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Using genomic data to study insecticide resistance in the house fly, *Musca domestica*

Richard P. Meisel[a], Jeffrey G. Scott[b,*]

[a] Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA
[b] Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY, USA

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ABSTRACT

The house fly, *Musca domestica*, is a major pest at livestock facilities throughout the world. Insecticides have been the most common control strategy for flies, but many populations have evolved resistance. The speed by which we are able to identify the mutations responsible for resistance has been a major challenge for the development of high throughput resistance monitoring assays as new insecticides are introduced for control. This is particularly true for mutations that cause *trans* regulation of a gene, which then results in resistance. In this paper we take advantage of the conserved homology of dipteran chromosomes to assign 3069 genes to chromosomes. Of these, 234 were of toxicological interest (CYPs, esterases/hydrolases, glutathione *S*-transferases (GSTs) and target sites). The chromosomal location of genes known from linkage analysis studies matched the location predicted by homology mapping in ten out of ten cases, indicating a high reliability of our approach. The CYPs, esterases/hydrolases and GSTs were not randomly distributed throughout the genome. They clustered on chromosomes, but the pattern was different between the CYPs, esterases/hydrolases and GSTs. Examples are provided for how the availability of the house fly genome, combined with an ability to assign genes to chromosomes, will help to accelerate research in house flies.

1. Introduction

The house fly, *Musca domestica*, is a pest of economic and medical importance. At animal production facilities, house flies have negative effects on animal health and productivity. House flies are also the mechanical vectors of human pathogens, including antibiotic-resistant bacteria [1–7] and the causative agents of numerous diseases such as trachoma, yaws and leprosy [8–10]. As a consequence of the economic and health problems associated with house flies, insecticides have been used to control populations for > 70 years. However, house flies have evolved resistance to nearly all of these insecticides, and this has compromised our ability to control this pest.

Many investigations into insecticide resistance in the house fly included efforts to map traits to specific chromosomes (the house fly has five autosomes, plus an X/Y). Some linkage maps were developed [11], but they were not highly detailed. With the rapid progress in genome sequencing we are in the midst of a significant change in how we will be able study insecticide resistance. In 2014 the house fly genome was sequenced [12], but the genome assembly is fragmented across 20,487 scaffolds which were not associated with particular chromosomes. Availability of the genome sequence is revolutionizing our understanding of many aspects of house fly biology, but a method of assigning scaffolds to chromosomes would further facilitate research in this important pest.

Herein, we exploit the conserved homology of dipteran chromosomes [13–15] to assign genes of primary toxicological interest in house fly (detoxification enzymes and neural target sites) to chromosomes, and illustrate the uneven distribution of these genes throughout the genome. Knowing the chromosomal location of detoxification genes in an organism, where resistance and gene expression can also be readily mapped to a chromosome, will accelerate our understanding of *cis* vs. *trans* regulation of these genes. We also present information about how the genome sequencing of a resistant strain can be used to identify alleles that confer resistance, informing future toxicological and evolutionary studies. With the continued sequencing of other non-model organism genomes, including many insect pests, the approaches we present here demonstrate unprecedented opportunities to accelerate the discovery of important aspects of insect biology, including insecticide resistance.

2. Materials and methods

Assigning sequences from the house fly genome assembly, and other

* Corresponding author.
E-mail address: jgs5@cornell.edu (J.G. Scott).

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Table 1
Chromosomal assignments of scaffolds and genes in the house fly genome.

	Muller element (chromosome)						Unmapped
	A (3)	B (1)	C (5)	D (4)	E (2)	F (X)	
Scaffolds	315	333	264	313	397	7	56
Genes	2524	2323	3117	2838	3586	51	3069

fly genomes, to chromosomes is more straightforward than in other taxa because of the remarkable conservation of gene content of chromosomes across the higher dipterans, or Brachycera. Most fly genomes are organized into 6 chromosomes arms, known as Muller elements A–F [16]. Elements A–E are autosomal in most fly species, including house fly, and F is the ancestral X chromosome of Brachycera [14,15,17,18]. Genes have been mapped to chromosome arms in *Drosophila* [19], which allows assignment of homologous genes to chromosome arms in other species. First,

Table 2
CYPs in the house fly genome for which homology mapping predicted a chromosomal linkage.

