Transcriptional differences between the two host strains of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Marion Orsucci[1,2], Yves Moné[1], Philippe Audiot[2], Sylvie Gimenez[1], Sandra Nhim[1], Rima Naït-Saïdi[1], Marie Frayssinet[1], Guillaume Dumont[1], Jean-Paul Boudon[3], Marin Vabre[3], Stéphanie Rialle[4], Rachid Koual[4], Gael J. Kergoat[2], Rodney N. Nagoshi[5], Robert L. Meagher[5], Emmanuelle d'Alençon[1] & Nicolas Nègre[1§]

[1]DGIMI, Univ Montpellier, INRA, Montpellier, France

[2]CBGP, Univ Montpellier, CIRAD, INRA, IRD, Montpellier SupAgro, Montpellier, France

[3]UE Diascope, INRA, 34130 Mauguio, France

[4] MGX, Univ Montpellier, CNRS, INSERM, Montpellier, France

[5]USDA-ARS, Gainesville, Florida, USA

[§] to whom correspondence should be addressed: nicolas.negre@umontpellier.fr

Abstract

Spodoptera frugiperda, the fall armyworm (FAW), is an important agricultural pest in the Americas and an emerging pest in sub-Saharan Africa, India and East-Asia, causing damage to major crops such as corn, sorghum and soybean. While FAW larvae are considered polyphagous, differences in diet preference have been described between two genetic variants: the corn strain (sf-C) and the rice strain (sf-R). These two strains are sometimes considered as distinct species, raising the hypothesis that host plant specialization might have driven their divergence. To test this hypothesis, we first performed controlled reciprocal transplant (RT) experiments to address the impact of plant diet on several traits linked to the fitness of the sf-C and sf-R strains. The phenotypical data suggest that sf-C is specialized to corn. We then used RNA-Seq to identify constitutive transcriptional differences between strains, regardless of diet, in laboratory as well as in natural populations. We found that mitochondrial transcription is the main difference between the two strains. Since mitochondrial genotypes are also the main genetic variation between the strains, we propose that the mitochondrial genome is the main target of selection between the two strains.

Introduction

The relatively recent development of agroecosystems modified the ecological niches in many ways (O'Brien and Laland 2012). First and foremost, artificial selection used by early farmers in south-west Asia as of 10,000 years ago to improve their crops, elicited the rapid apparition of new domesticated varieties in the biosphere (Zohary, Hopf, and Weiss 2012). Whilst being selected for human favored traits, cultivated plants concomitantly lost or gained additional properties and thus plant-interacting organisms were prone to exploit these new niches. For example, some phytophagous insects were able to adapt to cultivated plants and, with the intensification of production based on monoculture activities, these insects eventually became agricultural pests. This adaptation to agricultural plants provides an interesting model system to observe evolution at a relatively small time-scale and assess the genetic changes that may promote speciation in relation to environmental changes (Yoder et al. 2010).

Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae: Hadeninae), also known as the fall armyworm (FAW), constitutes a good model to study adaptation of phytophagous insects to agricultural plants. Its native distribution range spans a vast amount of the Americas from Brazil to Canada (Pogue 2002). The FAW has no winter diapause (Sparks 1979) and its wintering range is constrained to warmer regions such as southern Florida and southern Texas in the United States (Nagoshi and Meagher 2004). In 2016 it became invasive on the African continent where massive crop damages have been observed across sub-Saharan Africa in less than a year (Goergen et al. 2016; Jeger et al. 2017). It has since been reported in India, South-East Asia and China (see the most recent report maps at https://www.cabi.org/isc/fallarmyworm), threatening to become a world-wide menace.

The FAW is a polyphagous species, being documented on over 100 plants from 27 different families (Pogue 2002). However, using allozymes electrophoresis monitoring, a significant genetic heterogeneity has been observed in FAW populations that was associated with feeding preferences (Pashley et al. 1985; Pashley 1986). One genetic haplotype was mostly found on corn (*Zea mais*), sorghum (*Sorghum* spp.) and cotton (*Gossypium* spp.) and was named the corn strain (sf-C). Another haplotype was found associated to individuals collected on smaller grasses such as turf, pasture (*Cynodon dactylon*) grasses and rice (*Oryza* spp.), and has been named the rice strain (sf-R) (Pashley 1988). Subsequent studies have confirmed these genetic differences on markers such as the mitochondrial gene cytochrome oxidase c subunit I (COI) (Lu and Adang 1996; Meagher and Gallo-Meagher 2003; Nagoshi et al. 2006; Machado et al. 2008), but also nuclear loci, such as the sex-linked FR1 repeat element (Nagoshi and Meagher 2003a; Nagoshi and Meagher 2003b; Lu et al. 1994) and the Z chromosome-linked *Tpi* gene (Nagoshi 2010). Phylogenetic analyses based on COI only (Dumas et al. 2015a) or on several mitochondrial and nuclear markers (Kergoat et al. 2012) showed that sf-C and sf-R separate in two distinct clades that could represent incipient species. While some degree of hybridization has been reported in field samples (Prowell, McMichael, and Silvain 2004; Nagoshi and Meagher 2003a; Nagoshi et al. 2006; Machado et al. 2008), it has also been shown that pre- and post-zygotic reproductive isolation mechanisms exist between the strains (Groot et al. 2010), with a loss of viability of the hybrids (Dumas et al. 2015b; Kost et al. 2016). Differences in reproductive behavior were also documented, such as the timing of mating being shifted earlier in the night for sf-C compared to sf-R (Schöfl, Heckel, and Groot 2009; Groot et al. 2010; Pashley and Martin 1987; Pashley, Hammond, and Hardy 1992). In order to detect post-zygotic reproductive barriers, many studies tried

to quantify the impact of the diet on the general fitness of the FAW larvae (Groot et al. 2010; Roy et al. 2016; Meagher et al. 2004; Silva-Brandão et al. 2017; Pashley 1988; Whitford et al. 1988). The results of these studies are sometimes contrasted but seem to agree about a better performance of sf-C on corn indicating that sf-C might be specializing to corn (Groot et al. 2010).

In order to understand if plant adaptation is indeed at the origin of the differences between the strains, we first conducted phenotypical experiments in the context of oviposition choice (OV) to different plants and of a reciprocal transplant (RT) during which we surveyed fitness associated traits (also called Life History Traits or LHT; Stearns 2012) to estimate the preference-performance of both strains. In parallel, we performed RNA-Seq experiments to search for genes constitutively differently transcribed between strains, in laboratory as well as in natural populations, that could indicate which selective pressure led to strains divergence. Surprisingly, we identified a major difference in the transcription of the mitochondrial genome. Since mitochondrial genotypes are also the main genetic variation between the strains, we propose that the mitochondrial genome was the primary target of selection between the two strains.

RESULTS AND DISCUSSION

Difference in oviposition choice between sf-C and sf-R

Under the preference-performance hypothesis, the choice of host plants by adult females to lay their eggs should reflect the host plants on which the larval performance is higher (Thompson 1988; Jaenike 1990; Gripenberg et al. 2010; Clark, Hartley, and Johnson 2011). We conducted an oviposition choice experiment where *S. frugiperda* adult females of each strain (sf-C or sf-R) were set free to lay eggs in a cage containing

either their preferred host plant, their alternative host plant ("no-choice" trial) or both ("choice" trial). We recorded the number of egg masses laid by females in each cage, depending on the substrate (the plant type or the cage net). Analysis by a generalized linear model (see **Methods**) showed that the interaction between the strain and the experimental factors was not significant (LRT, F = 1.29, df = 2, *P* = 0.1644). Indeed, we found that the number of egg masses laid by females (Mean fertility) was similar between trials (LRT, F = 0.29, df = 2, *P* = 0.75) but significantly different according to the strain (LRT, F = 24.73, df = 1, *P* < 0.001). Effectively, sf-C laid almost double the number of egg masses than sf-R (Mean fertility of 3.89 for sf-C against 2.06 for sf-R across all trials; **Fig. S1A**). When we analyzed the percentage of egg masses hatching within each trial, we observed no significant difference between strains (LRT, χ^2 = 0.17, df = 1, *P* = 0.68) or laying sites (LRT, χ^2 = 6.39, df = 6, *P* = 0.38), with 55% to 83% of egg masses in average giving rise to a larva (**Fig. S1B-C**).

By contrast, we observed a striking difference in the distribution of egg masses between the two strains. For each experimental trial ("choice", "corn" and "rice"), sf-C laid between 33% to 52% and sf-R laid almost 85% of their egg masses on the cage net rather than on a plant (**Fig. 1**). Neither strain showed a preference for the expected host-plant in female's oviposition choice (*i.e.* corn for sf-C and rice for sf-R*)*. Behavior difference between strains was indicated by the highly significant interaction between strain and laying site in all trials (LRT for maize trial :χ^2 = -68.35, df = 1, *P* < 0.001; LRT for rice trial :χ^2 = -90.10 ,df = 1, *P* < 0.001.; LRT for choice trial :χ^2 = -39.53 , df = 2, *P* < 0.001) . For sf-C, our model shows no difference in the proportion of egg masses between the net and corn plants in corn trial (LRT, χ^2 = -1.30, df = 1, *P* = 0.25) but did show a significantly (LRT, χ^2 = -20.03, df = 1, P < 0.001) higher number of egg masses on rice plants than on the net in rice trials (**Fig. 1A-B**). For sf-R, in the no

133 `choice` trial, the females laid more eggs on the net than on plants (LRT for maize trial
134 :χ^2 = -83.99, df = 1, *P* < 0.001; LRT for rice trial :χ^2 = - 72.95, df = 1, *P* < 0.001; **Fig.**
135 **1C-D**). In the `choice` trial, both strains exhibited the same preference pattern. Indeed,
136 the proportion of egg masses for both strains was higher on the net than on corn (sf-C
137 strain :χ^2= -8.2766, df = 1, *P* < 0.01; LRT for sf-R strain :χ^2 = -60.65, df = 1, *P* < 0.001)
138 or on rice (sf-C strain :χ^2= -44.949, df = 1, *P* < 0.001; LRT for sf-R strain :χ^2 = -98.30,
139 df = 1, *P* < 0.001) and lower proportions on rice than on corn (sf-C strain :χ^2= -15.23,
140 df = 1, *P* < 0.001; sf-R strain :χ^2= -7.28, df = 1, *P* < 0.01; **Fig. 1E-F**).
141 While these results did not detect a plant host preference for egg laying,
142 behavioral differences between strains were observed, with sf-C laying more egg
143 masses than sf-R, and sf-R placing more egg masses on the cage surface than on
144 plants. This lack of preference for their preferred host plant is surprising because *S.*
145 *frugiperda* is a species subdivided into two strains according to the host plant on which
146 the individuals were found preferentially (*i.e.* sf-R on *Oriza sativa*, *Bermuda grass*,
147 *Cynodon* spp. and *Medicago sativa* whereas sf-C consumes mainly *Zea mays*,
148 *Sorghum* spp. and *Gossypium hirsutum*; Pashley 1986). The question of qualifying
149 them as two distinct species has already been raised (Dumas et al. 2015). However,
150 although two variants are defined, *S. frugiperda* is mainly qualified as a polyphagous
151 species found on about 100 different host plants belonging to 27 different families
152 (Pogue 2002). Despite these host plant preferences observed in natural populations,
153 both strains can be sampled on the same plants (Juárez et al. 2012). About 19% of sf-
154 R individuals are present on maize and 5% of sf-C individuals are present on various
155 herbaceous plants (Prowell et al. 2004). This lack of striking female preferences could
156 be accentuated by working on laboratory strains, forced for several generations to lay
157 on filter paper.

158

159 ### Larval fitness in RT experiment

160 To test whether different plant diets have an effect on the fitness of *S. frugiperda* larvae,
161 we performed a series of reciprocal transplant (RT) experiments in which larvae freshly
162 hatched of both strains were deposited in cages containing either their current or their
163 alternative host plant. Larvae were allowed to develop on their plants, with the food
164 source being regularly supplied as to avoid deprivation. A control population was
165 reared in parallel on the "Poitout" artificial diet normally used to culture the insects in
166 the laboratory (Poitout and Bues 1974). During the experiment, we recorded several
167 phenotypic traits: the weight (`wt`), the developmental stage to measure the time
168 intervals (`dt`) and the survival (`sv`).

169 After hatching, *S. frugiperda* larvae of the first stage (L1) have to undergo five
170 molts to reach their 6th and final stage (L6) prior to metamorphosis. The time intervals
171 between stage (`dt`) was explained only by the host plant (LRT, χ^2 = -37.41, df = 1, P
172 < 0.001) and there was no strain effect (LRT, χ^2 = -0.93, df = 1, *P* = 0.335; **Fig. S2E-**
173 **F)**. In sf-C, the larvae took about 11 to 12 days to complete their larval cycle feeding
174 on artificial diet. We obtained the same duration (11 days) with larvae feeding on corn.
175 Remarkably, development of sf-C larvae feeding on rice took 6 to 7 days longer
176 compared to the other diets (**Fig. S2E**). The sf-R larvae took 11 to 13 days after
177 hatching to complete their larval development on corn compared to 17 days for artificial
178 diet and rice (**Fig. S2F**). Finally, both strains exhibited a similar pattern for `dt` from 1st
179 larval instar to adult emergence, with both strains having a longer `dt` feeding on rice
180 than on corn (LRT, F = 28.88, df = 1, *P* < 0.0001; **Fig. S2E-F**). Development on corn
181 was similar for both strains (17 days), but sf-R grew faster on rice than sf-C (22 against
182 24 days, LRT: F = 182.38, df = 1, *P* < 0.0001).

Weight (wt) at the pupal stage was explained by host plant (LRT: χ^2 = -555.25, df = 1, P < 0.001), moth strain and sex, with a significant interaction between the last two variables (LRT: χ^2 = -6.61, df = 1, P = 0.012). Indeed, we observed, except for sf-C on corn, that males were heavier than females (**Fig. 2**). Both strains had heavier pupae from feeding on corn than feeding on rice (for sf-C: LRT, χ^2 = 67.107, df = 2, P < 0.001; for sf-R: LRT, χ^2 =27.18, df = 2, P < 0.0001, **Fig. 2**). Pupal weights were higher on corn condition (around 260 mg) than on rice (around 185 mg; **Fig. 2A-C**). Overall, sf-R larvae and pupae were much lighter than sf-C larvae. In all feeding regimes, the maximum larval weight was between 260 mg and 410 mg, while the pupal weight was between 115 mg and 180 mg. Larvae did best feeding on corn, with higher weight gain than on the artificial diet or on rice (**Fig. 2B-D**).

The survival (sv) of both strains was linked to the host plant on which the larvae developed. There was a significant interaction to sv between strain and host plant (LRT, χ^2 = -24.22, df = 1, P < 0.0001; **Fig. 2C-D**). The survival of sf-C was significantly greater on corn (about 34%) than on rice (about 7.5%; **Fig. 2C**). However, although sf-R tended to have higher survival on rice (LRT, χ^2 = 2.53, P = 0.11), sv was not significantly different between the two host plants (7.5% on "corn" *vs* 12.5% on "rice"; **Fig. 2D**). We noted that the survival rates on plant experimental set-ups were relatively low. These absolute numbers cannot be related to controlled conditions where artificial rearing is designed to provide as much survival of the population as possible (**Figure S3**). Similarly, it can not be compared to survival rates in the wild, for which we have no estimate. Host-plant, but also variable environmental parameters and interactions with competitors, predators, parasites and pathogens can affect the survival and are an essential component of the host-plant as an ecological niche. Here, we can only

conclude on the relative survival rates between similar experimental conditions, which we think reveals intrinsic adaptation to the host-plant.

In brief, this analysis indicates that under our laboratory conditions, there is a clear effect of the host plant on the fitness of *S. frugiperda*. Individuals of both strains grew faster and gained more weight feeding on corn than on rice. We observed one major difference between strains, with sf-C surviving better on corn than sf-R, suggesting a specialization of sf-C to corn. However, we didn't find the reciprocal trend for sf-R, which survived equally on both plants. Once again, as noted in the plant preference, the absence of plant cues during laboratory breeding over several generations could have allowed a relaxed selection of host plant characteristics. Moreover, the artificial diet is based on corn flour and therefore Sf-R has not been confronted with rice compounds for many years~~. Sf-R has therefore been able to adapt to certain compounds of corn explaining~~ which might explain ~~that~~ why differences between these two plants are not detected.

Gene expression in RT experiment

When confronted with different host plants, polyphagous insects will respond by expressing different sets of genes, some of them can be associated to a better adaptation to the host plant. Such adaptation genes in insect are known to be involved in chemosensory, digestion, detoxification and immunity processes among others (Simon et al. 2015; Celorio-Mancera et al. 2016). In order to understand if the two *S. frugiperda* strains express different adaptation genes to host plant diet, we performed RNA-Seq experiments from the larvae of the RT experiments. RNA was extracted from 4th instar larvae from the same RT experimental setup as the one on which LHT were measured. We could perform for each strain two replicates on the corn diet, one replicate for the rice diet and one replicate for the artificial diet. We recovered between

30 to 71 million reads per sample (**Table S1**), which we aligned on the OGS2.2 reference transcriptome for sf-C (Gouin et al. 2017) containing 21,778 sequences. The percentages of reads mapped were similar between the two moth strains, with 72.1% to 73.3% of alignments for sf-C under any diet (**Table S1**). For sf-R samples on corn the alignment percentages were similar (71% and 71.2%), and slightly less for the other samples (68.6% on artificial diet and 68.9% on rice; **Table S1**).

Constitutive transcriptional differences between sf-C and sf-R

PCA analysis of the RNA-Seq data shows that the samples are grouped by strain (29% of explained variance on PC2; **Fig. 3A**), suggesting there may be fundamental differences between sf-C and sf-R that could explain their plant preferences. However, this observation was contrasted by PC1, which explained 53% of the variance and revealed a pattern of separation by preferred diets. Indeed, an important part of the variance was explained by the sample sf-R on rice, clustering with sf-C on corn (**Fig. 3A**). We used DESeq2 (Love, Anders, and Huber 2014) to identify constitutive differences between the two strains regardless of the diet trial. We identified 1,697 (7.8%; $p.adj < 0.05$) genes overexpressed in sf-R compared to sf-C and 2,016 (9.3%; $p.adj < 0.05$) genes overexpressed in sf-C compared to sf-R (**Fig. 3B**). We verified by q-PCR on independent samples raised on artificial diet that this strain-specific difference of expression is stable. We selected and annotated (**Fig. S4**) 50 genes overexpressed in sf-R compared to sf-C in our RNA-Seq experiments (**Fig. S5-S6**), all except one (peroxidase), were systematically overexpressed in sf-R when measured by qPCR (**Table S2-S3 & Fig. S7**).

The GO enrichment analysis did not detect any significant enrichment of either Biological Process or Molecular Function terms in both gene lists. sf-R expresses some enzymes involved in digestion, metabolism and detoxification as well as, intriguingly,

ribonucleoproteins involved in mRNA splicing (**Fig. S5**) but no coherent pattern emerges. While no GO enrichment has been observed for sf-C, manual re-annotation of the 50 most expressed genes showed that at least 13/50 genes correspond to transposable elements (TE) (**Fig. S6**). Other genes encode putative endonucleases that could also be of TE origin, such as the Harbinger transposase-derived nuclease, HARBI. In addition, we could not find evidence for gene annotation by homology or protein domain analysis for 16/50 genes. Other genes encode proteins that could be linked to plant adaptation. For example, sf-C shows a strong expression of fatty acid synthase, suggesting that sf-C is constitutively more efficient at energy production and storage. We also found two peptidases, and the cytochrome P450: CYP9A31 indicating inherent digestive and detoxification potential for sf-C. While we have detected no transcriptional regulators in our plant adaptation datasets, we could at this time detect one important transcription factor (TF), expressed only in sf-C: *apterous-1*. This homeodomain (HD)-containing TF is known in Drosophila to be involved in wing development (http://flybase.org/reports/FBgn0267978.html). Annotation of HD genes in *Spodoptera* (Gouin et al. 2017) showed that *apterous* has two paralogs, suggesting a yet-to-be-determined potential shift in function for this TF. Finally, we detected overexpression of a small genomic sequence corresponding to a fragment of the mitochondrial gene cytochrome oxidase c subunit III (COIII). Genomes often contain insertions of mitochondrial sequences (Hazkani-Covo, Zeller, and Martin 2010). Such insertions are termed *numts*. Around 95 *numts* can be identified in the *Spodoptera frugiperda* genomes. They sometimes confound gene prediction because they contain the open reading frame (ORF) sequence of the original mitochondrial gene. However, *numts* are usually not transcribed, lacking the promoter region sequence and ~~. In the case of the COIII-*numt*,~~ the measured differential expression ~~we measured~~ comes from

messenger RNAs of mitochondrial origin, whose reads also align on the *numt* region (**Fig. S16**). Thus, in practice, *numts* can be used to measure the expression of portions of the mitochondrial genome.

Exploration of strain transcriptional differences in natural populations

We wanted to know if the transcriptional differences between *S. frugiperda* strains measured in the laboratory conditions can also be observed in the wild. We performed a field collection of FAW larvae in a sweet corn field (Citra, FL), in a volunteer corn field (Tifton, GA) and in a pasture grass field (Jacksonville, FL). We performed both DNA and RNA extractions from individual L4 larvae. DNA was used to genotype the individuals (see **Methods**). Based on the detection of mitochondrial Cytochrome Oxidase I (COI) polymorphism (Nagoshi et al. 2006), the Citra corn field contained 32/33 sf-C associated genotypes, the Tifton corn field contained 14/18 sf-C strains and the Jacksonville field contained 6/6 sf-R strains (**Fig. S8)**. We selected some sf-R and sf-C individuals from each field to genotype according to one SNP on the *Tpi* gene located on the Z chromosome (Nagoshi 2010) and presence of the FR1 repeat (Lu et al. 1994; Nagoshi and Meagher 2003a). Interestingly, most sf-R haplotypes recovered from corn fields seem to be hybrids from a sf-R mother. We didn't detect any potential hybrids in the pasture grass field (**Fig. S9-S10**).

