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

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

Running title: *D. suzukii* stress response and Jheh

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Keywords: invasive species, gene expression, rapid adaptation, paraquat, genotypes

30 Summary statement

31 The responses to oxidative stress of the invasive species, *Drosophila suzukii*, show variability
32 between genotypes related to their invasion status. The genes of the juvenile hormone epoxide
33 hydrolase cluster are involved in this response.

35 ABSTRACT

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

47 INTRODUCTION

48 The rapid spread of invasive species in a huge spectrum of environments relies on multiple
49 factors, from genetics to phenotypic plasticity, probably including fine molecular mechanism such
50 as hormonal production or epigenetic gene regulation (Beldade *et al.*, 2011; Marin *et al.*, 2019;
51 Stapley *et al.*, 2015). Phenotypic plasticity, i.e., the ability of a genotype to express different
52 phenotypes in different environments (Ghalambor *et al.*, 2015) has been proposed as one of the
53 most promising explanations for invasive success, particularly in the case of founder population
54 depleted of genetic variation (Estoup *et al.*, 2016; Marin *et al.*, 2019). Among deleterious
55 environments that can be encountered by invasive species, oxidative stress caused by
56 phytosanitary products is one of them. The invasive pest, *Drosophila suzukii*, is a good model to
57 investigate the adaptive process during invasion (Gibert *et al.*, 2016). This species which belong
58 to the group of the fruit fly *D. melanogaster*, originally comes from Asia and was detected
59 simultaneously both in North America (U.S.A) and in Europe in 2008. North America was invaded
60 by native Japan populations derived from Hawaii. In Europe, several introductions were detected
61 from U.S.A and from China (Fraimout *et al.*, 2017). Currently, *D. suzukii* is present in both North
62 and South America, in Europe from the south (Spain) to the East (Poland, Ukraine) and it has
63 also been observed in Russia (CABI, 2020; Lavrinienko *et al.*, 2017).

64 Characterization of the phenotypic and molecular responses of *D. suzukii* to changing
 65 environmental conditions may provide information to the mechanisms involved in the ability of
 66 invasive species to cope with environmental variation. Paraquat (N,N'-dimethyl-4,4'-bipyridinium
 67 dichloride) is one of the most widely used herbicide in the world leading to the production of ROS
 68 (reactive oxygen species) (Tsai, 2018). Oxidative stress due to the use of paraquat in the field
 69 has also been used in the laboratory as a good proxy for studying stress resistance (Bus J S and
 70 Gibson J E, 1984; Rzezniczak *et al.*, 2011). Paraquat was banned since 2007 in Europe but is
 71 still used in many other regions like in U.S.A or Japan. Paraquat exposition is known to induce a
 72 reduction in the lifespan associated with changes in gene expression (Finkel and Holbrook, 2000;
 73 Liguori *et al.*, 2018; Vermeulen *et al.*, 2005). One of the candidate genes involved in paraquat
 74 resistance is the cluster of *Jheh* (*Juvenile hormone epoxide hydrolase*) genes, which are not only
 75 involved in the lifespan but also in response to the oxidative environment (Flatt and Kawecki,
 76 2007; Guio *et al.*, 2014). Moreover in *D. melanogaster*, an insertion of a transposable element
 77 (TE) *Bari-Jheh*, near the cluster of the *Jheh* genes has been described as driving an increase of
 78 resistance in presence of paraquat (Guio *et al.*, 2014).

79 Using several strains of *D. suzukii*, we measured responses to oxidative stress at the phenotypic
 80 and molecular level. We made the hypothesis that different genetic backgrounds from native and
 81 invasive populations will have different responses to oxidative stress and that the *Jheh* cluster
 82 may be involved on it. Due to the over-representation of TEs in the genome of *D. suzukii* (33% of
 83 the repeated elements, (Sessegolo *et al.*, 2016)), compared to other *Drosophila* species, we
 84 looked for the presence of TEs in this region in the different lines. We monitored lifespan after
 85 paraquat exposure and measured the expression of three genes of *Jheh* cluster *Jheh-1*, *Jheh-2*
 86 and *Jheh-3* in six isofemale lines, four from the invasive regions, North America (Watsonville and
 87 Dayton) and France (Paris and Montpellier) and two from the native area, Japan (Sapporo and
 88 Tokyo). We evaluated the genetic diversity within and between lines by sequencing introns of the
 89 *Jheh* genes, searched for TEs and for transcription factor binding sites (TFBS). Our results
 90 suggest a strong effect of the genotype on the resistance to stress and changes in *Jheh*
 91 expression levels, with no link with TEs.

