Lovisa Eriksson

Explorative genomic surveillance of Diamondback moth

Jordbruksverket
Swedish Board of Agriculture

Diamondback moth (*Plutella xylostella*) in Sweden

Jordbruks verket

Swedish Board of Agriculture

- Diamondback moth doesn’t overwinter in Sweden
- Populations are introduced by warm, southeasterly winds
- Normally a rare pest, but has become more frequent in recent years, especially in horticultural crops
- No registered insecticides in agricultural crops. Horticultural crops have effective products registered
- Autumn 2023 was initially wet and it was impossible to sow winter oilseed rape in large areas of mid-Sweden. Instead an unusaul large area of spring oilseed crops were sown
- At the end of May we had a large inflight of diamondback moth to the eastern parts of Sweden

Situation in 2024 season

- In most fields inflight came inbetween the usual time to control flea beetles and pollen beetles
- Experience has shown it important to control the first generation of diamondback moth, otherwise coming generations easily get out of hand
- Two products were given emergency authorisations, Carnadine (almost in time) and Exirel (too late)
- Would they work? How could we find out? What traits did the population carry with them?

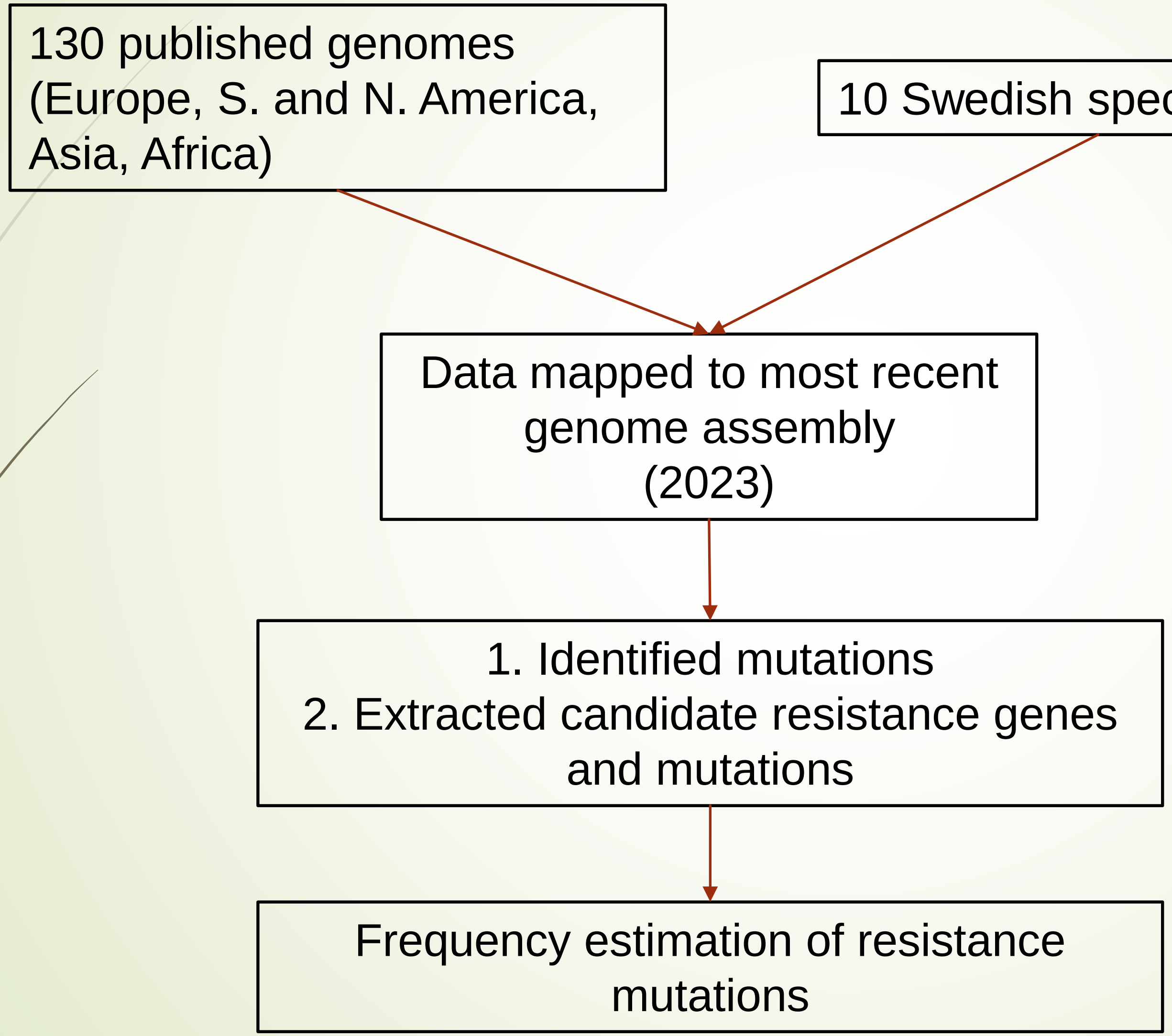
Options to test susceptibility to products