From the 20 most differentially expressed genes between sf-C and sf-R on corn, we selected 15 genes to perform qPCR measurements of their expression in individual L4 larvae from the laboratory strains raised the artificial diet as well as in individual L4 larvae from the Tifton field where we recovered both sf-C and sf-R mitochondrial haplotypes. The qPCR analysis showed that the genes we selected from RNA-Seq studies are concordantly differentially expressed between laboratory strains. However, for the genes we selected, we detected no difference in expression between natural

populations of sf-C and sf-R (**Fig. S11**). This result seems to indicate that studies of plant adaptation in laboratory conditions might not be directly applicable to natural conditions. Indeed, in laboratory conditions, we can control the genetic background of insects, the environmental conditions as well as the plant types and supply, while natural populations experience many more variables. Their genetic background might be different from one another, they may be infected or parasitized, they may be individually stressed by climate conditions, predators, competitors or parasites. In these conditions, to identify transcriptional differences between strains, one might want to turn to RNA-Seq experiments, which allow interrogating all genes at once.

Transcriptomic studies of natural *S. frugiperda* populations

We thus decided to produce a dataset (named FL15) of RNA-Seq experiments with 3 sf-C individuals from Tifton, 3 sf-R individuals from Tifton and 3 sf-R individuals from Jacksonville (**Fig. 4A**). We recovered from 23 to 74 million reads per sample (**Table S1**) with alignment percentages ranging from 45.32% to 58.40%, slightly less than in laboratory experiments. On a PCA analysis of FL15 dataset only, replicates of the same "`trial + strain`" individuals group well together with the FL15_B1J individual being slightly outlier (**Fig. S12A-B**). When integrating all FL15, and RT experiments, it becomes impossible to group together all Sf-C genotypes independently of trials (**Fig. S12C**). Moreover, when we looked at the expression of the 50 most differentially expressed genes in sf-R versus sf-C in RT2 experiments and observed the expression of these genes in two independent RT experiments RNA-Seq from our laboratory (RT1), a previously published study on the midgut Roy-RT (Roy et al. 2016) and the FL15 natural populations, we observed that most transcriptional response detected in RT2 was not recapitulated in the other experiments (**Figs. S13-S14**).

Strain specific expression in laboratory and in field collections

We took advantage of a large dataset to ask again a simple question: what are the genes whose expression is constitutive of one strain compared to the other? We performed a differential expression analysis across our laboratory RT experiment and our FL15 collection to identify these genes. We found 76 genes consistently overexpressed in sf-R compared to sf-C and 73 genes overexpressed in sf-C compared to sf-R (**Fig. 47B**). To verify the validity of these genes we again surveyed their expression across all the RT-RNA-Seq data at our disposal. We could see that for the majority of these genes their strain specific overexpression is confirmed in the different laboratory populations as well as in natural populations (**Fig. 4C** and **Fig. S15**). While many genes in this list have functions of potential interest to study the molecular basis of ecological speciation (**Tables S4 & S5**). As noted with laboratory sample RNAseq experiments, the sf-C associated overexpression points to many genes whose manual annotation reveal transposable elements of the PiggyBac and Ty1/Copia families (**Table S4**) suggesting a recent reactivation of transposition events in this strain. We also note many genes that could be linked to plant adaptation such as fatty-acyl CoA reductase, OBP36, glucose dehydrogenase, fatty acid synthase, cytochrome P450 and glyoxalase, as well as immunity genes such as a GNBP, a lectin and a cecropin. Finally, this list comprises some potential regulators of expression such as the homeobox transcription factor apterous-1, the DNA helicase Pif1, Orc4, the Mcm complex, a HMG box factor, NUP62 and Leo1. The genes associated to sf-R overexpression (**Table S5**) have a wider array of function but, interestingly, some members in this list also have the same molecular functions as the sf-C expressed such as Fatty acyl-CoA reductase and Glucose dehydrogenase. We also noted a strong pathway of hormonal regulation with the overexpression of the ecdysteroid

kinase and the broad complex, as well as the takeout gene which is a juvenile hormone binding protein involved in foraging behavior in Drosophila and NGFI-A-binding protein co-factor, involved in neuron regulation.

To verify the validity of this gene list, we ~~noticed, when applyinged~~ a hierarchical clustering ~~to this list of genes~~analysis of their expression across all the RNAseq data at our disposal. We noticed~~,~~ ~~a~~ peculiar outliers with strong expression associated to sf-R corresponding to the previously mentioned *numts* (**Fig 4C, S16**). ~~What~~ As mentioned, these *numts* reveal ~~are~~ parts of the mitochondrial genome that are differentially expressed according to the strain. Two of these *numts* ~~in particular,~~ correspond~~ing~~ to fragments ~~in~~ of the mitochondrial genes COI and COIII ~~are clearly differentially expressed in sf-C compared to sf-R in all the RNA-Seq datasets we analyzed~~ (**Fig. S16**). To rule out any effect of genome misassembly, we amplified both *numts* and mitochondrial sequence for COI and COIII and sequence them. We could confirm the presence of these *numts* within the genome of sf-C and sf-R strains with a sequence slightly different than the one from mitochondria. To rule out any sequence specific alignment bias, we retrieve from NCBI the reference genome sequence from *S. frugiperda* mitochondrion (accession KM362176.1) and realigned our RNA-Seq data on it. It was obvious that, in the regions corresponding to *numts*, there was a clear underexpression in the sf-C strain (**Fig. 4D**). The implication of this result on the metabolism of the larvae remains to be established, but nevertheless, it may explain why the mitochondrial haplotypes in the COI gene are the principal marker for strain discrimination. It may very well be that a difference in energy production between these two strains was linked at some point of their evolutionary history to a shift in host plant preference.

CONCLUSION

In this study, we wanted to determine if the differentiation of *S. frugiperda* in two strains - sf-C and sf-R - is a result of their adaptation to different host plant diet. First, we measured a combination of Life History Traits in the context of an oviposition preference experiment (OV) and of a reciprocal transplant (RT) experiment in controlled environments to characterize the specialization to host plants. Then we performed RNA-Seq measurements of gene expression variations of L4 larvae during controlled RT experiments in the laboratory and in natural populations. The integration of these datasets allowed us to reveal constitutive differences between sf-C and sf-R.

From this set of experiments, we concluded that the LHT of our laboratory colonies are consistent with a specialization of sf-C to corn, but does not provide evidence that rice is the preferred plant for sf-R, which showed only a slight trend to survive better on this plant than on corn. Interestingly, however, RNA-Seq experiments show that both strains express a similar set of genes, involved in growth and nutriment storage, when confronted to their main host-plant (corn for sf-C and rice for sf-R). This similarity in the transcriptional responses suggests that rice is indeed recognized as a suitable host for sf-R but maybe not its most preferred one.

We found several candidate genes that are differentially expressed between the strains regardless of the diet. However, when we looked at natural populations, almost none of these genes were differentially expressed between strains. But by combining the analysis of RNA-Seq data from laboratory populations as well as from natural populations, we detected a narrower set of genes constitutively differentially expressed between strains. Among those, one candidate stood out and turned out to be the mitochondrial gene COI. This gene is used as a genetic marker for strain identification in all fall armyworm related publications, including the survey of invasive populations

in Africa (Rodney N. Nagoshi et al. 2018). The fact that it is also constitutively differentially expressed may indicate that the COI gene, and potentially other mitochondrial genes, may be the original target of selection between the strains (Meiklejohn, Montooth, and Rand 2007). Changes in mitochondrial functions are associated to changes in energy demand or supply (Jose et al. 2013). In addition, variations in mitochondrial sequences can be the cause of mitonuclear incompatibilities between species (Hill 2015). The evolution of mitonuclear interactions can maintain the segregation of various mitochondrial haplotypes in the context of ecological speciation (Morales et al. 2016). These features are consistent with a model of ecological speciation for *S. frugiperda*, in which divergence in mitochondrial functions have been selected on plants with different nutritive values. For example, the sf-C haplotype, which has a lesser expression of mitochondrial genes might have a reduced energy production efficiency compared to sf-R. This reduced efficiency may be compensated by the higher nutritive value of the corn plant. Consistent with this explanation, we found sf-R haplotype in corn fields but almost no sf-C haplotype on pasture grass fields. Alternative explanations might involve adaptation to the redox state imposed by the host-plant xenobiotic compounds. Several insect proteins such as UGTs and P450s catalyze oxidation-reduction reactions to resist against these natural pesticides. Consistent with this second hypothesis, we also detected plastic and evolved differential expression of several P450 proteins. Finally, it is possible that variations in mitochondrial function reflect variations in energy demand associated with the different field environments. Indeed, corn plants, especially the hideouts within the whorl or the ear, may also provide more protection against competitors, predators and parasites than grass lands, which are more open spaces. Thus sf-R strain, that has a higher level of expression in mitochondrial genes might require more energy to move

433 around. Consistent with this explanation, sf-R larvae are consistently smaller than sf-
434 C larvae (**Fig. 2A-D**). Energy consumptions at adult stage, especially regarding
435 migratory capacities should also be considered.

436 Compared to other studies using a similar RT experimental design to identify
437 adaptation genes or evolved genes in *Spodoptera frugiperda*, our study highlighted
438 one important point that could explain the inconsistencies observed over the years in
439 the determination of the plant adaptation process in *S. frugiperda*. Traditionally, two
440 different RT strategies were used, either by using colonies from natural populations or
441 long maintained laboratory colonies and each approach has its pros and cons. Working
442 with laboratory colonies allows one to control for genetic background variations as well
443 as environmental conditions. But in turn, they might be subject to genetic drift or
444 adaptation to the artificial diet used to maintain them. Here, we show that by combining
445 the two approaches, we revealed a smaller set of genetic events that could explain the
446 differentiation of the two strains. In particular, we identified COI as both a genetic
447 marker and a functionally different locus between the two strains. The consequences
448 of functional variations in the mitochondrial genome on the shift of host-plant range in
449 *S. frugiperda* remains to be elucidated.
450

451 ## Material and Methods

452 ### Biological material: Moths and Plants

453 We used individuals from the two strains of *S. frugiperda*: corn (sf-C) and rice strain
454 (sf-R). Those strains were seeded with around 50 pupae sampled in Guadeloupe in
455 2001 for sf-C and in Florida (Hardee County) in 2012 for sf-R. From the time of their
456 collection they have been reared under laboratory conditions on artificial diet (from
457 Poitout et al. 1972, principal components: 77% H_2O, 2% Agar-agar, 13% maize flour,

458 6% other nutrients, 1% vitamins; 1% antibiotics), at 24°C with a 16h:8h Light:Dark
459 photoperiod (L:D) and 70 % Relative Humidity (R:H). The individuals that seeded the
460 corn strain came from French Guadeloupe whereas those that founded the rice strain
461 came from Florida (U.S.A.).
462 Corn (Corn line B73) and rice (Arelate variety from CFR, Centre Français du
463 Riz) were produced from organic seed at the DIASCOPE experimental research
464 station (INRA, Mauguio, France, 43°36'37"N, 3°58'35"E) in plastic pots (7 x 8cm for
465 both plants in RT and 6L plastic pots for maize in OV) filled with conventional substrate.
466 Corn and rice cultivation was carried out in a warm chamber at 25°C 2, 60% RH and
467 16:8 h (L:D) under organic conditions. Corn and rice plants were used 15 days or a
468 month after seeding, respectively, to have an equivalent of two biomass plants.

469 ## Experimentation

470 ### *Experimental trials*

471 *Spodoptera frugiperda* is not present in France and considered as a quarantine pest.
472 Consequently experiments on this study model are regulated. Our experiment
473 described hereafter was conducted in confined environment on insect quarantine
474 platform (PIQ, University of Montpellier, DGIMI laboratory).

475 ### *Oviposition experiment*

476 The oviposition (OV) experiment consisted in release of 12 to 20 virgin females and
477 males of the same strain per cage, and for three nights (72 hours) in three different
478 set-ups: *choice*, *corn-only* and *rice-only*. All individuals released had emerged the night
479 before the oviposition choice experiment. For the choice modality, each cage
480 contained five maize plants and 15 rice plants (the number of maize and rice were
481 adjusted to provide an equivalent biomass) arranged in two patches in two opposite

corners of the cage. For the rice- and corn-only modalities, we used either 10 maize or 30 rice plants. Plants were arranged in two equal patches (2 x 5 maize or 2 x 15 rice) located in two opposite corners of each cage. The experiment was conducted in insect rearing cages covered by an insect-proof net (175 x 175 cm) and 4 replicates of each set-up were done under the same climatic conditions, within the quarantine platform (22°C, 50% humidity, natural dark-light conditions - in November around 14h dark:10h light- with fluorescent light bulbs).

In each cage, at the end of the third night, all egg masses were counted and immediately individualized. We measured three variables for each cage:

(1) The number of egg masses laid by females in a given cage (on plants and on the net) to measure the fecundity. As the adult number was not similar in cages, it was important to balance the number of egg masses per the number of females in the cage. Indeed, the number of adults had a significant effect on the egg masses number ($P < 0.01$), so we decide to create a variable, *Mean Fecundity*, which take account the egg masses number divided by the number of females in the replicate. The following variables were the strain (sf-C and sf-R) and the trials (choice, rice-only, corn-only).

(2) The proportion of egg masses laid by females on one particular site (one given plant species or the net). This percentage was calculated in three set-ups to estimate the preference of each moth species according to present substrates in the cage. We performed the analysis on each set-up independently with two following factors, the strain and the oviposition site.

(3) The hatching proportion is the number of egg masses hatching on one particular site (one given plant species or the net) whatever the set-up. This percentage provides an estimate of the fertility of both strains according to the choice of

oviposition site by the females. The following factors are the strain and the oviposition site (nested in set-up).

Reciprocal transplant experiment

The reciprocal transplant (RT) experiment consisted in controlled infestations of corn and rice plants with first instar larvae in 8 insect rearing cages (32.5 x 32.5 cm) covered by an insect-proof veil to prevent contaminations and escapes in the incubator (24°C, 16h:8h L:D cycle and 70% R. H.). The RT experiment was conducted in the same incubator for four modalities: 1) corn plants infested by sf-C (native condition); 2) rice plants infested by sf-C (alternative condition); 3) corn plants infested by sf-R (alternative condition); 4) rice plants infested by sf-R (native condition). We realized two replicates by modality. Each cage contained four corn or rice pots, which were changed before the 4th larval instar and each day after this instar until the pupation.

From a batch of eggs reared on artificial diet, we subdivided the progeny on the three different diets (corn plant, rice plant and artificial diet). A total of 80 larvae (which hatched the morning of the experiment) were deposited in each cage.

Two generations have been conducted on plants; during the first generation we measured life history traits (LHT) for each strain in native and alternative conditions and during the second generation, the larvae had been sampled at 4th larval instar for RNA-Seq experiments.

As of the 2nd larval instar, we measured several LHT every other day until pupation, during which we determined the sex of each individual. In addition, at each counting, we determined the larval stage by the width of the head capsule. To limit the possible contamination between strains, we isolated two floors of the incubator with an insect proof net (150 µm) and to avoid a floor and edge effect, rotations between floor were

conducted and cages were randomly deposed after counting. We measured three variables:

- Survival (`sv`) is the number of emerging adults counted over the initial number of larvae;
- Developmental time (`dt`) is the number of days between the beginning of the experimental start until adult emergence (mean on all emerging adult in same cage);
- The weight (`wt`) of individual larvae and of individual pupae of each sex in mg. The day of plant infestation, we weighed the pool of 80 larvae. Then, from the 2nd larval stage, the weight was quantified every other day and each larva was individually weighed.

For all variables from RT, we analyzed by following factors: the strain (sf-C or sf-R) and the host plant (corn or rice). Replicate effect was negligible.

In parallel, and as a reference point, we performed the same experimental design and measurements on standard rearing conditions on artificial diet (Poitout and Bues 1974). Two replicates of each strain on artificial diet have been set-up from the same batch of L1 larvae from our laboratory strains. Compared to plant conditions, rearing has been performed in a square plastic box with mesh filter for aeration and food supplied *ad libitum*. Since the rearing conditions differ significantly from plant assays, we considered those experiments as reference and not as control.

Statistical analysis of LHT

All computations were performed using “lme4” package (Bates et al. 2015) of the R software version 3.0.3. We used different generalized linear models depending on the distribution of the residuals. For all the variables, we analyzed by following factors and

we also included the interaction between the following factors. If the replicates had a negligible influence on model outcome, they were not included in the models (using "glm" function), or if the replicates had a significant effect, they were added as a random factor (using "glmer" with replicate factor in random effect). Model selection was performed as follows: the significance of the different terms was tested starting from the higher-order terms using likelihood-ratio-tests (LRT). Non-significant terms ($P > 0.05$) were removed and factor levels of qualitative variables that were not significantly different were grouped (LRT; Crawley 2007).

Genomic

Sample preparation and sequencing

We collected 4th instar larvae of the second generation on native and alternative plants, corresponding to offspring of the larvae used to estimate the different components of fitness (survival, weight and developmental time). The larvae number was variable between experimental set-ups (n = 3 to 12 larvae). Larval instar was determined by the width of the head capsule (**Figure S.17**), if the larvae were considered like 4th instar, three larvae of the same experimental set-up were pooled. We weighed the pools and crushed them in liquid nitrogen to obtain a fine powder, which was placed in TRIzol® Reagent (Invitrogen) and stored at -80°C. After collection of samples in all experimental set-ups, total RNA was extracted using a TRIzol® Reagent, according to the manufacturer's RNA protocol. To remove contaminating DNA from RNA preparations, we used DNase from TURBO DNA-free™ Kit (Ambion). Bioanalyzer using 1 µl of total RNA from each sub-pool of three larvae permitted to estimate RNA quantity. The ratio of absorbance 260/280 and 260/230 was used to assess the purity of RNA in each sample. The sub-pools of three larvae, having a good quality (between

581 1.35 and 2) and quantity (>200 ng/µl), were pooled again to obtain samples
582 corresponding to the four experimental set-ups. On the one hand, the samples from
583 rice plant containing only three larvae because of the survival problem on rice for both
584 strains. On the other hand, the samples on artificial diet and on maize contained 12
585 larvae (*i.e.* 4 sub-pools of 3 larvae).

586 High throughput sequencing was performed for the pool samples using Illumina
587 technologies to obtain single-end 50-bp reads. Library construction and sequencing
588 were performed by MGX-Montpellier GenomiX (Montpellier, France) on a HiSeq 2000
589 (Illumina). For each pool, tagged cDNA libraries were generated using the TruSeq
590 Stranded mRNA Sample Preparation Kit (Illumina) following manufacturer's protocol.

591 ***Reference and annotation***

592 All RNA-Seq experiments were aligned against a common reference. This reference
593 is OGS2.2 (Gouin et al. 2017), generated from the sequencing and annotation of the
594 C-strain genome. Gene models result from direct ORF prediction, guided by
595 expression data published earlier (Legeai et al. 2014) and the mapping of RNA-Seq
596 reads. Gene models for selected gene families also underwent an expert annotation
597 by manual curators.

598 ***Differential expression analysis***

599 To identify differentially expressed genes, we first mapped reads on gene prediction
600 using Bowtie2 (Langmead and Salzberg 2012). We chose to use the same reference
601 for both the sf-C and the sf-R strain samples. For read mapping we used "very
602 sensitive" parameter setting in Bowtie2, which allowed searching extensively for the
603 best alignment for each read. Counting of aligned reads number to each gene is
604 produced by SAMtools program (Li et al. 2009). Then to detect the genes differentially
605 expressed we used DESeq2 (R package; Love, Anders, and Huber 2014). To measure

606 gene expression variations between conditions, DESeq2 uses a negative binomial
607 generalized mixed model. The estimates of dispersion and the logarithmic fold-
608 changes incorporate data-driven prior distributions. Genes were considered
609 differentially expressed if they satisfy a false discovery rate lesser than 1%.

610 ### *Characterizing gene function and comparison between two strains*

611 After identifying differentially expressed genes between two strains for the same food
612 resource, we used the Fisher's exact test (cut-off of FDR < 0.01) to identify GO
613 categories possibly involved in corn specialization. The resulting list of GO-terms may
614 contain redundant categories (*i.e.* there was a parent-child relationship in enriched
615 function or process). We used REVIGO (http://revigo.irb.hr/) that summarizes and
616 regrouped terms by a clustering algorithm based on semantic similarities (Supek et al.
617 2011). We used the default parameter ("medium").

618 ### Natural Populations collections

619 *Spodoptera frugiperda* wild larvae were collected in Florida and Georgia between
620 September, 18th and September, 25th 2015 in three different field locations. One
621 sweet corn field in Citra (Marion County, Florida), one volunteer corn in Tifton, (Tift
622 County, Georgia) and one pasture grass field in Jacksonville (Duval County, Florida).
623 In corn fields, plants were cut and larvae collected *in situ*. In the pasture grass field,
624 collections were made using a sweeping net. After confirming their identification as
625 *Spodoptera frugiperda* according to LepIntercept
626 (http://idtools.org/id/leps/lepintercept/frugiperda.html), larvae were placed in individual
627 plastic cups with cut leaves (either corn or grass) as a food source and brought back
628 in a cooler to the laboratory after a few hours of collection. Once in the laboratory,
629 larvae were sorted according to stage. Stages were measured according to the chart
630 in **Fig. S17**, where the width of the cephalic capsule should match the width of the line

631 for each stage. This chart has been determined based on rearing conditions of lab
632 strains in Montpellier and confirmed with a similar chart based on the rearing of lab
633 strains in Gainesville, Florida. L4 larvae were sacrificed with a razor blade and
634 immediately placed individually in a screw-cap 2ml tube containing 1ml of RNAlater
635 (Sigma; R0901).

636 ### *DNA/RNA extractions*

637 Larvae from field collections were placed in a 1.5ml Eppendorf tube with RLT buffer
638 from Qiagen. Individual larvae were ground using a TissueLyser II from Qiagen (Cat
639 No./ID: 85300) using one bead (size 5mm) by tube and processed for dual DNA and
640 RNA extraction using an AllPrep DNA/RNA Mini Kit (50) (Qiagen Cat. 80204).

