar 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 control conditions, this genotype had the highest amount of DE genes. At the phenotypic level, the Japanese genotype had the lowest lifespan under standardized control conditions but was one of the genotypes most resistant to oxidative stress.

Together, these results suggested that the Japanese genotype maintained some constitutive defences to oxidative stress. In the absence of oxidative stress, the expression of constitutive defence may come at a cost of reduced lifespan but it 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 indicate they are experiencing a greater amount of oxidative damage and therefore 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 not found in the other genotypes. Previous studies, ranging from bacteria to mammals, have demonstrated a trade-off between copper tolerance and sensitivity to paraquat [55,56]. 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.

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 involved in response to local environmental conditions then analysis of genes with GEI may provide insight into the mechanisms of local adaptation [57]. Genes with GEI have often been identified in studies of oxidative stress responses (see *e.g.,* Jordan *et al.,* 2012) [58].

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 [52,59–63]. We founded 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 across 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 [52] and also to oxidative stress (see review [49]). In general, GO analysis revealed an enrichment in 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 $\log_2$FC > 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 in our data [52,62–65].

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 [13,66]. A recent review cited several examples of TE family activation following stress, which may depend on the type of stress and the TE family [17,67]. Horváth *et al.* (2017) 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 [17]. 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 deregulated following exposure to paraquat in the French genotype. This result is not related to the potential activity of TE in *D. suzukii*, since a greater number of TE families are DE between genotypes in control conditions, suggesting that TEs in *D. suzukii* are capable of being expressed.

Most TE insertions are neutral or slightly deleterious, but some may be beneficial and implicated in adaptation [13,14,66,67]. The impact of TE insertions is often achieved by their effect on gene expression, likely due to the addition of regulatory sequences, present in the TE, that can modulate genes expressed, particularly during stress [20]. While TEs have been revealed 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 to support this hypothesis [3,6,16,66].

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 insertions along the chromosome 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 [68]. However, when we specifically analyzed DE genes, we observed a depletion of TE insertions, suggesting that TE insertions in stress response genes may be eliminated by strong purifying selection. This paucity of TE insertions was also observed for DE genes that were shared by the three genotypes. A gene encoding a glutathion-s-transferase was the only one to display a shared TE insertion in its flanking region. Finally, we tested for the enrichment 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 gene may be more permissive to TE insertions. Several insertions were found in 3’UTR and 5’UTRs and could have regulatory impacts on those genes. 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 sampling sites on the same country. 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 functional 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. Our data also reveal that gene expression patterns first depend on the genotype, and on the stress condition to a lesser extent. Finally, we showed that contrary to expectations, oxidative stress does not induce significant activation of TEs and that DE genes under stress conditions are depleted of TE insertions in the three genotypes of *D. suzukii* studied. Our results highlight that it is important to focus on several genotypes in performing phenotypic or transcriptomic analysis, and that we should consider the neglected role of TEs in adaptive evolution. Also, phenotypic and molecular approaches should complement each other to better understand the evolution of biological traits.

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 S8). 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 S9) in a controlled environment: 22.5°C ± 1°C, 70 % ± 5% RH (relative hygrometry) and a 16:8 (Light/Day) [69]. 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, [70]). First, for each replicate (10 survival times), we used the fitdistcens function from the fitdistrplus package (v.1.0-14,

[71]) to determine which of several distribution models (Weibull, lognormal and gamma) were most appropriate to r fit our right censored data (33 flies) data. The Weibull distribution was chosen after graphical comparison with others, also confirmed using loglikelihoods of the fitted models. For each replicate the fitted distribution was summarized using its theoretical median. Second, a linear mixed model was fitted to the log transformed medians using the lmer function of the lme4 R package (v.1.1-21, [72]), and p-values were estimated using lmerTest (v.3.1-0, [73]) 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 correlation coefficient in R (Fig. S1).

DNA extraction and sequencing

We sequenced genomic DNA for 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 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 [74].

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 to remove gonads, and the remaining somatic tissue was frozen in liquid nitrogen and stored at -80°C.

We used the RNAeasy Plus Mini Kit (Qiagen) to extract total RNA from the somatic tissues, following the protocol provided by manufacturer. Samples were treated with DNAse (ref AM2224, AMbion^{TM}) according to manufacturer instructions and stored at -80°C. RNA amount and quality was estimated using Qubit^{TM} (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 120.12 million paired-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 checked again using FastQC [75,76]. RNA-seq 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 [77–79]. 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 [80]. 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 performed using the DESeq2 package (v. 1.24.0) on R (v. 3.6.0) [81]. 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 [82]. 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*) [77,83]. The reads were then mapped to the reference genome using bwa bwasw (v0.7.17) [84]. Aligned bases were masked using bedtools, bamtobed, and bedtools maskfasta (v2.20.0) [85]. 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) [83]. 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 TE 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 [86]. 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 [77]. 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 [41]. 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) [87,88]. PFM (Position Frequency Matrix) matrices were extracted (CNC:MA0530.1, HSF:MA0486.2, DL:MA0022.1) before a PWM (Position Weight Matrix) 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 [89].

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 [54].

P-values were calculated using a Fisher test for enriched GO terms and adjusted with the Benjamini-Hochberg correction for multi-testing [89]. GO terms with FDR ≤ 0.05 were defined as significantly enriched. The GO terms were reduced to representative non-redundant terms using the REVIGO tool and manual curation [90].

Declarations

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Contribution

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. supervised the statistical analysis and revised manuscript. M.F. helped on transcriptomic analysis and manuscript correction. M.G.F. performed gene ontology analysis. V.M. produced all related TE informations (genome annotations, frequency insertions). All authors proofread and approved the manuscript

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data

DNA and RNA-seq data are available on NCBI with the Sequence Read Archive (SRA) number: SUB7319267 & SUB7021358.

Survival data of the populations are available to:

ftp://pbil.univ-lyon1.fr/pub/datasets/Marin2020/surv_suzukii_marin2020/surv_suzukii_marin2020.txt

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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.

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Fig. 7 Reaction norms between control and paraquat for DE genes using normalized log2 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)

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

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. Partial chi-square are in brackets.

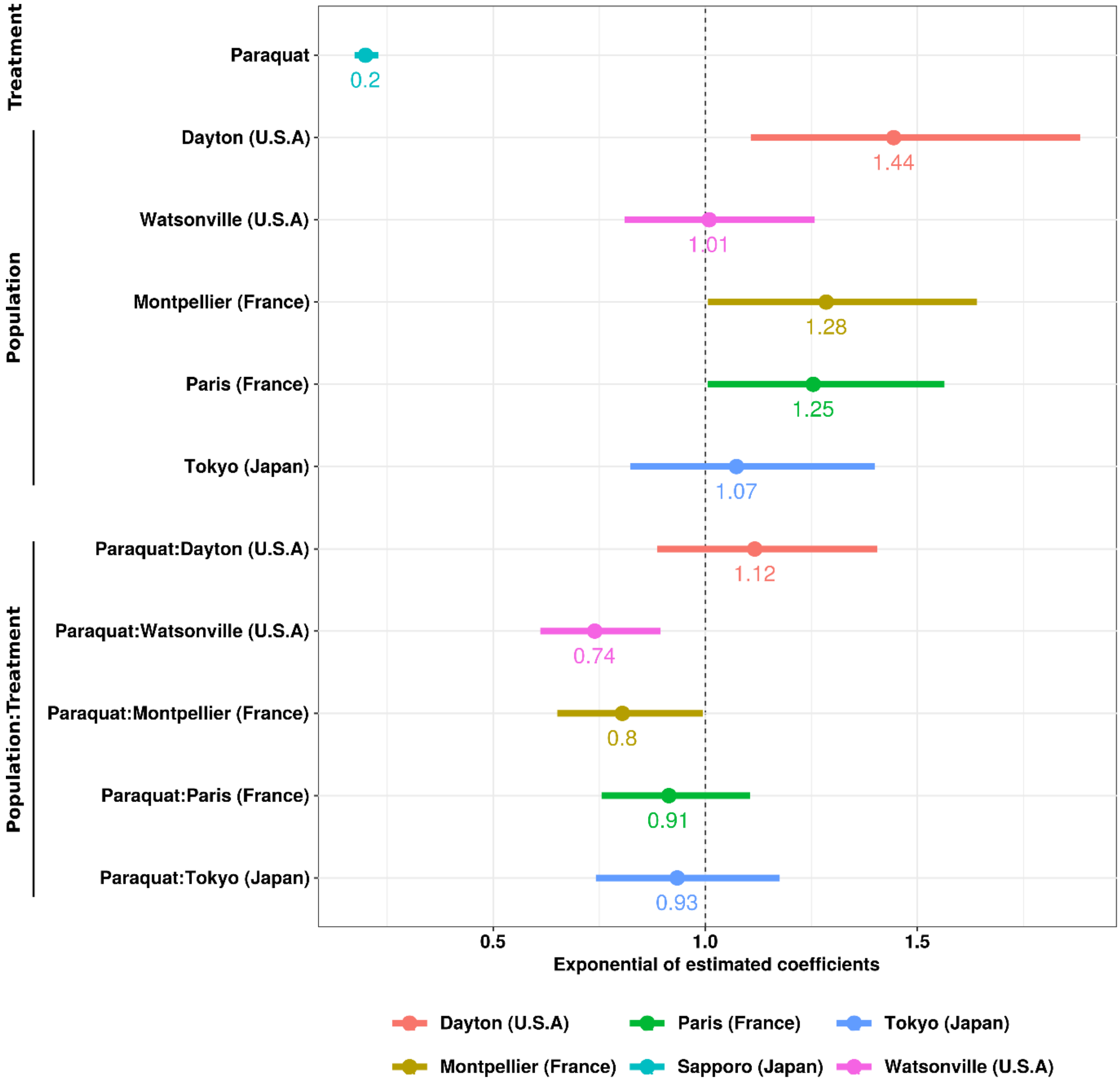
Table S6 Insertion position of TEs in genes differentially expressed. (XLSX)

Table S7 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.

Table S8 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.

Table S9 Recipe of diet medium modified from Dalton *et al.*, 2011.

Figures



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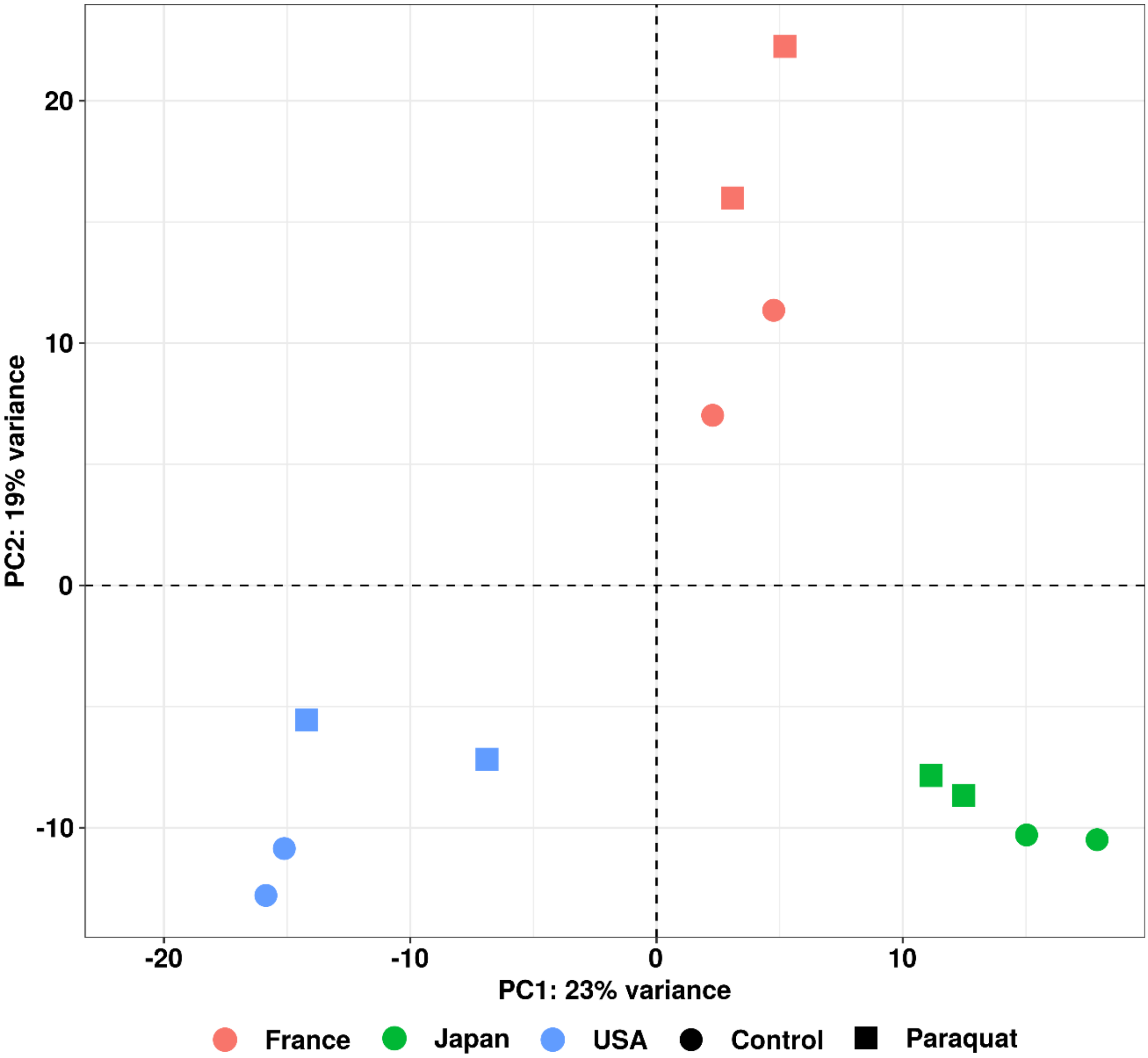