92

93 MATERIAL AND METHODS

94 *Drosophila suzukii* lines and rearing conditions

95

96 *D. suzukii* lines were sampled in 2014 from one native country (Japan: Sapporo and Tokyo) and
 97 2 invaded areas (USA: Watsonville and Dayton and France: Montpellier and Paris). Field-
 98 inseminated females were isolated to establish half-sib families called isofemale lines commonly

used to investigate *Drosophila* natural populations (David *et al.*, 2005). Flies were reared in modified medium (drosophila agar type, ref.66-103, ApexTM, 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, ApexTM 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. 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. We made three replicates per sex and treatment and used four flies per replicate.

RNA extraction was made using Direct-zolTM-96 RNA Kits (Zymo Research), following the manufacturer recommendations and RNA was treated with DNase. cDNA were obtained from 0.5 µg of RNA using SuperScriptTM IV VILLOTM Master Mix (Invitrogen). RT negative control was made with RNA but without the reverse transcriptase to control for genomic DNA contamination. cDNA were stored at -80°C before the quantification step. Gene expression was then quantified by quantitative PCR and *Rp49* was used as housekeeping gene. 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 the cDNA sample were supplemented with 5 µL of SsoADV Universal SYBR Green Supermix (BioRad) mix 2X, 0.3

33 μ l of each primer (10 μ M) and 2.4 μ l of pure water. We made technical duplicates for each sample.
 34 PCR reactions were made in a BioRAAd CFX-96 with a program consisted of an initial activation
 35 of 95°C for 10 minutes and then 40 cycles each comprising 15 seconds at 95°C, 10 seconds at
 36 60°C and 72°C .

37 **Genetic diversity of isofemale lines**

38 We sequenced intronic regions of *Jheh* gene cluster of the six lines used in this study. DNA was
 39 extracted individually from 10 females per line with the 96-Well Plate Animal Genomic DNA
 40 Miniprep kit (ref. BS437, Biobasic) following the manufacturer instructions. Primers were designed
 41 to flank the intronic regions for the three *Jheh* genes (Table S1) and Phusion high fidelity DNA
 42 Polymerase (2 U/ μ L) (F-530XL Thermofisher Scientific) was used to amplify sequences. The
 43 same PCR program was used for all primers pairs: 98°C for 10 minutes, followed by 40 cycles
 44 composed of 30 seconds at 98°C, 1 minute at 56°C and 20 seconds at 72°C and a final elongation
 45 step for 1 minute at 72°C. The sequencing of the two strands was done directly from the PCR
 46 product by BIOFIDAL sequencing company (Vaulx en Velin, France). Sequences were manually
 47 curated with CLC Main Workbench 8 software (Qiagen) before being aligned with the Muscle
 48 program implemented in the workbench to generate haplotypes by line for each intron. MEGA X
 49 software was used to calculate pairwise comparison and nucleotide diversity using p-method
 50 option (Table S2-S3) (Kumar *et al.*, 2018).

51 **Detection of Transposable elements and transcription factor binding sites**

52 We sequenced the intergenic regions of the *Jheh* gene cluster, plus the 5' and 3' regions
 53 of the cluster (Table S1). DNA was extracted from one female per population as described above.
 54 Classical PCR method was used with the following program, 10 minutes at 95°C followed by
 55 several cycles composed of 30 seconds at 95°C, 30 seconds at 63°C, 3 minutes at 72°C and a
 56 final elongation of 15 minutes at 72°C. The number of cycles was 25 for the region before *Jheh-1*
 57 and between *Jheh-1* and *Jheh-2*, 35 cycles for the region between *Jheh-2* and *Jheh-3* and 30
 58 cycles after *Jheh-3*. We identified TEs in the intergenic regions by a blast against a homemade
 59 data base of the TE sequences from the *D. suzukii* reference genome (Paris *et al.*, 2020, Mérel
 60 *et al.*, in prep.).