641 ### *Genotyping*

642 We used the COI genotype described in (Meagher Jr. and Gallo-Meagher 2003) to
643 discriminate between the sf-C and the sf-R strains. A PCR on genomic DNA was
644 performed using the following primer sequences (JM-77: ATC ACC TCC ACC TGC
645 AGG ATC and JM-76: GAG CTG AAT TAG GGA CTC CAG G) to amplify a DNA
646 fragment of 550bp corresponding to the mitochondrial cytochrome oxidase c subunit I.
647 The MspI enzyme is used to reveal a polymorphism between the 2 strains. The COI
648 fragment of the C-strain is digested by MspI to produce a 500bp and a 50bp fragment
649 (**Fig. S8A**).
650 For the Tpi genotyping we used the following primers as described (Rodney N.
651 Nagoshi 2010): *Tpi*-56 F (5'-CAAAATGGGTCGCAAATTCG-3') and *Tpi*-850gR (5'-
652 AATTTTATTACCTGCTGTGG-3'). Digestion of the PCR product was made with the
653 AvaII enzyme (**Fig. S9A**).
654 FR1 repeat genotyping was based on PCR amplification only, as described (Rod N.
655 Nagoshi and Meagher 2003a) with the following primers : FR-c (5'-

TCGTGTAAAACGTACTTTCTT- 3'), and FR-2 (5'-GACATAGAAGAGCACGTTT-3'). Amplification is then analyzed on agarose gel (**Fig. S10**)

Quantitative PCR

For reverse transcription quantitative PCR, we used the candidate transcript sequence, as retrieved from BIPAA platform* -for example by searching GSSPFG00029721001-RA from **Table S2**- as a template for primer design using Primer3 and asking for a 50 nt amplicon. Primers used are specified in **Table S3**.

qPCR have been performed on a LightCycler 480 (Roche) with SYBR green. Program used was 95°C for 10min and then 40 cycles of 94°C 10s, 60°C 10s, 72°C 10s. Relative expression was calculated using the ΔΔCt method with the laboratory sf-C strain as a reference point for each gene.

* https://bipaa.genouest.org/sp/spodoptera_frugiperda_pub/

Data availability

Spodoptera frugiperda reference genome and reference transcriptome can be publicly accessed *via* the BIPAA (BioInformatics Platform for Agroecosystem Arthropods) interface (http://bipaa.genouest.org/is/lepidodb/spodoptera_frugiperda/). fastq files and RNAseq counts from this study are accessible in ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) with the following accession number : E-MTAB-6540.

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Authors Contributions

NN, EA and MO designed the project. JPB and MV produced the corn and rice plants used in the RT experiments. MO, PA, NN and performed the RT and OV experiments. MO, GD performed the statistical analyses of LHT in the RT experiments. MO, GD, RNS and NN performed the RT-qPCR experiments. MO performed the RNA extractions for the RNA-Seq experiments. RK and SR produced the Illumina libraries, performed the Illumina sequencing and realized the computational analyses and quality control necessary to produce .fastq files of sequences. MO, YM, SN and NN performed the RNA-Seq analyses. MF, GJK, RNN, RLM and NN performed the field collections. RNS and NN performed the genotyping and RNA extractions of field samples. MO and NN wrote the manuscript and produced the figures. YM, SR, GJK, RNN, RLM and EA edited the current manuscript. All authors approved the present manuscript submission.

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Figure Legends

Figure 1: *oviposition choice of sf-C and sf-R.*

Proportion of egg masses laid in the three experimental trials (corn-only, rice-only and choice) by sf-C (**A-C-E**) and sf-R (**B-D-F**) according to the site of oviposition. There are three oviposition sites available: ~~-~~the net (light gray), the corn plant (~~yellow~~red) and the rice plant (~~green~~blue). Here, the relative proportions on each laying site represented the mean of proportions obtained about the four replicates.

Figure 2: *Fitness traits of sf-C and sf-R according to the diet.*

(**A-D**) Pupal weight (`wt`) is measured in duplicate for sf-C (**A-C**) and sf-R (**B-D**) according to plant diet: corn (~~yellow~~red) and rice (~~green~~blue). We measured separately females (**A-B**) and male (**C-D**) pupae.

(**E, F**) The survival (`sv`) rate is measured from the 1st larval instar to adult emergence for sf-C (**E**) and sf-R (**F**) according to plant diet. Bars represent the mean of survival rate of the two experimental replicates with the standard error. Different letters above bars indicate significant differences of survival between plant diets for each strain ($P < 0.05$).

Figure 3: *Transcriptional response of sf-R versus sf-C regardless of the diet.*

A. Principal component analysis on normalized RNA-seq reads for all RT samples of sf-R and sf-C when the larvae feed on corn (red), on rice (blue) or on artificial diet (green). **B.** Multidimensional scaling plot (MA-plot) reporting the log2 fold changes between the strains (sf-R vs sf-C) over the mean of normalized counts. Each dot represents a gene either with a non-significant differential expression between trials (gray dots) or with a significant differential of expression (red dots).

Figure 4: *RNA-Seq of individual larvae from the fields*

A. Genotypes of the individual L4 larvae from natural populations used for RNAseq studies. CoI, tpi and FR1 repeat genotyping has been done by PCR-RFLP (**Supplementary Figures 8-10**). Color code is dark green for presumptive C-strain genotype according to the literature while purple is for presumptive R-strain genotypes. Sex has been determined *post-facto* by examining the alignments of reads on the Z-associated *tpi* locus. If all SNP positions within the scaffold are homozygous, we assumed the individual was female. Heterozygosity indicates a male. **B**. Multidimensional scaling plot (MA-plot) reporting the log2 fold changes between the strains (sf-R vs sf-C) over the mean of normalized counts when combining FL15 and MORT2 experiments. Each dot represents a gene either with a non-significant differential expression between conditions (gray dots) or with a significant differential of expression (red dots). 76 genes are overexpressed in sf-R and 73 in sf-C. **C**. Heatmap of expression variations (expressed as z-scores) of the sf-R specific expressed genes across all RNAseq experiments. For each gene, red indicates a higher expression and blue a lesser expression across the experimental dataset. Genes have been hierarchically clustered as indicated by the dendrogram on the left by similarity of expression variation. The red asterisk identifies the COI-*numt* expression. **D**. View of the mitochondrial genome corresponding to the COI-numt sequence and alignment coverage of reads corresponding to sf-R (red) or sf-C (green) samples of the MORT2 experiment. We can observe a trough of expression in this region associated with sf-C strain.

Supplementary Information

Fig. S1 - A. Fertility represented by the number of egg-masses divided by the number of females present in mating cages. Values represent the mean of fertility with the standard error for sf-C (green) and sf-R (red) according to different experimental trials: choice (in presence of corn and rice plants), no-choice (either in presence of corn only or in presence of rice only). The letters above the bars means indicated the significant differences in the mean fertility ($P < 0.05$). For sf-C (**B**) and sf-R (**C**), we counted the percentage of eggs (y-axis) that gave rise to a live larva for sf-C and sf-R in each trial. Error bars represent the variations between egg-masses. No statistical differences were observed between trials.

Fig. S2 - Fitness traits in sf-C and sf-R according to the diet: corn plant (~~yellow~~red), rice plant (~~green~~blue). Bars represent the developmental time until adult emergence for sf-C (**A**) and sf-R (**B**). The variation between replicates is represented by the standard error (except for the developmental time which are exactly the same for both strain on corn plant) and the different letters above bars indicate significant differences between plant diets for each strain ($P < 0.05$).

Fig. S3 - Survival from egg hatching for 50 individuals reared on artificial diet with low (exp. #1) or high (exp. #2) hygrometry.

Fig. S4 - Example of manual gene annotation

A. In the *S. frugiperda* genome (Gouin et al., 2017) the gene GSSPFG00032711001 is differentially expressed between sf-C and sf-R, however its function is unknown. In this WebApollo browser screenshot, the predicted gene of the official gene set

(OGS2.0) is shown in green. The alignment of RNAseq reads in this region, shown in gray, reveals an intron darker gray. We used this support to correct the structure of this gene in the yellow track. **B**. The corrected sequence is now used to perform blastp annotations and reveal that this gene has in fact been identified as *polycalin* in other Lepidoptera (Mauchamp et al. 2006).

Fig. S5 - 50 most expressed genes in laboratory sf-R strain

This heatmap displays the relative gene expression of the top 50 most differentially expressed gene in sf-R across the MORT2 experimental datasets, where red is overexpressed and blue underexpressed (z-scores). The columns on the right indicate the gene identification name and its manual reannotation. Genes are ordered from most overexpressed (top) to less.

Fig. S6 - 50 most expressed genes in laboratory sf-C strain

This heatmap displays the relative gene expression of the top 50 most differentially expressed gene in sf-C across the MORT2 experimental datasets, where red is overexpressed and blue underexpressed (z-scores). The columns on the right indicate the gene identification name and its manual reannotation. Genes are ordered from most overexpressed (top) to less.

Fig. S7 - qPCR validation of RT RNAseq experiments

This figures shows two examples of strain associated gene expressions. The first one (top left: slack-LINE1) is a series of 3 LINE-type transposable elements expressed in sf-R. The IGV browser screenshot shows the RNA-Seq coverage across this region. On the right are the qPCR measurements ($\Delta\Delta$Ct values on the y-axis) of expression

1024 associated to slack-LINE1 in three independent individual larvae of each strain,
1025 confirming its overexpression in sf-R.
1026 At the bottom, another example is shown for the Fatty Acid Binding protein 10 (FABP-
1027 10), a member of a cluster of similar genes involved in fatty acid transport in the midgut,
1028 whose expression is associated to sf-R.
1029
1030 **Fig. S8** - Genotyping of individual larvae using the COI diagnostic gene
1031 A diagnostic locus of 550 bp in the mitochondrial gene Cytochrome Oxidase I (COI)
1032 (Meagher Jr. and Gallo-Meagher 2003) has been amplified by PCR. **A.** Digestion by
1033 the MspI restriction enzyme is possible only in the sf-C strain and liberates one 500 bp
1034 fragment and a 50bp fragment. This PCR_RFLP is tested on individual L4 larvae from
1035 our laboratory colonies. All sf-C are digested, none of the sf-R. **B.** Test on 32 L4
1036 individual larvae from the Citra sweet corn field. **C.** Test on 18 larvae from the Tifton
1037 corn field and 6 larvae from the Jacksonville pasture grass field. **D.** Proportion of
1038 diagnosed sf-C and sf-R individuals in each field.
1039
1040 **Fig. S9** - Genotyping of individual larvae using the *tpi* gene SNP
1041 A diagnostic locus of 800 bp in the Z-linked gene *Triose Phosphate Isomerase* (*Tpi*)
1042 (Nagoshi 2010) has been amplified by PCR. The PCR fragment encompasses introns
1043 2 and 3 of the *tpi* gene. **A.** Digestion by the AvaII restriction enzyme is possible only in
1044 the sf-R strain and liberates one 500 bp fragment and one 300bp fragment. This PCR-
1045 RFLP method is tested on individual L4 larvae from our laboratory colonies. All sf-R
1046 are digested, none of the sf-C. **B.** Test of the marker in select individuals from each
1047 field. The names in red indicate the putative sf-R larvae according to COI genotype.
1048 An R is noted when individuals show a proper restriction. Only one individual from

Tifton (B25) is tested as sf-R with this marker. Individuals A11 and B20 show two amplified bands, indicating that they may be heterozygous for the intron length. It has been shown that intron length polymorphism exists at this gene (Nagoshi and Meagher 2016). All tested larvae from Jacksonville show the expected sf-R digestion pattern.

Fig. S10 - Genotyping of individual larvae using the FR1 repeat

The FR1 repeat is a sex-linked repeat element associated with the sf-R strain. It is present in sf-C but with less copies (Nagoshi and Meagher 2003b; Nagoshi and Meagher 2003a). **A.** In the laboratory population, some sf-R individuals show a strong multiband amplification, indicative of the presence of this repeat. These copies are supposedly on the W chromosome and as such can only be detected in males. In natural populations, only two individuals from the Tifton field show this amplification. The B25 individual, that was genotyped as sf-R with COI and *Tpi* markers, doesn't show the FR1 amplification, probably because it is a male. **B.** Low copy numbers are detected in the Jacksonville individuals, except for the B5 individual, which might be the only female.

Fig. S11 - qPCR measurement of DE genes in natural populations

Examples shown here are qPCR expression measurements ($\Delta\Delta$Ct values on the y-axis) examples for two sf-R strain associated gene expressions: slack-LINE1 and ngf1a, a nervous system associated transcription factor. We tested the expression of these 2 genes in individual larvae from our laboratory colonies (Lab) and from the Florida collections of sf-C or sf-R genotypes. The overexpression is observed only in laboratory sf-R larvae.

Fig. S12 - **A**. Principal component analysis (PCA) of normalized RNA-seq reads of sf-R and sf-C individual larvae sampled in Tifton (blue) or Jacksonville fields (red). The samples cluster by collection groups. **B**. Correlogram of the FL15 RNAseq experiments showing no clear overall correlation per genotype. **C**. PCA of all RNA-seq samples from the laboratory and field conditions. The laboratory sf-R experiments cluster with field individuals while laboratory sf-C samples cluster away.

Fig. S13 - Heatmap of 50 most DE genes overexpressed in laboratory sf-R strain (same as **Fig. S5**) across all RNAseq experiments.
Each raw represents z-score normalized expression for one gene across all RT and field samples. Genes are ordered from top to bottom, from the most significant to the 50th most significant and the blue-white-red color scale indicates lower, no and higher variation of gene expression for each gene. These genes are clearly overexpressed in laboratory sf-R and underexpressed in laboratory sf-C. But no clear pattern is observable in other RNAseq experiments or from field collections.

Fig. S14 - Heatmap of 50 most DE genes overexpressed in laboratory sf-C strain (same as **Fig. S6**) across all RNAseq experiments.
Each raw represents z-score normalized expression for one gene across all RT and field samples. Genes are ordered from top to bottom, from the most significant to the 50th most significant and the blue-white-red color scale indicates lower, no and higher variation of gene expression for each gene. These genes are clearly overexpressed in laboratory sf-C but are mostly underexpressed in all other experiments.

Fig. S15 – Constitutive Sf-C associated gene expression across all RNAseq experiments.

These genes have a sf-C specific expression in laboratory experiments as well as in field collection samples. This heatmap shows the relative expression of each of these genes across all RNAseq samples analyzed (z-scores).

Fig. S16 - Annotation of COI-*numt* in the *S. frugiperda* genome

A. Webapollo screenshot showing the GSSPFG00006578001-RA predicted gene on scaffold-722 and RNAseq coverage underneath. In the yellow track, the part that has a sequence homology with mitochondrial COI gene is shown in magenta. **B**. log2 fold changes of expression of the COI-numt in all RNAseq samples showing their sf-R associated expression.

Fig. S17 - Staging of L4 larvae

A. Actual size chart that was used after calibration in laboratory conditions to stage *S. frugiperda* larvae. The width of the lines should correspond to the width of cephalic capsule. **B**. In field collections, larvae were placed on the chart printouts so that their body follows a line. To be considered an L4 larva, the width of the head should be the same size or slightly bigger than the width of the line.

Table S1 - Sequencing and alignment statistics of RNAseq experiments

This table is presenting the number of reads processed per sample and their different alignment statistics with bowtie2 (Langmead and Salzberg 2012).

Table S2 - Comparison of RNAseq data and qPCR

This table is a list of 30 genes that are found overexpressed in sf-R compared to sf-C in the RT experiment. Last two columns on the right indicate the log2 Fold Change observed in RNAseq experiments and the ΔΔCt values obtained by qPCR. Except for peroxidase, all genes tested show a confirmed overexpression of these genes in sf-R.

Table S3 - Candidate genes primers sequences for qPCR used in **Table S2**

Table S4 – Manual annotation of the 50 genes with the most constitutive sf-R associated expression

Table S5 – Manual annotation of the 50 genes with the most constitutive sf-C associated expression

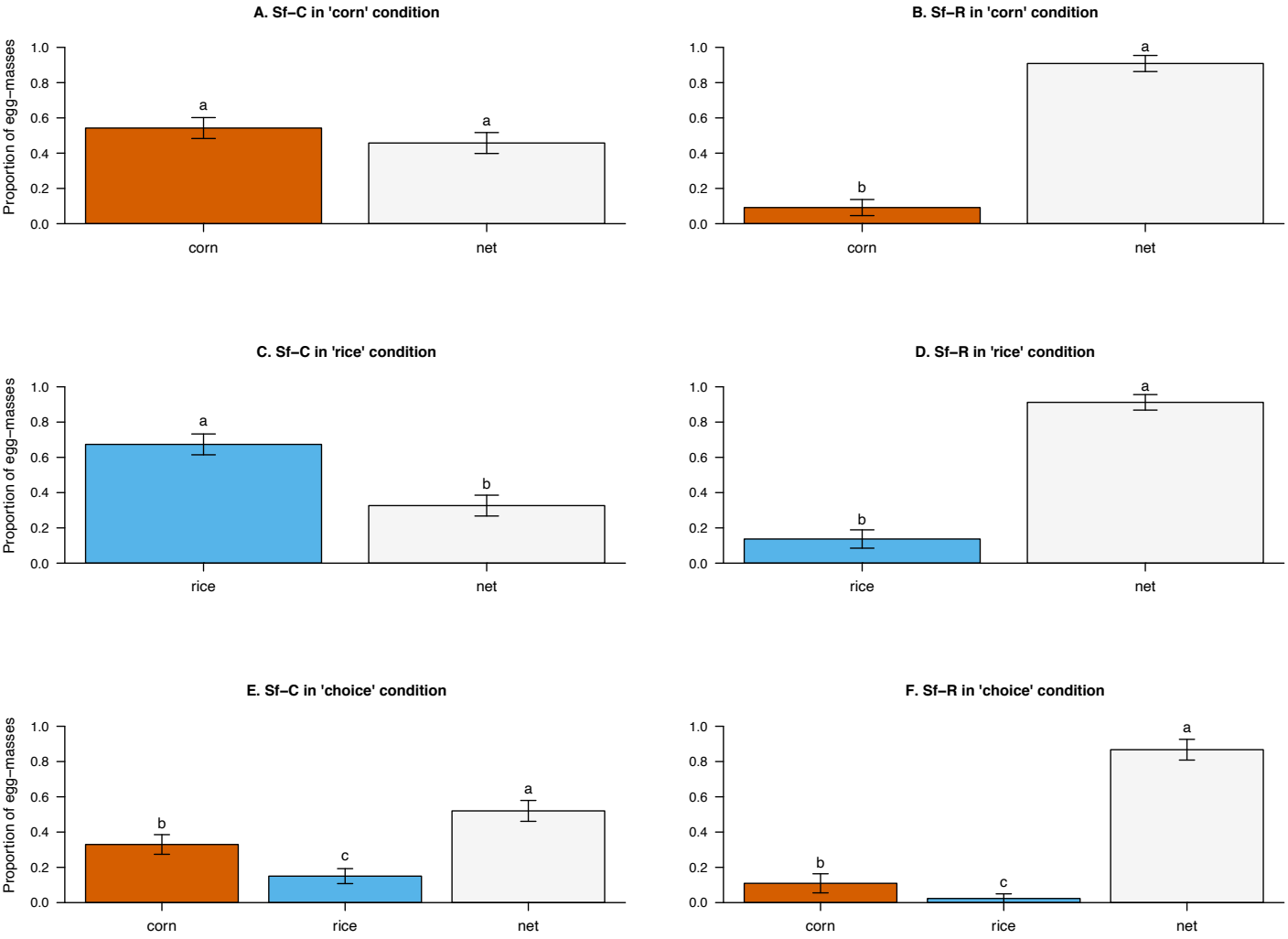

A. Sf–C in 'corn' condition
B. Sf–R in 'corn' condition
C. Sf–C in 'rice' condition
D. Sf–R in 'rice' condition
E. Sf–C in 'choice' condition
F. Sf–R in 'choice' condition
Proportion of egg–masses
0.0
0.2
0.4
0.6
0.8
1.0
a
b
c
corn
rice
net

FIGURE 1

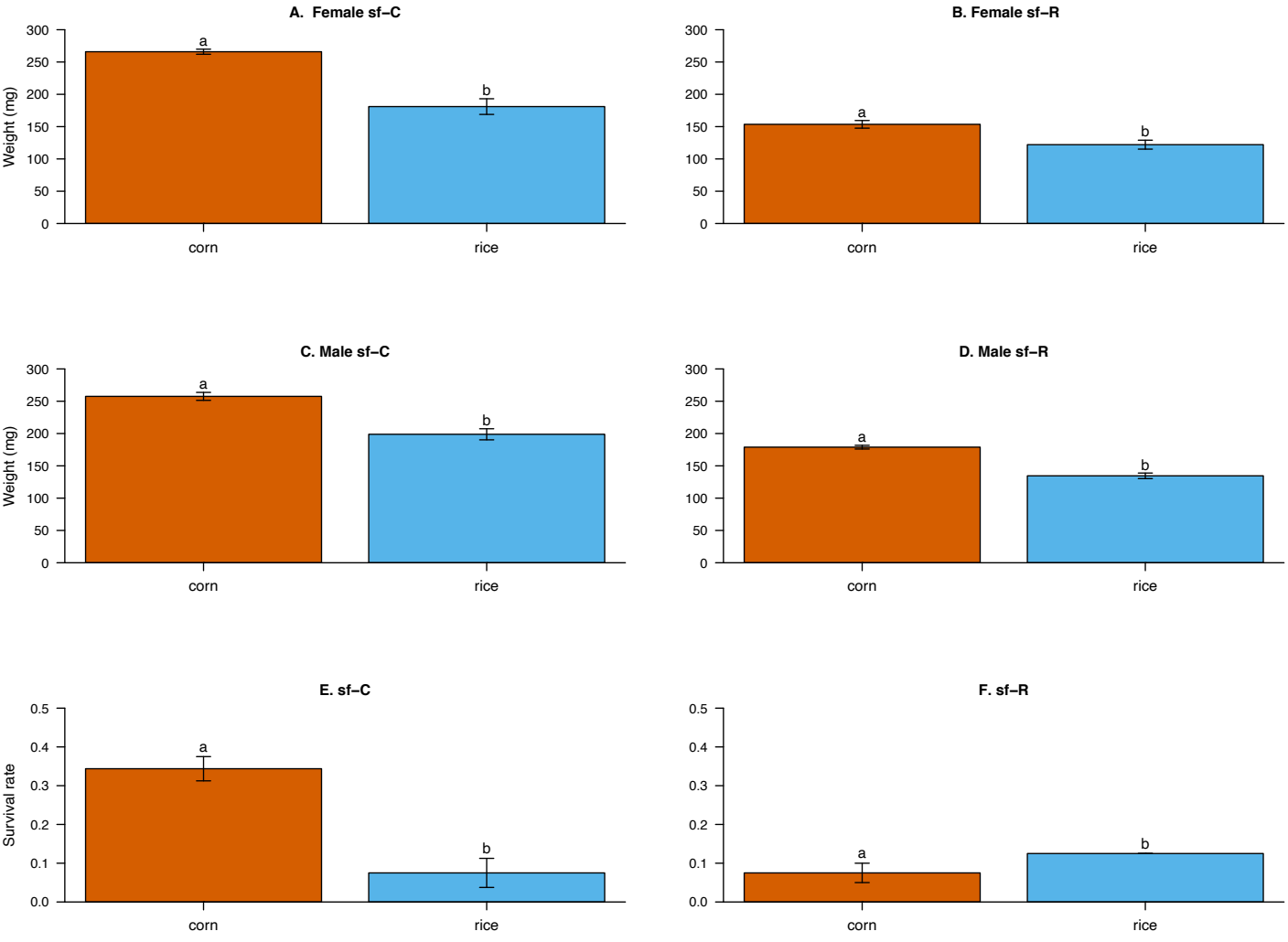
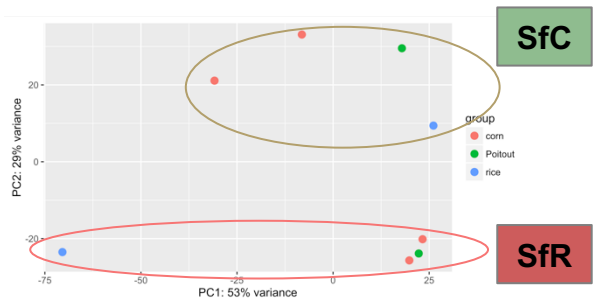

