



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

EAAP

Insect-IMP

Athens, Jan. 29-31, 2025

Population genomics from a food matrix: estimating *csd* variability using next generation sequencing on *Apis mellifera* DNA present in honey



Luca Fontanesi

Animal and Food Genomics Group

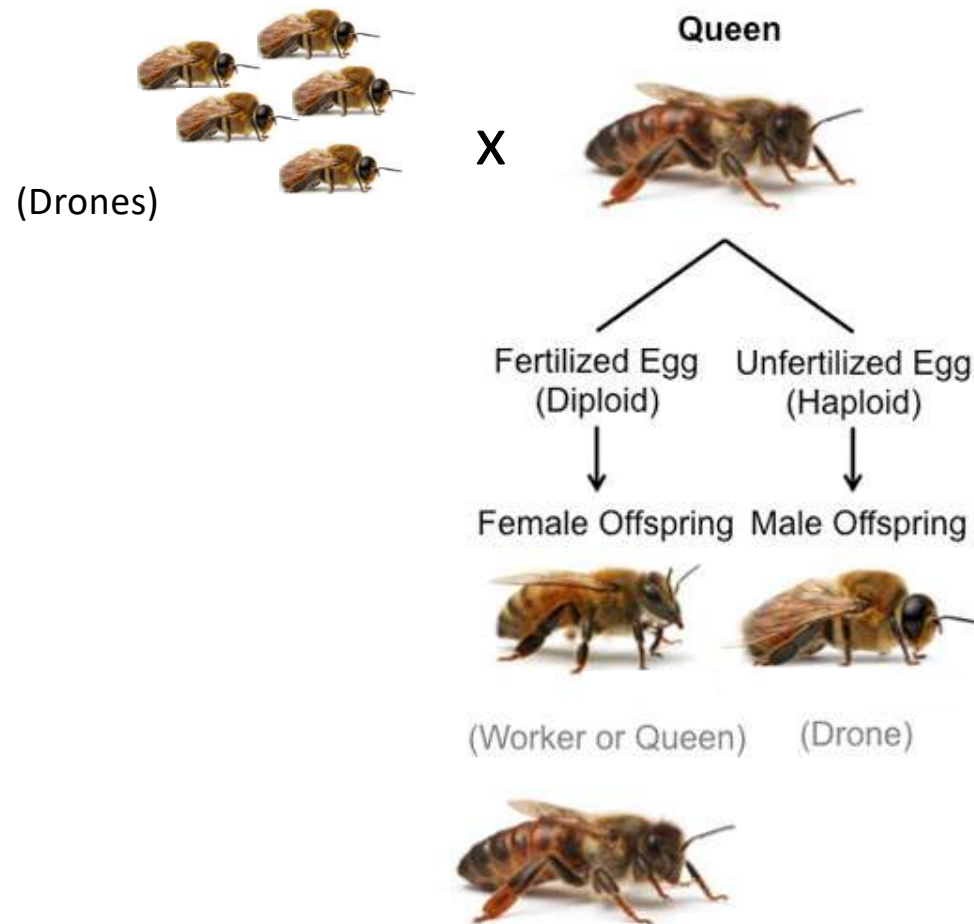
Department of Agricultural and Food Sciences

University of Bologna, Bologna Italy

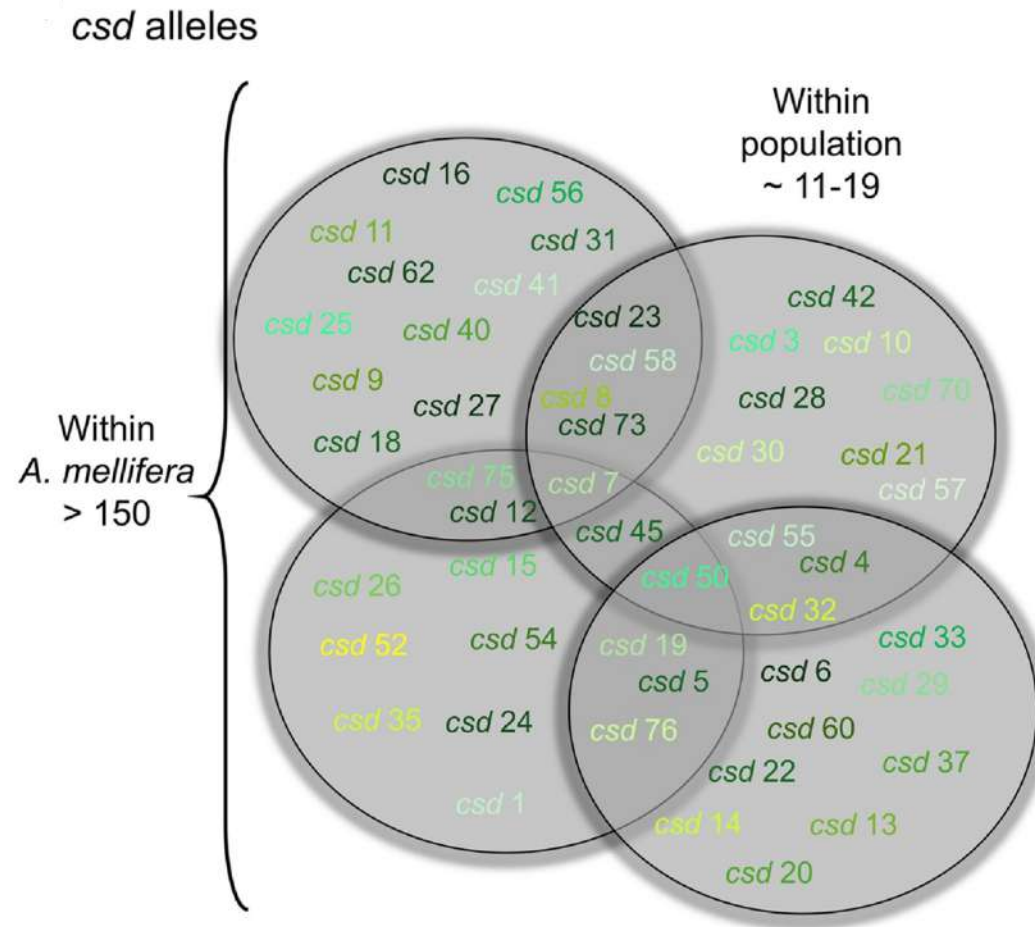
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Apis mellifera sex determination: haplodiploidism