61 For TFBS (Table 1), we used conSite website to screen all TFBS from insect in our sequences
 62 (Sandelin *et al.*, 2004). To complete our analysis, we used the TFBS obtained from Villanueva-
 63 Cañas *et al.* (2019) and we extracted PFM (position frequency matrix) of the 14 TFBS from the
 64 JASPAR2018 database (v.1.1.1) (Parcy *et al.*, 2017). Then, we used TFBSTools (v.1.22.0)
 65 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* (Paris *et al.*, 2020; R Core Team, 2019; Tan and Lenhard, 2016).

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) 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 Tukey test.

RESULTS

D. suzukii wild type lines have significant differences in life span

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 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).

***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 (Guio *et al.*, 2014; Oesch *et al.*, 2000). 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 the three gene. 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 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. This corresponds to a residual polymorphism that is still present 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 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 & 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 and González, 2015; Guio *et al.*, 2014). 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 and González, 2015; Guio *et al.*, 2014). 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 (Finkel and Holbrook, 2000; Liguori *et al.*, 2018). 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 (Campos *et al.*, 2014; Shaw, 2000).

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 (Guio *et al.*, 2014; Weber *et al.*, 2012). 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 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

00 is therefore unlikely that the residual polymorphism in the *Jheh* gene region can explain the
01 differences in gene expression.

02 The striking result in our analysis is the similar pattern of changes in the expression of
03 *Jheh-1* and *Jheh-2* in females of European genotypes, with an increase in expression, which was
04 associated with lower resistance to oxidative stress, since these are the most sensitive genotypes.
05 On the contrary, the Sapporo genotype systematically showed a reduction in the expression levels
06 of the three genes, which could also be associated with increased resistance in the presence of
07 paraquat, but this was not observed for the Tokyo genotype.

08

09

10 **Conclusion**

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

19

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28

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33

34 Competing interests

35 The authors declare that they have no competing interests.

36

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36 List of the figures

37 Fig. 1. **Mean ($\pm$ SD) of survival time (days) of *D. suzukii* genotypes (A) and reaction norm (log of the**
38 **mean) (B).** (A) mean values under control and paraquat condition for females (pink) and males (blue)
39 with associated SD. (B) reaction norm between treatments (control and paraquat) for both females and
40 males for the six genotypes using log transformed mean value. The slope differences between the
41 curves highlight the difference of sensitivity between genotypes.

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

44 Fig. 3. **Representation of the *Jheh* gene cluster on the reference genome and the relative position on**
45 **the other six genotypes.** The blue squares represent the exons and the blue lines the intronic regions.
46 Red triangles represent TE detected in the intergenic regions and TFBS are indicated by vertical lines.
47 The scale is shown below the figure in base pairs.

48 Fig. 4. **TFBS detected in the four intergenic regions of the *Jheh* gene cluster in the reference genome**
49 **and in the six genotypes of *D. suzukii*.** Of the 14 TFBS examined, 8 had at least one positive result. The
50 intergenic regions are upstream *Jheh-1*(CG18190-*Jheh-1*), between *Jheh-1* and -2 (*Jheh1-Jheh2*),
51 between *Jheh2* and -3 (*Jheh2-Jheh3*) downstream of *Jheh-3* (*Jheh3*-CG43069).

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53 List of the tables

54 Table 1. **Transcription factor analyzed with the PWM matrix ID from JASPAR2018.** Mainly matrix come
55 from *D. melanogaster* model, but several as HSF, HIF1 and XBP1 come from human, while MTF1 come
56 from mice.

57
58 Table 2. **Mean ($\pm$ SD) survival time (days) for male and female lines of *D. suzukii* under**
59 **control and paraquat conditions.** Sensitivity represents the exponential of the interaction values
60 in the model (i.e., the difference in slope between the Sapporo reference and the other genotypes,
61 see Figure S1). * indicate a significant difference with the reference (p-value < 0.05).

62 Table 3. **Within-line diversity (π) for the six genotypes of *D. suzukii* for the seven sequenced intronic**
63 **regions.** The mean diversity per intron was calculated using the most common sequence of the six
64 genotypes.

65 Table 4. **Size differences (bp) between the six genotypes and the reference genome.** TE insertions are
66 indicated by their size or abs if they are absent.

68 List of supplementary figures

69 Fig. S1. **Representation of the parameters estimated by the model for treatment, genotypes and**
70 **interactions for females (left) and males (right).** Values were transformed exponentially to be
71 interpreted as a multiplicator effect. The vertical line corresponds to the reference. Associated p-values
72 are greater than 0.05 when the confidence interval includes the vertical line.