A. Female sf–C
B. Female sf–R
C. Male sf–C
D. Male sf–R
E. sf–C
F. sf–R
Weight (mg)
Survival rate
corn
rice
a
b
0
50
100
150
200
250
300
0.0
0.1
0.2
0.3
0.4
0.5

FIGURE 2

A


SfC
SfR
group
corn
Poitout
rice
PC2: 29% variance
PC1: 53% variance
20
0
-20
-75
-50
-25
0
25

B

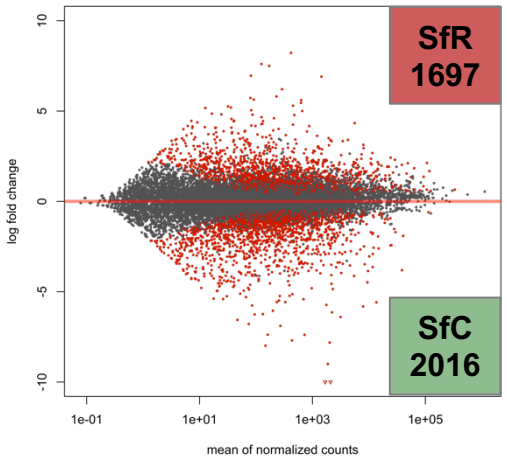

SfR
1697
SfC
2016
log fold change
mean of normalized counts
10
5
0
-5
-10
1e-01
1e+01
1e+03
1e+05

FIGURE 3

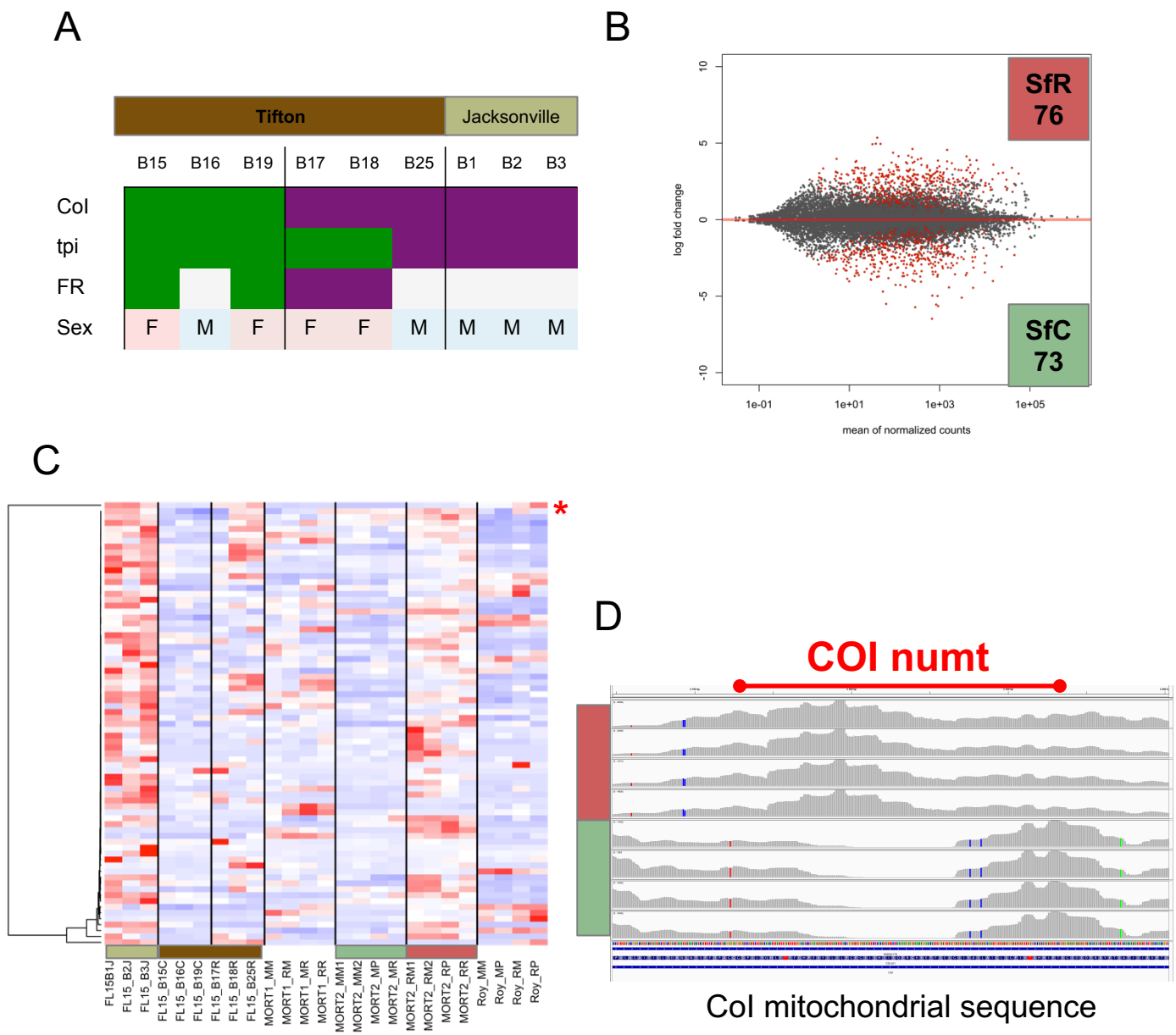
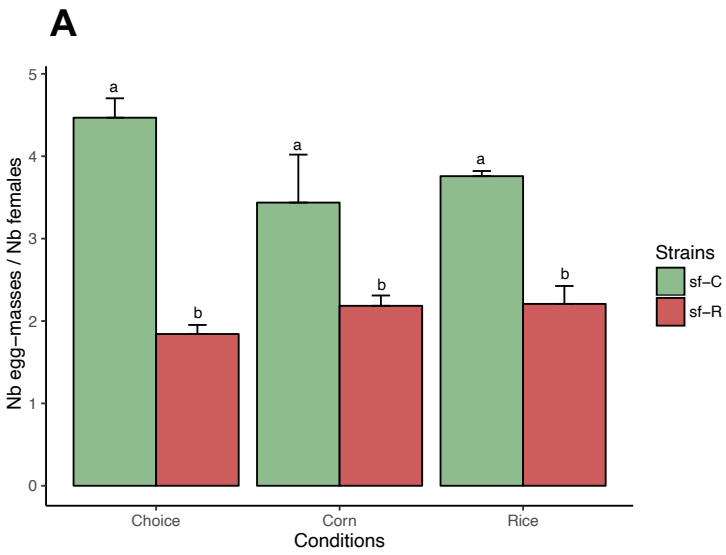

A
Tifton
Jacksonville
B15
B16
B19
B17
B18
B25
B1
B2
B3
ColI
tpi
FR
Sex
F
M
F
F
F
M
M
M
M
B
SfR
76
SfC
73
log fold change
mean of normalized counts
1e-01
1e+01
1e+03
1e+05
C
*
FL15B1J
FL15_B2J
FL15_B3J
FL15_B15C
FL15_B16C
FL15_B19C
FL15_B17R
FL15_B18R
FL15_B25R
MORT1_MM
MORT1_RM
MORT1_MR
MORT1_RR
MORT2_MM1
MORT2_MM2
MORT2_MP
MORT2_MR
MORT2_RM1
MORT2_RM2
MORT2_RP
MORT2_RR
Roy_MM
Roy_MP
Roy_RM
Roy_RP
D
COI numt
ColI mitochondrial sequence

FIGURE 4

FIGURE S1


A
Nb egg−masses / Nb females
0
1
2
3
4
5
a
b
a
b
a
b
Choice
Corn
Rice
Conditions
Strains
sf−C
sf−R

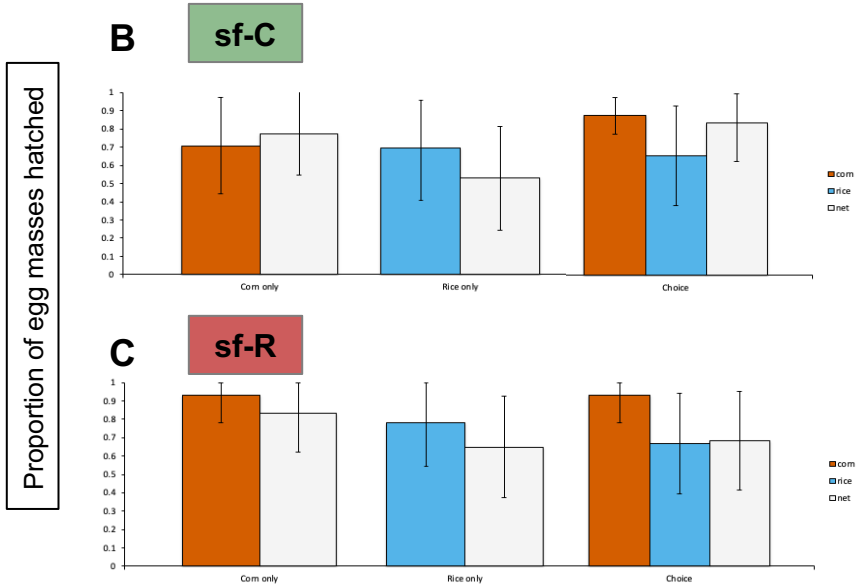

Proportion of egg masses hatched
B
sf-C
C
sf-R
Corn only
Rice only
Choice
corn
rice
net

FIGURE S2

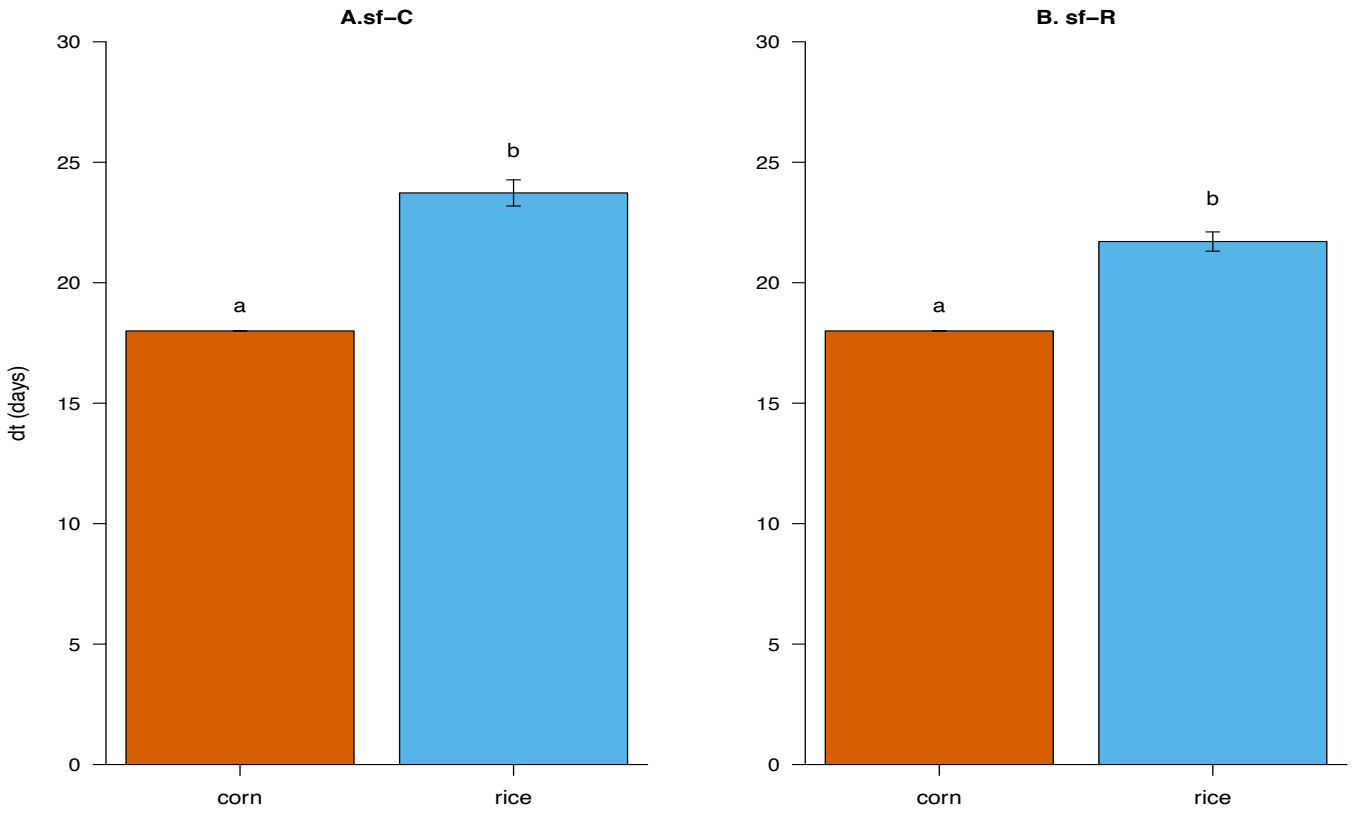


FIGURE S3

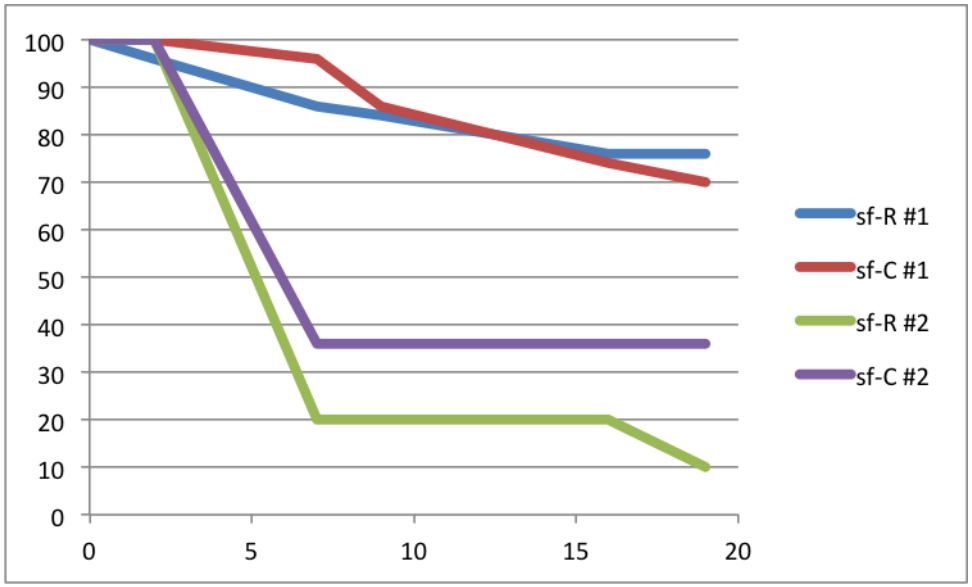
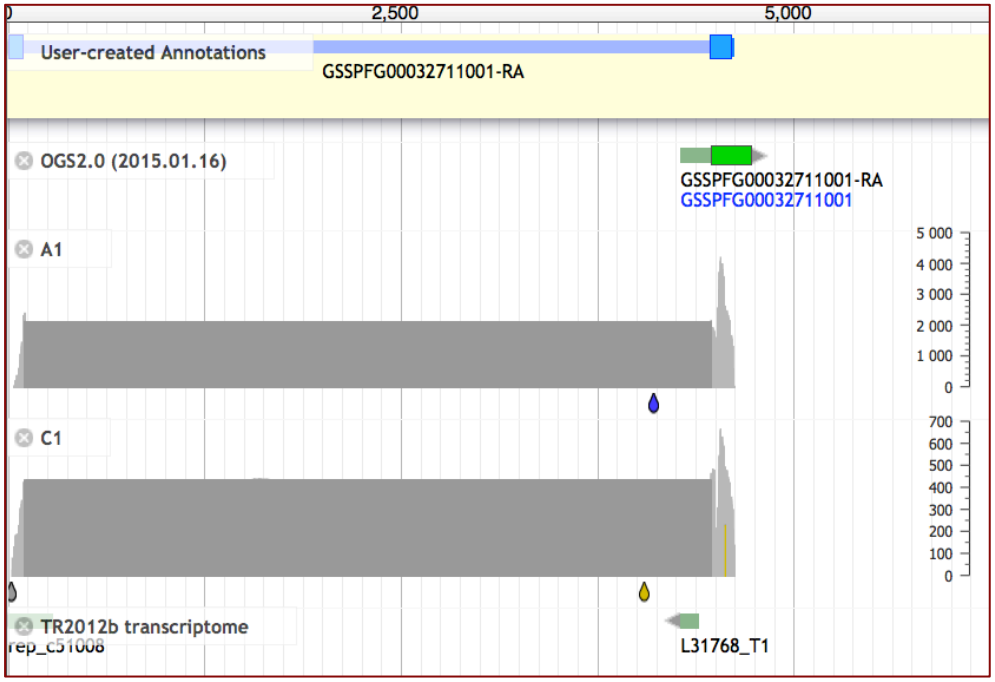


FIGURE S4

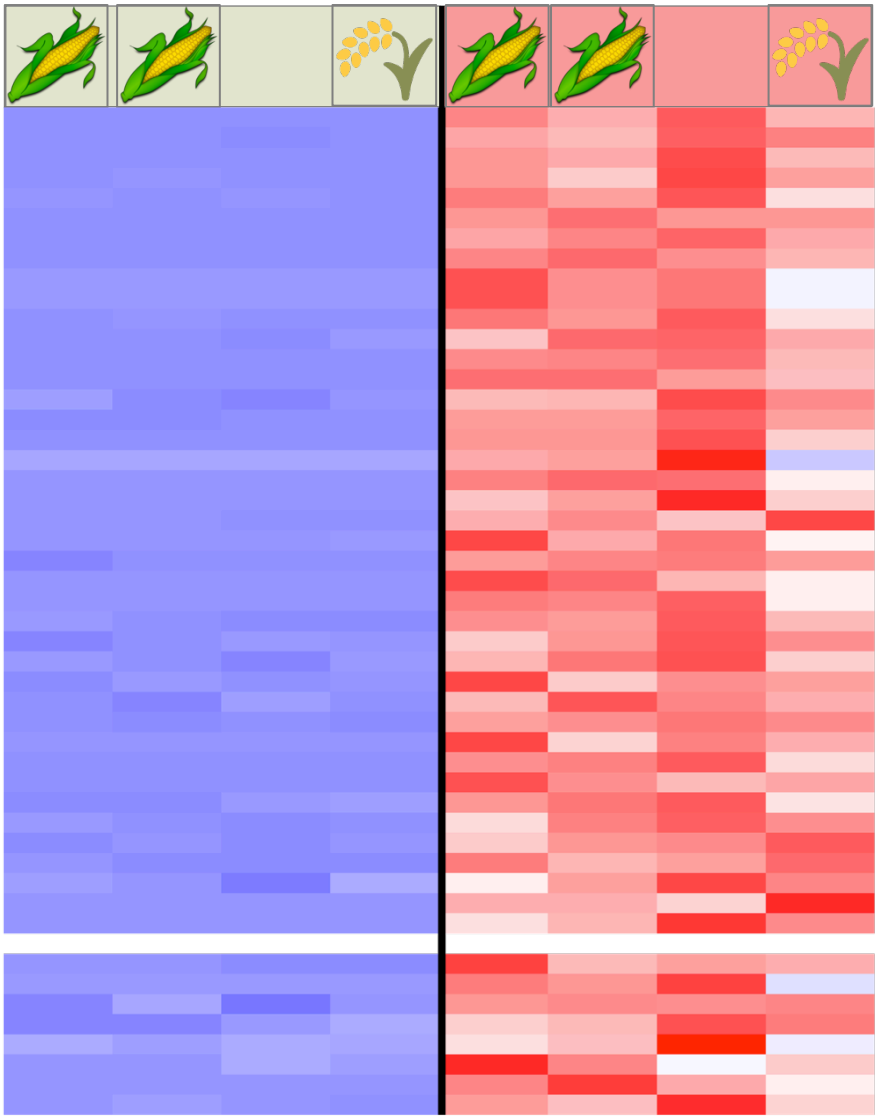
A.



B.