Apis mellifera complementary sex determiner (*csd*) gene

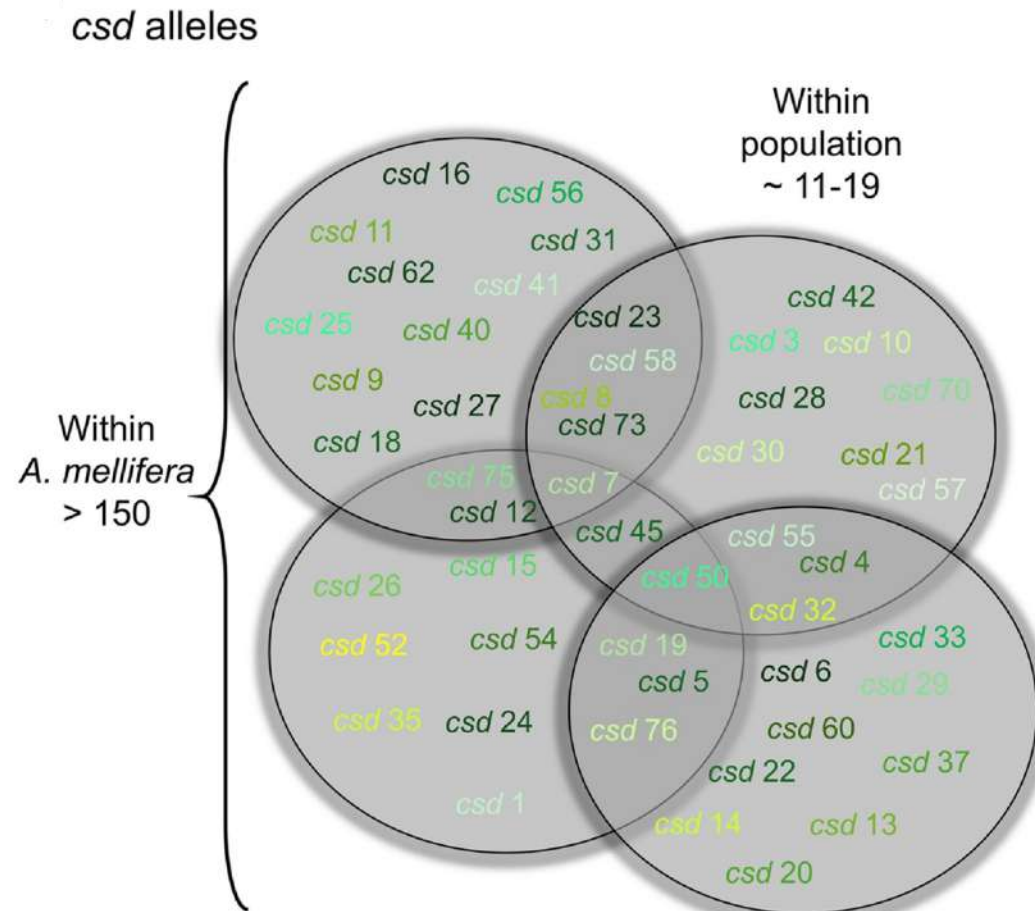


From Seiler & Beye (2024)



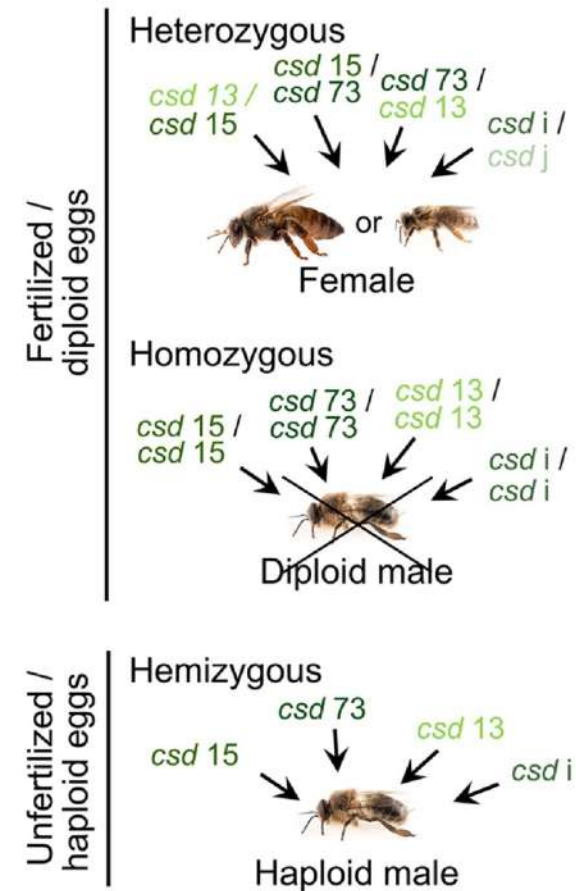
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Apis mellifera complementary sex determiner (csd) gene