- Initiated testing according to IRAC method no.18 for six products at three different rates
- Would require collection of ~3000 larvaes in L2 or L3 stage – not feasible
- Instead, initiated an explorative genomic testing of Diamondback moth
- How could that be used? Useful for future surveillance?

Project – Genomic testing of diamondback moth

- List all genes that are known to convey resistance
- Test collected Swedish specimens
- Map data to the most recent genome assembly
- Set it up as a searchable ”database” where it’s possible to add data in the future
- Project carried out by the Swedish Museum of Natural History (Nicolas Dussex and Niclas Gyllenstrand). The following five slides are theirs.

Bioinformatic analyses



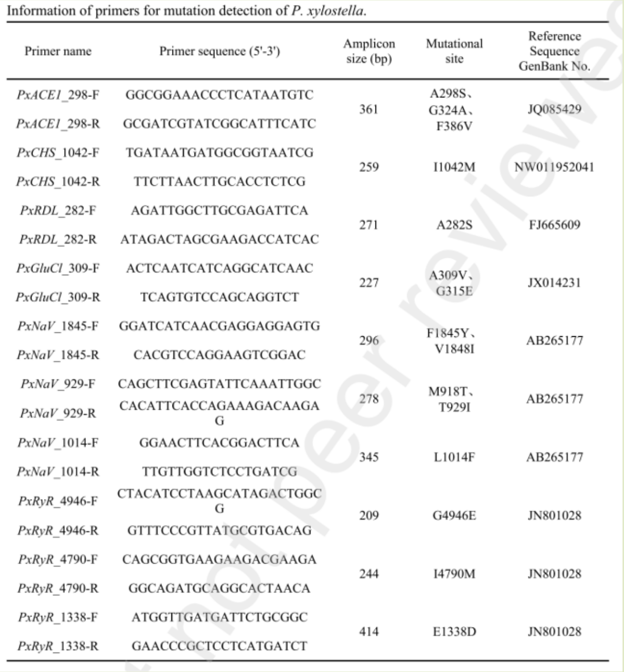
130 published genomes (Europe, S. and N. America, Asia, Africa)

10 Swedish specimens

Data mapped to most recent genome assembly (2023)