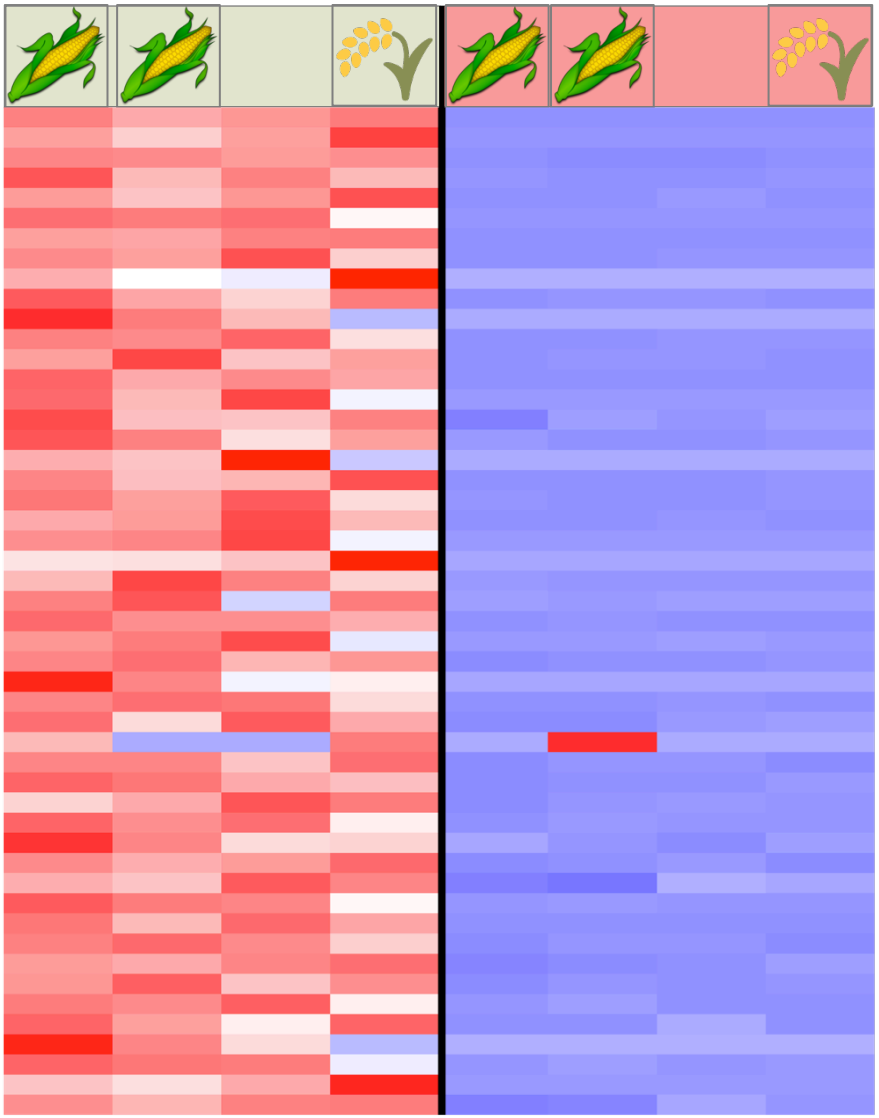
Sequences producing significant alignments:	(Bits)	Value
gb|AEA76321.1| polycalin [Mamestra configurata]	62.8	4e-09
gb|AJQ81210.1| polycalin [Helicoverpa armigera]	56.6	4e-07
ref|XP_012553082.1| PREDICTED: chlorophyllide A binding prote...	57.0	4e-07
ref|NP_001037071.1| chlorophyllide A binding protein precurso...	57.0	4e-07
gb|AGM34046.1| chlorophyllide A binding protein precursor [Bo...	57.0	4e-07
ref|XP_012553081.1| PREDICTED: chlorophyllide A binding prote...	57.0	4e-07
gb|ACB54957.2| polycalin [Helicoverpa armigera]	56.2	5e-07
gb|ACB54956.1| polycalin [Helicoverpa armigera]	56.2	6e-07
gb|ACB54951.1| polycalin [Helicoverpa armigera]	56.2	6e-07
gb|ABU98612.1| multi-domain lipocalin [Helicoverpa armigera]	55.8	7e-07

FIGURE S5



GSSPFG00010063001-RA	xaa-Pro aminopeptidase 1-like
GSSPFG00022625001-RA	NA
GSSPFG00004617001-RA	Uncharacterized membrane associated protein
GSSPFG00024658001-RA	Uncharacterized protein
GSSPFG00006224001-RA	Unknown Smc-like protein
GSSPFG00029999001-RA	Protein kinase C-like, phorbol ester/diacylglycerol-binding
GSSPFG00028883001-RA	Ubiquitin-conjugating enzyme E2
GSSPFG00003434001-RA	Uncharacterized membrane associated protein
GSSPFG00029716001.2-RA	**Fatty Acid Binding Protein 2**
GSSPFG00034702001-RA	Fatty Acid Binding Protein 2
GSSPFG00024077001-RA	Unknown Smc-like protein
GSSPFG00008178001-RA	Ecdysteroid kinase
GSSPFG00009245001-RA	apyrase
GSSPFG00010065001-RA	Uncharacterized secreted protein
GSSPFG00019823001-RA	Uncharacterized membrane associated protein
GSSPFG00022152001-RA	Glycosyl hydrolase family 47
GSSPFG00017532001-RA	Unknown Smc-like protein
GSSPFG00031881001.1-RA	**UGT33-11**
GSSPFG00034426001-RA	NADH:ubiquinone oxidoreductase subunit B14.5a
GSSPFG00024881001-RA	adenylate cyclase/proton-coupled folate transporter
GSSPFG00006331001.1-RA	NGFI-A-binding protein
GSSPFG00025955001-RA	TE
GSSPFG00007887001-RA	GMC oxidoreductase
GSSPFG00019552001-RA	pancreatic lipase
GSSPFG00025957001-RA	TE
GSSPFG00029884001-RA	NA
GSSPFG00015107001-RA	Glucose/ribitol dehydrogenase family
GSSPFG00004160001-RA	seminal fluid Aldo/keto reductase
GSSPFG00009249001-RA	kurz homolog
GSSPFG00025226001-RA	U1 small nuclear ribonucleoprotein C [SNRPC]
GSSPFG00021627001-RA	Chymotrypsin serine protease family (S1
GSSPFG00019502001-RA	U3 small nucleolar RNA-associated protein 10
GSSPFG00022995001-RA	RING finger protein, mdlc
GSSPFG00019425001-RA	alpha-tocopherol transfer protein
GSSPFG00024304001-RA	Transmembrane proteins 14C
GSSPFG00019445001-RA	Uncharacterized membrane associated protein
GSSPFG00032667001-RA	zinc finger protein weckle-like
GSSPFG00028547001-RA	zinc finger protein weckle-like
GSSPFG00023392001-RA	SP208
GSSPFG00020932001-RA	Growth-Arrest-Specific Protein 2
GSSPFG00009246001-RA	apyrase
GSSPFG00020720001-RA	fatty acyl-CoA reductase
GSSPFG00034771001-RA	fatty acyl-CoA reductase
GSSPFG00026559001-RA	Glucose/ribitol dehydrogenase family
GSSPFG00000341001-RA	NA
GSSPFG00015363001-RA	Calcineurin
GSSPFG00019185001.2-RA	**CYP9APARTIAL**
GSSPFG00008822001-RA	Cuticular protein
GSSPFG00008858001.3-RA	**Death-related protein**
GSSPFG00006953001-RA	alcohol dehydrogenase

FIGURE S6



GSSPFG00009092001-RA	TE
GSSPFG00021758001-RA	TE
GSSPFG00000055001-RA	NA
GSSPFG00015492001-RA	TE
GSSPFG00026424001-RA	Cytochrome P450
GSSPFG00028010001-RA	carboxypeptidase Q-like
GSSPFG00004899001-RA	NA
GSSPFG00027485001-RA	Uncharacterized protein
GSSPFG00033999001-RA	Uncharacterized protein
GSSPFG00018415001-RA	Uncharacterized protein
GSSPFG00012179001.1-RA	fatty-acid synthase
GSSPFG00003930001-RA	NA
GSSPFG00002576001-RA	TE
GSSPFG00033078001-RA	TE
GSSPFG00009529001-RA	CUB domain peptidase
GSSPFG00009290001-RA	TE
GSSPFG00005332001-RA	ATP-dependent DNA helicase PIF1
GSSPFG00033049001-RA	TE
GSSPFG00005622001-RA	nuclear pore complex protein Nup88
GSSPFG00030212001-RA	TE
GSSPFG00005743001-RA	Uncharacterized protein
GSSPFG00021504001-RA	TE
GSSPFG00009526001-RA	NA
GSSPFG00025034001-RA	TE
GSSPFG00008862001-RA	Harbinger transposase-derived nuclease, HARBI1
GSSPFG00023363001-RA	CG8420
GSSPFG00017311001-RA	TE
GSSPFG00013057001-RA	Uncharacterized protein
GSSPFG00027329001-RA	NA
GSSPFG00021422001-RA	NA
GSSPFG00029033001-RA	TE
GSSPFG00002062001-RA	mitochondrial insertion
GSSPFG00027050001.2-RA	**apterous 1**
GSSPFG00027757001-RA	Uncharacterized protein
GSSPFG00006076001-RA	ras-related GTPase Rap-2
GSSPFG00025780001-RA	CG8420
GSSPFG00026735001-RA	NA
GSSPFG00008269001.4-RA	**CYP9A31PARTIAL**
GSSPFG00025492001-RA	Histidine phosphatase
GSSPFG00003829001-RA	zona pellucida (ZP) domain secreted eukaryotic glycoproteins
GSSPFG00025374001-RA	Endonuclease/Exonuclease/phosphatase
GSSPFG00011985001-RA	Uncharacterized protein
GSSPFG00007980001-RA	Uncharacterized protein
GSSPFG00012726001-RA	TE
GSSPFG00018593001-RA	NA
GSSPFG00019843001-RA	Harbinger transposase-derived nuclease, HARBI1
GSSPFG00027466001-RA	**fatty acid synthase**
GSSPFG00003828001-RA	divergent subfamily of APPLE domains (Zona pellucida (ZP) domain
GSSPFG00026205001-RA	Histidine phosphatase
GSSPFG00006538001-RA	dsRNAse

FIGURE S7

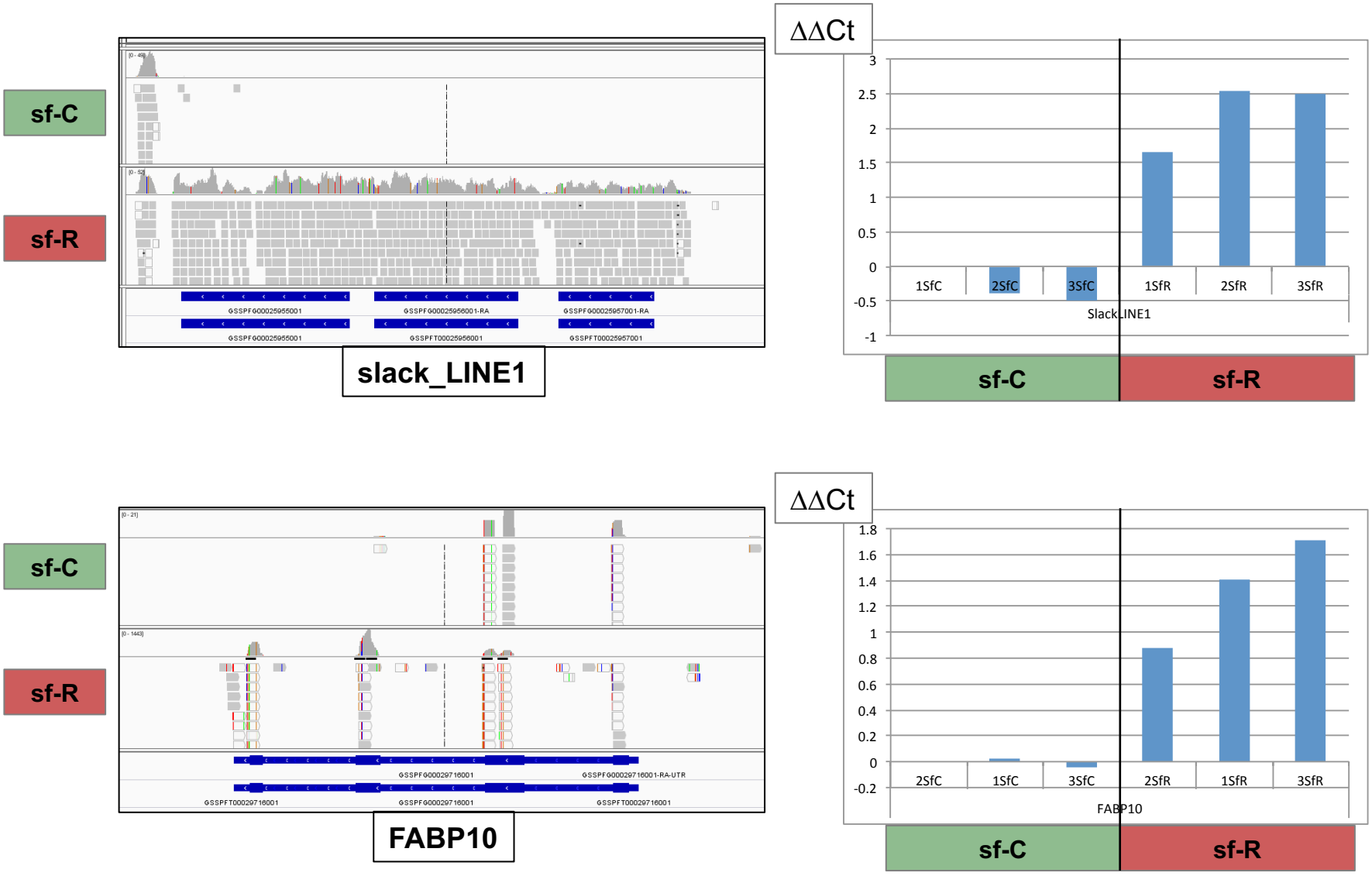
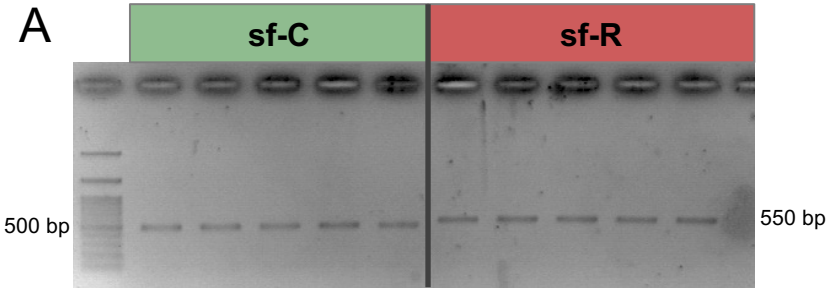
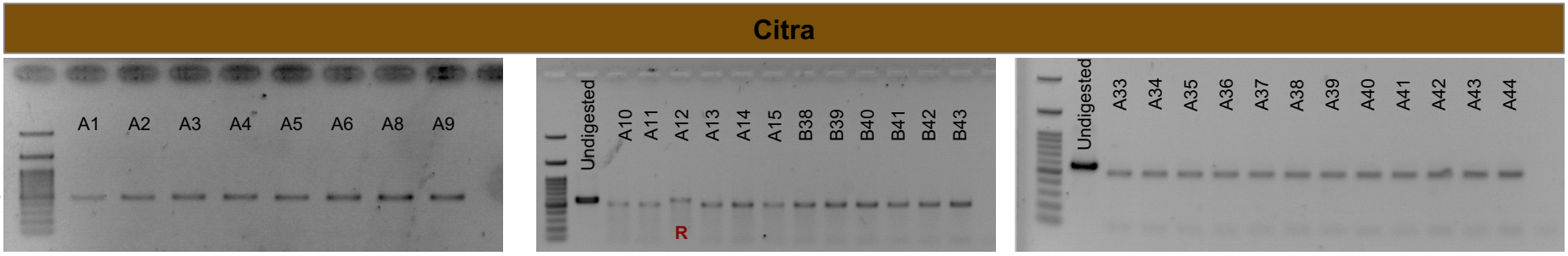

sf-C
sf-R
GSSPFG00025955001
GSSPFG00025956001-RA
GSSPFG00025957001-RA
GSSPFG00025955001
GSSPFT00025956001
GSSPFT00025957001
slack_LINE1
ΔΔCt
3
2.5
2
1.5
1
0.5
0
-0.5
-1
1SfC
2SfC
3SfC
1SfR
2SfR
3SfR
Slack LINE1
sf-C
sf-R
sf-C
sf-R
GSSPFG00029716001
GSSPFG00029716001-RA-UTR
GSSPFT00029716001
GSSPFG00029716001
GSSPFT00029716001
FABP10
ΔΔCt
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
2SfC
1SfC
3SfC
2SfR
1SfR
3SfR
FABP10
sf-C
sf-R

FIGURE S8

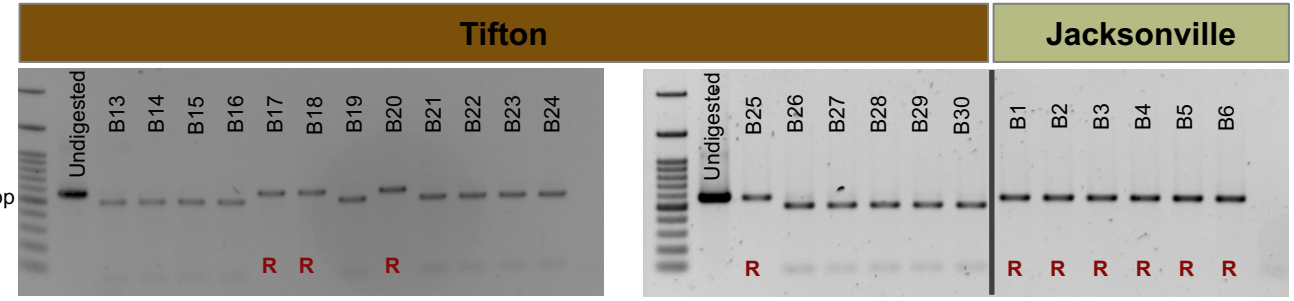
A



B



C

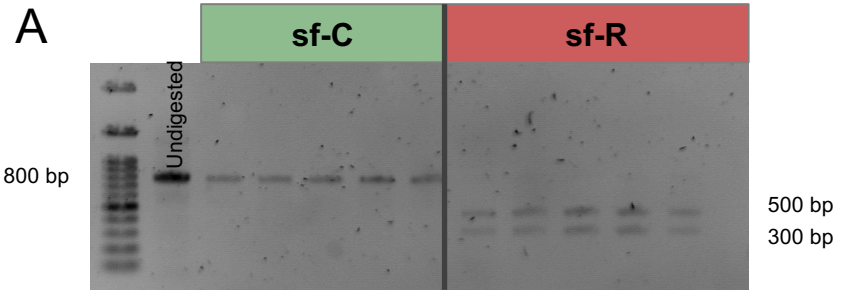


D

	Citra		Tifton		Jacksonville	
	SfC	SfR	SfC	SfR	SfC	SfR
Col	31	1	14	4	0	6

FIGURE S9

A


sf-C
sf-R
Undigested
800 bp
500 bp
300 bp

B

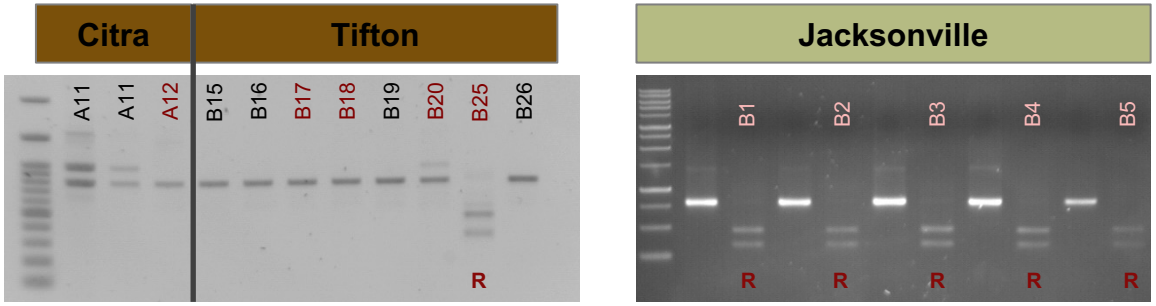
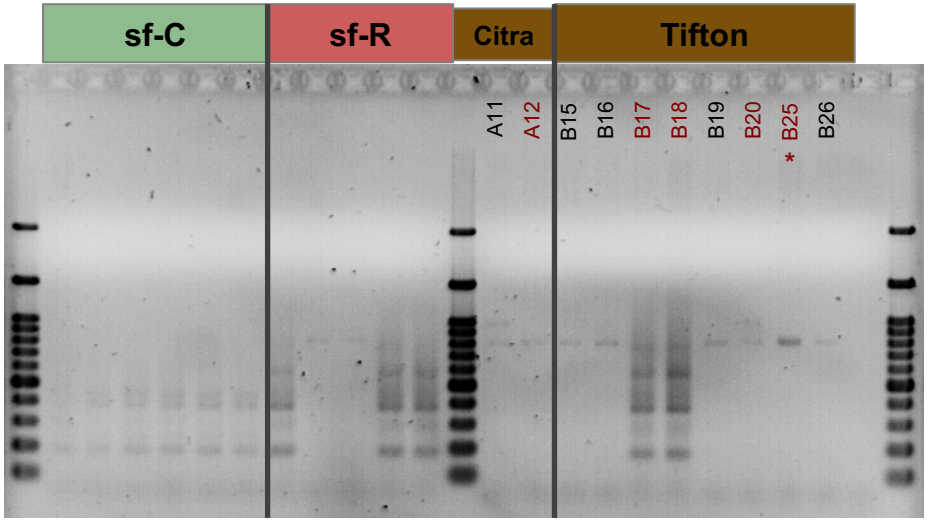

Citra
Tifton
Jacksonville
A11
A11
A12
B15
B16
B17
B18
B19
B20
B25
B26
B1
B2
B3
B4
B5
R

FIGURE S10

A



Tpi: 800bp

B

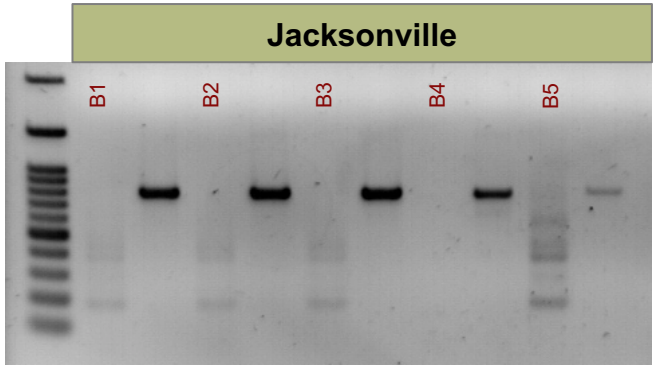
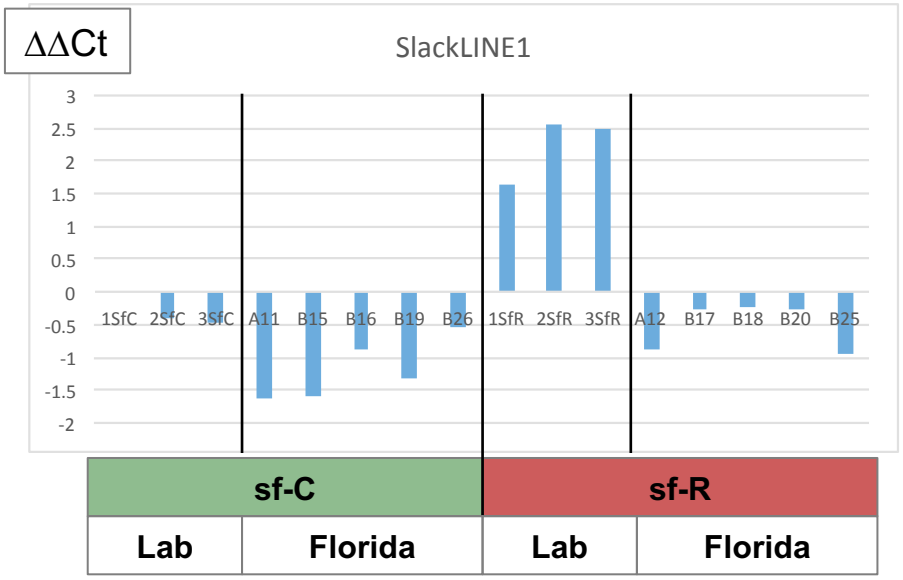