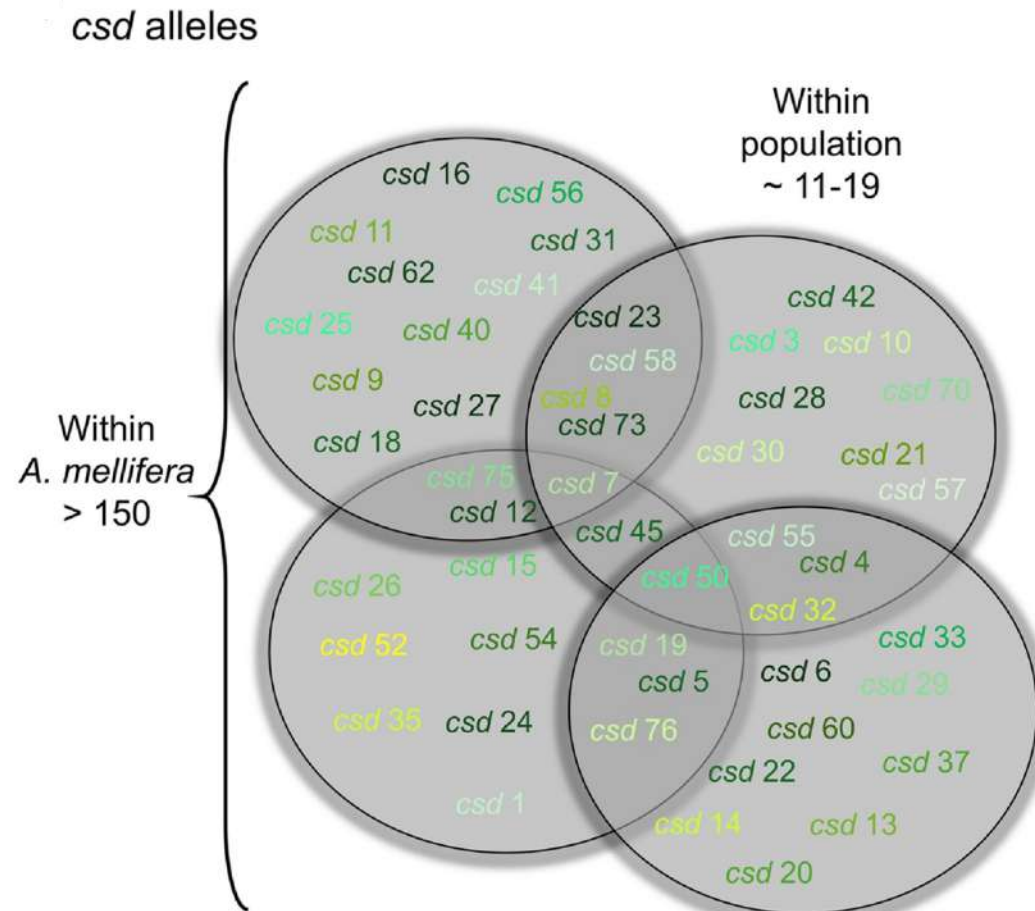


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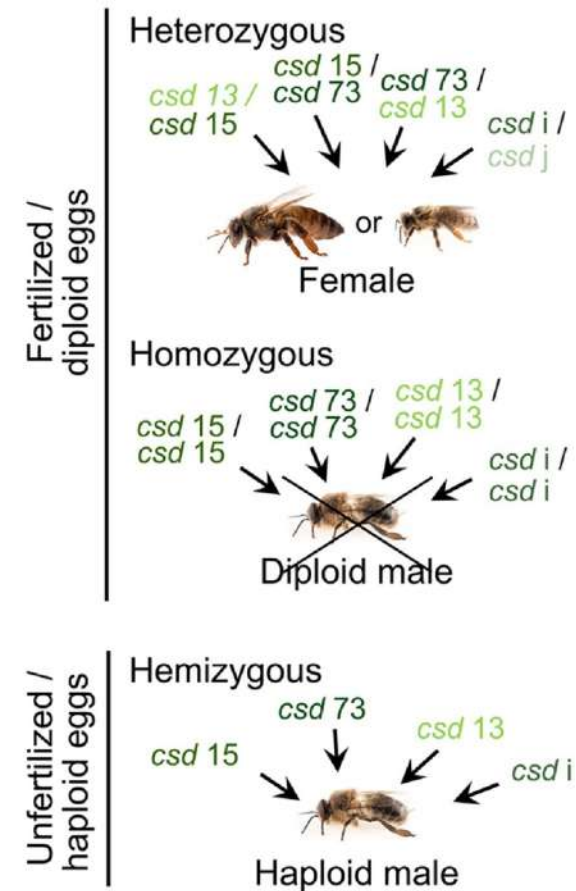
Example of genotypes



Apis mellifera complementary sex determiner (*csd*) gene

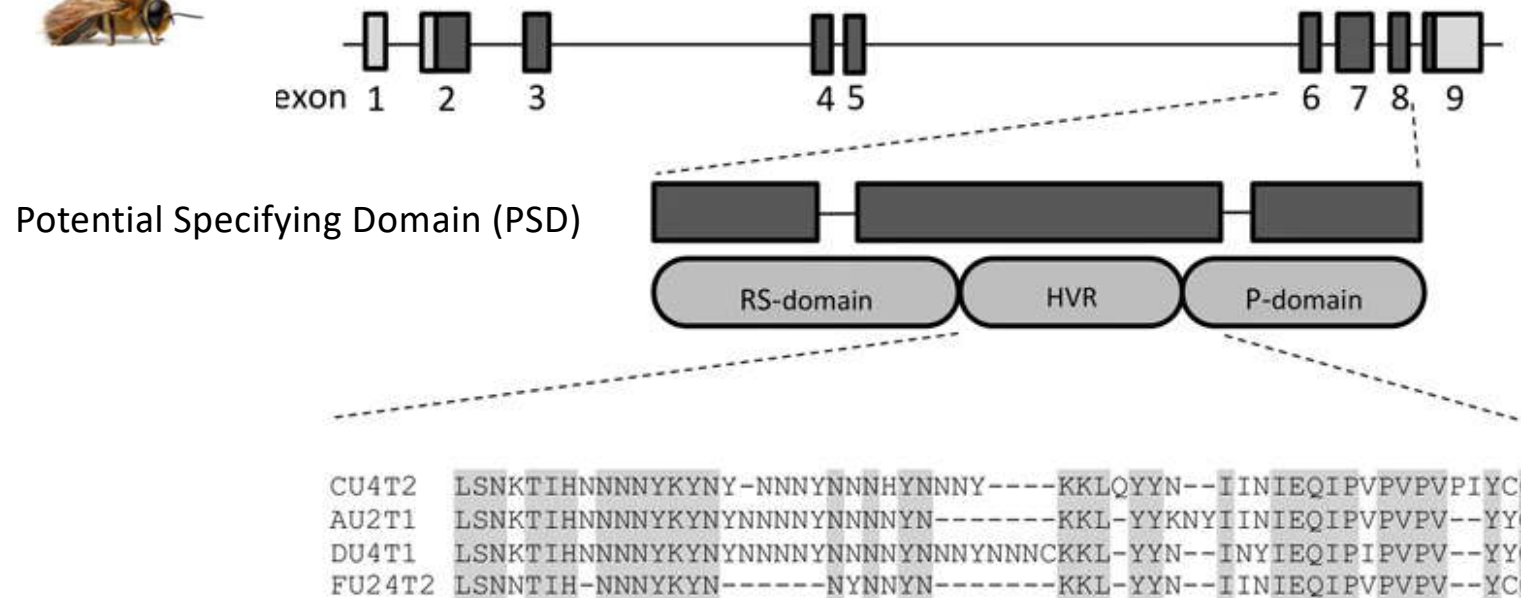


Example of genotypes



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Apis mellifera complementary sex determiner (*csd*) gene

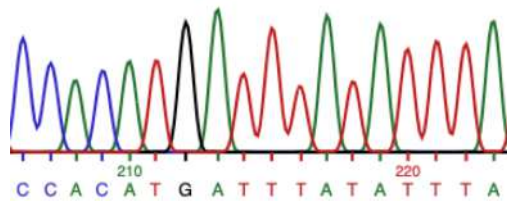


Zareba et al. (2017)



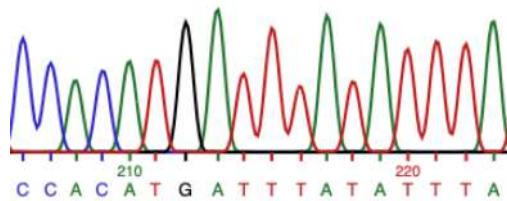
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(Drones)



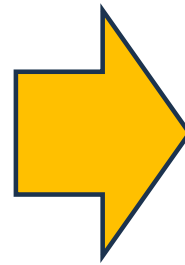
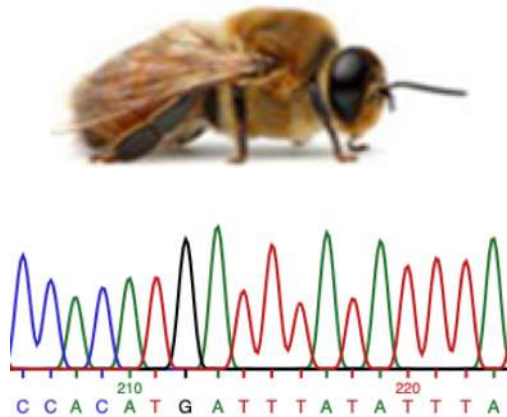
?