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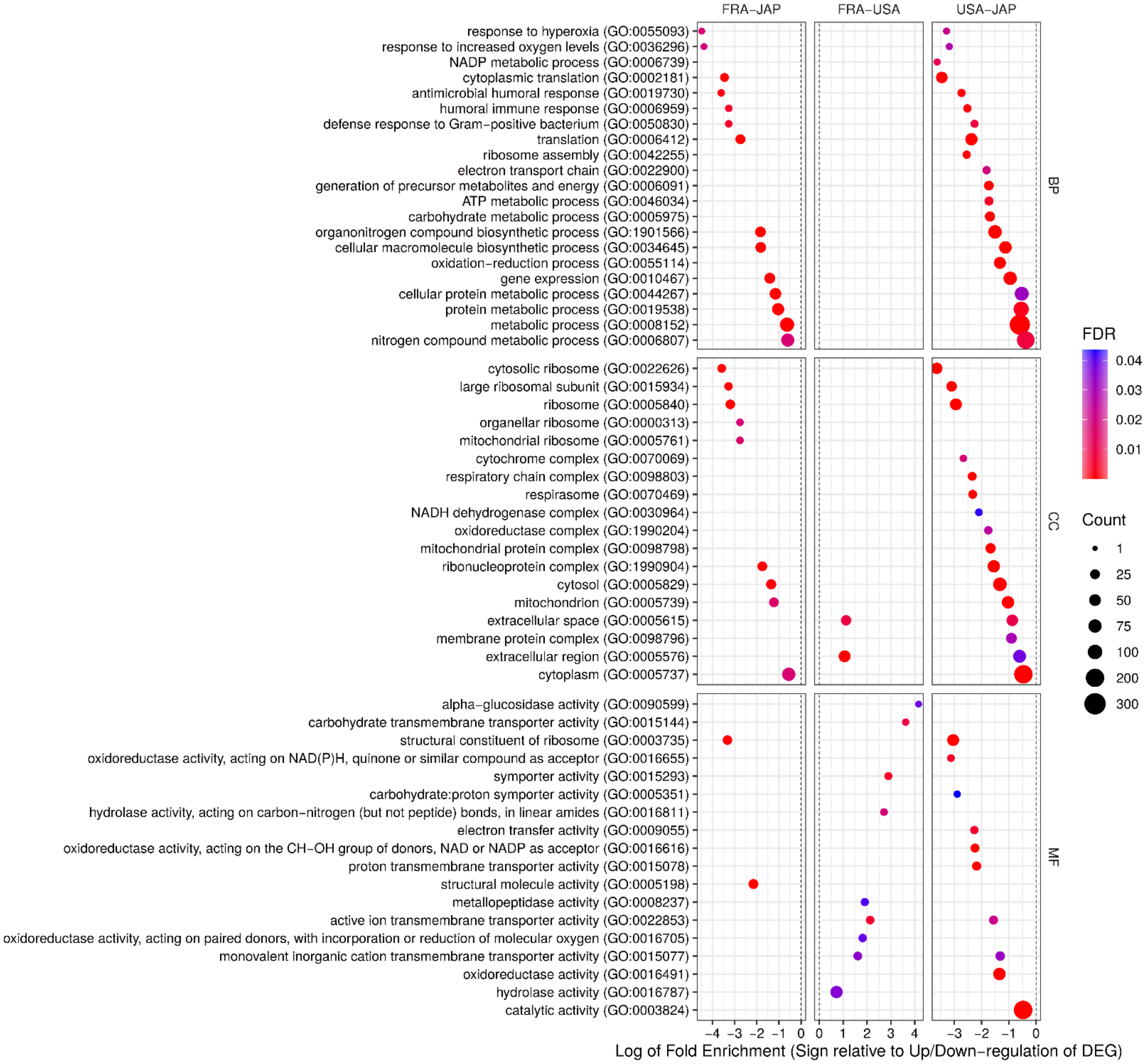

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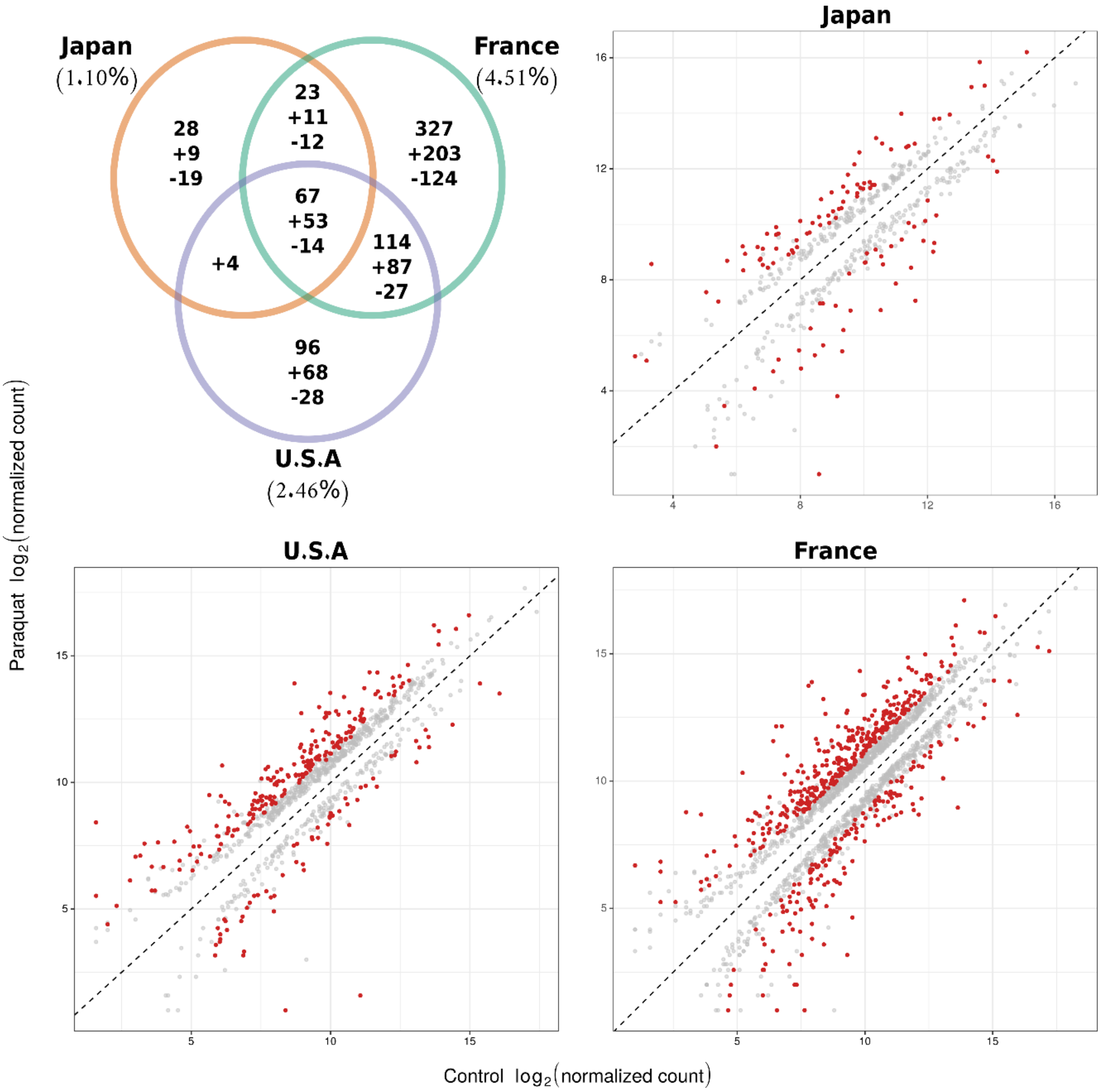

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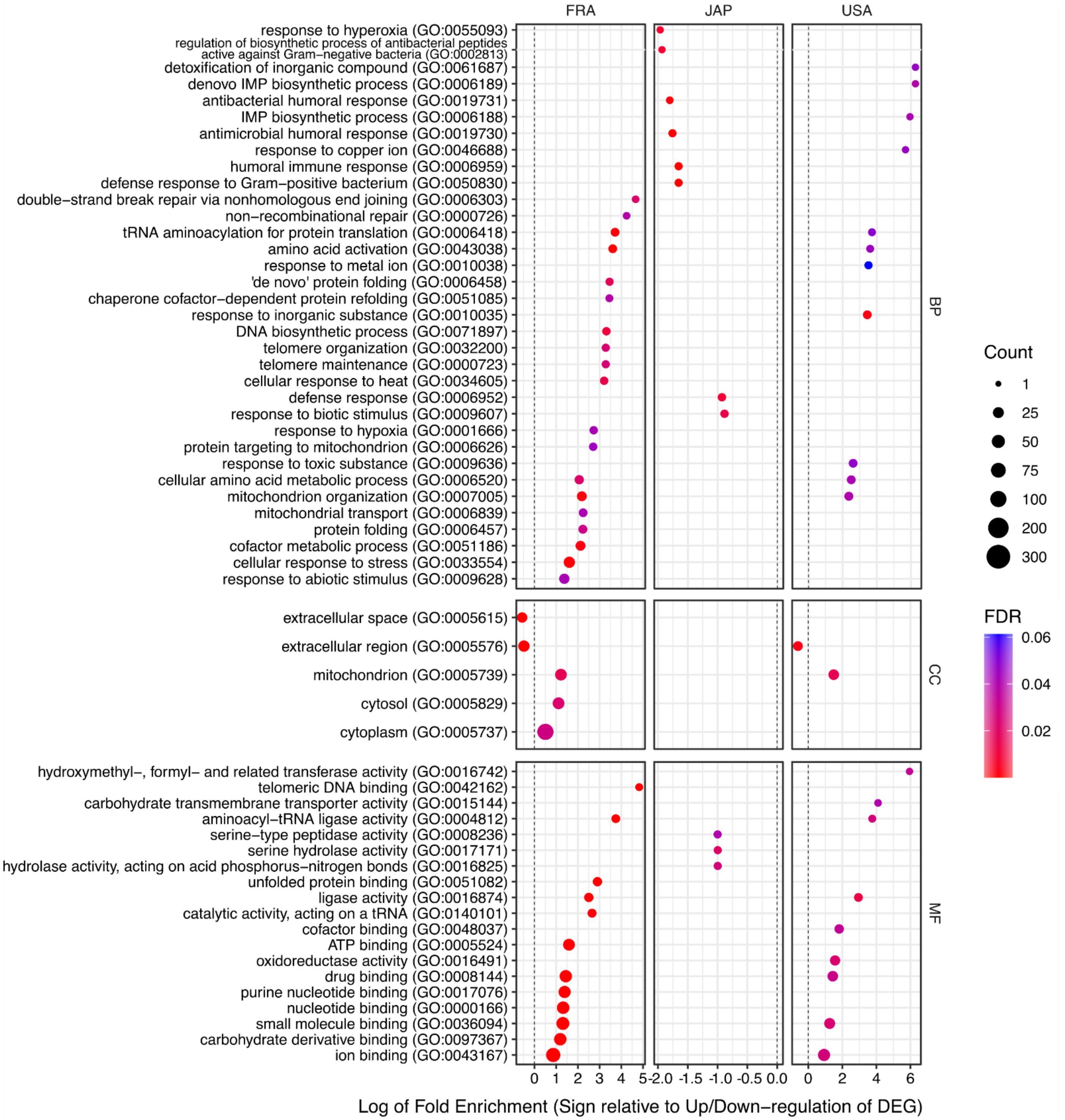

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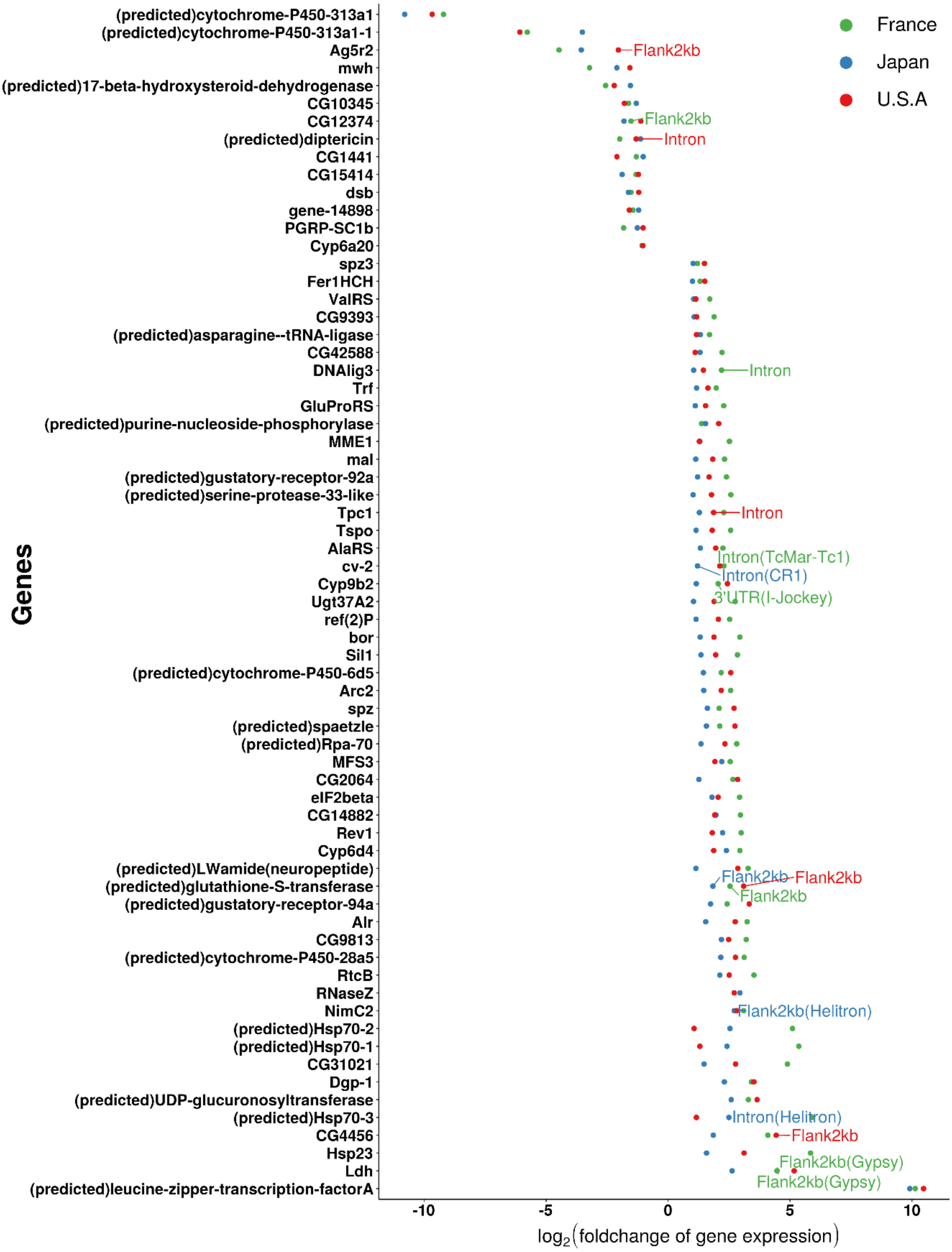