1. Identified mutations
2. Extracted candidate resistance genes and mutations

Frequency estimation of resistance mutations



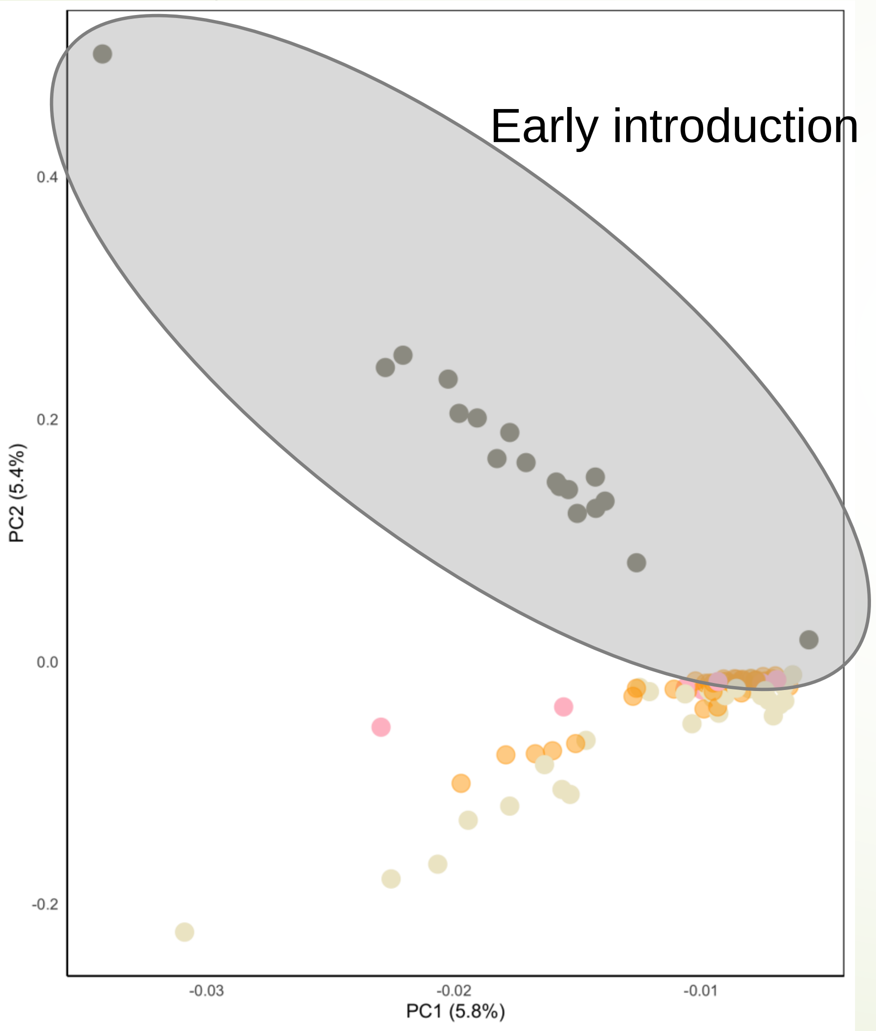
Information of primers for mutation detection of *P. xylostella*.

Primer name	Primer sequence (5'-3')	Amplicon size (bp)	Mutational site	Reference Sequence GenBank No.
*PxACE1*_298-F	GGCGGAAACCCTCATAATGTC	361	A298S、G324A、F386V	JQ085429
*PxACE1*_298-R	GCGATCGTATCGGCATTTCATC			
*PxCHS*_1042-F	TGATAATGATGGCGGTAATCG	259	I1042M	NW011952041
*PxCHS*_1042-R	TTCTTAACTTGCACCTCTCG			
*PxRDL*_282-F	AGATTGGCTTGCGAGATTCA	271	A282S	FJ665609
*PxRDL*_282-R	ATAGACTAGCGAAGACCATCAC			
*PxGluCl*_309-F	ACTCAATCATCAGGCATCAAC	227	A309V、G315E	JX014231
*PxGluCl*_309-R	TCAGTGTCCAGCAGGTCT			
*PxNaV*_1845-F	GGATCATCAACGAGGAGGAGTG	296	F1845Y、V1848I	AB265177
*PxNaV*_1845-R	CACGTCCAGGAAGTCGGAC			
*PxNaV*_929-F	CAGCTTCGAGTATTCAAATTGGC	278	M918T、T929I	AB265177
*PxNaV*_929-R	CACATTCACCAGAAAGACAAGAG			
*PxNaV*_1014-F	GGAACTTCACGGACTTCA	345	L1014F	AB265177
*PxNaV*_1014-R	TTGTTGGTCTCCTGATCG			
*PxRyR*_4946-F	CTACATCCTAAGCATAGACTGGCG	209	G4946E	JN801028
*PxRyR*_4946-R	GTTTCCCGTTATGCGTGACAG			
*PxRyR*_4790-F	CAGCGGTGAAGAAGACGAAGA	244	I4790M	JN801028
*PxRyR*_4790-R	GGCAGATGCAGGCACTAACA			
*PxRyR*_1338-F	ATGGTTGATGATTCTGCGGC	414	E1338D	JN801028
*PxRyR*_1338-R	GAACCCGCTCCTCATGATCT			