(Drones)



We aimed to transform a food matrix
into a population genomic tool

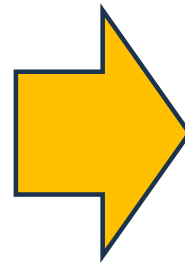
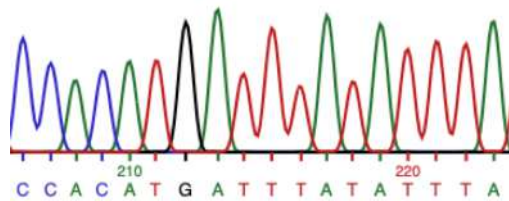
(Drones)



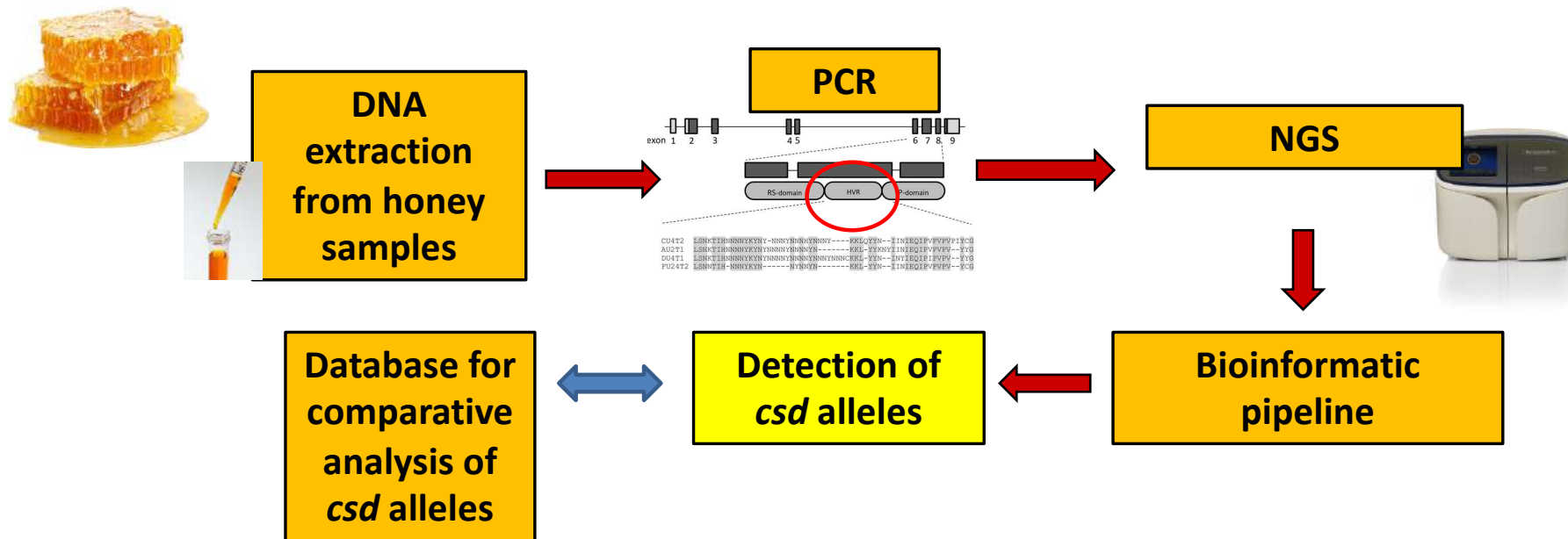
(Worker bees)



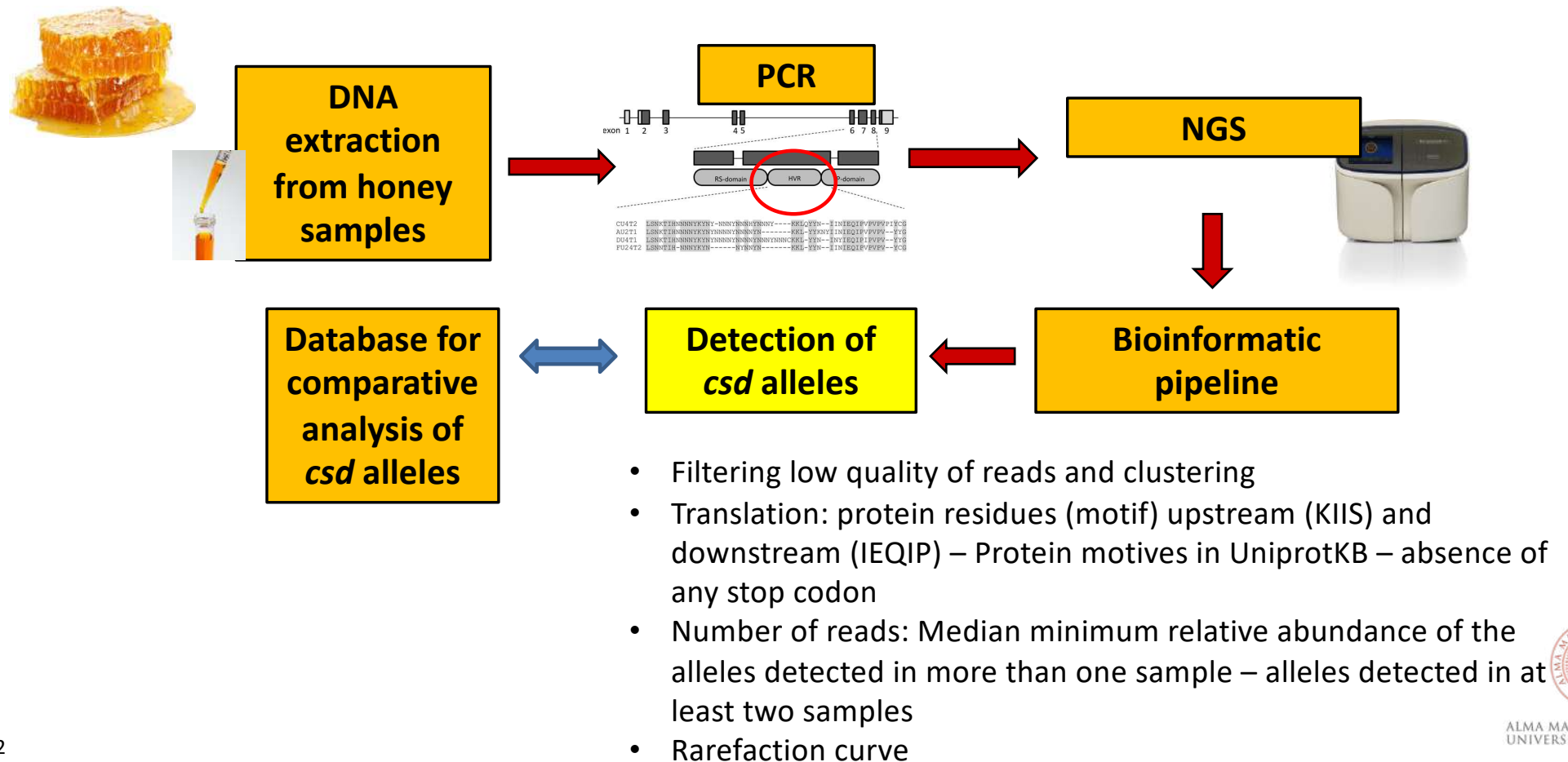
(Drones)