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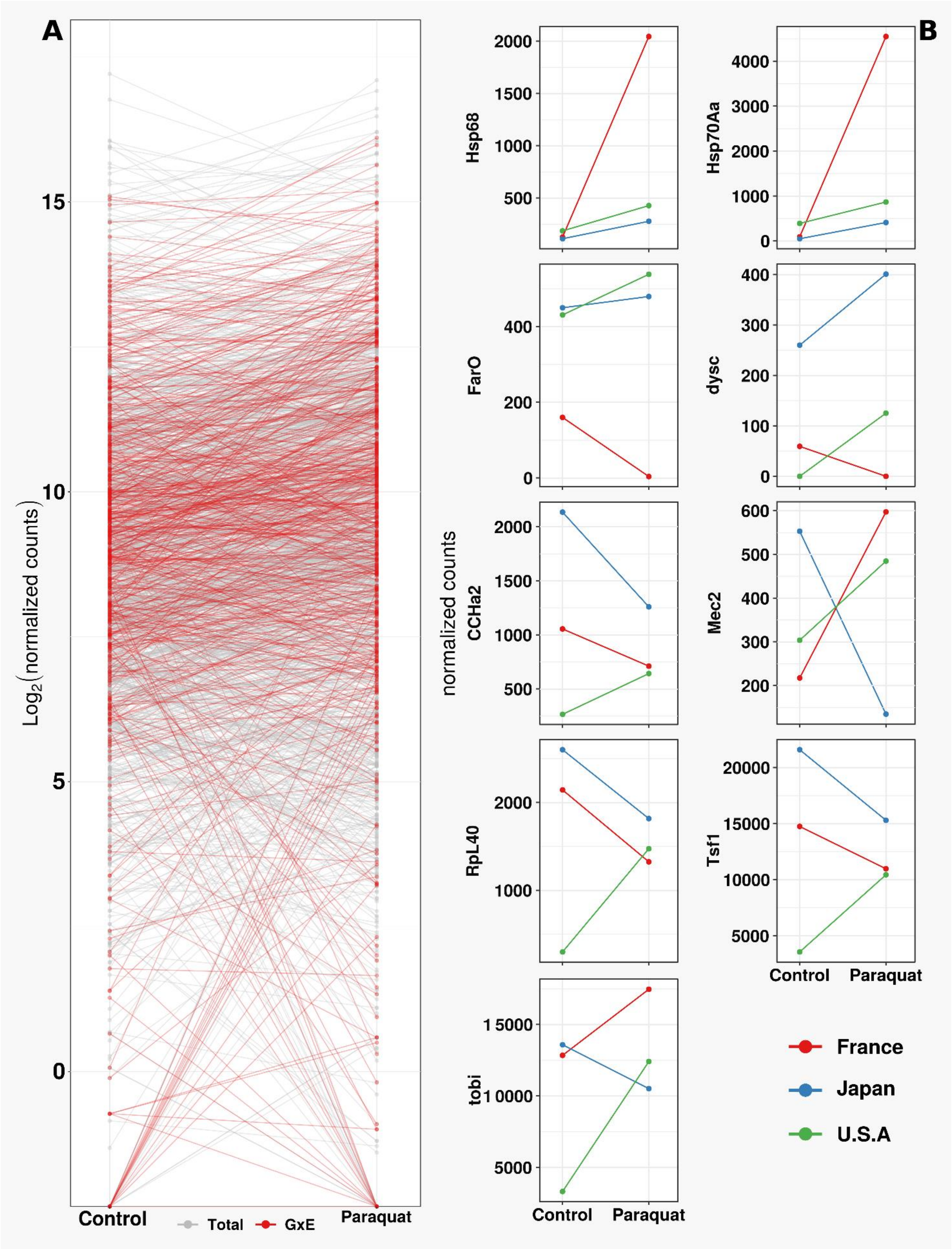

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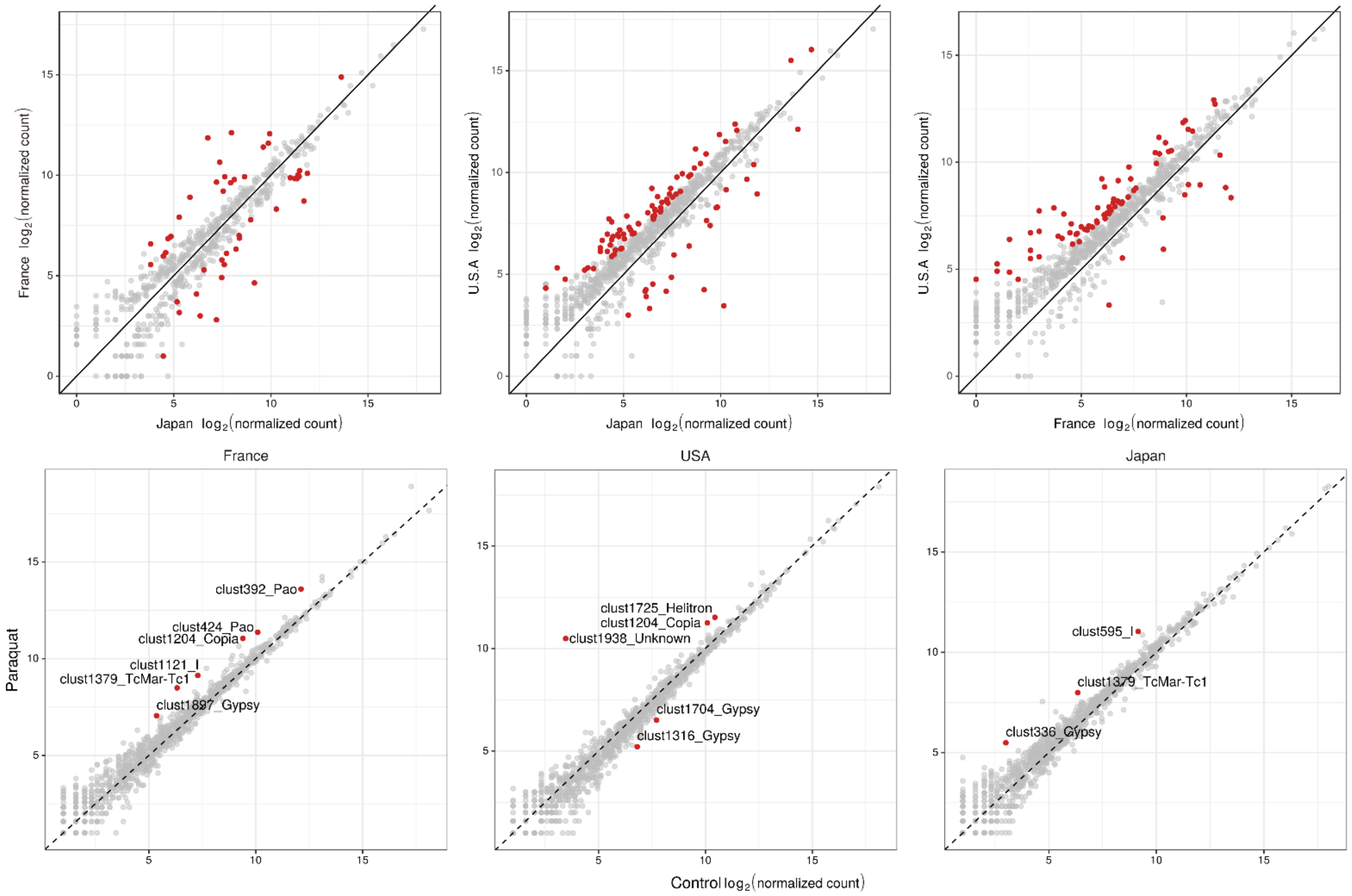

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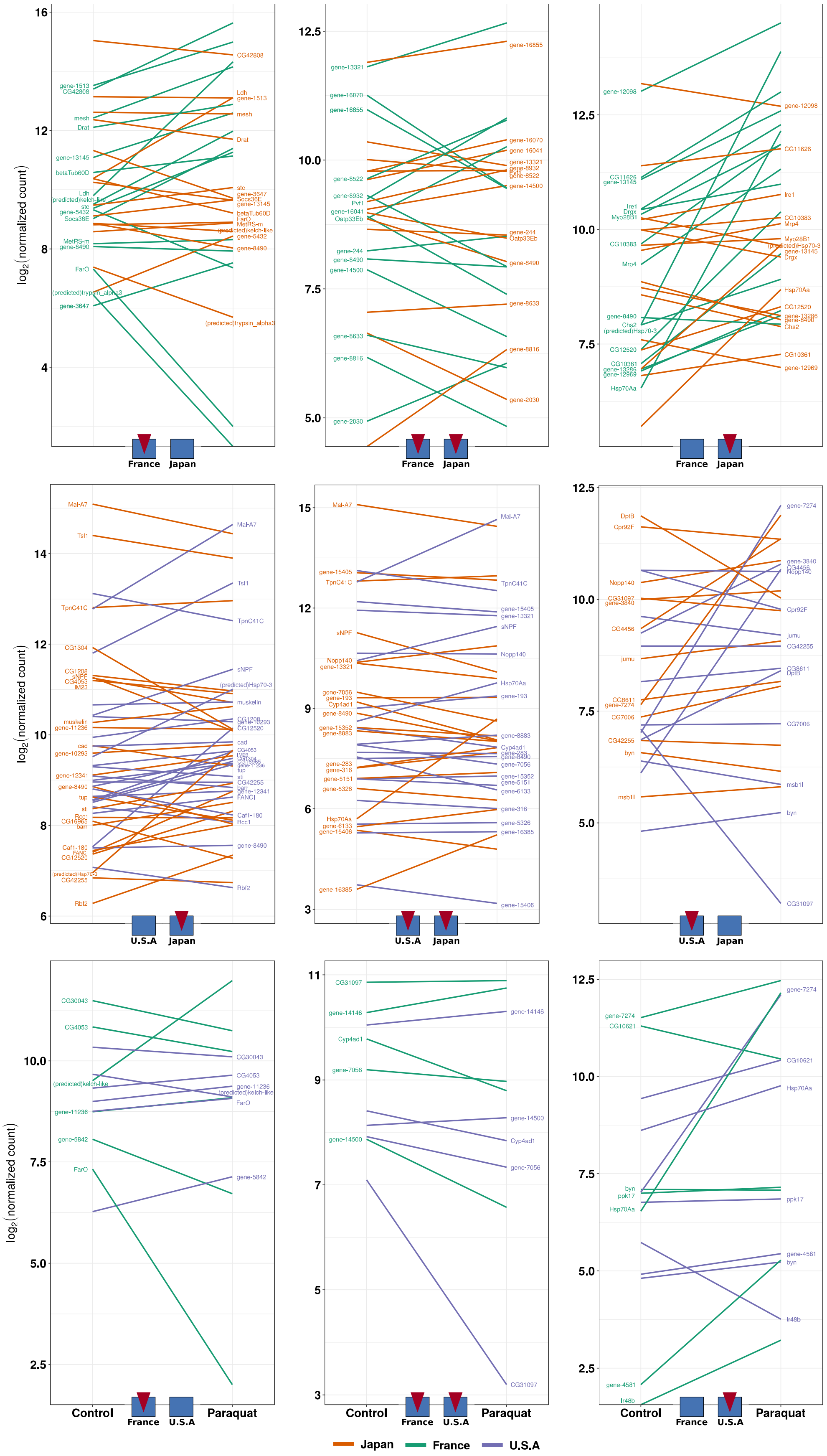

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Tables

Table 1 Median lifespan (days) by sex, treatment, and population with bracketed 0.25 & 0.75 quantiles. Values were calculated from estimated median (see M&M) at population level (line effects into the populations were estimated as a random effect from the linear mixed model).