Liu et al. 2024

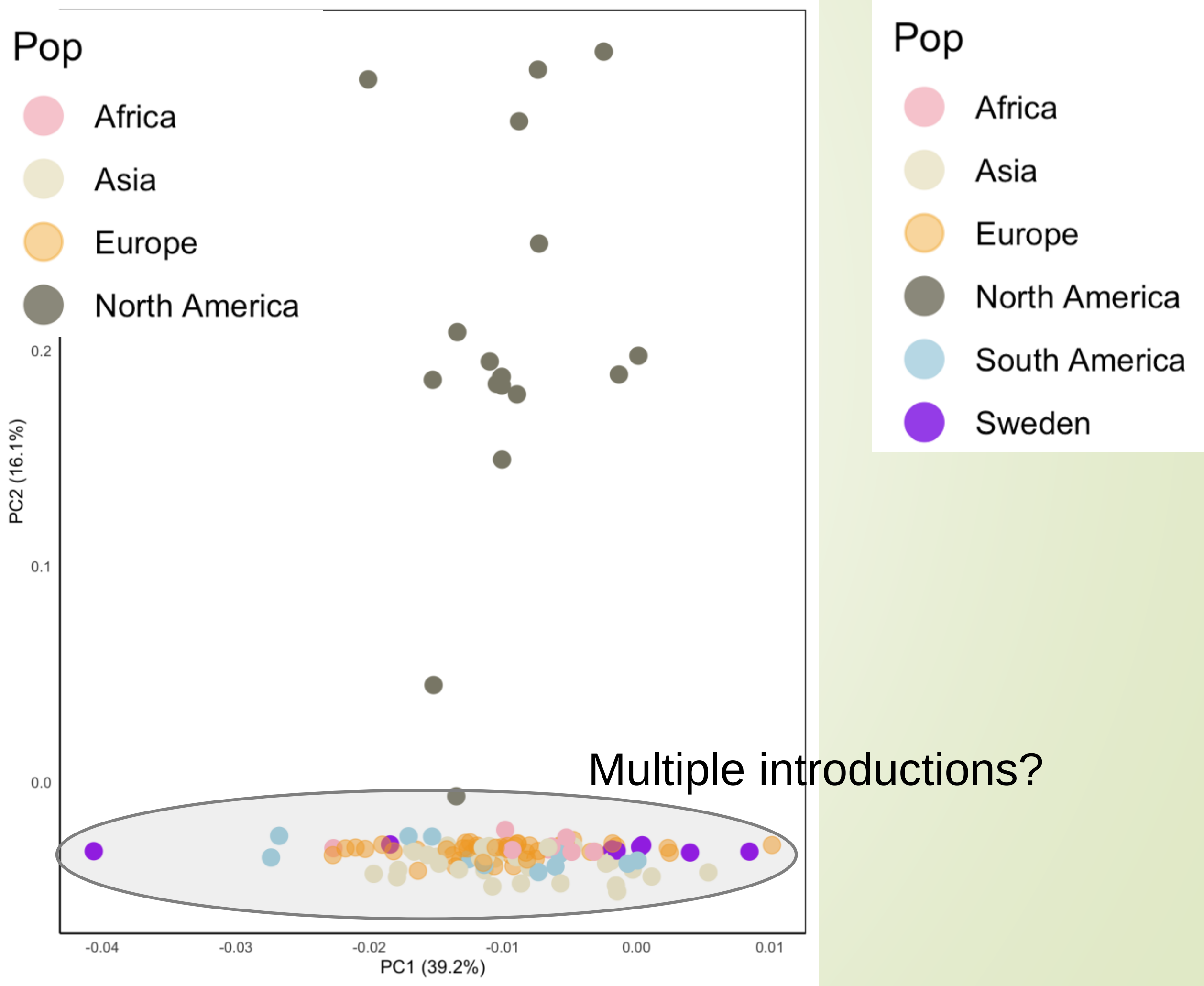
Population structure and origin of specimens