73 Fig. S2. **Distribution of the between genotype (blue) and within genotype (pink) genetic diversity using**
74 **π values for all intronic regions.**

76 List of supplementary tables

78 Table S1. **Primers used for the PCR experiments.**

79 Table S2. **Genotypes of *D. suzukii* with the number of haplotypes obtained.** The length of the sequence
80 corresponds to the size of the amplified fragment used to calculate the average diversity per population
81 per intron. The mean diversity was calculated as the average between the most common haplotypes of
82 the 6 lines.

83 Table S3. **Pairwise genetic distance between genotypes. Bold values represent genetic diversity within**
84 **lines.**

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

86

87 **Table S5. TFBS and TE detected in all six genotypes and the reference genome of *D.***
88 ***suzukii*.** We screened TFBS in the intergenic regions before, between and after the Jheh genes.
89 The names of the transcription factors (TF) and transposable elements (TE) are given with their
90 positions in the sequence (beginning and end).

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95 Figure 1

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Figure 2

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Figure 3

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Figure 4

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38 Table 1. **Transcription factor analyzed with the PWM matrix ID from JASPAR2018.** Mainly
39 matrix come from *D. melanogaster* model, but several as HSF, HIF1 and XBP1 come from human,
40 while MTF1 come from mice.

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

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44 Table 2. **Mean ($\pm$ SD) survival time (days) for male and female lines of *D. suzukii* under**
 45 **control and paraquat conditions.** Sensitivity represents the exponential of the interaction values
 46 in the model (i.e., the difference in slope between the Sapporo reference and the other genotypes,
 47 see Figure S1). * indicate a significant difference with the reference (p-value < 0.05).

Females				Males		
Lines	Control	Paraquat	sensitivity	Control	Paraquat	sensitivity
Sapporo (Japan)	31 $\pm$ 13.3	5.4 $\pm$ 2.3	1.0	25.4 $\pm$ 5.9	6.2 $\pm$ 2.1	1.0
Tokyo (Japan)	27.1 $\pm$ 9.1	5.1 $\pm$ 1.9	1.06	29.2 $\pm$ 11.5	7.7 $\pm$ 4.9	1.02
Dayton (U.S.A)	53 $\pm$ 8.4	9.5 $\pm$ 3	0.98	42.8 $\pm$ 16.3	11 $\pm$ 4.1	1.23
Watsonville (U.S.A)	34.9 $\pm$ 13.8	6.6 $\pm$ 2.3	1.13	34.5 $\pm$ 11.2	5.5 $\pm$ 2.1	0.68*
Paris (France)	55.4 $\pm$ 9.4	7.2 $\pm$ 1.8	0.72*	41.9 $\pm$ 14.8	8.1 $\pm$ 2.8	0.9
Montpellier (France)	37.9 $\pm$ 8.4	5.7 $\pm$ 2.2	0.83	44.7 $\pm$ 12.2	7.5 $\pm$ 3.8	0.66*

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Table 3 Within-line diversity (pi) for the six genotypes of *D. suzukii* for the seven sequenced intronic regions. The mean diversity per intron was calculated using the most common sequence of the six genotypes.

	<i>Jheh-1.1</i>	<i>Jheh-1.2</i>	<i>Jheh-1.3</i>	<i>Jheh-2.1</i>	<i>Jheh-2.2</i>	<i>Jheh-2.3</i>	<i>Jheh-3.2</i>
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

98 **Table 4. Size differences (bp) between the six genotypes and the reference genome. TE**
 99 insertions are indicated by their size or abs if they are absent.

		expected size (reference genome)	Sapporo (Japan)	Tokyo (Japan)	Dayton (U.S.A)	Watsonville (U.S.A)	Paris (France)	Montpellier (France)
X-Jheh1	Size	809	0	-2	0	+1	0	+12
	TE insertion	82	82	abs	82	82	82	abs
Jheh1/jheh2	Size	756	-17	-6	-2	+2	+1	+7
	TE insertion	abs	abs	abs	abs	abs	abs	abs
Jheh2/Jheh3	Size	1541	+31	-64	+35	-297	-1	+66
	TE insertion	41	48	49	91	abs	41	49
Jheh3-Y	Size	1021	+171	-8	+58	-55	+29	+122
	TE insertion	abs	58	abs	abs	abs	abs	36

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Fig. S1. Representation of the parameters estimated by the model for treatment, genotypes and interactions for females (left) and males (right). Values were transformed exponentially to be interpreted as a multiplier effect. The vertical line corresponds to the reference. Associated p-values are greater than 0.05 when the confidence interval includes the vertical line.