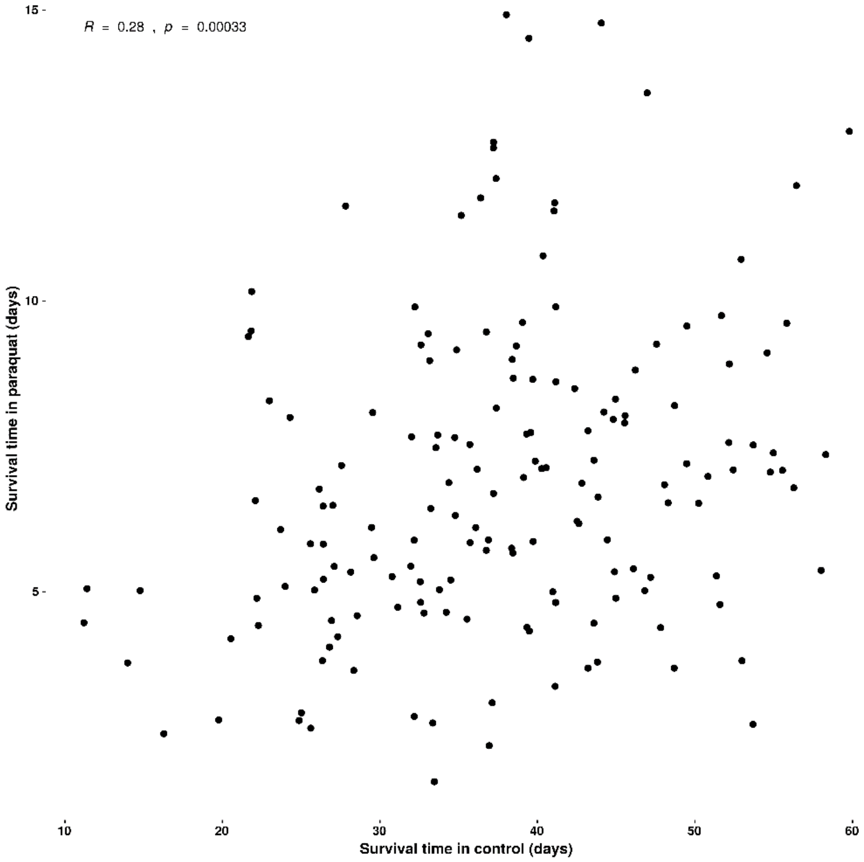
	Sapporo (Japan)	Tokyo (Japan)	Montpellier (France)	Paris (France)	Dayton (U.S.A)	Watsonville (U.S.A)
Females control	32.0 (26.0-39.8)	32.0 (29.6-41.0)	40.5 (35.3-46.3)	41.0 (34.5-49.3)	51.4 (39.7-54.6)	34.8 (27.4-42.4)
Females paraquat	6.8 (5.0-9.1)	5.3 (5.0-7.7)	5.5 (5.2-6.3)	7.1 (5.4-8.2)	9.1 (8.7-9.8)	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)

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

Supplementary figures



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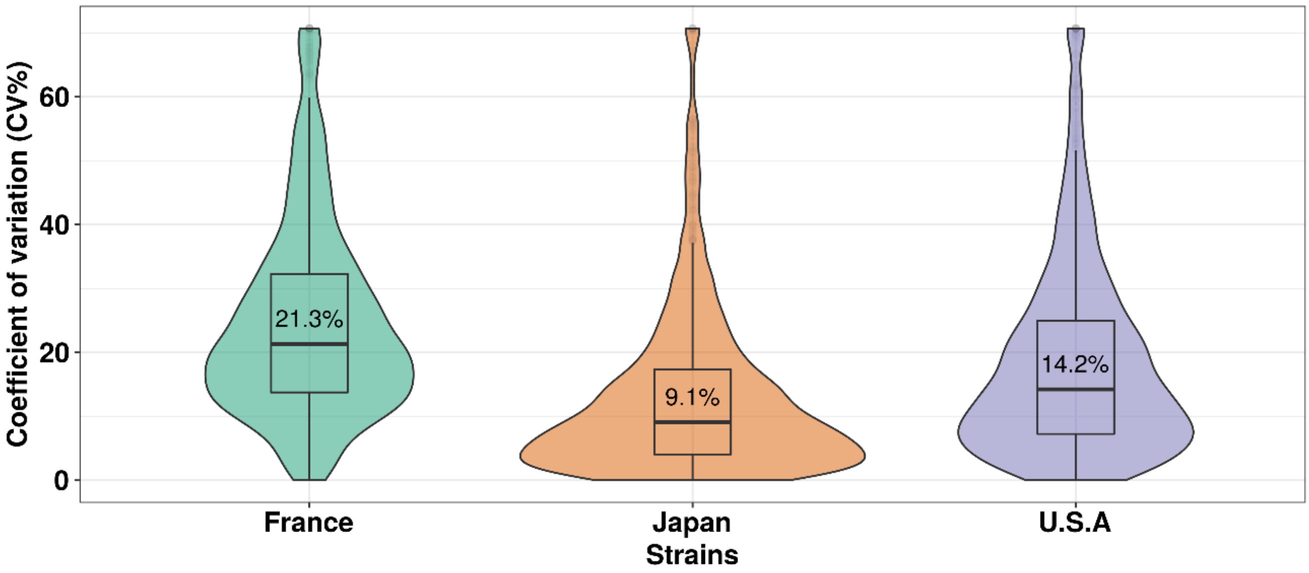

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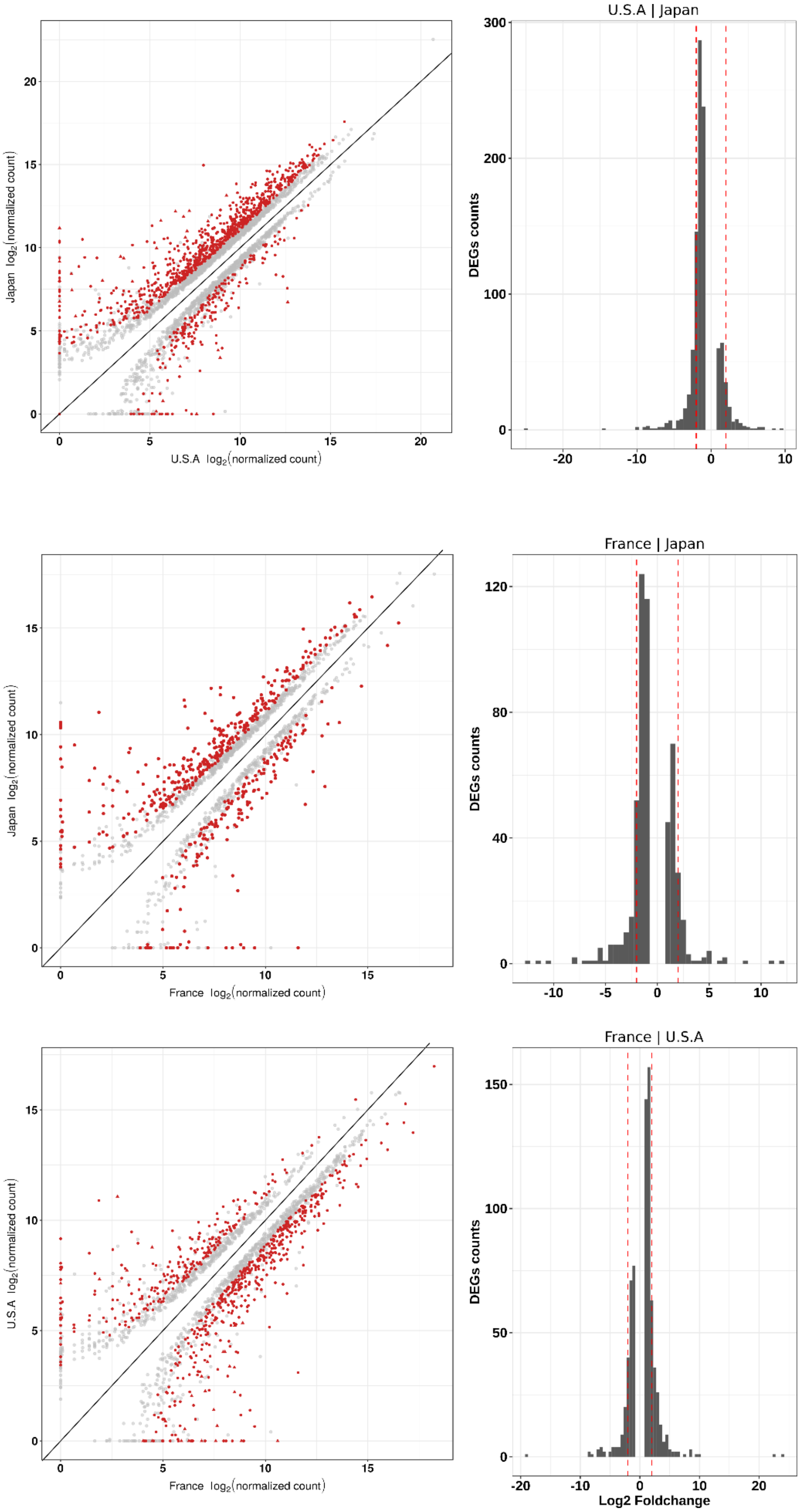

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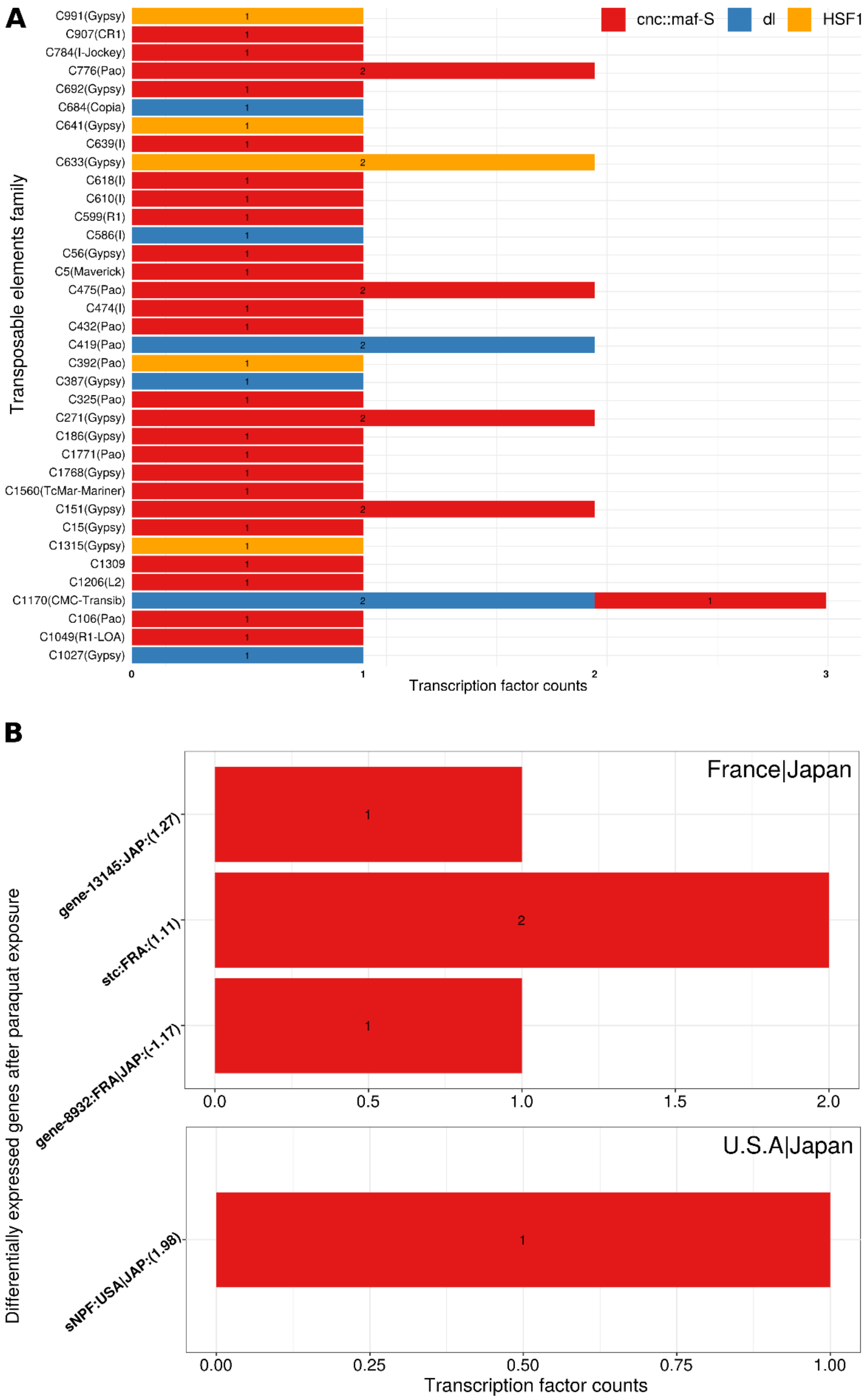

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Table S1 Table of linear mixed model with estimated coefficient and associated statistic. Model is centered on the Sapporo population reference, in untreated condition. Data were previously log transformed for normality. Isofemale lines were included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

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

Paraquat:Watsonville (U.S.A)	-0.302	0.097	297.00	-3.114	2.03e-03	0.611	0.895	0.739

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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	fatty-acyl-CoA reductase (alcohol-forming) activity, long-chain-fatty-acyl-CoA reductase activity

		growth, positive regulation of lipid storage	
Hsp68	Heat shock protein 68 (*Hsp*68) 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 (*Hsp*70Aa) 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

1

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 ≤0.01 and absolute $\log_2$foldchange ≥ 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. Partial chi-square are in brackets.

		Observed		Expected		
		TE-	TE+	TE-	TE+	p-value
France	DE-	11501	2506	11528.0	2479.0	0.00216
	DE+	464	67	437.0	94.0	
Japan	DE-	11664	2752	11675.2	2740.8	0.01321
	DE+	110	12	98.8	23.2	
U.S.A	DE-	11678	2579	11692.5	2564.5	0.02761
	DE+	245	36	230.5	50.5	
France | Japan	DE-	11358	3042	11352.2	3047.8	0.2678
	DE+	103	35	108.8	29.2	
France | U.S.A	DE-	11491	2982	11499.4	2973.6	0.01566
	DE+	60	5	51.6	13.4	
U.S.A | Japan	DE-	11383	3093	11385.2	3090.8	0.5894
	DE+	51	11	48.8	13.2	

Table S6 Insertion position 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

Table S7 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	$\log_2$FoldChange	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
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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