FIGURE S11


ΔΔCt
SlackLINE1
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
1SfC 2SfC 3SfC A11 B15 B16 B19 B26 1SfR 2SfR 3SfR A12 B17 B18 B20 B25
sf-C
sf-R
Lab
Florida
Lab
Florida

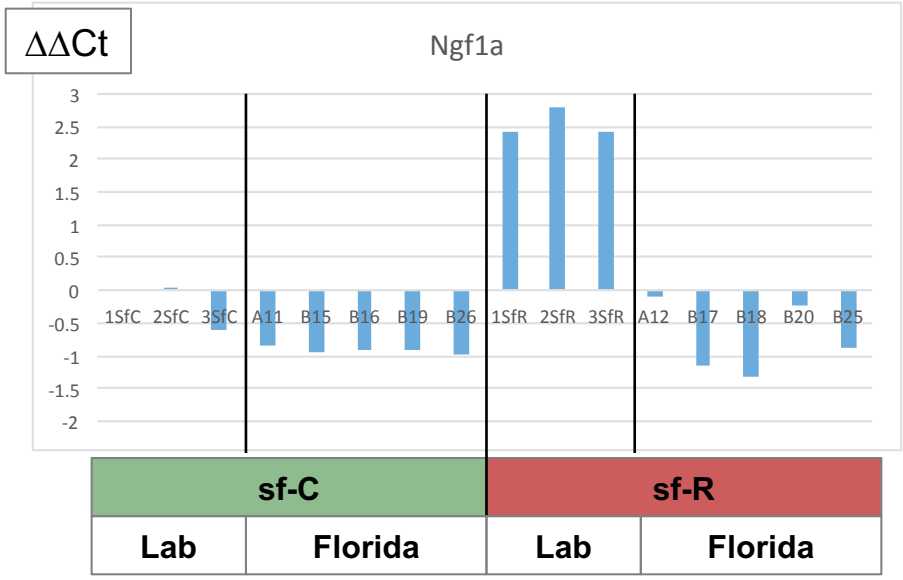
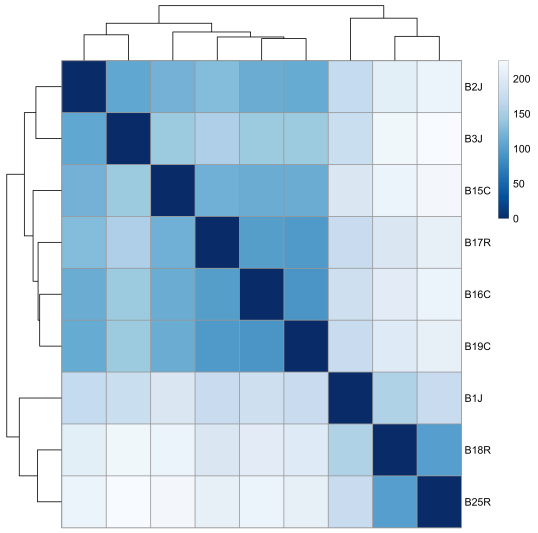

ΔΔCt
Ngf1a
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
1SfC 2SfC 3SfC A11 B15 B16 B19 B26 1SfR 2SfR 3SfR A12 B17 B18 B20 B25
sf-C
sf-R
Lab
Florida
Lab
Florida

FIGURE S12

A

B



C

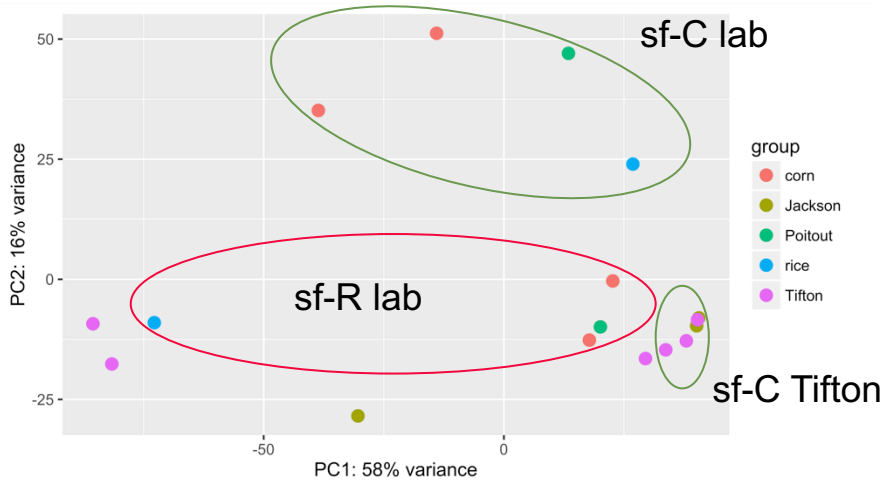
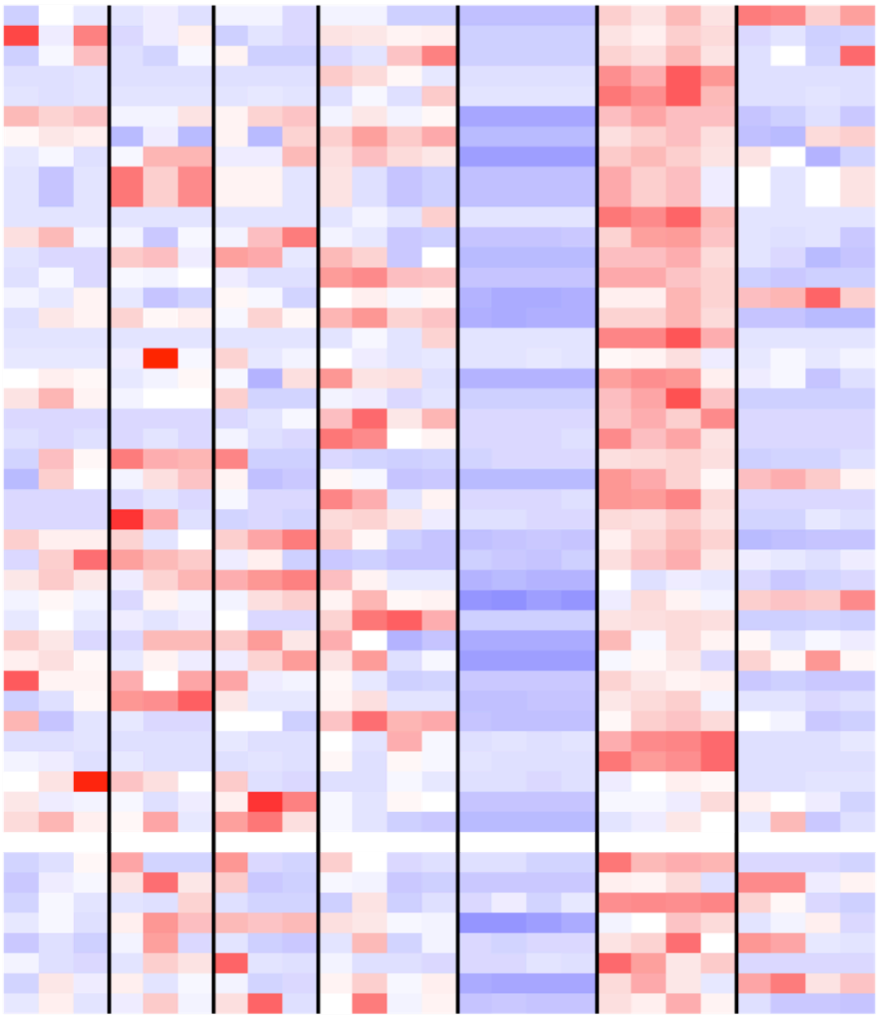
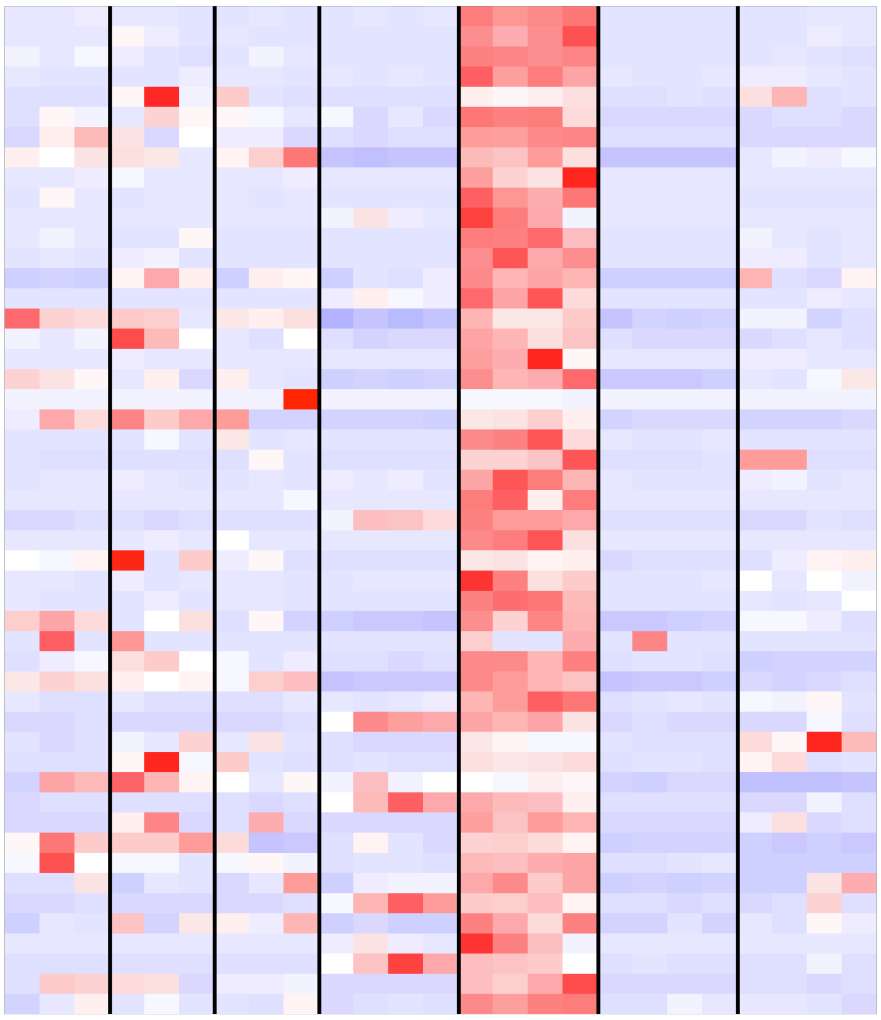


FIGURE S13



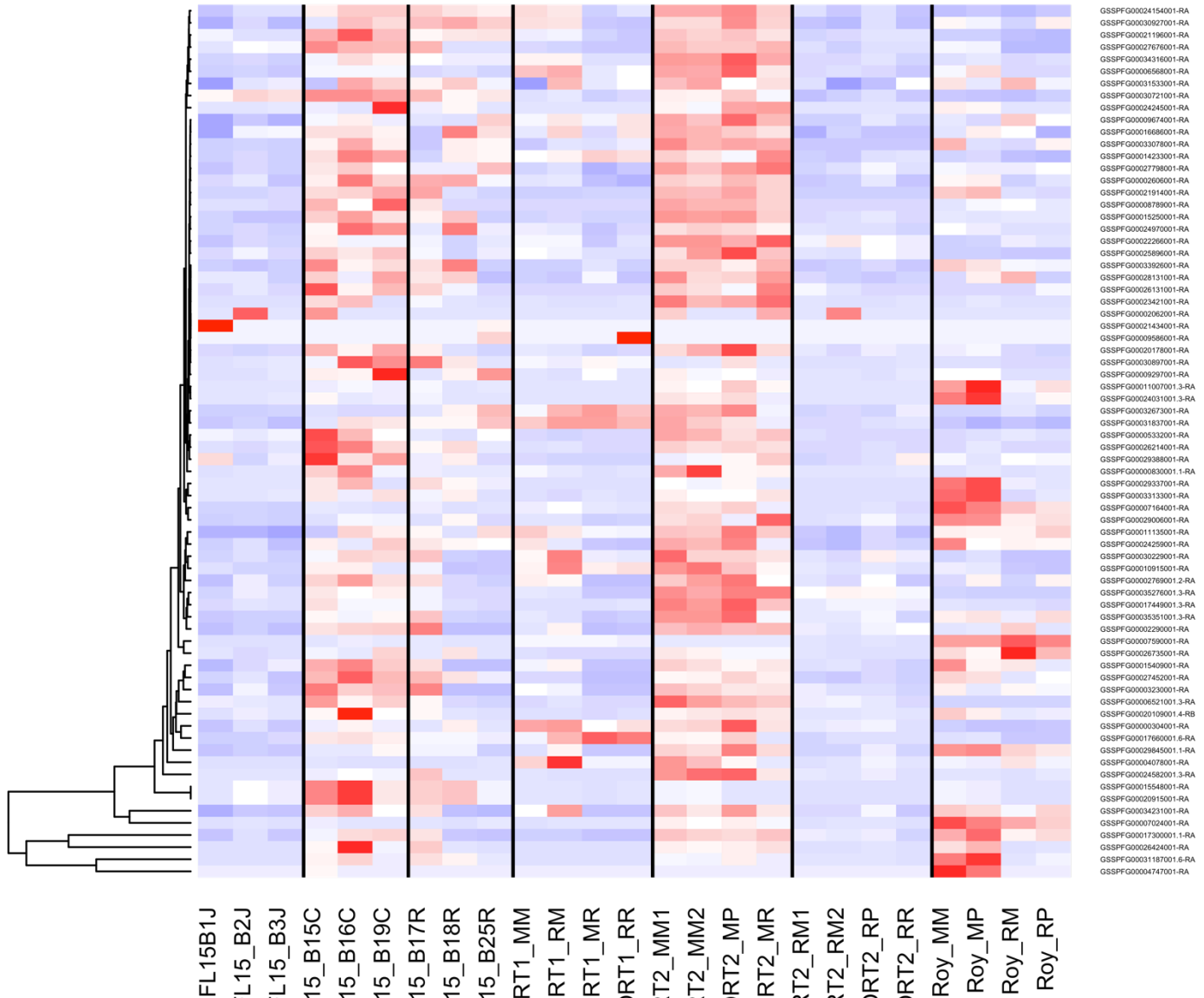
GSSPFG00010063001-RA	xaa-Pro aminopeptidase 1-like
GSSPFG00022625001-RA	NA
GSSPFG00004617001-RA	Uncharacterized membrane associated protein
GSSPFG00024658001-RA	Uncharacterized protein
GSSPFG00006224001-RA	Unknown Smc-like protein
GSSPFG00029999001-RA	Protein kinase C-like, phorbol ester/diacylglycerol-binding
GSSPFG00028883001-RA	Ubiquitin-conjugating enzyme E2
GSSPFG00003434001-RA	Uncharacterized membrane associated protein
GSSPFG00029716001.2-RA	**Fatty Acid Binding Protein 2**
GSSPFG00034702001-RA	Fatty Acid Binding Protein 2
GSSPFG00024077001-RA	Unknown Smc-like protein
GSSPFG00008178001-RA	Ecdysteroid kinase
GSSPFG00009245001-RA	apyrase
GSSPFG00010065001-RA	Uncharacterized secreted protein
GSSPFG00019823001-RA	Uncharacterized membrane associated protein
GSSPFG00022152001-RA	Glycosyl hydrolase family 47
GSSPFG00017532001-RA	Unknown Smc-like protein
GSSPFG00031881001.1-RA	**UGT33-11**
GSSPFG00034426001-RA	NADH:ubiquinone oxidoreductase subunit B14.5a
GSSPFG00024881001-RA	adenylate cyclase/proton-coupled folate transporter
GSSPFG00006331001.1-RA	NGFI-A-binding protein
GSSPFG00025955001-RA	TE
GSSPFG00007887001-RA	GMC oxidoreductase
GSSPFG00019552001-RA	pancreatic lipase
GSSPFG00025957001-RA	TE
GSSPFG00029884001-RA	NA
GSSPFG00015107001-RA	Glucose/ribitol dehydrogenase family
GSSPFG00004160001-RA	seminal fluid Aldo/keto reductase
GSSPFG00009249001-RA	kurz homolog
GSSPFG00025226001-RA	U1 small nuclear ribonucleoprotein C [SNRPC]
GSSPFG00021627001-RA	Chymotrypsin serine protease family (S1
GSSPFG00019502001-RA	U3 small nucleolar RNA-associated protein 10
GSSPFG00022995001-RA	RING finger protein, mdlc
GSSPFG00019425001-RA	alpha-tocopherol transfer protein
GSSPFG00024304001-RA	Transmembrane proteins 14C
GSSPFG00019445001-RA	Uncharacterized membrane associated protein
GSSPFG00032667001-RA	zinc finger protein weckle-like
GSSPFG00028547001-RA	zinc finger protein weckle-like
GSSPFG00023392001-RA	SP208
GSSPFG00020932001-RA	Growth-Arrest-Specific Protein 2
GSSPFG00009246001-RA	apyrase
GSSPFG00020720001-RA	fatty acyl-CoA reductase
GSSPFG00034771001-RA	fatty acyl-CoA reductase
GSSPFG00026559001-RA	Glucose/ribitol dehydrogenase family
GSSPFG00000341001-RA	NA
GSSPFG00015363001-RA	Calcineurin
GSSPFG00019185001.2-RA	**CYP9APARTIAL**
GSSPFG00008822001-RA	Cuticular protein
GSSPFG00008858001.3-RA	**Death-related protein**
GSSPFG00006953001-RA	alcohol dehydrogenase

FIGURE S14



GSSPFG00009092001-RA	TE
GSSPFG00021758001-RA	TE
GSSPFG00000055001-RA	NA
GSSPFG00015492001-RA	TE
GSSPFG00026424001-RA	Cytochrome P450
GSSPFG00028010001-RA	carboxypeptidase Q-like
GSSPFG00004899001-RA	NA
GSSPFG00027485001-RA	Uncharacterized protein
GSSPFG00033999001-RA	Uncharacterized protein
GSSPFG00018415001-RA	Uncharacterized protein
GSSPFG00012179001.1-RA	fatty-acid synthase
GSSPFG00003930001-RA	NA
GSSPFG00002576001-RA	TE
GSSPFG00033078001-RA	TE
GSSPFG00009529001-RA	CUB domain peptidase
GSSPFG00009290001-RA	TE
GSSPFG00005332001-RA	ATP-dependent DNA helicase PIF1
GSSPFG00033049001-RA	TE
GSSPFG00005622001-RA	nuclear pore complex protein Nup88
GSSPFG00030212001-RA	TE
GSSPFG00005743001-RA	Uncharacterized protein
GSSPFG00021504001-RA	TE
GSSPFG00009526001-RA	NA
GSSPFG00025034001-RA	TE
GSSPFG00008862001-RA	Harbinger transposase-derived nuclease, HARBI1
GSSPFG00023363001-RA	CG8420
GSSPFG00017311001-RA	TE
GSSPFG00013057001-RA	Uncharacterized protein
GSSPFG00027329001-RA	NA
GSSPFG00021422001-RA	NA
GSSPFG00029033001-RA	TE
GSSPFG00002062001-RA	mitochondrial insertion
GSSPFG00027050001.2-RA	**apterous 1**
GSSPFG00027757001-RA	Uncharacterized protein
GSSPFG00006076001-RA	ras-related GTPase Rap-2
GSSPFG00025780001-RA	CG8420
GSSPFG00026735001-RA	NA
GSSPFG00008269001.4-RA	**CYP9A31PARTIAL**
GSSPFG00025492001-RA	Histidine phosphatase
GSSPFG00003829001-RA	zona pellucida (ZP) domain secreted eukaryotic glycoproteins
GSSPFG00025374001-RA	Endonuclease/Exonuclease/phosphatase
GSSPFG00011985001-RA	Uncharacterized protein
GSSPFG00007980001-RA	Uncharacterized protein
GSSPFG00012726001-RA	TE
GSSPFG00018593001-RA	NA
GSSPFG00019843001-RA	Harbinger transposase-derived nuclease, HARBI1
GSSPFG00027466001-RA	**fatty acid synthase**
GSSPFG00003828001-RA	divergent subfamily of APPLE domains (Zona pellucida (ZP) domain
GSSPFG00026205001-RA	Histidine phosphatase
GSSPFG00006538001-RA	dsRNAse