Chromosome	Gene	Clan	Scaffold	XM_number
1	*CYP303A1*	2	NW_004765158	XM_005184116
1	*CYP310B2*	3	NW_004765160	XM_005184125
1	*CYP6A54*	3	NW_004760864	XM_005175565
1	***CYP6D1*** [46]	3	NW_004765160	XM_005184124
1	*CYP6D10*	3	NW_004765160	XM_005184128
1	*CYP6D11*	3	NW_004765015	XM_005183145
1	***CYP6D3*** [47]	3	NW_004765160	XM_005184123
1	*CYP6GV1*	3	NW_004766752	XM_005189530
1	*CYP6GV2*	3	NW_004766752	XM_005189529
1	*CYP6GZ1*	3	NW_004764064	XM_005175852
1	*CYP6HA1*	3	NW_004764539	XM_005177719
2	*CYP304A1*	2	NW_004765002	XM_005183058
2	*CYP304A2*	2	NW_004765002	XM_005183057
2	*CYP6D8*	3	NW_004765436	XM_005185673
2	*CYP9F10*	3	NW_004764700	XM_005180062
2	*CYP9F11*	3	NW_004764700	XM_005180054
2	*CYP9F12*	3	NW_004764700	XM_005180063
2	*CYP9F7*	3	NW_004764700	XM_005180052
2	*CYP9F8*	3	NW_004764700	XM_005180050
2	*CYP9F9*	3	NW_004764700	XM_005180053
2	*CYP313D1*	4	NW_004767316	XM_005189825
2	*CYP313D2*	4	NW_004766063	XM_005188279
2	*CYP313D3*	4	NW_004767316	XM_005189830
2	*CYP313D4*	4	NW_004764945	XM_005182545
2	*CYP4C74*	4	NW_004765515	XM_005185973
2	*CYP12A1*	Mitochondrial	NW_004764697	XM_005180004
2	*CYP12A12*	Mitochondrial	NW_004764697	XM_005180006
2	*CYP12A13*	Mitochondrial	NW_004764697	XM_005180007
2	*CYP12A14*	Mitochondrial	NW_004764697	XM_005179996
2	*CYP12A16*	Mitochondrial	NW_004764697	XM_005180005
2	*CYP12A2*	Mitochondrial	NW_004764697	XM_005179998
2	*CYP12A3*	Mitochondrial	NW_004764697	XM_005179997
2	*CYP315A1*	Mitochondrial	NW_004765301	XM_005184970
3	*CYP18A1*	2	NW_004765049	XM_005183375
3	*CYP306A1*	2	NW_004765049	XM_005183378
3	*CYP28B1*	3	NW_004764738	XM_005180394
3	*CYP28B2*	3	NW_004764738	XM_005180398
3	*CYP28K1*	3	NW_004764738	XM_005180399
3	*CYP438A4*	3	NW_004765049	XM_005183376
3	*CYP6V3*	3	NW_004764478	XM_005176346
3	*CYP311A1*	4	NW_004764740	XM_005180423
3	*CYP4AE3*	4	NW_004764514	XM_005177255
3	*CYP4D3*	4	NW_004764514	XM_005177259
3	*CYP4D54*	4	NW_004764514	XM_005177250
3	*CYP4D55*	4	NW_004764514	XM_005177252
3	*CYP4D56*	4	NW_004764514	XM_005177251
3	*CYP4D62*	4	NW_004764514	XM_005177344
3	*CYP4D9*	4	NW_004764514	XM_005177345
3	*CYP4G103*	4	NW_004764475	XM_005176293
3	*CYP4G13*	4	NW_004764475	XM_005176292
3	***CYP4G2*** [48]	4	NW_004764475	XM_005176294
3	*CYP4G96*	4	NW_004764475	XM_005176299
3	*CYP4G97*	4	NW_004764475	XM_005176300
3	*CYP4G98*	4	NW_004764475	XM_005176301
3	*CYP4G99*	4	NW_004764542	XM_005177736
3	*CYP4S23*	4	NW_004764524	XM_005177495
3	*CYP4S24*	4	NW_004764524	XM_005177488
4	*CYP305A1*	2	NW_004764745	XM_005180589
4	*CYP4AC6*	4	NW_004765632	XM_005186465
4	*CYP4D64*	4	NW_004769269	XM_005190767
4	*CYP12G2*	Mitochondrial	NW_004764745	XM_005180644
4	*CYP12G4*	Mitochondrial	NW_004765031	XM_005183241
4	*CYP302A1*	Mitochondrial	NW_004764628	XM_005179206

(*continued on next page*)

Table 2 (*continued*)

Chromosome	Gene	Clan	Scaffold	XM_number
4	*CYP314A1*	Mitochondrial	NW_004764603	XM_005178726
5	*CYP437A4*	3	NW_004765921	XM_005187739
5	*CYP6EK2*	3	NW_004765349	XM_005185208
5	*CYP6FS2*	3	NW_004764906	XM_005182161
5	*CYP6FT2*	3	NW_004768760	XM_005190444
5	*CYP6FT3*	3	NW_004768760	XM_005190452
5	*CYP6FT4*	3	NW_004768760	XM_005190451
5	*CYP6FT5*	3	NW_004768760	XM_005190450
5	*CYP6FT6*	3	NW_004768760	XM_005190449
5	*CYP6FT7*	3	NW_004768760	XM_005190425
5	*CYP6G4*	3	NW_004766190	XM_005188667
5	*CYP6GY1*	3	NW_004766190	XM_005188672
5	*CYP6HB1*	3	NW_004765735	XM_005186997
5	*CYP318B1*	4	NW_004766506	XM_005189277
5	*CYP4AA1*	4	NW_004764464	XM_005175977
5	*CYP4AD1*	4	NW_004765578	XM_005186278
5	***CYP4D4*** [48]	4	NW_004765144	XM_005183986
5	*CYP4D58*	4	NW_004765144	XM_005183988
5	*CYP4D61*	4	NW_004765144	XM_005183993
5	*CYP4E10*	4	NW_004765578	XM_005186272
5	*CYP4E11*	4	NW_004765578	XM_005186268
5	*CYP4E12*	4	NW_004765578	XM_005186277
5	*CYP4E7*	4	NW_004765578	XM_005186267
5	*CYP4P10*	4	NW_004764771	XM_005180896
5	*CYP4P11*	4	NW_004764771	XM_005180895
5	*CYP4P8*	4	NW_004764771	XM_005180909
5	*CYP12A17*	Mitochondrial	NW_004764512	XM_005177016
5	*CYP301A1*	Mitochondrial	NW_004764517	XM_005177409
5	*CYP49A1*	Mitochondrial	NW_004754940	XM_005174794

Genes that had been previously mapped to a linkage group are shown in bold. In all cases the homology mapping agreed with the previous results.

homologous genes are identified between a focal species (e.g., house fly) and *D. melanogaster* (e.g [12].,). The genes in the focal species are part of larger assembled genomic segments, known as scaffolds. Second, using a "majority rules" approach, these scaffolds are assigned to Muller elements (chromosome arms) if the majority of genes residing on the scaffold have homologs on the same element in *D. melanogaster* [20].