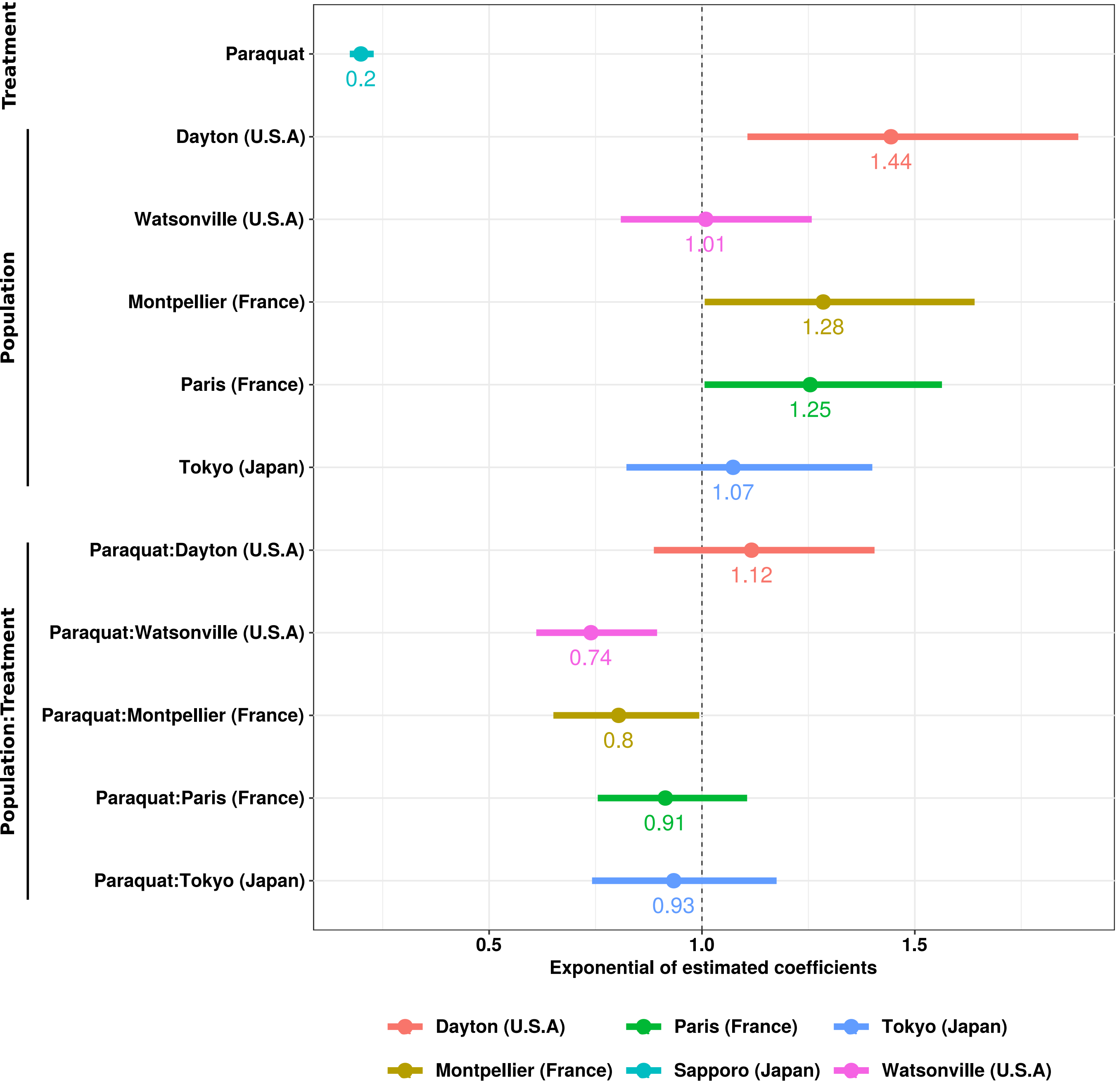
Table S8 Geographical location of isofemale lines. *D. suzukii* flies were sampled in 3 countries (Japan, U.S.A and France) with their location and invasive status. Line name is indicated with bold type for the line use in molecular analysis.

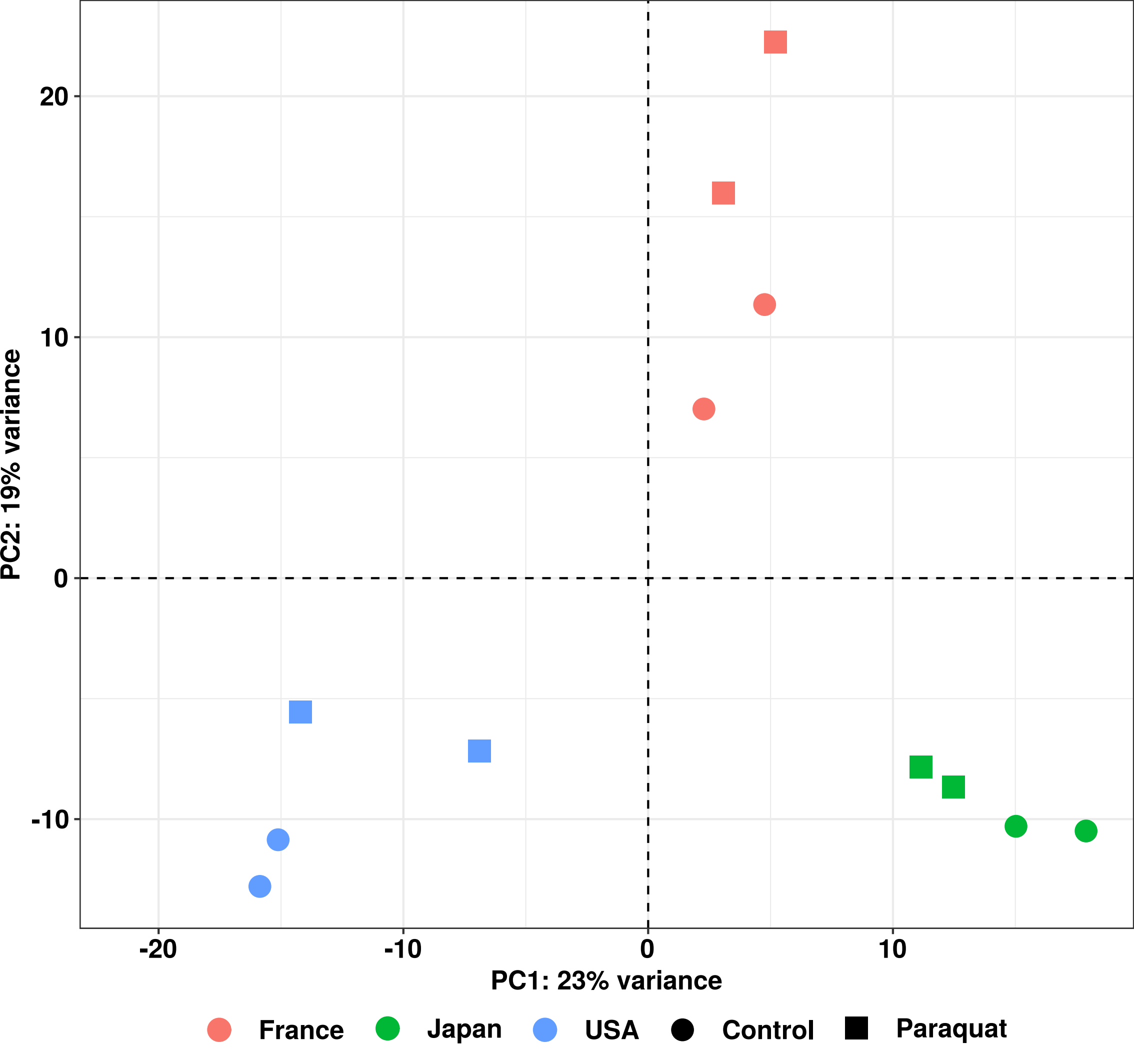
Location	Coordinates	Status	Lines
Sapporo (Hokkaido, Japan)	43° 3' 43.545"N 141° 21' 15.754" E	Native	S11, S20, S21, S24, **S29**
Tokyo (Honshu, Japan)	35° 41' 22.155" N 139° 41' 30.143" E	Native	T3, T11, T18
Watsonville (California, U.S.A)	36°54'51.8"N 121°45'27.7"W	Invasive	W106, W112, W113, **W120**, 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**

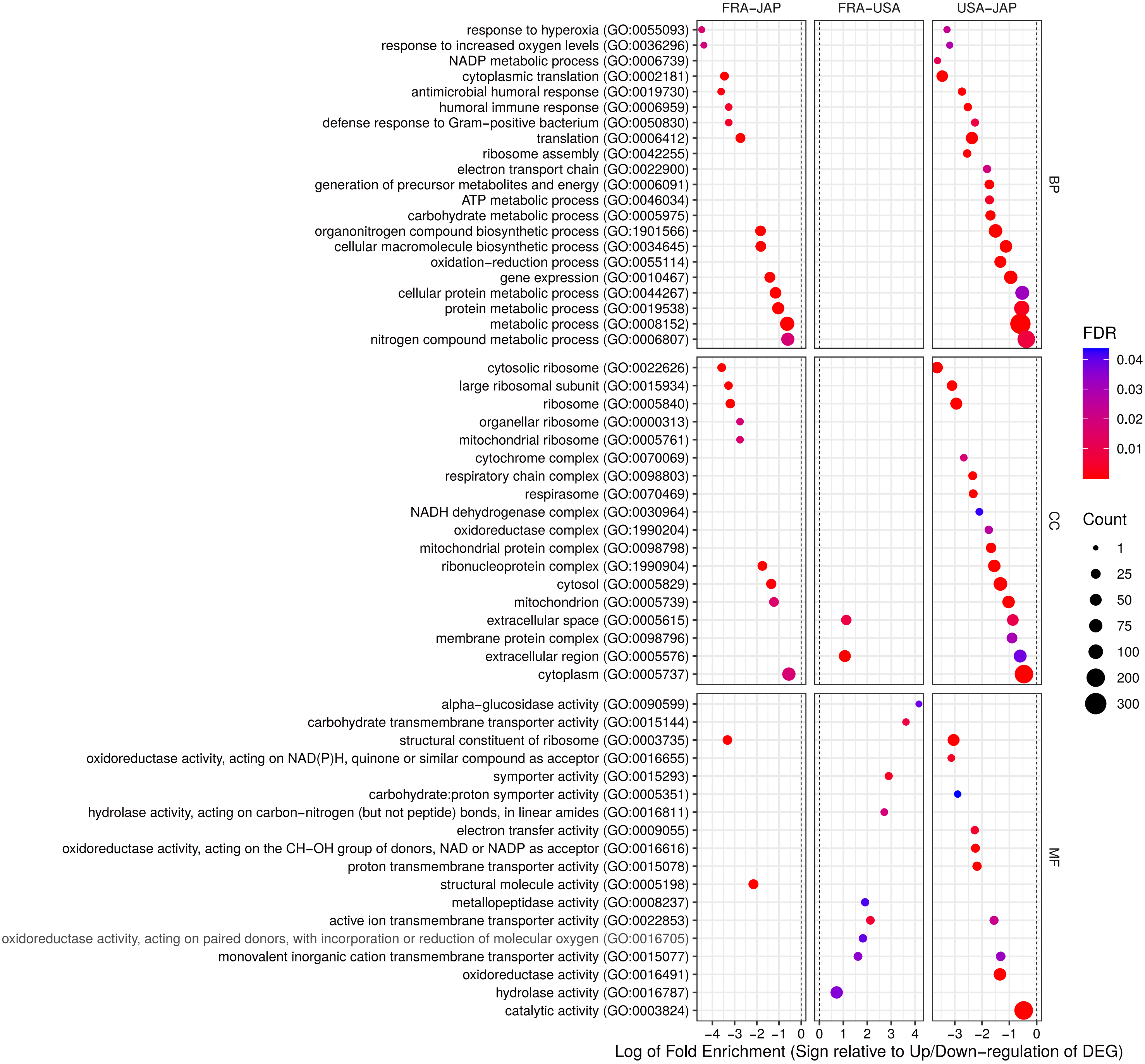
Table S9 Recipe of diet medium modified from Dalton *et al.*, 2011.

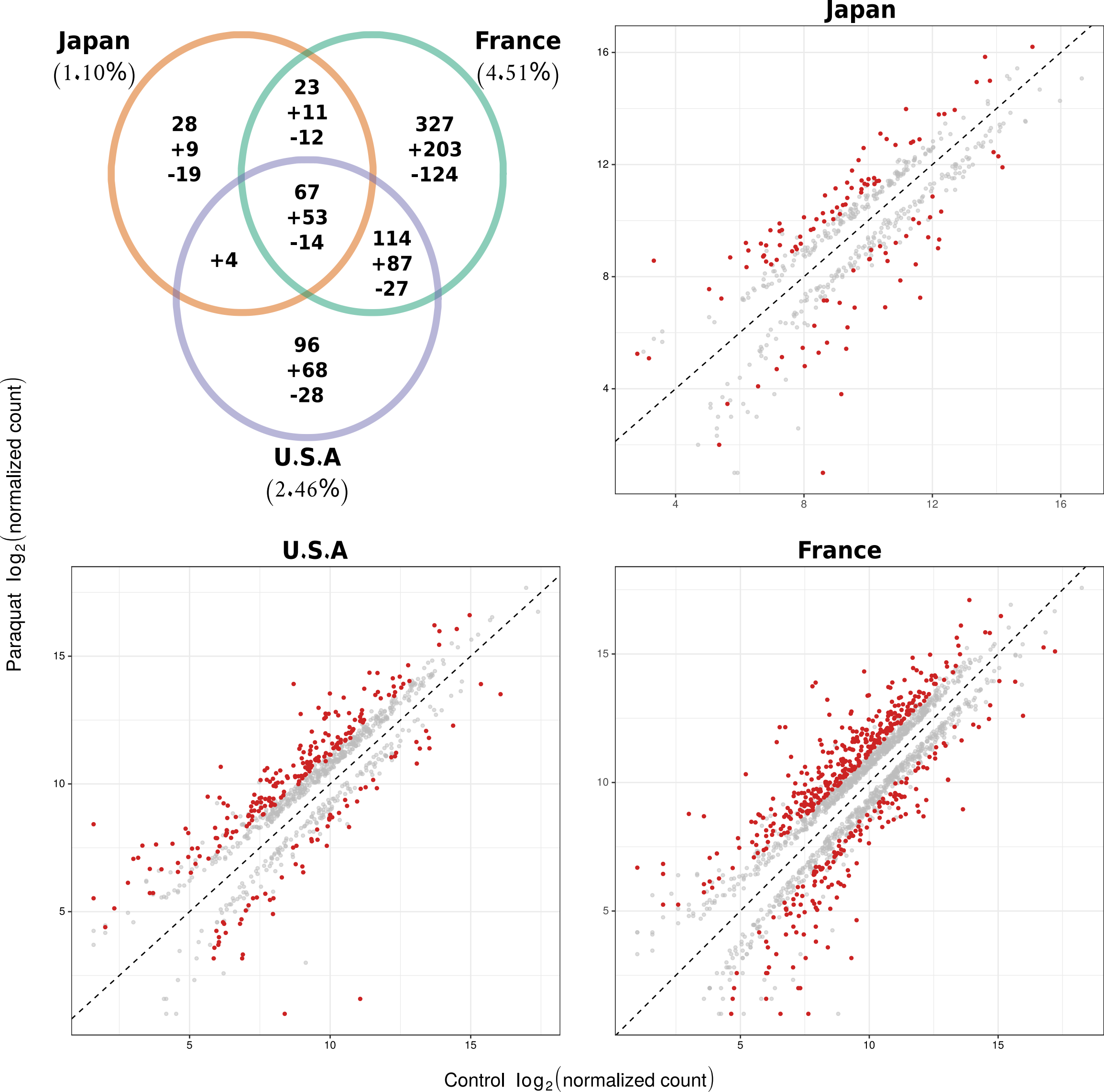
Distilled water: 1 L
Agar (Drosophila Agar Type, ref.66-103, Apex^{TM}): 9 g.L^{-1}
Cornmeal (Farine de gaudes, Moulin Giraud): 33 g.L^{-1}
Ethanol 96%: 40 ml.L^{-1}
Yeast (ref.75570, LYNSIDE®): 17 g.L^{-1}
Sugar (supermarket sugar): 50 g.L^{-1}

Nipagin (Tegosept,ref.20-258, Apex^{TM}): 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.