FIGURE S15


FL15B1J
FL15_B2J
FL15_B3J
FL15_B15C
FL15_B16C
FL15_B19C
FL15_B17R
FL15_B18R
FL15_B25R
MORT1_MM
MORT1_RM
MORT1_MR
MORT1_RR
MORT2_MM1
MORT2_MM2
MORT2_MP
MORT2_MR
MORT2_RM1
MORT2_RM2
MORT2_RP
MORT2_RR
Roy_MM
Roy_MP
Roy_RM
Roy_RP
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GSSPFG00021196001-RA
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GSSPFG00006568001-RA
GSSPFG00031533001-RA
GSSPFG00030721001-RA
GSSPFG00024245001-RA
GSSPFG00009674001-RA
GSSPFG00016686001-RA
GSSPFG00033078001-RA
GSSPFG00014233001-RA
GSSPFG00027798001-RA
GSSPFG00002606001-RA
GSSPFG00021914001-RA
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GSSPFG00015250001-RA
GSSPFG00024970001-RA
GSSPFG00022266001-RA
GSSPFG00025896001-RA
GSSPFG00033926001-RA
GSSPFG00028131001-RA
GSSPFG00026131001-RA
GSSPFG00023421001-RA
GSSPFG00002062001-RA
GSSPFG00021434001-RA
GSSPFG00009586001-RA
GSSPFG00020178001-RA
GSSPFG00030897001-RA
GSSPFG00009297001-RA
GSSPFG00011007001.3-RA
GSSPFG00024031001.3-RA
GSSPFG00032673001-RA
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GSSPFG00005332001-RA
GSSPFG00026214001-RA
GSSPFG00029388001-RA
GSSPFG00000830001.1-RA
GSSPFG00029337001-RA
GSSPFG00033133001-RA
GSSPFG00007164001-RA
GSSPFG00029006001-RA
GSSPFG00011135001-RA
GSSPFG00024259001-RA
GSSPFG00030229001-RA
GSSPFG00010915001-RA
GSSPFG00002769001.2-RA
GSSPFG00035276001.3-RA
GSSPFG00017449001.3-RA
GSSPFG00035351001.3-RA
GSSPFG00002290001-RA
GSSPFG00007590001-RA
GSSPFG00026735001-RA
GSSPFG00015409001-RA
GSSPFG00027452001-RA
GSSPFG00003230001-RA
GSSPFG00006521001.3-RA
GSSPFG00020109001.4-RB
GSSPFG00000304001-RA
GSSPFG00017660001.6-RA
GSSPFG00029845001.1-RA
GSSPFG00004078001-RA
GSSPFG00024582001.3-RA
GSSPFG00015548001-RA
GSSPFG00020915001-RA
GSSPFG00034231001-RA
GSSPFG00007024001-RA
GSSPFG00017300001.1-RA
GSSPFG00026424001-RA
GSSPFG00031187001.6-RA
GSSPFG00004747001-RA

FIGURE S16

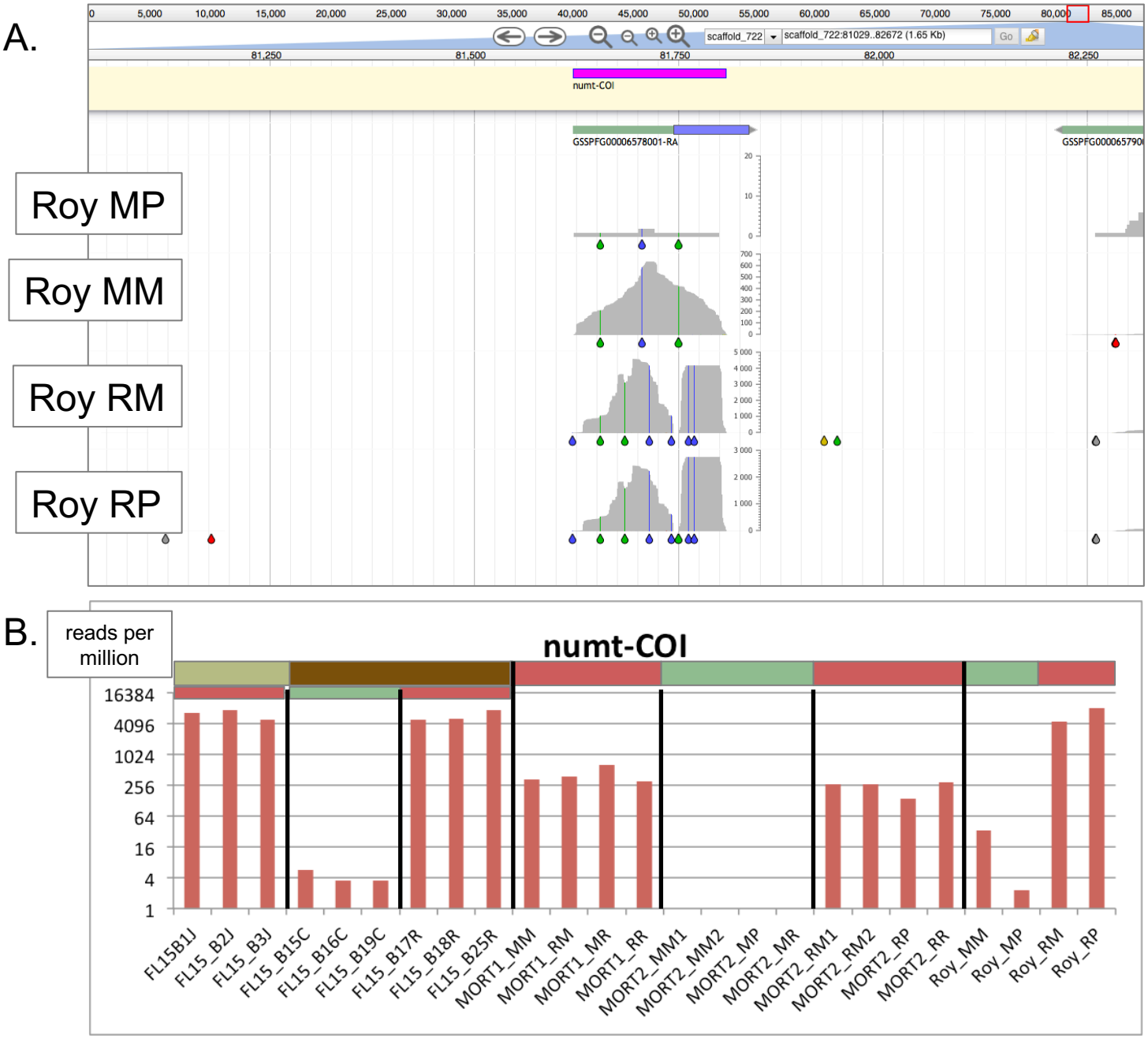
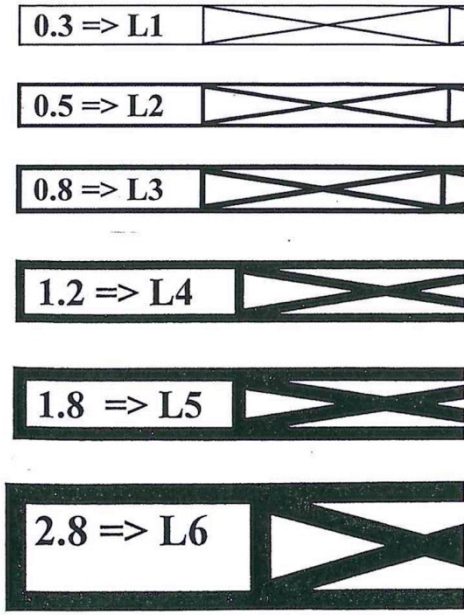

A.
Roy MP
Roy MM
Roy RM
Roy RP
numt-COI
GSSPFG00006578001-RA
B.
reads per million
numt-COI
16384
4096
1024
256
64
16
4
1
FL15B1J
FL15_B2J
FL15_B3J
FL15_B15C
FL15_B16C
FL15_B19C
FL15_B17R
FL15_B18R
FL15_B25R
MORT1_MM
MORT1_RM
MORT1_MR
MORT1_RR
MORT2_MM1
MORT2_MM2
MORT2_MP
MORT2_MR
MORT2_RM1
MORT2_RM2
MORT2_RP
MORT2_RR
Roy_MM
Roy_MP
Roy_RM
Roy_RP

FIGURE S17

A.



B.

Table S1

				Bowtie2 alignment			
sample	Strain	Diet	Total_reads	aligned 0 times	aligned exactly 1 time	aligned > 1 times	fraction_mapped
RT experiment							
MORT2_MM1	sf-C	Corn	67150390	18011798 (26.82%)	30746676 (45.79%)	18391916 (27.39%)	73.18%
MORT2_MM2	sf-C	Corn	43617452	11210535 (25.70%)	20344243 (46.64%)	12062674 (27.66%)	74.30%
MORT2_MP	sf-C	Poitout	31801441	8475906 (26.65%)	14912937 (46.89%)	8412598 (26.45%)	73.35%
MORT2_MR	sf-C	Rice	48323710	12899202 (26.69%)	22473995 (46.51%)	12950513 (26.80%)	73.31%
MORT2_RM1	sf-R	Corn	33742585	8997746 (26.67%)	15013813 (44.50%)	9731026 (28.84%)	73.33%
MORT2_RM2	sf-R	Corn	35347649	9334932 (26.41%)	15943900 (45.11%)	10068817 (28.49%)	73.59%
MORT2_RP	sf-R	Poitout	63139685	19832315 (31.41%)	27649786 (43.79%)	15657584 (24.80%)	68.59%
MORT2_RR	sf-R	Rice	70682628	21958766 (31.07%)	32163009 (45.50%)	16560853 (23.43%)	68.93%
Second generation RT experiment							
MORT1_MM	sf-R	Corn	36304954	9214731 (25.38%)	20459886 (56.36%)	6630337 (18.26%)	74.62%
MORT1_MR	sf-R	Rice	46719601	12461844 (26.67%)	27211195 (58.24%)	7046562 (15.08%)	73.33%
MORT1_RM	sf-R	Corn	41858774	9927539 (23.72%)	24195882 (57.80%)	7735353 (18.48%)	76.28%
MORT1_RR	sf-R	Rice	37354506	8593642 (23.01%)	22722574 (60.83%)	6038290 (16.16%)	76.99%
Natural populations							
FL15_B15C	sf-C	Corn	58940405	27961728 (47.44%)	23352881 (39.62%)	7625796 (12.94%)	52.56%
FL15_B16C	sf-C	Corn	74388159	28113552 (37.79%)	37128525 (49.91%)	9146082 (12.30%)	62.21%
FL15_B19C	sf-C	Corn	33627219	12941686 (38.49%)	16468020 (48.97%)	4217513 (12.54%)	61.51%
FL15_B17R	sf-R	Corn	39842098	15290479 (38.38%)	19573496 (49.13%)	4978123 (12.49%)	61.62%
FL15_B18R	sf-R	Corn	78623719	28419568 (36.15%)	41790045 (53.15%)	8414106 (10.70%)	63.85%
FL15_B25R	sf-R	Corn	23392758	8936331 (38.20%)	12240977 (52.33%)	2215450 (9.47%)	61.80%
FL15_B2J	sf-R	Grass	33537139	12307865 (36.70%)	17130166 (51.08%)	4099108 (12.22%)	63.30%
FL15_B3J	sf-R	Grass	42191185	16293397 (38.62%)	20834698 (49.38%)	5063090 (12.00%)	61.38%
FL15_B1J	sf-R	Grass	24904583	10145929 (40.74%)	11875708 (47.68%)	2882946 (11.58%)	59.26%

Table S2

OGS2.2	Annotation	Abbreviation	log2FC	ΔΔCt
GSSPFG00029721001-RA	S01.UNA + repeat motif	501VNA	4.841451941	3.362002334
GSSPFG00024881001-RA	adenylate cyclase	adenylate cyclase	4.636752333	1.816379812
GSSPFG00010063001-RA	xaa-Pro aminopeptidase 1-like	aminopeptidase	5.128397542	2.967979508
GSSPFG00035209001.5-RA	carboxylesterase 016c	carboxylesterase	6.776645953	2.548145672
GSSPFG00004817001.2-RA	Polycalin1_other-exons	cohesin4817	7.782372143	2.582019629
GSSPFG00031119001.2-RA	CYP340L	CYP	6.046285317	6.149297371
GSSPFG00017290001.2-RC	CYP340L1	CYP340L1	5.379910588	3.556942464
GSSPFG00002985001-RA	delta-24-sterol reductase	d245reductase	6.111188925	2.522842306
GSSPFG00029999001-RA	DEF8	Def8	4.771051161	2.347034643
GSSPFG00031106001.2-RA	DUF4602; C1orf131 homolog	DUF4601	6.932457522	1.390933337
GSSPFG00002727001-RA	Lipocalin - nitrobinding domain - DUF1794 protein	DVF1794	5.584081573	12.00773469
GSSPFG00029716001.2-RA	FABP	FABP10	6.361577058	1.334187653
GSSPFG00034702001-RA	FABP	FABP12	6.605986931	1.368224598
GSSPFG00020720001-RA	FAR	FAR-X	5.826344504	1.674146
GSSPFG00018006001-RA	Glycogen synthase	glyc synt	5.635375812	2.062591316
GSSPFG00024097001-RA	Hemicentin 2	hemicentin2	5.658948506	3.155072607
GSSPFG00006331001.1-RA	NGFI-A-binding protein	Ngf1a	5.296521038	2.729999007
GSSPFG00008932001-RA	intraflagellar transport protein 52 homolog isoform X2	p52	5.109329722	2.97790438
GSSPFG00022903001-RA	Peroxidase	peroxydase	5.70457573	-0.42053074
GSSPFG00020440001-RA	Polycalin1	polycalin	5.257370575	1.437914219
GSSPFG00035966001.2-RB	Polycalin1	polycalin1p3	7.373488645	1.753858172
GSSPFG00002897001-RA	putative inorganic phosphate cotransporter	Ptransporter	5.338207499	0.85946203
GSSPFG00014224001-RA	Rpb8	rbp8	5.334190603	1.498294088
GSSPFG00019426001-RA	phosphatidylinositol transfer protein (Sec14p)	Sec14P	5.052587086	0.438937448
GSSPFG00025955001-RA	Slack-LINE1	SlackLINE1	7.55220001	2.518786492
GSSPFG00025956001-RA	Slack-LINE2	SlackLINE2	8.408523346	3.316992707
GSSPFG00017532001-RA	putative cohesin	smc2	7.417473777	9.842471525
GSSPFG00004617001-RA	UGT33-11	UGT3311	8.168349428	4.136506264
GSSPFG00035441001.3-RA	UDP-glycosyltransferase-33-23	UGT3323	5.473349608	2.391054548
GSSPFG00031881001.1-RA	UDP-glycosyltransferase 33J2	UGT33J2	8.291597518	3.633597123

Table S3

OGS2.2	Abbreviation	Primer Orientation	Primer Seq	Primer Orientation	Primer Seq	Prod Size
GSSPFG00029721001-RA	501VNA	FORWARD	CCAAGGAACTGATGGATTGG	REVERSE	GGGATCATGACAGAGGACACA	56
GSSPFG00024881001-RA	adenylate cyclase	FORWARD	CACGGTGGACACACTACCAG	REVERSE	TCATAACCCCTCCCAGCATA	50
GSSPFG00010063001-RA	aminopeptidase	FORWARD	ACTGGACGCAATTTGAGGAG	REVERSE	GCTTCATCAGCTTCCAGAGG	54
GSSPFG00035209001.5-RA	carboxylesterase	FORWARD	TTGTGATACCTGGCGATGAA	REVERSE	GGGGGTGTAGACATTGAGGA	50
GSSPFG00004817001.2-RA	cohesin4817	FORWARD	CGGGTGTTCCTGGAGAATTA	REVERSE	TCGACTGTGCATCATTGGAT	51
GSSPFG00031119001.2-RA	CYP	FORWARD	GGGGTTTGATCGCTCATCTA	REVERSE	CGTCAAATGGCTCTTTACCC	51
GSSPFG00017290001.2-RC	CYP340L1	FORWARD	TTAAACCGGAGCGATGGTTA	REVERSE	GCATTCGGGTTTTCTGGTAA	52
GSSPFG00002985001-RA	d245reductase	FORWARD	ATCATCGTGATGGTGGCTCT	REVERSE	CCAGATCTTCCAAACCAAGG	51
GSSPFG00029999001-RA	Def8	FORWARD	GTGCCAAACCGCATTAACTT	REVERSE	ATAATCGCGGTTCATTCCAC	50
GSSPFG00031106001.2-RA	DUF4601	FORWARD	GTTTGGAATGTCGGGTTTTG	REVERSE	CTATCCGCGCTTCTTCTTTC	50
GSSPFG00002727001-RA	DVF1794	FORWARD	ATCAAACCTGGAACGAACGA	REVERSE	GCCCATGTTATGACTGACGA	51
GSSPFG00029716001.2-RA	FABP10	FORWARD	GTGTCCCCGATGACAAGATT	REVERSE	TCTGGTCTGGGGTGTAGCTC	51
GSSPFG00034702001-RA	FABP12	FORWARD	GTGTCCCCGATGACAAGATT	REVERSE	TCTGGTCTGGGGTGTAGCTC	51
GSSPFG00020720001-RA	FAR-X	FORWARD	CGGAGCTACCGTATTCCTGA	REVERSE	TGAGCTGCTTCCCAAGAAAT	53
GSSPFG00018006001-RA	glyc synt	FORWARD	GCTCCGACATGACAGTGGTA	REVERSE	TATTCGTCTTGGCAGGGAAG	51
GSSPFG00024097001-RA	hemicentin2	FORWARD	TGTGGTGCTGAAGAACACCT	REVERSE	TGGGCCCATATTTCCTATCA	50
GSSPFG00006331001.1-RA	Ngf1a	FORWARD	TTAATAACCCCGCCCTTTTC	REVERSE	CAGTTGGGCAGAGGTTAGGA	54
GSSPFG00008932001-RA	p52	FORWARD	ATCCAAAAGAATGCCACGTC	REVERSE	GGTGACGGCTCGGTTTAGTA	50
GSSPFG00022903001-RA	peroxydase	FORWARD	TAGCGCAATCTGGTGATGAG	REVERSE	GGTTGAGACGGACGGTTCTA	51
GSSPFG00020440001-RA	polycalin	FORWARD	GGGCCAAACGATTGTTTCTA	REVERSE	TATTGCCATGTCGGATCAAA	50
GSSPFG00035966001.2-RB	polycalin1p3	FORWARD	TGGTGGTGGCATCTCAGTAA	REVERSE	CGTTGCAAGTCTTTGGTTCA	55
GSSPFG00002897001-RA	Ptransporter	FORWARD	TCCAATTCTACTGAAGCCAGAG	REVERSE	TTACATCCTCAGCTCTTTCTACG	52
GSSPFG00014224001-RA	rbp8	FORWARD	AATGGCCGGTGTATTATTCG	REVERSE	CCGGGTCAATATCTTTCACG	53
GSSPFG00019426001-RA	Sec14P	FORWARD	ACCGCTGTTCCAAATTTCAT	REVERSE	TCCTAACGTCAAAACAGCTGAA	51
GSSPFG00025955001-RA	SlackLINE1	FORWARD	GGAGAAGGGTGGCAAAAGAT	REVERSE	GGCCTCCTCTAACGACTTCC	50
GSSPFG00025956001-RA	SlackLINE2	FORWARD	CCCCAACAGAGAAAGATCCA	REVERSE	TTGTGCATAGAATGGCCTTG	50
GSSPFG00017532001-RA	smc2	FORWARD	CCATGGCCAATGGTATTAGG	REVERSE	CATCACCTGTTTCCTCGACA	53
GSSPFG00004617001-RA	UGT3311	FORWARD	GGTGTTGCAAAAATGGGATT	REVERSE	CACGAGTCCAACCAAAACAA	57
GSSPFG00035441001.3-RA	UGT3323	FORWARD	CAGTTCCTTTGGTGGAGCTT	REVERSE	CTGAAGCGCCAATATTCTCA	50
GSSPFG00031881001.1-RA	UGT33J2	FORWARD	CTCTGGAAGTGGGACAAGGA	REVERSE	TCTGATGTTCGCTGATTTGC	51

Table S4 – Manual annotation of the 50 genes with the most constitutive sf-R associated expression