The insecticide susceptible aabys strain was used for sequencing of the house fly genome in 2014 [12]. We compared the genome sequence of the aabys strain with two other strains. LPR is a highly pyrethroid resistant strain because it carries the *kdr* allele (L1014F mutation in the *Voltage sensitive sodium channel* (*Vssc*)), and it has cytochrome P450 (CYP)-mediated resistance due to overexpression of *CYP6D1*[21–23]. The A3 strain [24] is resistant to pyrethroids due to *kdr*, but it also contains a low frequency of the *CYP6D1v1* resistance allele [25]. The genomes of LPR and A3 were recently sequenced [18]. This provided us with the first opportunity to examine genomes of resistant and susceptible house fly strains side-by-side. To do so, we aligned short sequencing reads (75 bp paired-end) from males and females of the LPR and A3 strains to the reference aabys genome using the BWA-MEM program [26]. We used the same approach to align 150 bp paired-end reads from aabys flies [11]. We next employed GATK to identify single nucleotide polymorphisms (SNPs) and small insertions/deletions (indels) that differentiate LPR or A3 from the aabys reference [27,28], as described previously [18]. We also identified variable sites within the aabys strain. We only retained variants that passed filtering to exclude SNP clusters and other sequencing/alignment artifacts. We then cross-referenced those sequence variants with a manual curation of the *Vssc* gene. These strains offered contrasting, yet mutually informative comparisons, because the A3 strain carries aabys chromosomes 1, 2, 4, and 5 (but not 3), while the LPR strain is not derived from aabys.

3. Results

3.1. Assignment of genes to chromosomes

We applied our homology mapping approach to map house fly

Table 3
*GST*s in the house fly genome for which homology mapping predicted a chromosomal linkage.

Chromosome	Name[a]	Scaffold	XM_number
1	1	NW_004764949	XM_005182646
2	2	NW_004764700	XM_005180042
2	3	NW_004764700	XM_005180043
2	4	NW_004764700	XM_005180044
2	5	NW_004764700	XM_005180045
2	6	NW_004764700	XM_005180046
2	7	NW_004764700	XM_005180058
2	8	NW_004765088	XM_005183587
2	9	NW_004765088	XM_005183588
2	10	NW_004765088	XM_005183589
2	11	NW_004765333	XM_005185108
2	12	NW_004765333	XM_005185109
2	13	NW_004765333	XM_005185112
2	14	NW_004767563	XM_005189970
2	15	NW_004767563	XM_005189971
3	16	NW_004764527	XM_005177543
3	17	NW_004764527	XM_005177555
3	18	NW_004764950	XM_005182675
3	19	NW_004765503	XM_005185937
3	20	NW_004765503	XM_005185939
3	21	NW_004766150	XM_005188597
3	22	NW_004772095	XM_005191498
4	23	NW_004764628	XM_005179225
5	24	NW_004764537	XM_005177693
5	25	NW_004764645	XM_005179451
5	26	NW_004764645	XM_005179452
5	27	NW_004764751	XM_005180696
5	28	NW_004764751	XM_005180697
5	29	NW_004765100	XM_005183706
5	30	NW_004765227	XM_005184605
5	31	NW_004765227	XM_005184606
5	32	NW_004765227	XM_005184607
5	33	NW_004765660	XM_005186599
5	34	NW_004770206	XM_005191032

[a] None of these genes have been named, so we provided numbers as putative names.

Table 4
Esterases/hydrolases in the house fly genome for which homology mapping predicted a chromosomal linkage.