Population structure (without Sweden)



Early introduction

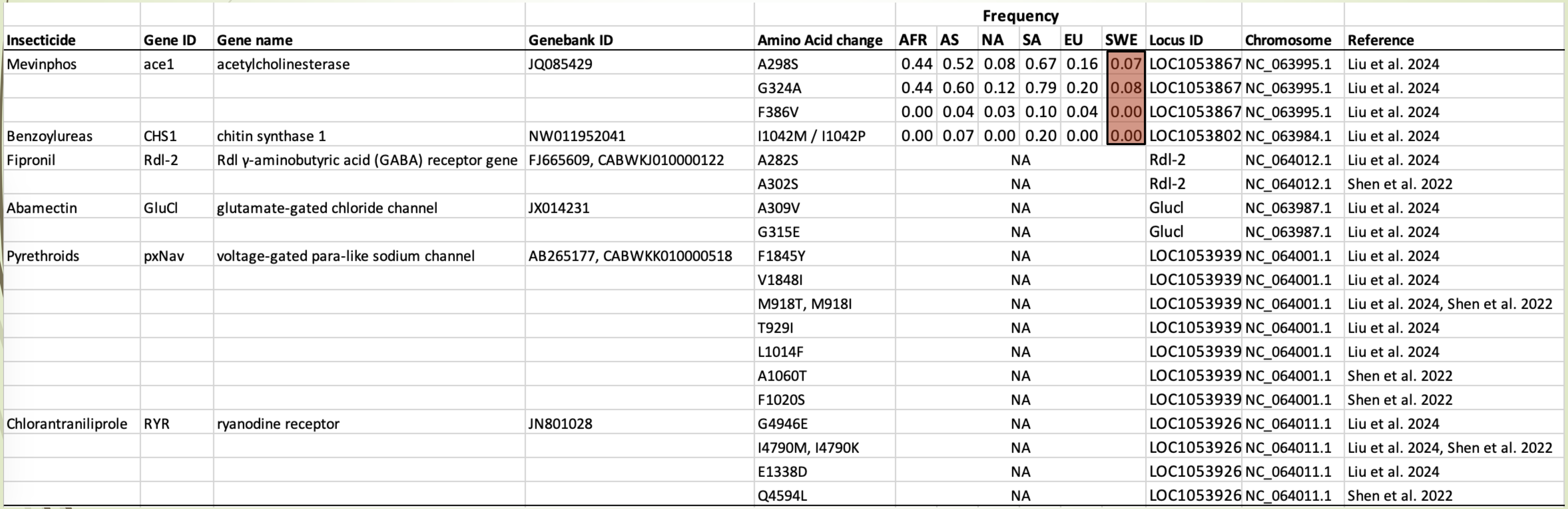
Population structure (with Sweden)



Multiple introductions?