OGS2.2	baseMean	log2FoldChange	padj	scaffold	start	end	strand	Annotation	Best Homology	InterPro
GSSPFG00012499001-RA	41.438	5.356	9.1E-17	scaffold_24562	2044	2184	PLUS	Partial peptidase S1A, chymotrypsin family	>XP_022827099.1 uncharacterized protein LOC111356844 [Spodoptera litura]	IPR009003 Peptidase S1, PA clan
GSSPFG00017312001-RA	72.309	2.778	1.9E-11	scaffold_5799	9191	11743	PLUS	TE	gi|1573721284|ref|XP_028042925.1| (piggyBac transposable element-derived protein 4-like isoform X2 [Bombyx	PiggyBac transposable element-derived protein
GSSPFG00006331001.1-RA	38.630	4.966	3.7E-09	superscaffold_207	307369	312517	PLUS	NGFI-A-binding protein	gi|199392082|ref|XP_021191057.1| (NGFI-A-binding protein homolog [Helicoverpa armigera])	NGFI-A BINDING PROTEIN
GSSPFG00033823001-RA	102.169	1.554	2.5E-08	scaffold_665	62519	63360	PLUS	NA	/	/
GSSPFG00033815001.4-RA	1204.896	2.556	3.6E-07	scaffold_665	1731	7761	PLUS	**PGRP**	gi|1274144291|ref|XP_022832520.1| (peptidoglycan recognition protein-like isoform X1 [Spodoptera litura])	Peptidoglycan recognition protein
GSSPFG00006224001-RA	277.127	3.794	4.2E-07	scaffold_8364	313	2849	PLUS	**Unknown Smc-like protein**	gi|1274132455|ref|XP_022830871.1| (coiled-coil domain-containing protein 40 isoform X2 [Spodoptera litura])	COILED-COIL DOMAIN-CONTAINING PROTEIN 40
GSSPFG00016090001-RA	179.903	3.750	2.3E-06	superscaffold_67	8782	9731	MINUS	alpha-tocopherol transfer protein	gi|1274144492|ref|XP_022832628.1| (alpha-tocopherol transfer protein-like [Spodoptera litura])	CRAL/TRIO N-terminal domain
GSSPFG00012333001-RA	202.416	4.183	2.6E-06	scaffold_419	24249	27320	MINUS	Fatty acyl-CoA reductase	gi|1274118142|ref|XP_022824237.1| (putative fatty acyl-CoA reductase CG5065 [Spodoptera litura])	Fatty acyl-CoA reductase
GSSPFG00004574001-RA	32.459	4.934	3.1E-06	scaffold_965	16341	25645	PLUS	**Serine protease. S01.034: transmembrane peptidase, serine 4**	gi|1274124282|ref|XP_022826384.1| (transmembrane protease serine 9-like [Spodoptera litura])	Peptidase S1A, chymotrypsin family
GSSPFG00018418001-RA	120.502	3.447	7.3E-06	scaffold_10763	1141	1281	PLUS	numt_COI_ND4	/	/
GSSPFG00008178001-RA	57.597	2.801	8.8E-06	scaffold_11019	4453	5244	PLUS	Ecdysteroid kinase	gi|1274103665|ref|XP_022837597.1| (uncharacterized protein LOC111364787 isoform X1 [Spodoptera litura])	Ecdysteroid kinase-like
GSSPFG00025164001-RA	249.863	4.622	8.9E-06	scaffold_9398	824	2510	PLUS	NA	gi|1274098509|ref|XP_022834526.1| (uncharacterized protein LOC111362190 [Spodoptera litura])	/
GSSPFG00017532001-RA	49.167	4.954	1.1E-05	scaffold_42011	32	1151	PLUS	**Unknown Smc-like protein**	gi|1274132453|ref|XP_022830870.1| (coiled-coil domain-containing protein 40 isoform X1 [Spodoptera litura])	/
GSSPFG00011475001-RA	1468.212	4.561	1.1E-05	superscaffold_515	270856	300209	MINUS	NA	gi|1274125088|ref|XP_022826827.1| (PAX-interacting protein 1-like [Spodoptera litura])	/
GSSPFG00030114001-RA	86.306	3.734	1.2E-05	scaffold_35751	459	1476	MINUS	alpha-tocopherol transfer protein	gi|1274144492|ref|XP_022832628.1| (alpha-tocopherol transfer protein-like [Spodoptera litura])	RETINALDEHYDE BINDING PROTEIN-RELATED
GSSPFG00015325001-RA	21.918	3.405	1.2E-05	scaffold_1961	38313	40593	PLUS	TE	gi|1486920932|ref|XP_026493425.1| (piggyBac transposable element-derived protein 2-like [Vanessa tameamea	PiggyBac transposable element-derived protein: DDE_Tnp_1_7
GSSPFG00019510001-RA	205.546	1.024	1.3E-05	scaffold_18956	70	1750	MINUS	Uncharacterized BTP/POZ transcription factor	gi|1274098409|ref|XP_022834473.1| (uncharacterized protein LOC111362155 [Spodoptera litura])	SKP1/BTB/POZ domain superfamily
GSSPFG00011838001-RA	2475.550	2.620	2.1E-05	scaffold_4541	7397	12803	PLUS	clavesin	gi|1274145132|ref|XP_022832976.1| (clavesin-2-like [Spodoptera litura])	CRAL-TRIO lipid binding domain superfamily
GSSPFG00024658001-RA	92.976	4.375	2.1E-05	scaffold_517	12864	14446	PLUS	Uncharacterized protein; s_517	gi|1549086025|gb|RVE41430.1| (hypothetical protein evm_013924, partial [Chilo suppressalis])	/
GSSPFG00021956001-RA	202.072	0.898	3.7E-05	scaffold_15156	3941	6160	PLUS	Tbk1 kinase	gi|1274100408|ref|XP_022835575.1| (LOW QUALITY PROTEIN: serine/threonine-protein kinase TBK1 [Spodopter	TANK binding kinase 1, ubiquitin-like domain
GSSPFG00014620001-RA	5.207	3.972	4.9E-05	superscaffold_813	83352	84278	PLUS	Zinc-finger protein	gi|1496238390|ref|XP_026745493.1| (uncharacterized protein LOC113506854 [Trichoplusia ni])	FYVE/PHD zinc finger + Baculovirus FP protein
GSSPFG00025955001-RA	122.996	4.151	5.2E-05	scaffold_183	169301	170656	MINUS	Slack_LINE1	gi|298204367|gb|ADI61832.1| (endonuclease-reverse transcriptase [Bombyx mori])	Reverse transcriptase domain
GSSPFG00007463001-RA	263.490	1.755	7.8E-05	scaffold_14772	1486	2878	MINUS	Ecdysteroid kinase	gi|1274103669|ref|XP_022837613.1| (uncharacterized protein LOC111364787 isoform X3 [Spodoptera litura])	Ecdysteroid kinase-like
GSSPFG00006526001-RA	90.653	1.572	1.1E-04	scaffold_19200	833	3186	PLUS	Protein artichoke	gi|1274104572|ref|XP_022816805.1| (protein artichoke [Spodoptera litura])	Leucine-rich repeat domain superfamily
GSSPFG00011681001.1-RA	264.791	3.249	1.1E-04	superscaffold_608	9468	12872	PLUS	Glucose dehydrogenase	gi|1274137345|ref|XP_022837607.1| (glucose dehydrogenase [FAD, quinone]-like isoform X2 [Spodoptera litura])	FAD/NAD(P)-binding domain superfamily - Glucose-methanol-choline oxidoreductase
GSSPFG00028883001-RA	307.920	2.094	1.1E-04	scaffold_2806	24600	28945	MINUS	Ubiquitin-conjugating enzyme E2	gi|1274144092|ref|XP_022832411.1| (ubiquitin-conjugating enzyme E2-22 kDa [Spodoptera litura])	Ubiquitin-conjugating enzyme E2
GSSPFG00017010001-RA	451.199	0.942	1.1E-04	scaffold_1685	43159	53355	MINUS	Rho guanine nucleotide exchange factor	gi|1496285056|ref|XP_026732580.1| (rho guanine nucleotide exchange factor 1-like isoform X1 [Trichoplusia ni]	Dbl homology (DH) domain
GSSPFG00013575001-RA	386.916	3.999	1.6E-04	scaffold_4472	2	8543	PLUS	**takeout**	gi|1275386485|gb|ATU07277.1| (takeout [Spodoptera litura])	Haemolymph juvenile hormone binding
GSSPFG00017882001.1-RA	916.677	3.863	2.4E-04	superscaffold_306	11517	13529	MINUS	yellow h2	gi|1274099564|ref|XP_022835105.1| (protein yellow-like, partial [Spodoptera litura])	Major royal jelly protein/protein yellow
GSSPFG00002468001-RA	328.535	3.930	2.4E-04	scaffold_190	16628	20217	MINUS	**takeout**	gi|1275386485|gb|ATU07277.1| (takeout [Spodoptera litura])	Haemolymph juvenile hormone binding
GSSPFG00026628001-RA	581.378	1.312	2.6E-04	superscaffold_106	38580	40189	PLUS	/	gi|1274098298|ref|XP_022834413.1| (uncharacterized protein LOC111362112 [Spodoptera litura])	/
GSSPFG00010450001-RA	52.899	2.135	2.6E-04	scaffold_1076	74513	75201	PLUS	NA	/	TM domain
GSSPFG00001797001-RA	873.270	2.536	2.7E-04	superscaffold_345	138911	146770	MINUS	endocuticle structural glycoprotein	gi|1274103967|ref|XP_022814927.1| (endocuticle structural glycoprotein SgAbd-3-like [Spodoptera litura])	Insect cuticle protein - Chitin-binding type R&R consensus
GSSPFG00007187001-RA	57.501	1.086	3.1E-04	superscaffold_259	121981	124396	MINUS	islet cell autoantigen	gi|1274131622|ref|XP_022830425.1| (islet cell autoantigen 1 [Spodoptera litura])	Arfaptin homology (AH) domain
GSSPFG00034405001-RA	3934.962	4.139	3.2E-04	scaffold_899	32079	34798	PLUS	neurofilament heavy polypeptide	gi|1274110658|ref|XP_022820115.1| (neurofilament heavy polypeptide isoform X1 [Spodoptera litura])	TM domain
GSSPFG00028547001-RA	13.673	3.778	3.2E-04	scaffold_1264	68168	71148	PLUS	Sp3-like glucocorticoid receptor	gi|1274113568|ref|XP_022821723.1| (transcription factor Sp3-like [Spodoptera litura])	Glucocorticoid receptor-like (DNA-binding domain) + 3 Zinc finger C2H2-type
GSSPFG00031080001-RA	21.142	3.018	3.3E-04	scaffold_7275	3039	4697	PLUS	NA	/	TM domain
GSSPFG00011415001-RA	1042.695	0.686	3.3E-04	scaffold_8188	30	3176	MINUS	NA	gi|1274113154|ref|XP_022821497.1| (uncharacterized protein LOC111352977 [Spodoptera litura])	/
GSSPFG00008177001-RA	353.888	1.624	3.5E-04	scaffold_11019	1065	1859	PLUS	Ecdysteroid kinase	gi|1274103667|ref|XP_022837605.1| (uncharacterized oxidoreductase dhs-27-like isoform X2 [Spodoptera litura]	Ecdysteroid kinase-like
GSSPFG00030139001-RA	763.770	1.947	3.7E-04	scaffold_2510	37531	39530	PLUS	/	gi|1274140375|ref|XP_022814957.1| (uncharacterized protein LOC111348539 [Spodoptera litura])	/
GSSPFG00008472001-RA	190.464	3.679	3.9E-04	scaffold_7187	6435	7951	PLUS	/	gi|1486899259|ref|XP_026500633.1| (glycine-rich cell wall structural protein-like [Vanessa tameamea])	/
GSSPFG00034784001-RA	611.883	3.246	3.9E-04	scaffold_5	338897	350259	MINUS	Fatty acyl-CoA reductase	gi|1274118142|ref|XP_022824237.1| (putative fatty acyl-CoA reductase CG5065 [Spodoptera litura])	*Male_sterile_NAD-bd*
GSSPFG00012223001.1-RA	395.574	3.579	4.1E-04	superscaffold_596	31792	36073	MINUS	Reeler domain protein	gi|1274122069|ref|XP_022825175.1| (putative defense protein 3 [Spodoptera litura])	Reeler domain superfamily
GSSPFG00011683001.1-RA	56.502	3.550	4.5E-04	superscaffold_608	2521	6717	PLUS	glucose dehydrogenase	gi|1274137343|ref|XP_022837606.1| (glucose dehydrogenase [FAD, quinone]-like isoform X1 [Spodoptera litura])	Glucose-methanol-choline oxidoreductase
GSSPFG00027105001-RA	113.987	1.098	5.0E-04	superscaffold_658	48126	55291	PLUS	Broad complex core protein	gi|1274137725|ref|XP_022837815.1| (broad-complex core protein isoforms 1/2/3/4/5 isoform X3 [Spodoptera lit	BTB/POZ domain - Zinc finger C2H2-type
GSSPFG00004390001-RA	241.490	4.076	5.4E-04	scaffold_8617	4109	4848	MINUS	/	gi|1274138702|ref|XP_022814045.1| (uncharacterized protein LOC111347889 [Spodoptera litura])	/
GSSPFG00012336001-RA	884.031	3.154	6.2E-04	scaffold_419	85316	93664	PLUS	Fatty acyl-CoA reductase	gi|1274117912|ref|XP_022824115.1| (putative fatty acyl-CoA reductase CG5065 [Spodoptera litura])	Fatty acyl-CoA reductase
GSSPFG00028400001-RA	528.332	3.534	6.2E-04	scaffold_696	88259	89695	MINUS	/	gi|1274125665|ref|XP_022827146.1| (uncharacterized protein LOC111356881 [Spodoptera litura])	/
GSSPFG00010616001-RA	31.503	3.032	6.6E-04	scaffold_26453	749	2860	PLUS	/	gi|1402415181|gb|PZC74914.1| (hypothetical protein B5X24_HaOG207044 [Helicoverpa armigera])	/
GSSPFG00030439001-RA	49.406	3.287	6.6E-04	scaffold_4057	20799	25587	PLUS	Acyltransferase	gi|1274134691|ref|XP_022831691.1| (nose resistant to fluoxetine protein 6-like isoform X1 [Spodoptera litura])	Acyltransferase 3

Table S5. Manual annotation of the 50 genes with the most constitutive sf-C associated expression

OGS2.2	baseMean	log2FoldChange	padj	scaffold	start	end	strand	Annotation	Homology	IP
GSSPFG00003930001-RA	65.426	-5.504	3.52E-15	superscaffold_328	16022	16834	PLUS	/	/	/
GSSPFG00014445001-RA	53.167	-2.872	2.89E-14	scaffold_404	31734	33821	PLUS	DNA helicase	uncharacterized protein LOC110380119 [Helicoverpa ...	DNA helicase Pif1-like
GSSPFG00009092001-RA	676.632	-6.477	6.66E-12	scaffold_1577	12569	14890	PLUS	TE	uncharacterized protein LOC113494593 [Trichoplusia...	Reverse transcriptase domain
GSSPFG00033049001-RA	287.649	-5.722	5.41E-11	superscaffold_601	170017	174234	PLUS	TE	hypothetical protein B5V51_5889 [Heliothis virescens]	Reverse transcriptase domain
GSSPFG00033999001-RA	836.982	-5.546	1.81E-10	superscaffold_1180	158004	158412	PLUS	/	/	Transmembrane region
GSSPFG00034206001.1-RA	925.406	-5.645	6.11E-09	superscaffold_816	59297	66958	PLUS	**fatty-acyl-CoA reductase**	fatty acyl-CoA reductase wat-like [Spodoptera litura]	Fatty acyl-CoA reductase
GSSPFG00009529001-RA	5513.420	-4.451	2.36E-08	scaffold_81	110099	118043	MINUS	Spermadhesin-like lectin	uncharacterized protein LOC111350041 [Spodoptera litura]	Spermadhesin, CUB domain superfamily
GSSPFG00010240001.3-RA	1233.191	-2.321	2.41E-08	scaffold_13709	3294	5629	PLUS	**Calcium-dependent lectin 4**	hemolymph lipopolysaccharide-binding protein-like, partial [Spodoptera litura]	C-type lectin-like
GSSPFG00000148001-RA	366.426	-2.175	3.03E-08	scaffold_29813	360	2044	MINUS	GNBP	beta-1,3-glucan-binding protein-like [Spodoptera litura]	GRAM-NEGATIVE BACTERIA-BINDING PROTEIN 1-RELATED
GSSPFG00027050001.2-RA	128.368	-1.786	5.34E-08	scaffold_3897	4633	19471	PLUS	**apterous 1**	protein apterous-like isoform X1 [Helicoverpa armigera]	Homeobox domain
GSSPFG00024233001-RA	50.412	-2.765	7.24E-08	scaffold_1362	17970	20057	MINUS	DNA helicase	uncharacterized protein LOC110380119 [Helicoverpa armigera]	DNA helicase Pif1-like
GSSPFG00013166001-RA	49.428	-2.753	1.98E-07	scaffold_882	51328	53415	MINUS	DNA helicase	uncharacterized protein LOC110380119 [Helicoverpa armigera]	DNA helicase Pif1-like
GSSPFG00003295001.3-RA	1828.842	-2.837	2.37E-07	scaffold_22553	1941	3508	PLUS	**odorant-binding protein 36**	odorant binding protein 17 [Spodoptera exigua]	Insect pheromone/odorant-binding proteins
GSSPFG00024351001-RA	451.875	-0.972	2.78E-07	scaffold_28552	59	2295	PLUS	TM protein	uncharacterized protein LOC111352652 [Spodoptera litura]	PMP-22/EMP/MP20/Claudin superfamily
GSSPFG00015043001-RA	55.248	-4.696	3.61E-07	scaffold_22559	265	2480	MINUS	/	fibrinogen silencer-binding protein-like [Spodoptera litura]	/
GSSPFG00027329001-RA	105.513	-4.262	4.07E-07	scaffold_6725	11537	12291	PLUS	/	/	/
GSSPFG00005332001-RA	187.315	-2.921	4.16E-07	superscaffold_751	153518	156286	MINUS	DNA helicase	uncharacterized protein LOC110380119 [Helicoverpa armigera]	DNA helicase Pif1-like
GSSPFG00021758001-RA	901.184	-4.987	7.32E-07	scaffold_1475	45647	49735	PLUS	TE	Retrovirus-related Pol polyprotein from transposon TNT 1-94 [Eumeta japonica]	Retrotransposon Ty1/copia-like
GSSPFG00021650001-RA	1036.158	-2.601	1.30E-06	scaffold_29236	64	1943	MINUS	glucose dehydrogenase	glucose dehydrogenase [FAD, quinone]-like [Spodoptera litura]	Glucose-methanol-choline oxidoreductase / FAD/NAD(P)-binding domain superfamily
GSSPFG00015431001.1-RA	3095.855	-5.096	1.62E-06	scaffold_25	365539	377676	PLUS	Fatty acid synthase	fatty acid synthase-like [Spodoptera litura]	Fatty acid synthase
GSSPFG00008269001.4-RA	10079.204	-3.445	2.01E-06	scaffold_11622	5807	8216	MINUS	**CYP9A31PARTIAL**	cytochrome P450 SE-CYP9A21v2, partial [Spodoptera exigua]	Cytochrome P450, E-class, group I
GSSPFG00029033001-RA	1946.912	-2.442	2.39E-06	superscaffold_334	42062	43502	PLUS	TE	hypothetical protein [Piscirickettsia salmonis]	/
GSSPFG00015492001-RA	134.477	-4.271	3.77E-06	scaffold_5015	1654	3189	PLUS	TE	uncharacterized protein LOC111359856 [Spodoptera litura]	/
GSSPFG00030456001.4-RA	371.255	-3.717	3.80E-06	superscaffold_667	32764	34262	PLUS	**Cecropin D2**	cecropin C [Spodoptera exigua]	Cecropin
GSSPFG00025034001-RA	47.979	-3.169	3.94E-06	scaffold_119	8323	11097	MINUS	TE	piggyBac transposable element-derived protein 4-like [Bombyx mandarina]	PiggyBac transposable element-derived protein
GSSPFG00011213001-RA	26.035	-1.501	4.45E-06	scaffold_1341	14383	19256	PLUS	Orc4	origin recognition complex subunit 4 [Spodoptera litura]	Origin recognition complex subunit 4
GSSPFG00017887001.1-RA	689.116	-4.965	4.70E-06	scaffold_25	334090	363141	PLUS	Fatty acid synthase	fatty acid synthase-like [Spodoptera litura]	FATTY ACID SYNTHASE 3
GSSPFG00028982001-RA	237.134	-0.908	9.57E-06	scaffold_11342	3664	7637	PLUS	RNA methyltransferase	putative methyltransferase NSUN6 [Helicoverpa armigera]	RNA (C5-cytosine) methyltransferase
GSSPFG00008611001-RA	182.154	-1.328	1.06E-05	scaffold_23545	1099	2682	PLUS	Mcm replication complex helicase	DNA replication licensing factor Mcm3 [Spodoptera litura]	/
GSSPFG00023421001-RA	14.073	-3.500	1.06E-05	scaffold_1914	33353	35936	MINUS	Major facilitator, sugar transmembrane transporter	facilitated trehalose transporter Tret1-like [Spodoptera litura]	Major facilitator, sugar transporter-like
GSSPFG00000830001.1-RA	394.040	-2.926	1.29E-05	superscaffold_636	59933	63312	PLUS	glucose dehydrogenase	glucose dehydrogenase [FAD, quinone]-like [Spodoptera litura]	Glucose-methanol-choline oxidoreductase
GSSPFG00018074001-RA	512.151	-0.557	1.82E-05	scaffold_7139	5677	12546	MINUS	HMG box protein	HMG domain-containing protein 4 isoform X1 [Spodoptera litura]	High mobility group box domain
GSSPFG00002576001-RA	73.215	-3.822	1.94E-05	scaffold_27664	441	2372	PLUS	TE	piggyBac transposable element-derived protein 4-like isoform X1 [Spodoptera litura]	PiggyBac transposable element-derived protein
GSSPFG00018367001-RA	175.907	-4.389	2.06E-05	scaffold_9785	5077	9577	PLUS	DUF1676	uncharacterized protein LOC111357194 isoform X1 [Spodoptera litura]	Protein of unknown function DUF1676
GSSPFG00011154001.1-RA	91.034	-1.228	2.38E-05	scaffold_924	9409	14940	PLUS	Claspin like	microtubule-associated protein futsch-like [Spodoptera litura]	Claspin
GSSPFG00023769001-RA	200.369	-1.346	2.62E-05	scaffold_12811	5528	7142	MINUS	Nucleoporin NSP1/NUP62	nuclear pore glycoprotein p62-like [Helicoverpa armigera]	Nucleoporin NSP1/NUP62
GSSPFG00003828001-RA	890.911	-3.717	2.65E-05	scaffold_1985	41560	42289	PLUS	/	/	/
GSSPFG00002062001-RA	279.560	-3.354	3.14E-05	scaffold_459	105497	105630	PLUS	numt-ND2	/	/
GSSPFG00025780001-RA	1050.775	-2.608	4.37E-05	scaffold_2063	30092	40049	PLUS	/	uncharacterized protein LOC111357139 [Spodoptera litura]	Signal Peptide
GSSPFG00016432001.1-RA	250.152	-3.953	4.50E-05	scaffold_1877	52654	54924	MINUS	/	uncharacterized protein LOC111348319 [Spodoptera litura]	TRANSMEMBRANE
GSSPFG00018669001.2-RB	162.057	-3.682	4.89E-05	scaffold_22469	1969	3330	MINUS	**CYP338A1**	cytochrome CYP338A2 [Spodoptera littoralis]	Cytochrome P450, E-class, group IV
GSSPFG00023363001-RA	279.368	-4.025	5.07E-05	scaffold_5632	3032	5922	PLUS	/	uncharacterized protein LOC111357139 [Spodoptera litura]	signal peptide
GSSPFG00002117001-RA	909.154	-3.597	5.21E-05	scaffold_9208	1816	4928	MINUS	/	uncharacterized protein LOC110384158 [Helicoverpa armigera]	Zona pellucida domain
GSSPFG00027037001-RA	258.729	-0.823	6.42E-05	scaffold_15135	344	2637	PLUS	Leo1	another transcription unit protein [Spodoptera litura]	Leo1-like protein
GSSPFG00024631001-RA	34.842	-1.601	7.74E-05	scaffold_13033	4861	5525	PLUS	/	/	TRANSMEMBRANE
GSSPFG00004275001-RA	14.350	-3.334	7.77E-05	scaffold_14274	196	2776	PLUS	/	uncharacterized protein LOC111357540 [Spodoptera litura]	/
GSSPFG00032900001-RA	17.524	-2.389	8.20E-05	scaffold_32	16629	17758	PLUS	Cog7	conserved oligomeric Golgi complex subunit 7-like [Hyposmocoma kahamanoa]	Conserved oligomeric Golgi complex subunit 7
GSSPFG00021626001-RA	305.017	-3.529	8.35E-05	scaffold_2114	4137	5142	MINUS	/	/	/
GSSPFG00003829001-RA	3809.960	-3.627	8.35E-05	scaffold_1985	42432	51963	MINUS	/	uncharacterized protein LOC111356160 [Spodoptera litura]	Zona pellucida domain
GSSPFG00024020001-RA	1179.432	-1.684	8.40E-05	scaffold_3326	660	6100	MINUS	Glyoxalase I	lactoylglutathione lyase [Spodoptera litura]	Glyoxalase/Bleomycin resistance protein/Dihydroxybiphenyl dioxygenase