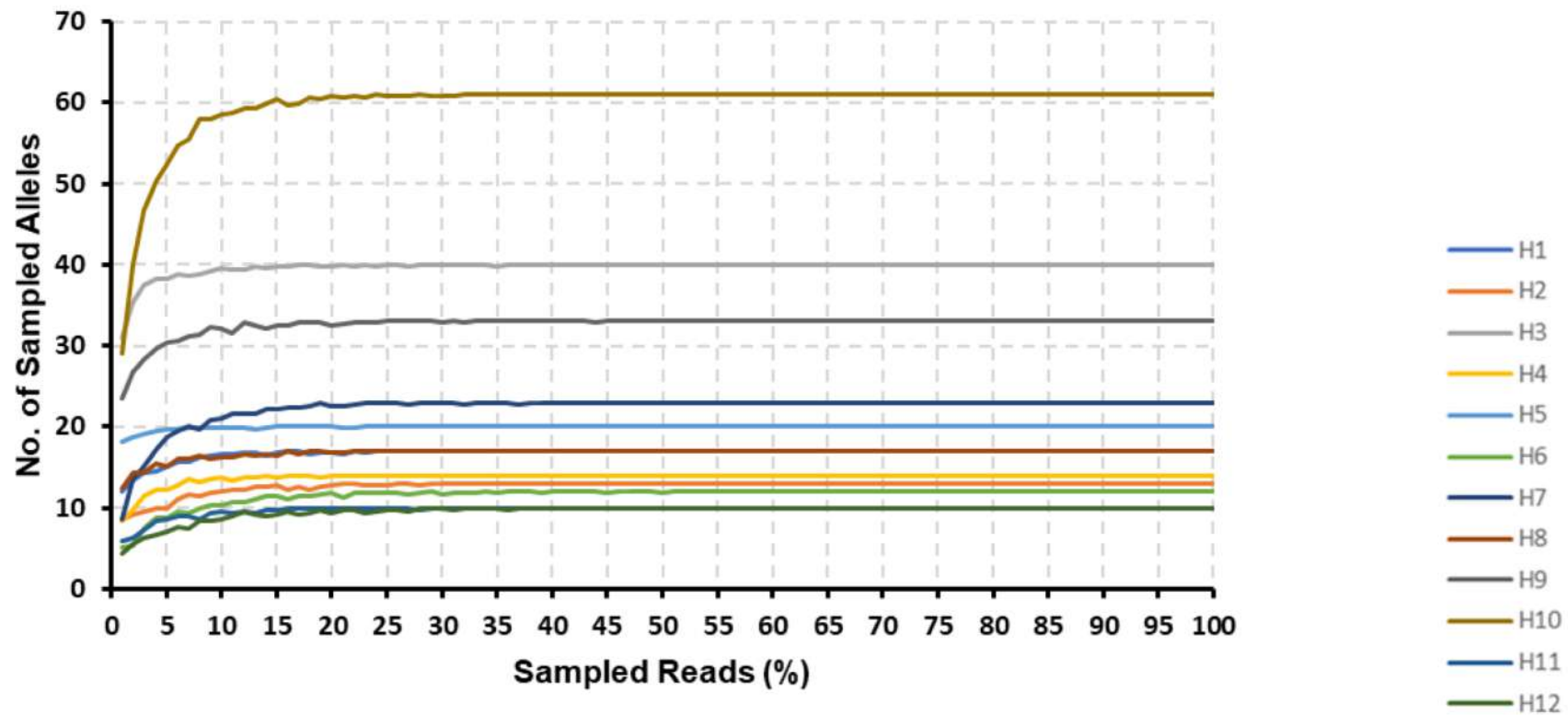
Analysis of *Apis mellifera* complementary sex determiner (*csd*) gene from honey DNA



Analysis of *Apis mellifera* complementary sex determiner (*csd*) gene from honey DNA



Rarefaction curves obtained for the honey samples



Results (1)



Sample ID ¹	Province	No. of Reads	No. of Retained Reads (%) ²
H1	Bologna	173,064	149,678 (86.4)
H2 °	Reggio Emilia	75,454	72,027 (95.4)
H3	Piacenza	414,002	269,785 (64.8)
H4 °	Reggio Emilia	84,307	82,056 (97.3)
H5 °	Reggio Emilia	397,333	296,982 (74.7)
H6	Ferrara	34,350	33,503 (97.5)
H7	Piacenza	99,979	48,369 (48.3)
H8	Bologna	41,741	37,431 (89.7)
H9 *	Piacenza	97,735	73,994 (75.7)
H10	Rimini	70,307	53,728 (75.1)
H11 *	Piacenza	35,183	29,865 (84.9)
H12	Piacenza	1341	1220 (91.0)

Overall: 75.3%



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Results (1)



Sample ID ¹	Province	No. of Reads	No. of Retained Reads (%) ²	No. of <i>csd</i> Protein Alleles ³	No. of Private <i>csd</i> Alleles (%) ⁴	No. of New <i>csd</i> Alleles (Private) ⁵
H1	Bologna	173,064	149,678 (86.4)	17	8 (47.1)	9 (7)
H2 °	Reggio Emilia	75,454	72,027 (95.4)	13	4 (30.8)	5 (3)
H3	Piacenza	414,002	269,785 (64.8)	40	16 (40.0)	13 (11)
H4 °	Reggio Emilia	84,307	82,056 (97.3)	14	2 (14.3)	5 (0)
H5 °	Reggio Emilia	397,333	296,982 (74.7)	20	7 (35.0)	5 (3)
H6	Ferrara	34,350	33,503 (97.5)	13	3 (23.1)	7 (3)
H7	Piacenza	99,979	48,369 (48.3)	23	11 (47.8)	12 (11)
H8	Bologna	41,741	37,431 (89.7)	17	4 (23.5)	3 (1)
H9 *	Piacenza	97,735	73,994 (75.7)	33	13 (39.4)	16 (11)
H10	Rimini	70,307	53,728 (75.1)	61	41 (67.2)	40 (37)
H11 *	Piacenza	35,183	29,865 (84.9)	10	2 (20.0)	3 (1)
H12	Piacenza	1341	1220 (91.0)	10	3 (30.0)	0 (0)