Results

Known resistance mutations identified in Sweden



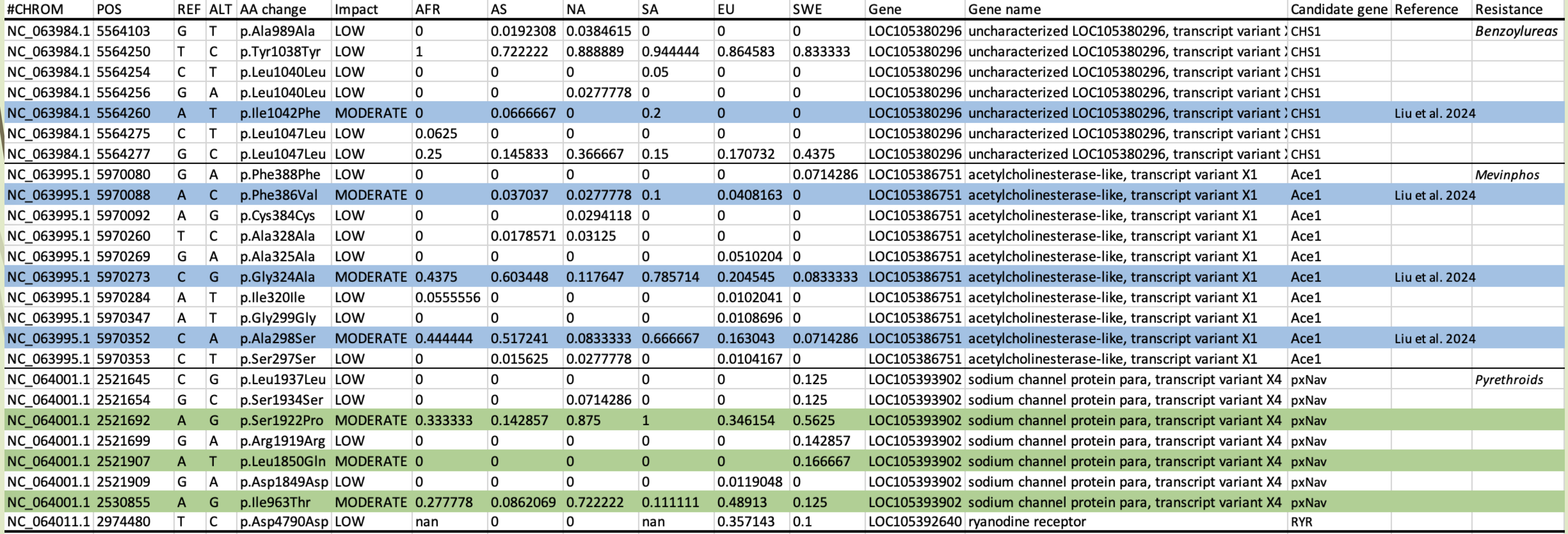
					Frequency								
Insecticide	**Gene ID**	**Gene name**	**Genebank ID**	**Amino Acid change**	**AFR**	**AS**	**NA**	**SA**	**EU**	**SWE**	**Locus ID**	**Chromosome**	**Reference**
Mevinphos	ace1	acetylcholinesterase	JQ085429	A298S	0.44	0.52	0.08	0.67	0.16	0.07	LOC1053867	NC_063995.1	Liu et al. 2024
				G324A	0.44	0.60	0.12	0.79	0.20	0.08	LOC1053867	NC_063995.1	Liu et al. 2024
				F386V	0.00	0.04	0.03	0.10	0.04	0.00	LOC1053867	NC_063995.1	Liu et al. 2024
Benzoylureas	CHS1	chitin synthase 1	NW011952041	I1042M / I1042P	0.00	0.07	0.00	0.20	0.00	0.00	LOC1053802	NC_063984.1	Liu et al. 2024
Fipronil	Rdl-2	Rdl γ-aminobutyric acid (GABA) receptor gene	FJ665609, CABWKJ010000122	A282S	NA						Rdl-2	NC_064012.1	Liu et al. 2024
				A302S	NA						Rdl-2	NC_064012.1	Shen et al. 2022
Abamectin	GluCl	glutamate-gated chloride channel	JX014231	A309V	NA						Glucl	NC_063987.1	Liu et al. 2024
				G315E	NA						Glucl	NC_063987.1	Liu et al. 2024
Pyrethroids	pxNav	voltage-gated para-like sodium channel	AB265177, CABWKK010000518	F1845Y	NA						LOC1053939	NC_064001.1	Liu et al. 2024
				V1848I	NA						LOC1053939	NC_064001.1	Liu et al. 2024
				M918T, M918I	NA						LOC1053939	NC_064001.1	Liu et al. 2024, Shen et al. 2022
				T929I	NA						LOC1053939	NC_064001.1	Liu et al. 2024
				L1014F	NA						LOC1053939	NC_064001.1	Liu et al. 2024
				A1060T	NA						LOC1053939	NC_064001.1	Shen et al. 2022
				F1020S	NA						LOC1053939	NC_064001.1	Shen et al. 2022
Chlorantraniliprole	RYR	ryanodine receptor	JN801028	G4946E	NA						LOC1053926	NC_064011.1	Liu et al. 2024
				I4790M, I4790K	NA						LOC1053926	NC_064011.1	Liu et al. 2024, Shen et al. 2022
				E1338D	NA						LOC1053926	NC_064011.1	Liu et al. 2024
				Q4594L	NA						LOC1053926	NC_064011.1	Shen et al. 2022