Treatment
Population
Population:Treatment
Paraquat
Dayton (U.S.A)
Watsonville (U.S.A)
Montpellier (France)
Paris (France)
Tokyo (Japan)
Paraquat:Dayton (U.S.A)
Paraquat:Watsonville (U.S.A)
Paraquat:Montpellier (France)
Paraquat:Paris (France)
Paraquat:Tokyo (Japan)
0.2
1.44
1.01
1.28
1.25
1.07
1.12
0.74
0.8
0.91
0.93
0.5
1.0
1.5
Exponential of estimated coefficients
Dayton (U.S.A)
Paris (France)
Tokyo (Japan)
Montpellier (France)
Sapporo (Japan)
Watsonville (U.S.A)


PC2: 19% variance
20
10
0
-10
-20
-10
0
10
PC1: 23% variance
France
Japan
USA
Control
Paraquat


FRA−JAP
FRA−USA
USA−JAP
response to hyperoxia (GO:0055093)
response to increased oxygen levels (GO:0036296)
NADP metabolic process (GO:0006739)
cytoplasmic translation (GO:0002181)
antimicrobial humoral response (GO:0019730)
humoral immune response (GO:0006959)
defense response to Gram−positive bacterium (GO:0050830)
translation (GO:0006412)
ribosome assembly (GO:0042255)
electron transport chain (GO:0022900)
generation of precursor metabolites and energy (GO:0006091)
ATP metabolic process (GO:0046034)
carbohydrate metabolic process (GO:0005975)
organonitrogen compound biosynthetic process (GO:1901566)
cellular macromolecule biosynthetic process (GO:0034645)
oxidation−reduction process (GO:0055114)
gene expression (GO:0010467)
cellular protein metabolic process (GO:0044267)
protein metabolic process (GO:0019538)
metabolic process (GO:0008152)
nitrogen compound metabolic process (GO:0006807)
BP
cytosolic ribosome (GO:0022626)
large ribosomal subunit (GO:0015934)
ribosome (GO:0005840)
organellar ribosome (GO:0000313)
mitochondrial ribosome (GO:0005761)
cytochrome complex (GO:0070069)
respiratory chain complex (GO:0098803)
respirasome (GO:0070469)
NADH dehydrogenase complex (GO:0030964)
oxidoreductase complex (GO:1990204)
mitochondrial protein complex (GO:0098798)
ribonucleoprotein complex (GO:1990904)
cytosol (GO:0005829)
mitochondrion (GO:0005739)
extracellular space (GO:0005615)
membrane protein complex (GO:0098796)
extracellular region (GO:0005576)
cytoplasm (GO:0005737)
CC
alpha−glucosidase activity (GO:0090599)
carbohydrate transmembrane transporter activity (GO:0015144)
structural constituent of ribosome (GO:0003735)
oxidoreductase activity, acting on NAD(P)H, quinone or similar compound as acceptor (GO:0016655)
symporter activity (GO:0015293)
carbohydrate:proton symporter activity (GO:0005351)
hydrolase activity, acting on carbon−nitrogen (but not peptide) bonds, in linear amides (GO:0016811)
electron transfer activity (GO:0009055)
oxidoreductase activity, acting on the CH−OH group of donors, NAD or NADP as acceptor (GO:0016616)
proton transmembrane transporter activity (GO:0015078)
structural molecule activity (GO:0005198)
metallopeptidase activity (GO:0008237)
active ion transmembrane transporter activity (GO:0022853)
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen (GO:0016705)
monovalent inorganic cation transmembrane transporter activity (GO:0015077)
oxidoreductase activity (GO:0016491)
hydrolase activity (GO:0016787)
catalytic activity (GO:0003824)
MF
−4 −3 −2 −1 0
0 1 2 3 4
−3 −2 −1 0
Log of Fold Enrichment (Sign relative to Up/Down−regulation of DEG)
FDR
0.04
0.03
0.02
0.01
Count
1
25
50
75
100
200
300


Japan
(1.10%)
France
(4.51%)
U.S.A
(2.46%)
28
+9
-19
23
+11
-12
327
+203
-124
67
+53
-14
+4
114
+87
-27
96
+68
-28
Japan
U.S.A
France
Paraquat log$_2$(normalized count)
Control log$_2$(normalized count)
16
12
8
4
4
8
12
16
15
10
5
5
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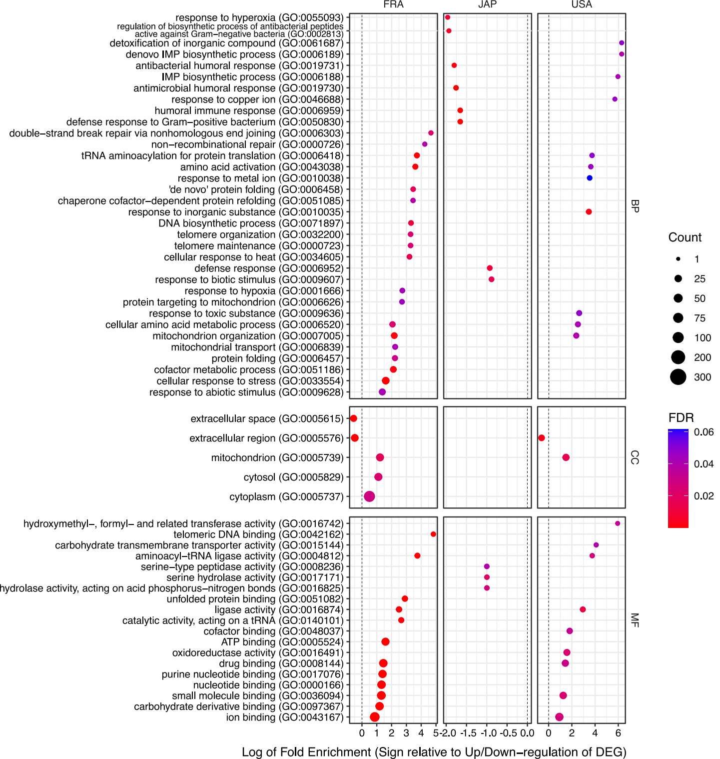
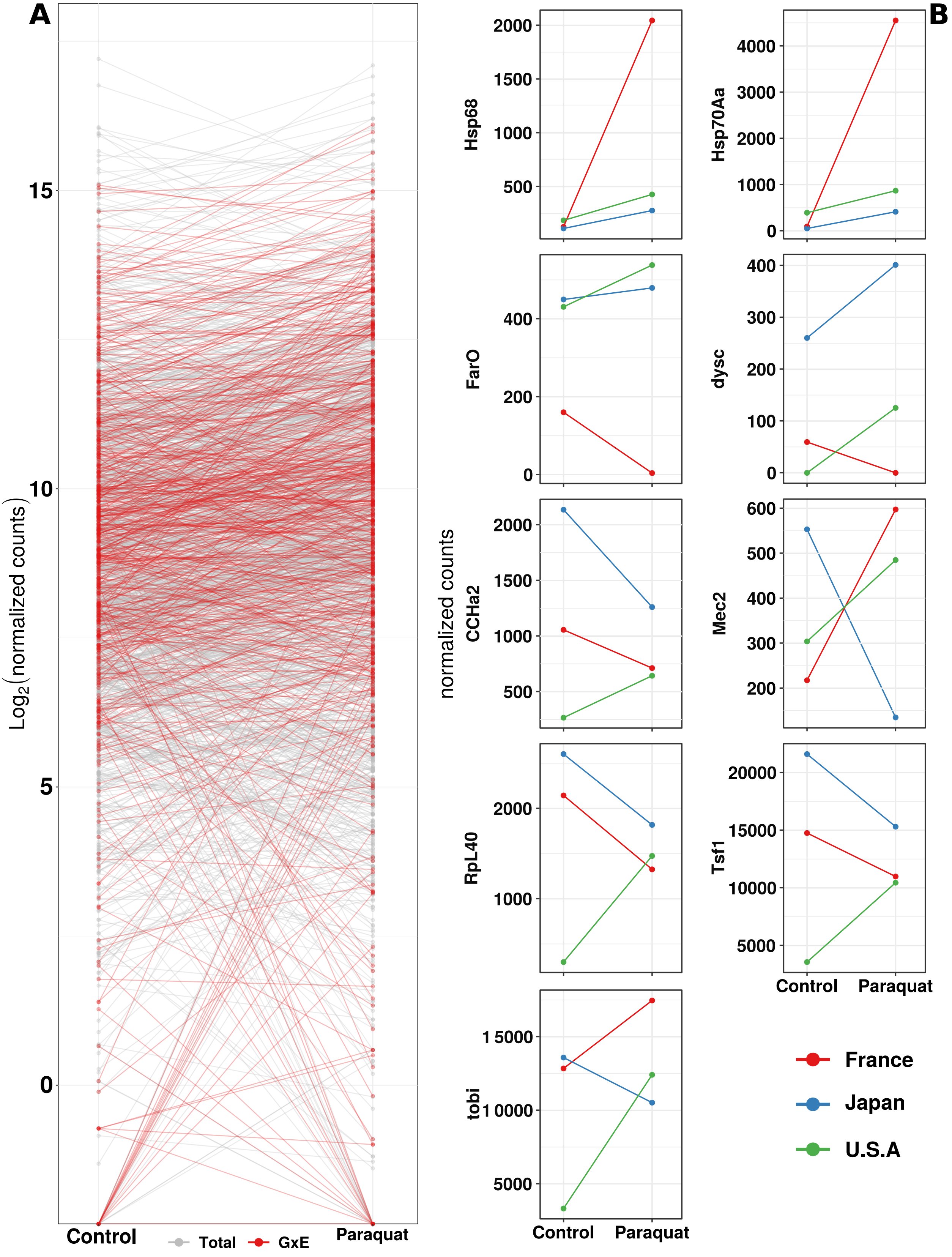
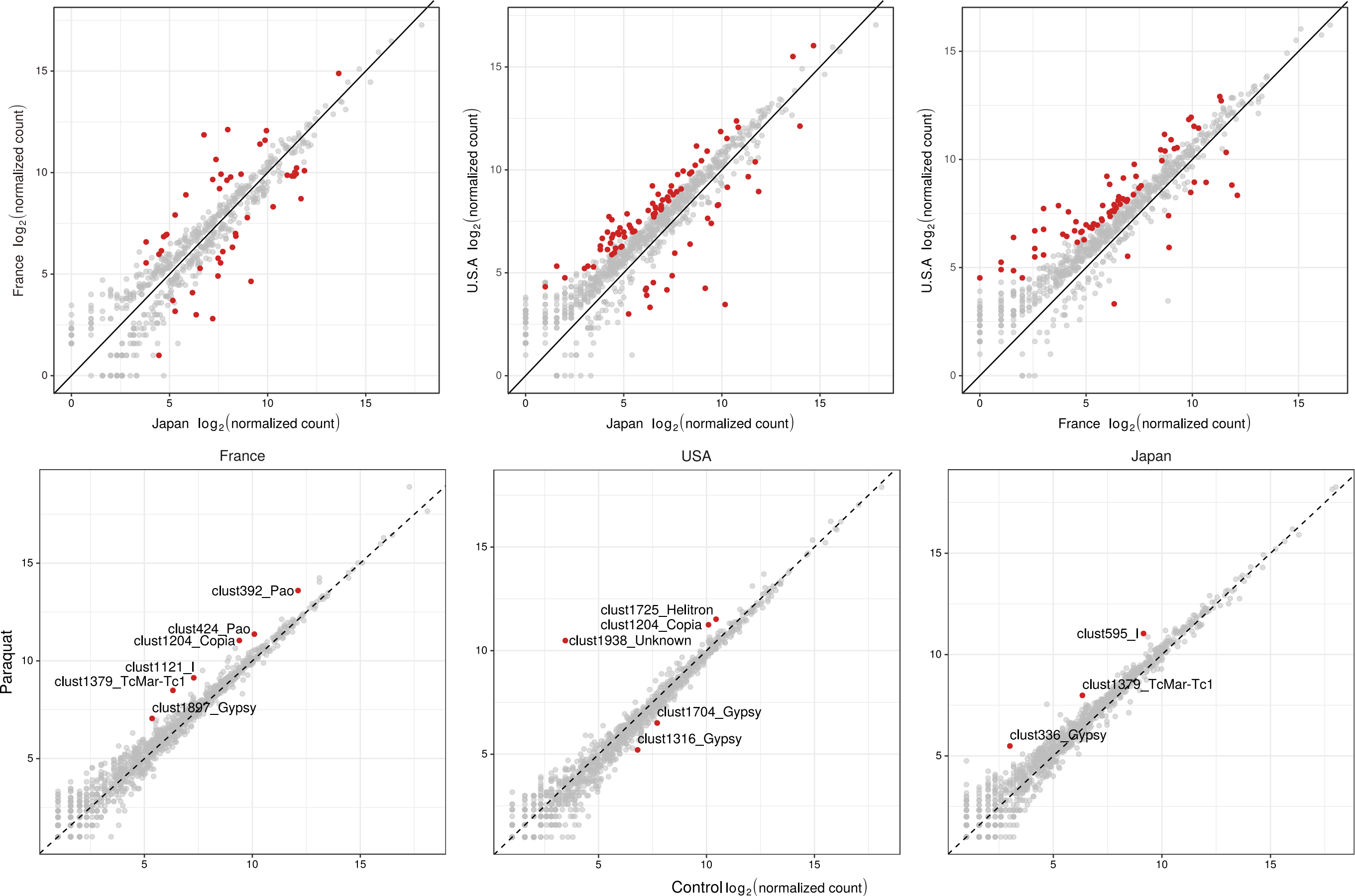
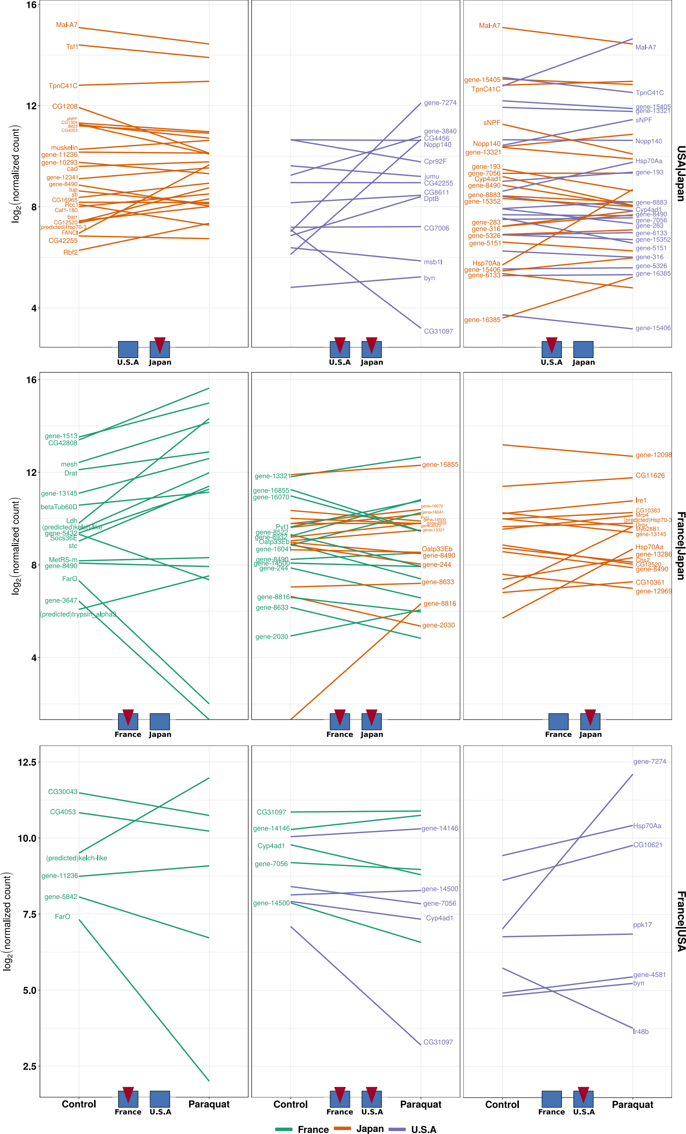
15
10
5
5
10
15