Chromosome	Gene[a]	Scaffold	XM_number
1	Carboxylesterase 1E	NW_004765371	XM_005185388
1	Esterase FE4	NW_004764515	XM_005177391
1	1	NW_004764515	XM_005177392
1	Phosphodiesterase	NW_004765798	XM_005187247
1	Ubiquitin thioesterase	NW_004764555	XM_005177980
1	Metallophosphoesterase	NW_004764064	XM_005175846
1	2	NW_004764526	XM_005177514
1	3	NW_004764526	XM_005177515
1	4	NW_004765348	XM_005185191
1	5	NW_004765348	XM_005185192
1	6	NW_004765348	XM_005185193
1	7	NW_004765684	XM_005186805
1	8	NW_004765285	XM_005184873
2	Esterase B1	NW_004764595	XM_005178638
2	9	NW_004764595	XM_005178640
2	10	NW_004764470	XM_005176152
2	11	NW_004764470	XM_005176156
2	12	NW_004764595	XM_005178642
2	13	NW_004764595	XM_005178634
2	14	NW_004764595	XM_005178635
2	15	NW_004764595	XM_005178636
2	16	NW_004764595	XM_005178637
2	17	NW_004764595	XM_005178639
2	18	NW_004764595	XM_005178643
2	19	NW_004764595	XM_005178644
2	Juvenile hormone esterase	NW_004764693	XM_005179982
2	20	NW_004759308	XM_005175393
2	21	NW_004764751	XM_005180691
2	22	NW_004764751	XM_005180692
2	23	NW_004764751	XM_005180693
2	24	NW_004764751	XM_005180695
2	25	NW_004765072	XM_005183480
2	26	NW_004760941	XM_005175571
2	Phosphodiesterase	NW_004765301	XM_005184969
2	27	NW_004765104	XM_005183772
2	28	NW_004765104	XM_005183790
2	29	NW_004765104	XM_005183791
2	30	NW_004764578	XM_005178312
2	31	NW_004766094	XM_005188471
2	32	NW_004766094	XM_005188472
2	33	NW_004765115	XM_005183852
2	34	NW_004770971	XM_005191152
2	35	NW_004764703	XM_005180087
2	36	NW_004764558	XM_005178055
3	Phosphodiesterase	NW_004765081	XM_005183542
3	Neuropathy target esterase	NW_004764802	XM_005181277
3	37	NW_004764802	XM_005181278
3	38	NW_004764802	XM_005181279
3	39	NW_004764779	XM_005180961
3	Palmitoyl-protein thioesterase	NW_004765718	XM_005186941
3	40	NW_004765718	XM_005186947
3	41	NW_004765196	XM_005184476
3	42	NW_004764514	XM_005177302
3	43	NW_004764514	XM_005177303
3	44	NW_004764514	XM_005177304
3	45	NW_004764514	XM_005177305
3	46	NW_004764514	XM_005177306
3	47	NW_004765081	XM_005183540
4	Acyl-protein thioesterase	NW_004774994	XM_005192151
4	48	NW_004767076	XM_005189687
4	49	NW_004767076	XM_005189688
4	50	NW_004764916	XM_005182230
4	Esterase P	NW_004765121	XM_005183882
4	Methylesterase	NW_004766410	XM_005189138
4	Acyl-coenzyme A thioesterase	NW_004765445	XM_005185709
4	51	NW_004769271	XM_005190788
4	52	NW_004769271	XM_005190789
4	53	NW_004765111	XM_005183831
4	54	NW_004765970	XM_005187899
4	55	NW_004765970	XM_005187900
4	56	NW_004765449	XM_005185716
4	57	NW_004764513	XM_005177167
4	58	NW_004764513	XM_005177219
4	Esterase 5A	NW_004765121	XM_005183883
4	Esterase 5B	NW_004765121	XM_005183881
5	59	NW_004754939	XM_005174719
5	Venom carboxylesterase	NW_004764825	XM_005181454
5	60	NW_004773204	XM_005191693
5	61	NW_004764648	XM_005179475
5	62	NW_004762819	XM_005175706
5	63	NW_004762819	XM_005175707
5	64	NW_004765349	XM_005185226
5	65	NW_004765349	XM_005185227
5	66	NW_004765349	XM_005185228

[a] For genes that have not been named we provided numbers as putative names.

Table 5
Insecticide target sites/ion channels in the house fly genome. Only genes for which homology mapping predicted a chromosomal linkage are shown.

Chromosome	Gene	Scaffold	NCBI reference seq.
1	***nAChRβ3*** [46]	NW_004765351	XP_005185318
2	***nAChRα2*** [47]	NW_004765458	XP_005185792
2	*nAChRβ2*	NW_004765458	XP_005185796
2	*HisCl1*	NW_004765072	XP_005183540
2	*HisCl2*	NW_004772093	XP_005191543
2	***Ace*** [48]	NW_004765027	XP_005183285
2	*GluCl*	NW_004765120	XP_005183932
3	*nAChRα3*	NW_004764616	XP_005178988
3	***nAChRα7***	NW_004765188	XP_005184466
3	*LCCH3*	NW_004765465	XP_005185818
3	***Vssc*** [49]	NW_004765908	NP_001273814.1
4	*Gly Receptor*	NW_004764498	XP_005176739
4	***Rdl*** [50]	NW_004766311	NP_001292048.1
4	*nAChRβ1*	NW_004764708	XP_005180169
5	*RyR*	NW_004764523	XP_005177532

Genes shown in bold were found to be located on the same chromosome by both linkage analysis and homology mapping.
Genes that had been previously mapped to a linkage group are shown in bold. In all cases the homology mapping agreed with the previous results.

genomic scaffolds to chromosome arms [20], assigning 1629/1685 scaffolds containing annotated genes with *D. melanogaster* homologs to chromosomes (Table 1). This allowed us to assign 14,439/17,508 annotated house fly genes to chromosomes (Table 1). In contrast, when we relied only on homology calls between genes, rather than scaffold-wide chromosome assignments, we were only able to map 11,253 house fly genes to chromosomes (data not shown). This same chromosome arm assignment strategy could readily be applied to any annotated brachyceran genome assembly.

Using homology mapping we were able to assign toxicologically relevant genes to chromosomes with a high degree of accuracy. Most of the genes of toxicological relevance could be mapped. For example, 92 of 163 CYPs (Table 2), 34 of 36 GSTs (Table 3), 84 of 90 esterases/hydrolases (Table 4), and 15 of 19 target sites/ion channels (Table 5) (only *nAChRα1, α4, α5, and α6* could not be assigned) were assigned to a chromosome. In total we were able to predict the chromosomal linkage of 216 toxicologically relevant genes. To test the reliability of homology mapping we compared our results to published studies that had identified the linkage of ten of these genes (Tables 2 and 5). In addition, two other genes, *Gfi-1* and *MdY* have been mapped to autosomes 1 [29] and 3 [30], respectively. Using homology mapping the linkage of all 12 genes were correctly predicted, indicating a high degree of accuracy in this technique.