Results

🟦 Known mutations

🟩 Unknown mutations

Database format



#CHROM	POS	REF	ALT	AA change	Impact	AFR	AS	NA	SA	EU	SWE	Gene	Gene name	Candidate gene	Reference	Resistance
NC_063984.1	5564103	G	T	p.Ala989Ala	LOW	0	0.0192308	0.0384615	0	0	0	LOC105380296	uncharacterized LOC105380296, transcript variant	CHS1		*Benzoylureas*
NC_063984.1	5564250	T	C	p.Tyr1038Tyr	LOW	1	0.722222	0.888889	0.944444	0.864583	0.833333	LOC105380296	uncharacterized LOC105380296, transcript variant	CHS1		
NC_063984.1	5564254	C	T	p.Leu1040Leu	LOW	0	0	0	0.05	0	0	LOC105380296	uncharacterized LOC105380296, transcript variant	CHS1		
NC_063984.1	5564256	G	A	p.Leu1040Leu	LOW	0	0	0.0277778	0	0	0	LOC105380296	uncharacterized LOC105380296, transcript variant	CHS1		
NC_063984.1	5564260	A	T	p.Ile1042Phe	MODERATE	0	0.0666667	0	0.2	0	0	LOC105380296	uncharacterized LOC105380296, transcript variant	CHS1	Liu et al. 2024	
NC_063984.1	5564275	C	T	p.Leu1047Leu	LOW	0.0625	0	0	0	0	0	LOC105380296	uncharacterized LOC105380296, transcript variant	CHS1		
NC_063984.1	5564277	G	C	p.Leu1047Leu	LOW	0.25	0.145833	0.366667	0.15	0.170732	0.4375	LOC105380296	uncharacterized LOC105380296, transcript variant	CHS1		
NC_063995.1	5970080	G	A	p.Phe388Phe	LOW	0	0	0	0	0	0.0714286	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1		*Mevinphos*
NC_063995.1	5970088	A	C	p.Phe386Val	MODERATE	0	0.037037	0.0277778	0.1	0.0408163	0	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1	Liu et al. 2024	
NC_063995.1	5970092	A	G	p.Cys384Cys	LOW	0	0	0.0294118	0	0	0	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1		
NC_063995.1	5970260	T	C	p.Ala328Ala	LOW	0	0.0178571	0.03125	0	0	0	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1		
NC_063995.1	5970269	G	A	p.Ala325Ala	LOW	0	0	0	0	0.0510204	0	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1		
NC_063995.1	5970273	C	G	p.Gly324Ala	MODERATE	0.4375	0.603448	0.117647	0.785714	0.204545	0.0833333	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1	Liu et al. 2024	
NC_063995.1	5970284	A	T	p.Ile320Ile	LOW	0.0555556	0	0	0	0.0102041	0	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1		
NC_063995.1	5970347	A	T	p.Gly299Gly	LOW	0	0	0	0	0.0108696	0	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1		
NC_063995.1	5970352	C	A	p.Ala298Ser	MODERATE	0.444444	0.517241	0.0833333	0.666667	0.163043	0.0714286	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1	Liu et al. 2024	
NC_063995.1	5970353	C	T	p.Ser297Ser	LOW	0	0.015625	0.0277778	0	0.0104167	0	LOC105386751	acetylcholinesterase-like, transcript variant X1	Ace1		
NC_064001.1	2521645	C	G	p.Leu1937Leu	LOW	0	0	0	0	0	0.125	LOC105393902	sodium channel protein para, transcript variant X4	pxNav		*Pyrethroids*
NC_064001.1	2521654	G	C	p.Ser1934Ser	LOW	0	0	0.0714286	0	0	0.125	LOC105393902	sodium channel protein para, transcript variant X4	pxNav		
NC_064001.1	2521692	A	G	p.Ser1922Pro	MODERATE	0.333333	0.142857	0.875	1	0.346154	0.5625	LOC105393902	sodium channel protein para, transcript variant X4	pxNav		
NC_064001.1	2521699	G	A	p.Arg1919Arg	LOW	0	0	0	0	0	0.142857	LOC105393902	sodium channel protein para, transcript variant X4	pxNav		
NC_064001.1	2521907	A	T	p.Leu1850Gln	MODERATE	0	0	0	0	0	0.166667	LOC105393902	sodium channel protein para, transcript variant X4	pxNav		
NC_064001.1	2521909	G	A	p.Asp1849Asp	LOW	0	0	0	0	0.0119048	0	LOC105393902	sodium channel protein para, transcript variant X4	pxNav		
NC_064001.1	2530855	A	G	p.Ile963Thr	MODERATE	0.277778	0.0862069	0.722222	0.111111	0.48913	0.125	LOC105393902	sodium channel protein para, transcript variant X4	pxNav		
NC_064011.1	2974480	T	C	p.Asp4790Asp	LOW	nan	0	0	nan	0.357143	0.1	LOC105392640	ryanodine receptor	RYR		