No correlation between the number of reads and the number of *csd* alleles

Overall: 75.3%



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Results (1)



Sample ID ¹	Province	No. of Reads	No. of Retained Reads (%) ²	No. of csd Protein Alleles ³	No. of Private csd Alleles (%) ⁴	No. of New csd Alleles (Private) ⁵
H1	Bologna	173,064	149,678 (86.4)	17	8 (47.1)	9 (7)
H2 °	Reggio Emilia	75,454	72,027 (95.4)	13	4 (30.8)	5 (3)
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H4 °	Reggio Emilia	84,307	82,056 (97.3)	14	2 (14.3)	5 (0)
H5 °	Reggio Emilia	397,333	296,982 (74.7)	20	7 (35.0)	5 (3)
H6	Ferrara	34,350	33,503 (97.5)	13	3 (23.1)	7 (3)
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H8	Bologna	41,741	37,431 (89.7)	17	4 (23.5)	3 (1)
H9 *	Piacenza	97,735	73,994 (75.7)	33	13 (39.4)	16 (11)
H10	Rimini	70,307	53,728 (75.1)	61	41 (67.2)	40 (37)
H11 *	Piacenza	35,183	29,865 (84.9)	10	2 (20.0)	3 (1)
H12	Piacenza	1341	1220 (91.0)	10	3 (30.0)	0 (0)

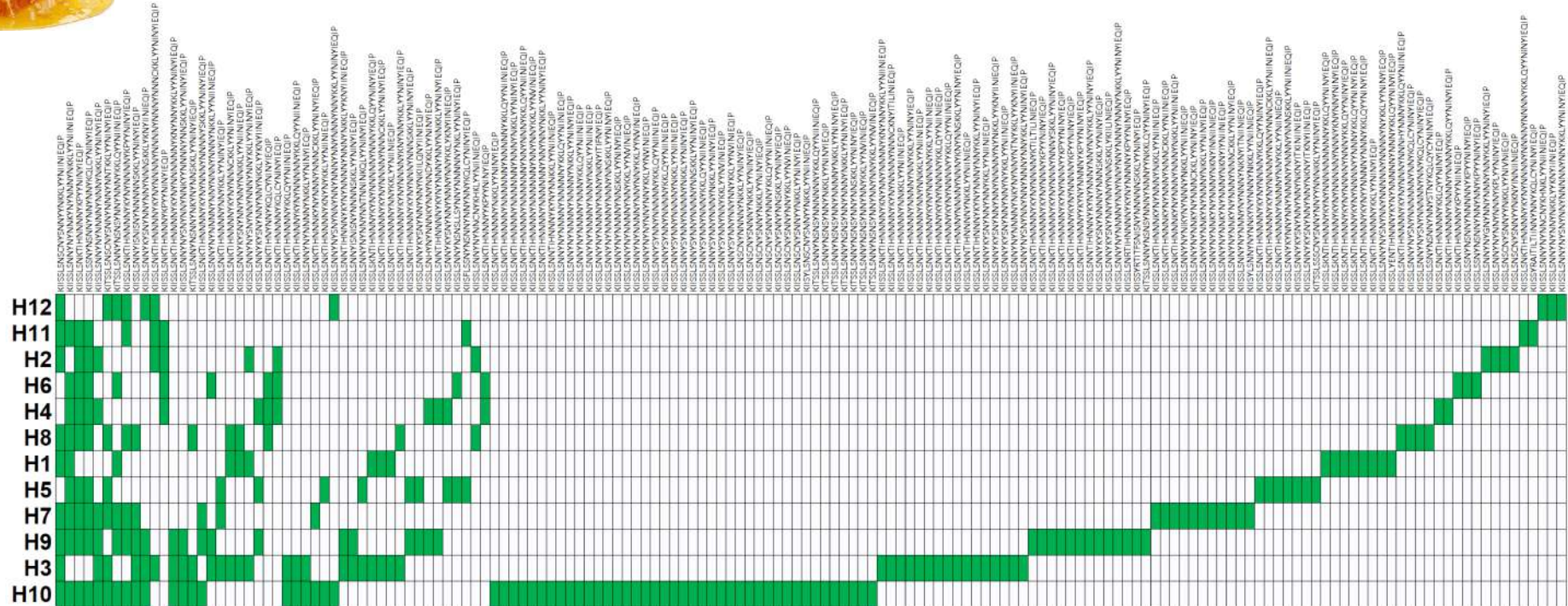
Overall: 75.3%



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Results (1)

Distribution of the 160 *csd* alleles across the honey samples



Results (2)

Most abundant *csd* alleles (>5%) identified in the analyzed honey samples

Sample ID ¹	<i>csd</i> Protein Allele Sequence ²	Abundance %	Novel ³
H1	KISSLKNTIHNNNYKYNYNNNNNNNNNYKLLQYYNINIEQIP	53.4	
H1	KISSLNKTIHNNNNYKLLYNINIEQIP	9.7	
H1	KISSLNNYNSYNNNNNNNNNYKLLYNINIEQIP ‡	8.9	
H2 °	KISSLNSCNSYNNNYKLLYNINIEQIP †	53.7	
H2 °	KISSLNNYNSYNNNNNNNNNYKLLYNINIEQIP ‡	30.2	
H2 °	KISSLNKTIHNNNNYKPPYNINIEQIP **	7.7	
H3	KISSLNNYKYSYNNNNNNNNNKLLYNINIEQIP	40.0	Yes
H3	KISSLNKTIHNNNNYKLLYNINIEQIP	12.4	Yes
H3	KISSLNKTIHNNNNYKYNYNNNNNNNYKLLQYYNINIEQIP	6.3	
H4 °	KISSLNKTIHNNNNYKPPYNINIEQIP **	83.6	
H5 °	KISSLNNYNSYNNNNNNNYKQLCYNINIEQIP @	39.6	
H5 °	KISSLNNYKYSYNNNNNNNKLLYKNYINIEQIP	12.0	
H5 °	KISSLNNYNNKYNNNNNNNKLLYNINIEQIP §	8.5	
H6	KISSLNKTIHNNNNYKPPYNINIEQIP **	93.1	
H7	KISSLNNYNNKYNNNNNNNKLLYNINIEQIP §	38.2	
H7	KISSLNKTIHNNNNKYNNNNNNNCKLLYNINIEQIP	8.9	Yes
H8	KISSLNKTIHNNNNYKPPYNINIEQIP **	33.3	
H8	KISSLNNYNSYNNNNNNNYKQLCYNINIEQIP @	25.9	
H8	KITSSLNNYNSYNNNKYNNNNSKLLYNINIEQIP	13.2	
H8	KISSLNKTIHNNNNYKYNNNNNNNYKLLYNINIEQIP	5.8	
H9 *	KISSLNKTIHNNNNYKYNNNNNNNYKPPYNINIEQIP	45.0	
H9 *	KISSLNKTIHNNNNYKYNNNNNNNNSKLLYNINIEQIP	10.3	Yes
H9 *	KISSLNNYISNISYNNNNNNSKLLYNINIEQIP	5.2	
H10	KISSLNSCNSYNNNYKLLYNINIEQIP †	17.6	
H11 *	KISSLNNYNSYNNNNNNNYKQLCYNINIEQIP @	43.8	
H11 *	KISSLNKTIHNNNNYKPPYNINIEQIP **	32.5	
H11 *	KISSLNNYNNKYNNNNNNNKLLYNINIEQIP §	6.7	
H12	KITSSLNNYNSYNNNNNNNYKLLQYYNINIEQIP	37.5	
H12	KISSLNNYNSYNNNNNNNNNNNNNNNNNNNYKLLYNINIEQIP	31.5	
H12	KISSLNKTIHNNNNYKYNNNNNNNNNNNNNNNNNCKLLYNINIEQIP	5.4	
H12	KISSLNNYKYSYNNNNNNNNNNSKLLYKNYINIEQIP	5.3	
H12	KISSLNSCNSYNNNYKLLYNINIEQIP †	5.1	