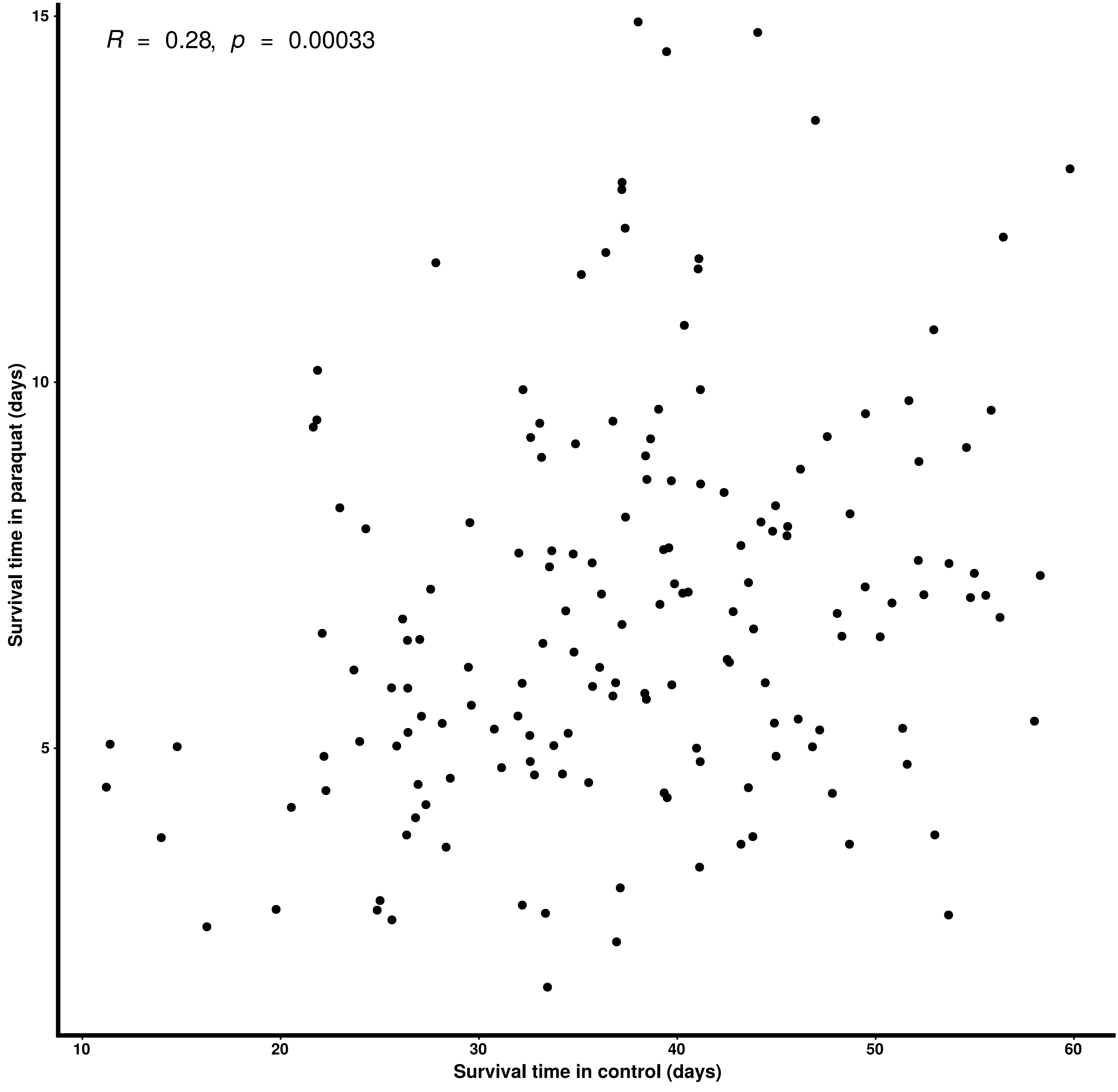

FRA
JAP
USA
response to hyperoxia (GO:0055093)
regulation of biosynthetic process of antibacterial peptides active against Gram−negative bacteria (GO:0002813)
detoxification of inorganic compound (GO:0061687)
denovo IMP biosynthetic process (GO:0006189)
antibacterial humoral response (GO:0019731)
IMP biosynthetic process (GO:0006188)
antimicrobial humoral response (GO:0019730)
response to copper ion (GO:0046688)
humoral immune response (GO:0006959)
defense response to Gram−positive bacterium (GO:0050830)
double−strand break repair via nonhomologous end joining (GO:0006303)
non−recombinational repair (GO:0000726)
tRNA aminoacylation for protein translation (GO:0006418)
amino acid activation (GO:0043038)
response to metal ion (GO:0010038)
'de novo' protein folding (GO:0006458)
chaperone cofactor−dependent protein refolding (GO:0051085)
response to inorganic substance (GO:0010035)
DNA biosynthetic process (GO:0071897)
telomere organization (GO:0032200)
telomere maintenance (GO:0000723)
cellular response to heat (GO:0034605)
defense response (GO:0006952)
response to biotic stimulus (GO:0009607)
response to hypoxia (GO:0001666)
protein targeting to mitochondrion (GO:0006626)
response to toxic substance (GO:0009636)
cellular amino acid metabolic process (GO:0006520)
mitochondrion organization (GO:0007005)
mitochondrial transport (GO:0006839)
protein folding (GO:0006457)
cofactor metabolic process (GO:0051186)
cellular response to stress (GO:0033554)
response to abiotic stimulus (GO:0009628)
BP
extracellular space (GO:0005615)
extracellular region (GO:0005576)
mitochondrion (GO:0005739)
cytosol (GO:0005829)
cytoplasm (GO:0005737)
CC
hydroxymethyl−, formyl− and related transferase activity (GO:0016742)
telomeric DNA binding (GO:0042162)
carbohydrate transmembrane transporter activity (GO:0015144)
aminoacyl−tRNA ligase activity (GO:0004812)
serine−type peptidase activity (GO:0008236)
serine hydrolase activity (GO:0017171)
hydrolase activity, acting on acid phosphorus−nitrogen bonds (GO:0016825)
unfolded protein binding (GO:0051082)
ligase activity (GO:0016874)
catalytic activity, acting on a tRNA (GO:0140101)
cofactor binding (GO:0048037)
ATP binding (GO:0005524)
oxidoreductase activity (GO:0016491)
drug binding (GO:0008144)
purine nucleotide binding (GO:0017076)
nucleotide binding (GO:0000166)
small molecule binding (GO:0036094)
carbohydrate derivative binding (GO:0097367)
ion binding (GO:0043167)
MF
0
1
2
3
4
5
-2.0
-1.5
-1.0
-0.5
0.0
0
2
4
6
Log of Fold Enrichment (Sign relative to Up/Down−regulation of DEG)
Count
1
25
50
75
100
200
300
FDR
0.06
0.04
0.02

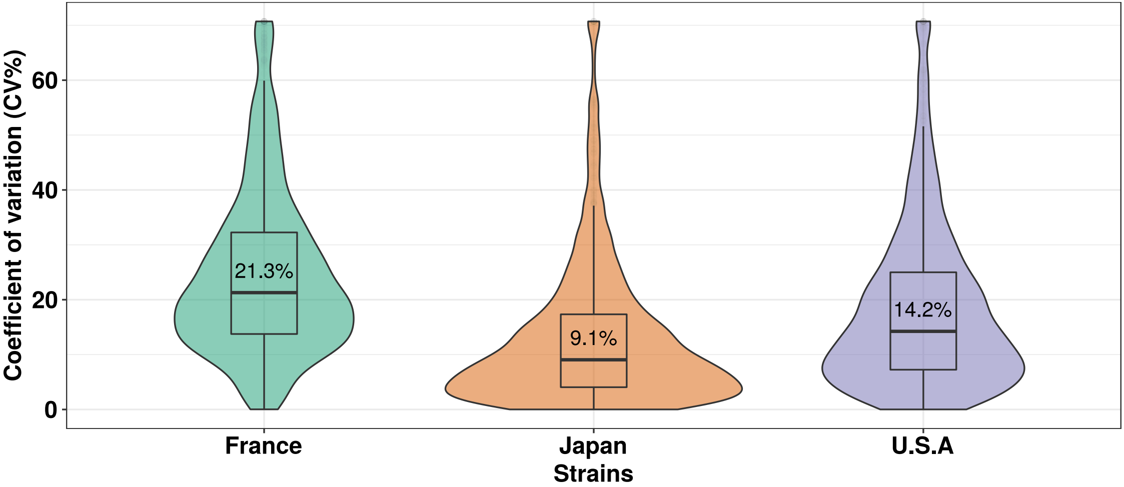

(predicted)cytochrome-P450-313a1
(predicted)cytochrome-P450-313a1-1
Ag5r2
mwh
(predicted)17-beta-hydroxysteroid-dehydrogenase
CG10345
CG12374
(predicted)diptericin
CG1441
CG15414
dsb
gene-14898
PGRP-SC1b
Cyp6a20
spz3
Fer1HCH
ValRS
CG9393
(predicted)asparagine--tRNA-ligase
CG42588
DNAlig3
Trf
GluProRS
(predicted)purine-nucleoside-phosphorylase
MME1
mal
(predicted)gustatory-receptor-92a
(predicted)serine-protease-33-like
Tpc1
Tspo
AlaRS
cv-2
Cyp9b2
Ugt37A2
ref(2)P
bor
Sil1
(predicted)cytochrome-P450-6d5
Arc2
spz
(predicted)spaetzle
(predicted)Rpa-70
MFS3
CG2064
eIF2beta
CG14882
Rev1
Cyp6d4
(predicted)LWamide(neuropeptide)
(predicted)glutathione-S-transferase
(predicted)gustatory-receptor-94a
Alr
CG9813
(predicted)cytochrome-P450-28a5
RtcB
RNaseZ
NimC2
(predicted)Hsp70-2
(predicted)Hsp70-1
CG31021
Dgp-1
(predicted)UDP-glucuronosyltransferase
(predicted)Hsp70-3
CG4456
Hsp23
Ldh
(predicted)leucine-zipper-transcription-factorA
P:flank2kb
P:flank2kb
P:intron
P:intron
P:intron
P:intron:clust738_CR1
P:intron:clust1431_TcMar-Tc1
P:3'UTR:clust423_I-Jockey
F:flank2kb
F:flank2kb
P:flank2kb
P:flank2kb:clust1730_Helitron
F:intron:clust1694_Helitron
P:flank2kb
F:flank2kb:clust1762_Gypsy
F:flank2kb:clust1762_Gypsy
Genes
-10
-5
0
5
10
log$_2$(foldchange of gene expression)
sample
France
Japan
U.S.A


A
B
$\mathrm{Log}_2\left(\text{normalized counts}\right)$
15
10
5
0
Control
Paraquat
Total
GxE
normalized counts
Hsp68
Hsp70Aa
FarO
dysc
CCHa2
Mec2
RpL40
Tsf1
tobi
France
Japan
U.S.A