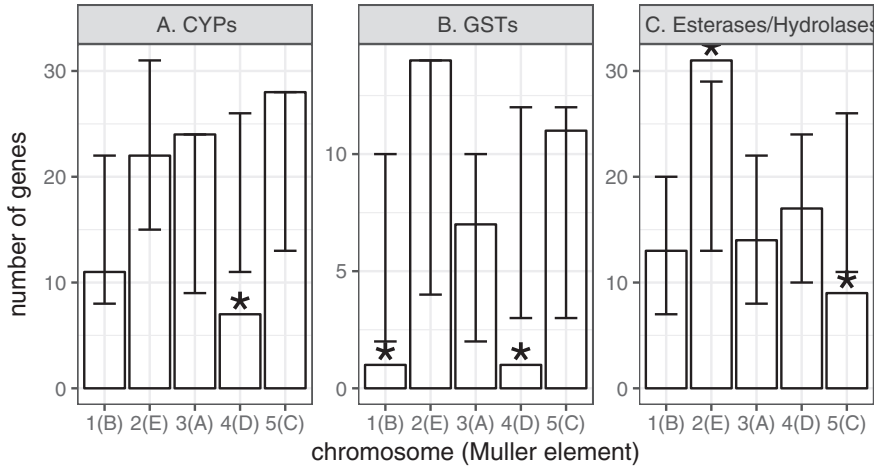

Fig. 1. Counts of genes encoding different classes of xenobiotic detoxification enzymes mapped to each autosome. Bars show the number of (A) CYPs, (B) GSTs, and (C) esterases/hydrolases mapped to each of the five autosomes (no genes were mapped to X chromosome). Error bars show 95% confidence intervals of the expected number of genes mapped to each chromosome from subsampling all genes on the five autosomes 1000 times to generate a null expectation of the random distribution of genes across the autosomes. Asterisks indicate significant differences between the observed counts and the null expectation.

We additionally tested if the distribution of CYPs, GSTs, and esterases/hydrolases across chromosomes deviates from a random distribution. The relative number of CYPs, GSTs and hydrolases on each chromosome are shown in Fig. 1 and Tables 2–4. We indeed find evidence for excesses and deficiencies of each category of xenobiotic detoxification genes on individual chromosomes (Fig. 1). Notably, there is a deficiency of CYPs and GSTs on chromosome 4. Additional work is necessary to determine if the over- or under-representation of functional classes on individual chromosomes is the result of selection or a bias in the rate of intra-chromosomal duplication.

3.2. Sequence variation within Vssc

We analyzed sequence polymorphism in the *Vssc* gene in two strains that exhibit pyrethroid resistance: A3 [31] and LPR [32,33]. LPR is also multi-resistant to several insecticides [21,32]. VSSC is the target site of pyrethroid insecticides, but is challenging to study because the gene encoding it is large (> 250,000 bp with 29 exons) [34], including two mutually exclusive exons (17a/b and 23a/b, also known as c/d and k/l), two optional exons [35], and five exons with 5′ or 3′ alternative splice sites. There are now > 16 full length cDNA sequences available from different house fly strains, but they all reflect the most common splicing variant. With the genome sequences of aabys, A3 and LPR we were able to identify allelic variants that are homozygous in A3 and/or LPR and not present in the insecticide susceptible genome reference strain, aabys [12], including comparisons of the optional and mutually exclusive exons (Supplementary Table 1). We found that, relative to exons in the aabys genome sequence, there were 12 synonymous SNPs in A3 and three in LPR. There was one non-synonymous SNP that LPR and A3 shared: *kdr,* a C to T transition in exon 18 of *Vssc* (position 7391 in scaffold NW_004765908) that confers resistance to pyrethroid insecticides [36,37]. We also confirmed one other non-synonymous SNP in A3 (R1940G) that was previously described [25]. These results are consistent with previous results that found very little variation in *Vssc* sequences between different strains of house flies [25,38].

4. Discussion and future directions

In the post-genomic era the house fly is an increasingly powerful organism for studying the evolution of insecticide resistance. Combining traditional genetics (being able to map resistance and/or expression profiles to a chromosome) with a genome sequence that includes chromosomal assignments for most genes will allow for rapid progress in the future. This is particularly true in at least three cases. The first is in the effort to identify when resistance due to over-expression of a gene is due to *trans*-regulation. *CYP6D1v1* (on chromosome 1), for example, is overexpressed in LPR by *trans* factors on chromosome 1 (Gfi-1 [29]) and 2 (unknown), resulting in resistance [29,39–41]. Being able to compare the linkage of the overexpression trait (e.g. transcript abundance affected by a *trans* factor) and know the location of the gene being overexpressed provides an avenue for rapid determination of *trans*-regulation of the gene [42,43]. Such an approach has recently been used to identify the factors (genes and the chromosomes responsible for their up-regulation) involved in imidacloprid resistance in house flies (Reid et al., unpublished) and may eventually lead to identification of the trans-regulatory factor(s). Leveraging new methods from population genomics to identify quantitative trait loci that affect gene expression (eQTL) has tremendous potential to elucidate the genetic basis of insecticide resistance that evolves through *trans* regulatory mutations [42,43]. Identification of mutations responsible for the enhanced *trans*-regulation would promote the development of novel insecticide resistance monitoring strategies. Second, some genes of toxicological relevance are very large (e.g. the ryanodine receptor ORF is > 15,000 bp). Knowing the sequence of such genes (from the genome) makes all investigations into resistance (from the design of PCR primers to studies on the molecular evolution of resistance) much easier. For example, efforts to genotype individual insects for the presence or absence of two *Vssc* mutations, M918 T (or T929I) and L1014F, led researchers to try in vain to PCR amplify a product from DNA. The genome sequence revealed the problem; there was a 8354 bp intron between exons 17b (site of the M918 T or T929I mutation) and 18 (site of the L1014F mutation) [25]. Thus, knowing the size, position and sequences of introns will make PCR detection of resistance mutations more tractable. Third, bulk segregant analysis combined with next generation sequencing [44,45] can be done on organisms with a sequenced genome and has the potential to rapidly identify novel loci involved in insecticide resistance.