¹ Samples provided by the same beekeeper are marked with the same symbol (* or °).

² The same alleles identified in different samples are marked with the same symbol.

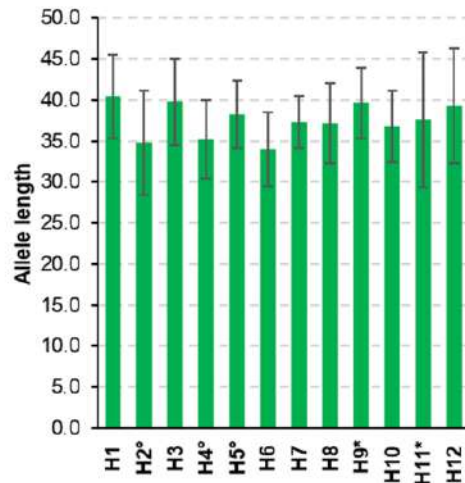
³ Alleles that were not present in NCBI Database.



Results (3)

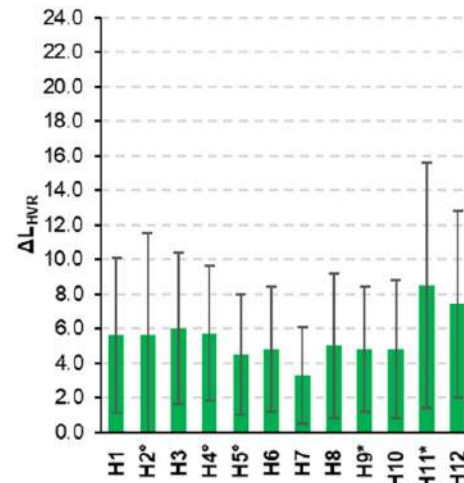
Study of the within sample HVR *csd* variability

(a)



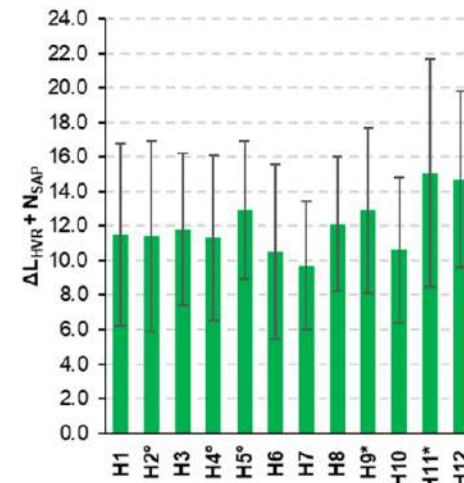
Allele length (mean $\pm$ s.d.)

(b)



ΔL_{HVR} (mean $\pm$ s.d.)

(c)



Total differences
($\Delta L_{HVR} + N_{SAP}$; mean $\pm$ s.d.)

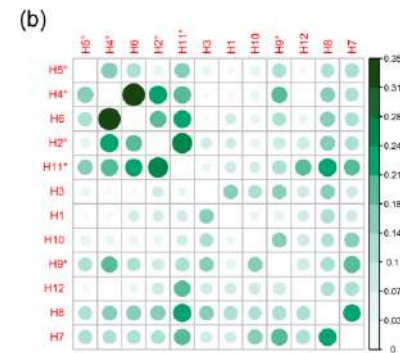
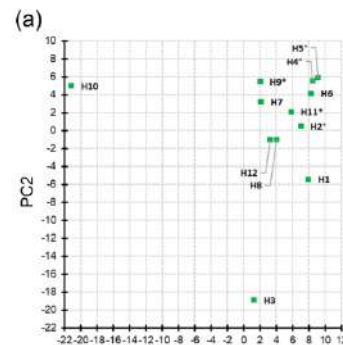


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Results (4)

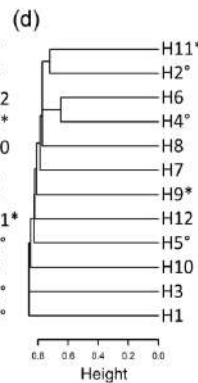
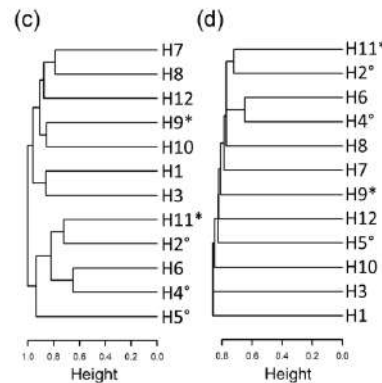
Analyses of honey sample similarity