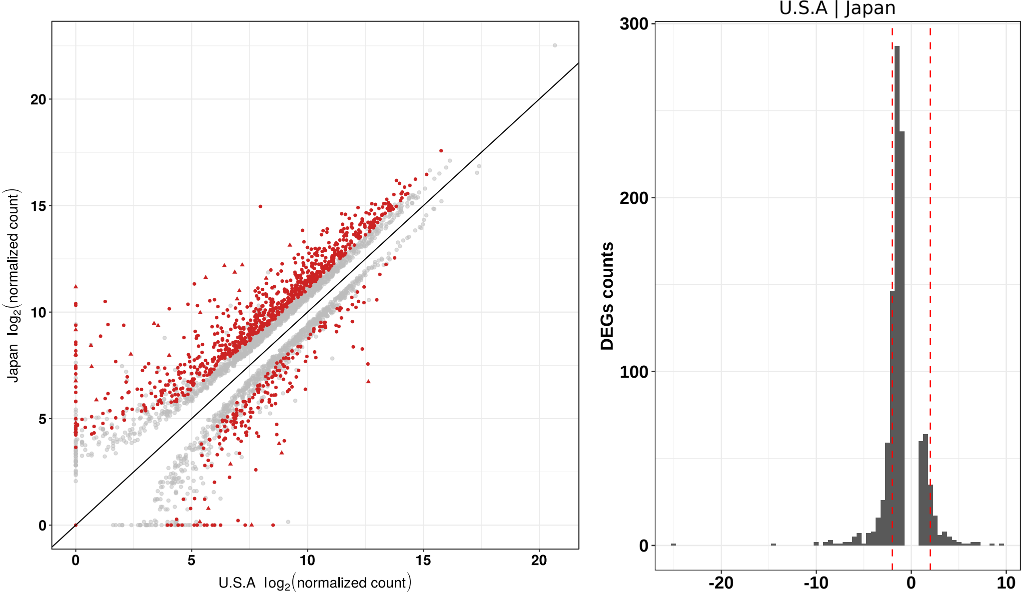

France log2(normalized count)
Japan log2(normalized count)
U.S.A log2(normalized count)
Japan log2(normalized count)
U.S.A log2(normalized count)
France log2(normalized count)
0
5
10
15
France
USA
Japan
Paraquat
Control log2(normalized count)
clust392_Pao
clust424_Pao
clust1204_Copia
clust1121_I
clust1379_TcMar-Tc1
clust1897_Gypsy
clust1725_Helitron
clust1204_Copia
clust1938_Unknown
clust1704_Gypsy
clust1316_Gypsy
clust595_I
clust1379_TcMar-Tc1
clust336_Gypsy

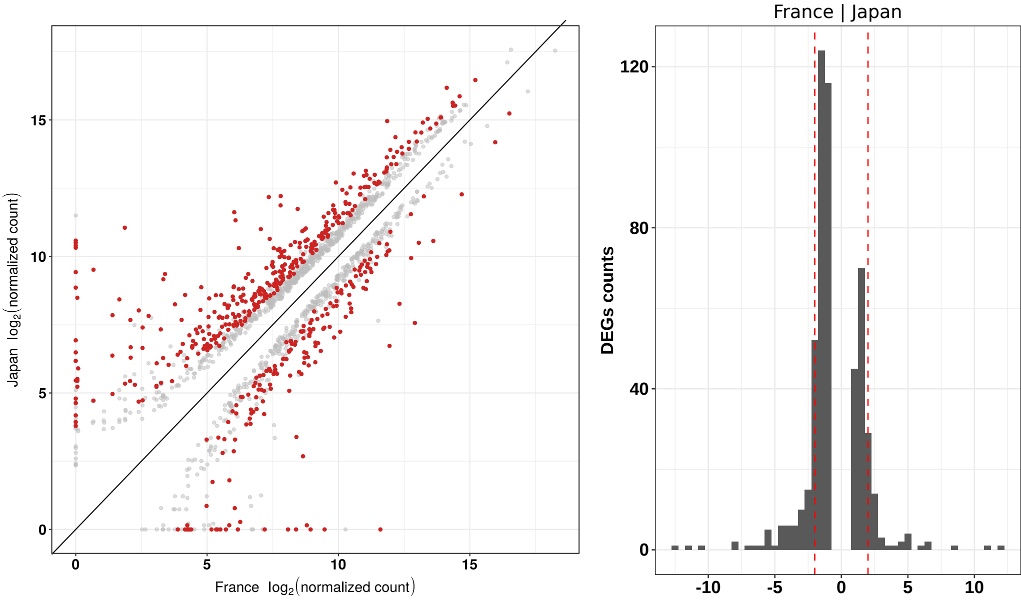

log2(normalized count)
16
12
8
4
12.5
10.0
7.5
5.0
2.5
USA|Japan
France|Japan
France|USA
Mal-A7
Tsf1
TpnC41C
CG1208
sNPF
CG1304
IM23
CG4053
muskelin
gene-11236
gene-10293
cad
gene-12341
gene-8490
tup
sti
CG16965
Rcc1
Caf1-180
barr
CG12520
(predicted)Hsp70-3
FANCI
CG42255
Rbf2
gene-7274
gene-3840
CG4456
Nopp140
Cpr92F
jumu
CG42255
CG8611
DptB
CG7006
msb1l
byn
CG31097
gene-15405
gene-13321
Hsp70Aa
gene-193
gene-7056
Cyp4ad1
gene-8883
gene-15352
gene-283
gene-316
gene-5326
gene-5151
Hsp70Aa
gene-15406
gene-6133
gene-16385
U.S.A
Japan
gene-1513
CG42808
mesh
Drat
gene-13145
betaTub60D
Ldh
(predicted)kelch-like
gene-5432
Socs36E
stc
MetRS-m
gene-8490
FarO
gene-3647
(predicted)trypsin_alpha3
gene-13321
gene-16855
gene-16070
Pvf1
gene-8522
gene-8932
Oatp33Eb
gene-16041
gene-8490
gene-14500
gene-244
gene-8816
gene-8633
gene-2030
gene-16041
gene-13321
gene-8633
gene-12098
CG11626
Ire1
CG10383
Mrp4
(predicted)Hsp70-3
Orgx
Myo28B1
gene-13145
gene-13286
Chs2
CG12520
gene-8490
CG10361
gene-12969
France
Japan
CG30043
CG4053
(predicted)kelch-like
gene-11236
gene-5842
FarO
CG31097
gene-14146
Cyp4ad1
gene-7056
gene-14500
CG31097
gene-7274
Hsp70Aa
CG10621
ppk17
gene-4581
byn
Ir48b
Control
Paraquat
France
U.S.A
France
Japan
U.S.A

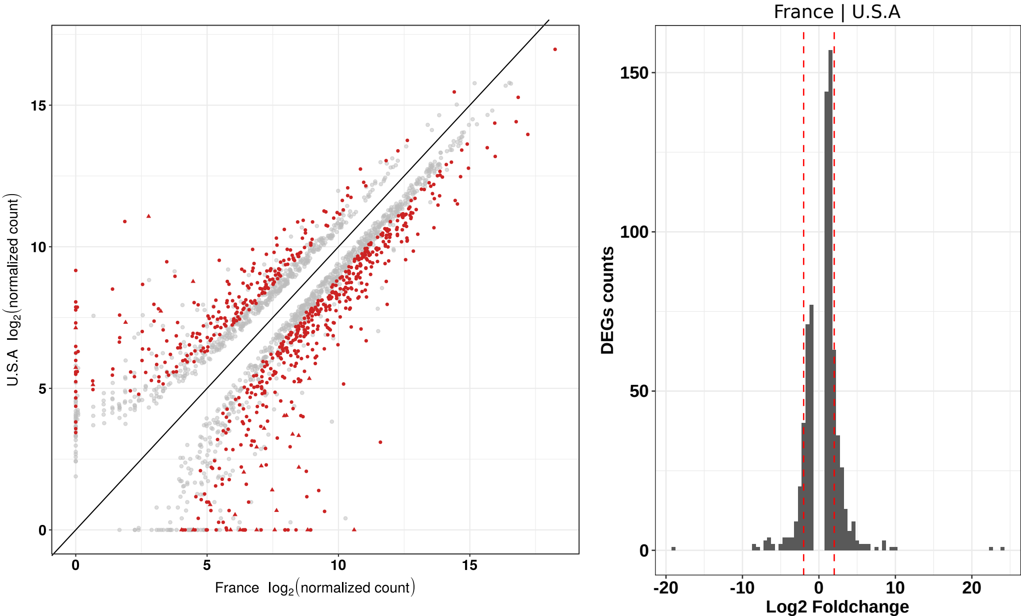

R = 0.28, p = 0.00033
Survival time in paraquat (days)
15
10
5
Survival time in control (days)
10
20
30
40
50
60

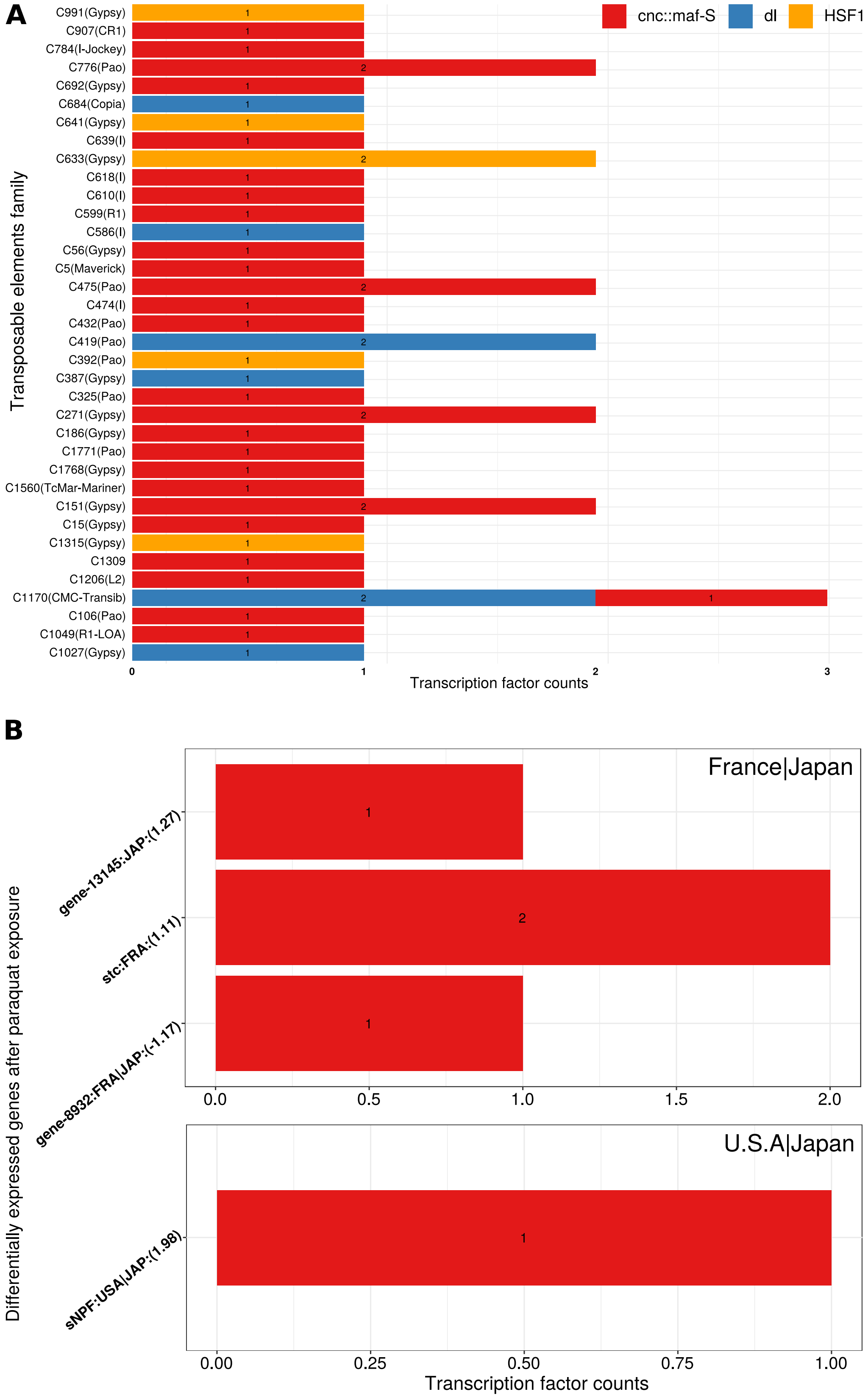


Coefficient of variation (CV%)
60
40
20
0
21.3%
9.1%
14.2%
France
Japan
U.S.A
Strains


Japan log2(normalized count)
U.S.A log2(normalized count)
0
5
10
15
20
U.S.A | Japan
DEGs counts
0
100
200
300
-20
-10
0
10


Japan log2(normalized count)
France log2(normalized count)
0
5
10
15
France | Japan
DEGs counts
0
40
80
120
-10
-5
0
5
10


U.S.A log2(normalized count)
France log2(normalized count)
0
5
10
15
France | U.S.A
DEGs counts
0
50
100
150
-20
-10
0
10
20
Log2 Foldchange


A
cnc::maf-S
dl
HSF1
Transposable elements family
C991(Gypsy) 1
C907(CR1) 1
C784(I-Jockey) 1
C776(Pao) 2
C692(Gypsy) 1
C684(Copia) 1
C641(Gypsy) 1
C639(I) 1
C633(Gypsy) 2
C618(I) 1
C610(I) 1
C599(R1) 1
C586(I) 1
C56(Gypsy) 1
C5(Maverick) 1
C475(Pao) 2
C474(I) 1
C432(Pao) 1
C419(Pao) 2
C392(Pao) 1
C387(Gypsy) 1
C325(Pao) 1
C271(Gypsy) 2
C186(Gypsy) 1
C1771(Pao) 1
C1768(Gypsy) 1
C1560(TcMar-Mariner) 1
C151(Gypsy) 2
C15(Gypsy) 1
C1315(Gypsy) 1
C1309 1
C1206(L2) 1
C1170(CMC-Transib) 2 1
C106(Pao) 1
C1049(R1-LOA) 1
C1027(Gypsy) 1
0
1
2
3
Transcription factor counts
B
Differentially expressed genes after paraquat exposure
France|Japan
gene-13145:JAP:(1.27) 1
stc:FRA:(1.11) 2
gene-8932:FRA|JAP:(-1.17) 1
0.0
0.5
1.0
1.5
2.0
U.S.A|Japan
sNPF:USA|JAP:(1.98) 1
0.00
0.25
0.50
0.75
1.00
Transcription factor counts