For decades the field of insecticide resistance genetics struggled because it took many years to determine the mutations causing resistance, and thus high throughput resistance monitoring could not be done in a timely manner. This is no longer the case. In the post-genomic era, identification of mutations and development of monitoring techniques can be done in a fraction of the time it previously took. Thus, there is renewed optimism that monitoring programs can be established before resistance levels have achieved unmanageable levels. For example, recently two new *Vssc* mutations were found that confer resistance to pyrethroid insecticides [38]. The availability of genome sequences will help in the design of *Vssc* specific primers, which will optimize our abilities to detect these alleles and will facilitate other studies on the molecular evolution of *Vssc* mediated resistance. Furthermore, genome-wide analyses are now possible using these data to identify candidate sequence variants that could affect insecticide resistance.

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References

[1] N. Rahuma, K.S. Ghenghesh, R. Ben-Aissa, A. Elamaari, Carriage by the housefly (*Musca domestica*) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospital and other urban environments in Misurata, Libya, Ann. Trop. Med. Parasitol. 99 (2005) 795–802.
[2] L. Macovei, B. Miles, L. Zurek, Potential of houseflies to contaminate ready-to-eat food with antibiotic-resistant enterococci, J. Food Protec. 71 (2008) 435–439.
[3] L. Macovei, L. Zurek, Ecology of antibiotic resistance genes: characterization of enterococci from houseflies collected in food settings, Appl. Environ. Microbiol. 72 (2006) 4028–4035.
[4] G. Boulesteix, P. Le Dantec, B. Chevalier, M. Dieng, B. Niang, B. Diatta, Role of *Musca domestica* in the transmission of multiresistant bacteria in the centres of intensive care setting in sub-Saharan Africa, Annales-Francaises-d'Anesthesie-et-de-Reanimation 24 (2005) 361–365.
[5] J.P. Graham, L.B. Price, S.L. Evans, T.K. Graczyk, E.K. Silbergeld, Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations, Sci. Total Environ. 407 (2009) 2701–2710.
[6] T.K. Graczyk, R. Knight, R.H. Gilman, M.R. Cranfield, The role of non-biting flies in the epidemiology of human infectious diseases, Microbes Infec. 3 (2001) 231–235.
[7] G.W. Sundin, Evolution and selection of antibiotic and pesticide resistance: a molecular genetic perspective, in: T.M. Brown (Ed.), Molecular Genetics and Evolution of Pesticide Resistance, American Chemical Society, Washington, D.C., 1996, pp. 97–105.
[8] H.G. Scott, K.S. Lettig, Flies of Public Health Importance and their Control, U.S. Government Printing Office, Washington, D.C., 1962.
[9] J. Keiding, The House Fly – Biology and Control, World Health Organization (WHO), Vector Biology and Control Division, 1986 WHO/VBC/86.937.
[10] B. Greenberg, Flies and disease, Sci. Am. 213 (1965) 92–99.
[11] M. Tsukamoto, Methods of genetic analysis of insecticide resistance, in: G.P. Georghiou, T. Saito (Eds.), Pest Resistance to Pesticides, Plenum Press, New York, 1983, pp. 71–98.
[12] J.G. Scott, W.C. Warren, L.W. Beukeboom, D. Bopp, A.G. Clark, S.D. Giers, M. Hediger, A.K. Jones, S. Kasai, C.A. Leichter, M. Li, R.P. Meisel, P. Minx, T.D. Murphy, D.R. Nelson, W.R. Reid, F.D. Rinkevich, H.M. Robertson, T.B. Sackton, D.B. Sattelle, F. Thibaud-Nissen, C. Tomlinson, L. van de Zande, K.K.O. Walden, R.K. Wilson, N. Liu, Genome of the house fly (*Musca domestica* L), a global vector of diseases with adaptations to a septic environment, Genome Biol. 15 (2014) 466.
[13] J.A. Sved, Y. Chen, D. Shearman, M. Frommer, A.S. Gilchrist, W.B. Sherwin, Extraordinary conservation of entire chromosomes in insects over long evolutionary periods: brief communication, Evolution 70 (2016) 229–234.
[14] B. Vicoso, D. Bachtrog, Numerous transitions of sex chromosomes in diptera, PLoS Biol. 13 (2015) e1002078.
[15] G.G. Foster, M.J. Whitten, C. Konovalov, J.T.A. Arnold, G. Maffi, Autosomal genetic maps of the Australian sheep blowfly, *Lucilia cuprina dorsalis* R.-D. (Diptera: Calliphoridae), and possible correlations with the linkage maps of *Musca domestica* L. and *Drosophila melanogaster* (Mg.), Genet. Res. Camb. 37 (1981) 55–69.