Low = no change in protein structure

Moderate = change in protein structure

Will not induce resistance

Could induce resistance

Long-term surveillance

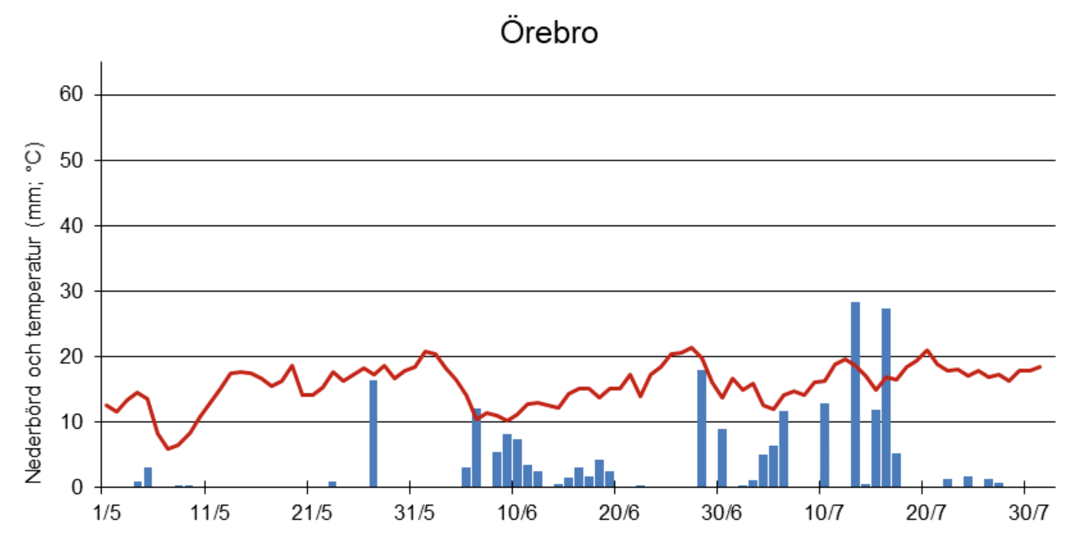
- Few (4) candidates for resistance
- Low frequency (for now)
- Candidate mutations in NaV gene not previously identified for pesticide (e.g., pyrethroids) resistance at high frequency in Sweden

What have we learned?

- Genomic testing can be a tool to detect resistance in a population
- Will not directly answer how severe the resistance is within the population
- Results are achieved faster compared to test with living samples
- Will not detect metabolic resistance
- Could highlight possible future problematic mutations
- We have to be faster on either method, otherwise we can’t give advice to growers

What came out of the inflight?

- Majority of spring oilseed crops were saved by rainshowers
- Average yields of 2,4 ton/ha, 13% above five-year average



Thank’s for the attention

E-mail: lovisa.eriksson@jordbruksverket.se

Phone: + 46 (0)73 3878423