Logistic
PCA

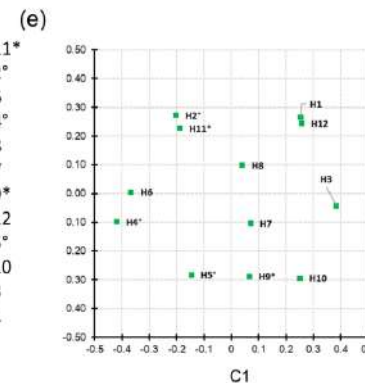


Jaccard similarity coefficient

Single-linkage
clustering based on
Jaccard distance



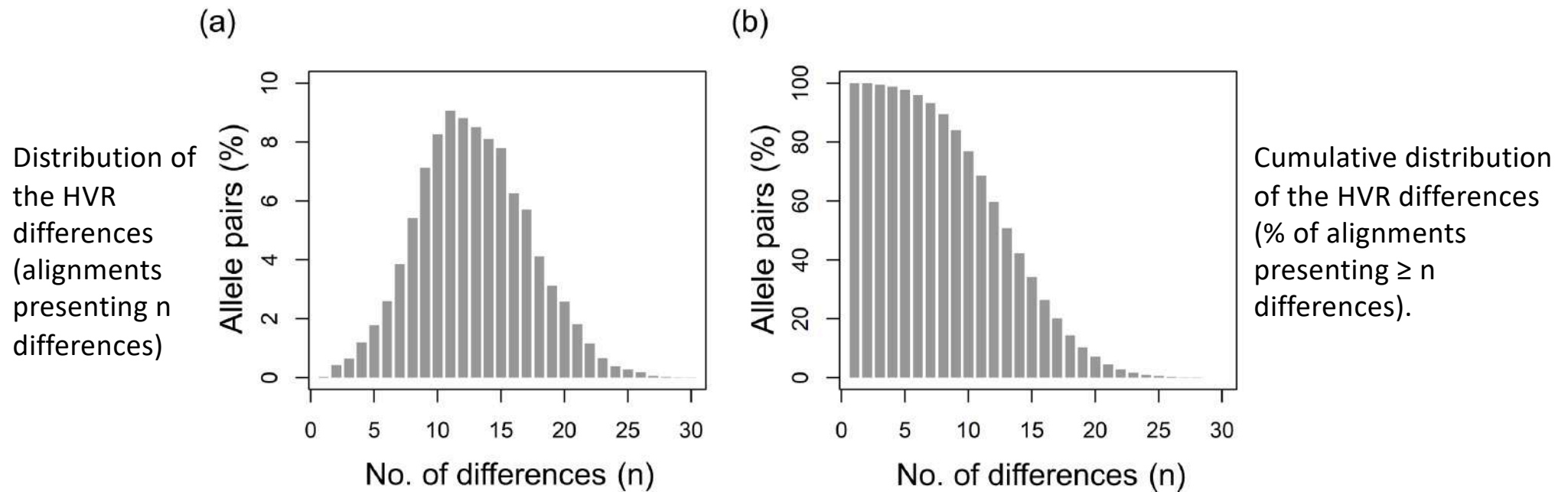
Complete-linkage
clustering based on
Jaccard distance



Multidimensional scaling
of the Jaccard distance
matrix

Results (5)

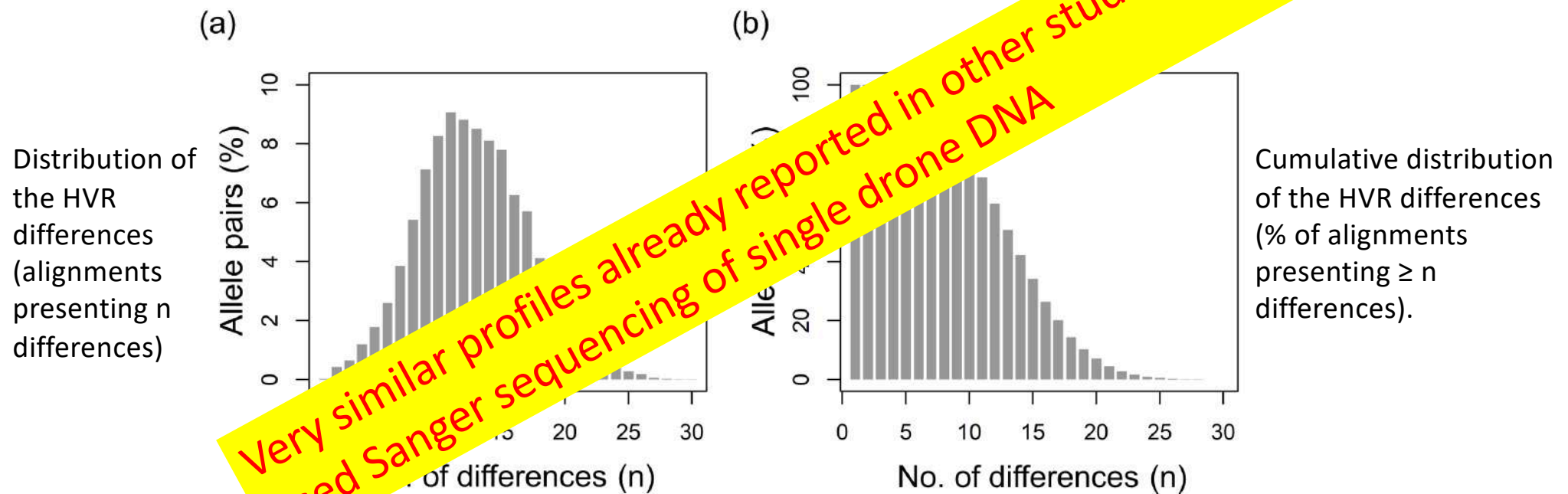
Study of the HVR *csd* variability via pairwise alignment of 160 non-identical alleles identified in the investigated honey samples.



Differences considered the $\Delta L_{\text{HVR}} + N_{\text{SAP}}$ values

Results (5)

Study of the HVR *csd* variability via pairwise alignment of 100 non-identical alleles identified in the investigated honey bee colonies.



Differences considered the $\Delta L_{HVR} + N_{SAP}$ values

Conclusions

1



Technical issues can be managed and considered to correctly interpret the final results



Conclusions

1



Technical issues can be managed and considered to correctly interpret the final results

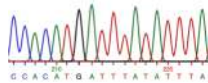
2



≈



For honey bee population genetic studies, including analysis of the *csd* gene



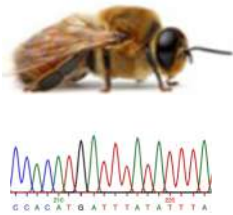
Conclusions

1



Technical issues can be managed and considered to correctly interpret the final results

2



≈



For honey bee population genetic studies, including analysis of the *csd* gene

3



Can be more effective in capturing all *csd* alleles present in a colony

Co-authors of the Animal and Food Genomics Group of the University of Bologna



Samuele Bovo
Junior Assistant Professor



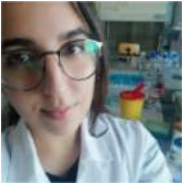
Francesca Bertolini
Associate Professor



Anisa Ribani
Junior Assistant Profess



Luca Fontanesi
Full Professor



Valeria Taurisano
Post-doc



Giuseppina Schiavo
Junior Assistant Professor



Regione Emilia-Romagna



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