[16] H.J. Muller, Bearings of the 'Drosophila' work on systematics, in: J. Huxley (Ed.), The New Systematics, Oxford University Press, Oxford, 1940, pp. 185–268.
[17] B. Vicoso, D. Bachtrog, Reversal of an ancient sex chromosome to an autosome in *Drosophila*, Nature 499 (2013) 332–337.
[18] R.P. Meisel, C.A. Gonzales, H. Luu, The house fly Y Chromosome is young and minimally differentiated from its ancient X Chromosome partner, Genome Res. 27 (2017) 1417–1426.
[19] S.W. Schaeffer, A. Bhutkar, B.F. McAllister, M. Matsuda, L.M. Matzkin, P.M. O'Grady, C. Rohde, V.L.S. Valente, M. Aguade, W.W. Anderson, K. Edwards, A.C.L. Garcia, J. Goodman, J. Hartigan, E. Kataoka, R.T. Lapoint, E.R. Lozovsky, C.A. Machado, M.A.F. Noor, M. Papaceit, L.K. Reed, S. Richards, T.T. Rieger, S.M. Russo, H. Sato, C. Segarra, D.R. Smith, T.F. Smith, V. Strelets, Y.N. Tobari, Y. Tomimura, M. Wasserman, T. Watts, R. Wilson, K. Yoshida, T.A. Markow, W.M. Gelbart, T.C. Kaufman, Polytene chromosomal maps of 11 drosophila species: the order of genomic scaffolds inferred from genetic and physical maps, Genetics 179 (2008) 1601–1655.
[20] R.P. Meisel, J.G. Scott, A.G. Clark, Transcriptome differences between alternative sex determining genotypes in the house fly, *Musca domestica*, Genome Biol. Evol. 7 (2015) 2051–2061.
[21] J.G. Scott, G.P. Georghiou, Mechanisms responsible for high levels of permethrin resistance in the house fly, Pestic. Sci. 17 (1986) 195–206.
[22] G.D. Wheelock, J.G. Scott, The role of cytochrome $P450_{lpr}$ in deltamethrin metabolism by pyrethroid resistant and susceptible strains of house flies, Pestic. Biochem. Physiol. 43 (1992) 67–77.
[23] T. Tomita, N. Liu, F.F. Smith, P. Sridhar, J.G. Scott, Molecular mechanisms involved in increased expression of a cytochrome P450 responsible for pyrethroid resistance in the housefly, *Musca domestica*, Insect Mol. Biol. 4 (1995) 135–140.
[24] N. Liu, J.W. Pridgeon, Metabolic detoxication and the *kdr* mutation in pyrethroid resistant house flies, *Musca domestica* (L.), Pestic. Biochem. Physiol. 73 (2002) 157–163.
[25] H. Sun, K.P. Tong, S. Kasai, J.G. Scott, Overcoming *super-kdr* mediated resistance: multi-halogenated benzyl pyrethroids are more toxic to *super-kdr* than *kdr* house flies, Insect Mol. Biol. 25 (2016) 126–137.
[26] H. Li, Aligning Sequence Reads, Clone Sequences and Assembly Contigs With BWA-MEM, (2013) arXiv.org > q-bio > arXiv:1303.3997v2.
[27] A. McKenna, M. Hanna, E. Banks, S. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, M. Daly, M.A. DePristo, The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data, Genome Res. 20 (2010) 1297–1303.
[28] G.A. Van der Auwera, M.O. Carneiro, C. Hartl, R. Poplin, G. del Angel, A. Levy-Moonshine, T. Jordan, K. Shakir, D. Roazen, J. Thibault, E. Banks, K.V. Garimella, D. Altshuler, S. Gabriel, M.A. DePristo, From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline: the genome analysis toolkit best practices pipeline, in: A. Bateman, W.R. Pearson, L.D. Stein, G.D. Stormo, J.R. Yates (Eds.), Current Protocols in Bioinformatics, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2013(pp. 11.10.11-11.10.33).
[29] J. Gao, J.G. Scott, Role of the transcriptional repressor *mdGfi-1* in *CYP6D1v1*-mediated insecticide resistance in the house fly, *Musca domestica*, Insect Biochem. Mol. Biol. 36 (2006) 387–395.
[30] S.D. Heinze, T. Kohlbrenner, D. Ippolito, A. Meccariello, A. Burger, C. Mosimann, G. Saccone, D. Bopp, CRISPR-Cas9 targeted disruption of the yellow ortholog in the housefly identifies the brown body locus, Sci. Rep. 7 (2017).
[31] L. Tian, C. Cao, L. He, M. Li, L. Zhang, L. Zhang, H. Liu, N. Liu, Autosomal interactions and mechanisms of pyrethroid resistance in house flies, *Musca domestica*, Int. J. Biol. Sci. 7 (2011) 902–911.
[32] J.G. Scott, G.P. Georghiou, Rapid development of high-level permethrin resistance in a field-collected strain of house fly (Diptera: Muscidae) under laboratory selection, J. Econ. Entomol. 78 (1985) 316–319.
[33] N. Liu, J.G. Scott, Genetics of resistance to pyrethroid insecticides in the house fly, *Musca domestica*, Pestic. Biochem. Physiol. 52 (1995) 116–124.
[34] S. Kasai, H. Sun, J.G. Scott, Diversity of knockdown resistance alleles in a single house fly population facilitates adaptation to pyrethroid insecticides, Insect Mol. Biol. 26 (2017) 13–24.