Fig. S2. Distribution of the between genotype (blue) and within genotype (pink) genetic diversity using pi values for all intronic regions.

30 Table S1. Primers used for the PCR experiments.

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Name	Sequence
<i>Jheh-1</i>	Forward: GAGCAACCTGGACAAGAACAAC Reverse: TATCCCAAGCGCTGCATAAG
<i>Jheh-2</i>	Forward: AGAAGCTGGACCACTACCAAAC Reverse: AGAACCTTCTTGGGCTTCTGG
<i>Jheh-3</i>	Forward: AGTACGCTTTTGAGGTCGTG Reverse: AGACGCAGCATCAAGTTTCG
<i>RP49</i>	Forward: CCGCTTCAAGGGACAGTATC Reverse: GACGATCTCCTTGCCTTCT
<i>DS10_00005800-Jheh-1</i>	Forward: GTGTCCCTGGACCATGTTGT Reverse: GGAGGACACTTTGCGGCTAT
<i>Jheh-1-Jheh-2</i>	Forward: GCCAATGGCCAGTACACAGA Reverse: GCCCCAGAAGCTGTACGATG
<i>Jheh-2-Jheh-3</i>	Forward: GCAAAGTGAGCATGATTTGGC Reverse: CAACCCTGTGAACCGAGCTA
<i>DS10_0005804-Jheh-3</i>	Forward: GCAATTAGCTCCCACTCGGT Reverse: CGTGACACTGCAGTTTATGGC
<i>Jheh-1_intr1</i>	Forward: GAGCGGATCCTAGACCCTTC Reverse: GCTGGTCGGAGGTAAGTTGT
<i>Jheh-1_intr2</i>	Forward: AAGAAAGTGCATGCGTAGCC Reverse: TGGCAGTTCAACCACTTCAC
<i>Jheh-1_intr3</i>	Forward: ATTGAGGCGGCTCTTTAGGT Reverse: CGGAGGTGATAAACAACAACTT
<i>Jheh-2_intr1</i>	Forward: GAGGCCTGGAATTGGAAAAT Reverse: TCTCGAGGGAATAAGAGGTTCA
<i>Jheh-2_intr2</i>	Forward: CGGCTTGGCATGAATAAAGT Reverse: ACGGAGATCCAGGGGTAAGT
<i>Jheh-2_intr3</i>	Forward: CCTCAATTACCTGTGGGGTAAA Reverse: CCCGAGGTAAGCTATGTTTCA
<i>Jheh-3_intr2</i>	Forward: GCCTTCTCGTGAACGTAGTGA Reverse: CAAGCAGTACACGACCGAGA

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38 Table S2. **Genotypes of *D. suzukii* with the number of haplotypes obtained.** The length of
 39 the sequence corresponds to the size of the amplified fragment used to calculate the average
 40 diversity per population per intron. The mean diversity was calculated as the average between
 41 the most common haplotypes of the 6 lines.
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Lineage		Jheh-1 intron 1		
	# of haplotypes	# of flies	Size of the alignment	Mean diversity
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	4-2-1		
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	4-3-2		
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	7-2-1		
Dayton (U.S.A)	1	9		
Watsonville (U.S.A)	1	10		

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44 Table S3. Pairwise genetic distance between genotypes. Bold values represent genetic diversity within
45 lines.

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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

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

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Gene	Sex	Estimate	Df	Sum Sq	Mean Sq	F value	Pr(>F)
<i>Jheh-1</i>	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
<i>Jheh-2</i>	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
<i>Jheh-3</i>	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

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55 Table S5. **TFBS and TE detected in all six genotypes and the reference genome of *D.***
56 ***suzukii***. We screened TFBS in the intergenic regions before, between and after the Jheh genes.
57 The names of the transcription factors (TF) and transposable elements (TE) are given with their
58 positions in the sequence (beginning and end).

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-	TE	3991670	3991701

B2608-Map1					
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	Jheh-1-Jheh-2	cad	TF	3985692	3985702

(U.S.A)						
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	Jheh-3-CG43069	Ubx	TF	3991351	3991358

(U.S.A)					
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

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