[35] S.H. Lee, P.J. Ingles, D.C. Knipple, D.M. Soderlund, Developmental regulation of alternative exon usage in the house fly *Vssc1* sodium channel gene, Invertebr. Neurosci. 4 (2002) 125–133.
[36] M. Williamson, D. Martinez-Torres, C. Hick, A. Devonshire, Identification of mutations in the housefly *para*-type sodium channel gene associated with knockdown resistance (*kdr*) to pyrethroid insecticides, Mol. Gen. Genet. 252 (1996) 51–60.
[37] M. Miyazaki, K. Ohyama, D. Dunlap, F. Matsumura, Cloning and sequencing of the *para*-type sodium channel gene from susceptible and *kdr*-resistant German cockroaches (*Blattella germanica*) and house fly (*Musca domestica*), Mol. Gen. Genet. 252 (1996) 61–68.
[38] H. Sun, S. Kasai, J.G. Scott, Two novel house fly *Vssc* mutations, D600N and T929I, give rise to new insecticide resistance alleles, Pestic. Biochem. Physiol. 143 (2017) 116–121.
[39] S. Kasai, J.G. Scott, Overexpression of cytochrome P450 CYP6D1 is associated with monooxygenase-mediated pyrethroid resistance in house flies from Georgia, Pestic. Biochem. Physiol. 68 (2000) 34–41.
[40] N. Liu, J.G. Scott, Increased transcription of *CYP6D1* causes cytochrome P450-mediated insecticide resistance in house fly, Insect Biochem. Mol. Biol. 28 (1998) 531–535.
[41] N. Liu, J.G. Scott, Inheritance of CYP6D1-mediated pyrethroid resistance in house fly (Diptera: Muscidae), J. Econ. Entomol. 90 (1997) 1478–1481.
[42] J. Bryois, A. Buil, D.M. Evans, J.P. Kemp, S.B. Montgomery, D.F. Conrad, K.M. Ho, S. Ring, M. Hurles, P. Deloukas, G. Davey Smith, E.T. Dermitzakis, Cis and trans effects of human genomic variants on gene expression, PLoS Genet. 10 (2014) e1004461.
[43] H.-J. Westra, M.J. Peters, T. Esko, H. Yaghootkar, C. Schurmann, J. Kettunen, M.W. Christiansen, B.P. Fairfax, K. Schramm, J.E. Powell, A. Zhernakova, D.V. Zhernakova, J.H. Veldink, L.H. Van den Berg, J. Karjalainen, S. Withoff, A.G. Uitterlinden, A. Hofman, F. Rivadeneira, P.A.C. Hoen, E. Reinmaa, K. Fischer, M. Nelis, L. Milani, D. Melzer, L. Ferrucci, A.B. Singleton, D.G. Hernandez, M.A. Nalls, G. Homuth, M. Nauck, D. Radke, U. Völker, M. Perola, V. Salomaa, J. Brody, A. Suchy-Dicey, S.A. Gharib, D.A. Enquobahrie, T. Lumley, G.W. Montgomery, S. Makino, H. Prokisch, C. Herder, M. Roden, H. Grallert, T. Meitinger, K. Strauch, Y. Li, R.C. Jansen, P.M. Visscher, J.C. Knight, B.M. Psaty, S. Ripatti, A. Teumer, T.M. Frayling, A. Metspalu, J.B.J. van Meurs, L. Franke, Systematic identification of trans eQTLs as putative drivers of known disease associations, Nat. Genet. 45 (2013) 1238–1243.
[44] P. Demaeght, E.J. Osborne, J. Odman-Naresh, M. Grbi, R. Nauen, H. Merzendorfer, R.M. Clark, T. Van Leeuwen, High resolution genetic mapping uncovers chitin synthase-1 as the target-site of the structurally diverse mite growth inhibitors clofentezine, hexythiazox and etoxazole in *Tetranychus urticae*, Insect Biochem. Mol. Biol. 51 (2014) 52–61.
[45] T. Van Leeuwen, P. Demaeght, E.J. Osborne, W. Dermauw, S. Gohlke, R. Nauen, M. Garbic, L. Tirry, H. Merzendorfer, R.M. Clark, Population bulk segregant mapping uncovers resistance mutations and the mode of action of a chitin synthesis inhibitor in arthropods, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 4407–4412.
[46] J.-R. Gao, J.M. Deacutis, J.G. Scott, Characterization of the nicotinic acetylcholine receptor subunits *Mdalpha5* and *Mdbeta3* on autosome 1 of *Musca domestica* indicate they are not involved in spinosad resistance, Insect Mol. Biol. 16 (2007) 691–701.
[47] J.-R. Gao, J.M. Deacutis, J.G. Scott, Characterization of the nicotinic acetylcholine receptor subunit gene *Mda2* from the house fly, *Musca domestica*, Arch. Insect Biochem. Physiol. 64 (2007) 30–42.
[48] M. Ugaki, T. Shono, M. Tsukamoto, F.I. Fukami, Linkage group analysis of glutathione S-transferase and acetylcholinesterase in an organophosphorus resistant strain of Musca domestica L. (Diptera: Muscidae), Appl. Ent. Zool. 20 (1985) 73–81.
[49] R. Milani, A. Travaglino, Ricerche genetiche sulla resistenza al DDT in *Musca domestica* concatenazione del gene *kdr* (*knockdown-resistance*) con due mutanti morfologigi, Riv. Parassitol. 18 (1957) 199–202.
[50] F.J. Oppenoorth, G.E. Nasrat, Genetics of dieldrin and gamma-BHC resistance in the housefly, Ent. Exp. Appl. 9 (1966) 223–231.