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Variation in secondary metabolite production potential in the *Fusarium incarnatum-equiseti* species complex revealed by comparative analysis of 13 genomes

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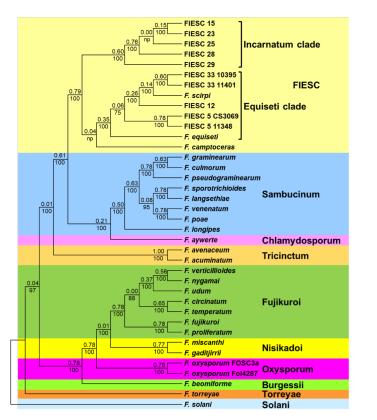
FUSARIUM INCARNATUM-EQUISETI SPECIES COMPLEX

Members of FIESC

- > are cosmopolitan soil inhabitants
- are regularly identified as co-occurring with other Fusarium species in cereals, fruits, and vegetables
- can cause human and insect infections
- can produce multiple SM/mycotoxins including trichothecenes, zearalenone, beauvericin, and equisetin







FIESC is a lineage of *Fusarium* that consists of over 35 phylogenetically distinct species

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- diversity and distribution of BGCs in 13 FIESC genomes
- evolutionary processes that have likely contributed to the observed distribution

Villani et al. 2019, BMC genomics

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Which evolutionary force has contributed to the distribution of SM biosynthetic genes among species?

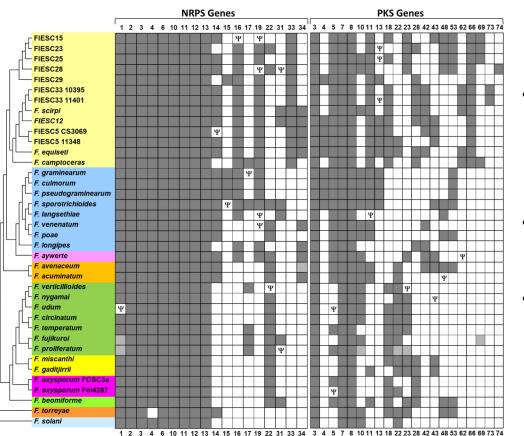
Vertical inheritance, gene loss, and HGT are major contributors to the variation in the content of BGCs

Bioinformatics-based evidence for gene exchange between fungal species is based on analyses of genomic sequence data

• SPECIES TREES

COMPARISON OF GENES IN NEIGHBORING SPECIES

• COMPUTATIONAL METHODS FOR DETECTION OF HGT, LOSS, AND DUPLICATION



33–42 SM biosynthetic gene clusters per FIESC genome, comparable to the numbers of clusters in other *Fusarium* genomes

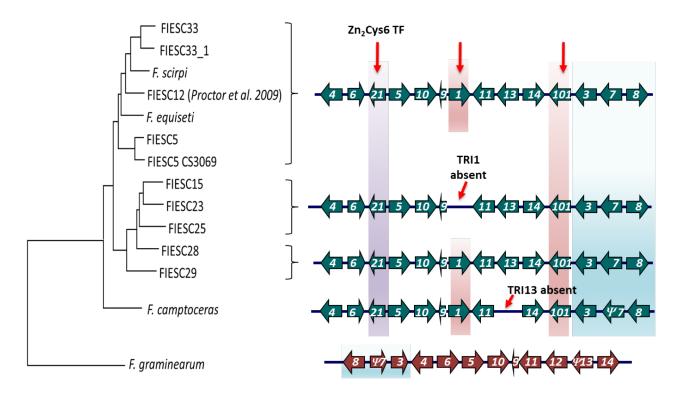
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- 6 PKSs and 9 NRPSs present in all genomes
- 3 PKSs and one NRPS are uniquely present in FIESC



TRICHOTHECENE CLUSTER IN FIESC





Evolutionary forces acting upon distribution of SM gene clusters within FIESC

- 1) assessment of the occurrence of the genes in FIESC and other *Fusarium* lineages
- 2) manual comparison of single NRSP/PKS gene trees to the species tree
- 3) reconciliation analysis (NOTUNG)
- 4) genetic divergence analysis (dS analysis)
- 5) constraint analyses (Shimodaira-Hasegawa and SH-AU tests)



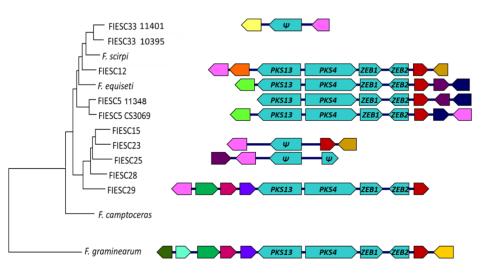
Evolutionary forces acting upon distribution of SM gene clusters within FIESC

Vertical Inheritance

- widespread occurrence of NRPS/PKS genes in FIESC and other *Fusarium* lineages
- topological similarities between NRPS/PKS gene trees and the species tree
- reconciliation between the gene tree and rooted species tree
- dS values for NRPS/PKS genes significantly higher than dS values for HK genes

Evolutionary forces acting upon distribution of SM gene clusters within FIESC

Gene loss



Zearalenone biosynthetic gene cluster among FIESC members

 sporadically distribution of NRPS/PKS among FIESC genomes

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- presence of pseudogenized NRPS/PKS genes
- NOTUNG may have overestimated the number of losses



Horizontal gene transfer

Gene	HGT Donor	HGT Recipient	Identification ^a		Additional Evidence for HGT ^b		
			Manual Tree Comparison	NOTUNG	Bootetrap	SH-AU	ds
			Companson	NOTUNG	воотегар	3H-AU	us
		HGT Events with F	IESC Recipient				
NRPS4	Tricinctum complex	FIESC (Incarnatum clade)	÷	+	100	+	+
NRPS11	Sambucinum complex (or close relative)	FIESC	-	+	-	-	-
NRPS14	Sambucinum complex (or close relative)	FIESC	-	+	100°	+	-
NRPS16	F. longipes (or close relative)	FIESC	+	+	77	-	±
NRPS22	Fujikuroi complex (African clade)	FIESC (Incarnatum clade)	+	+	100	+	+
PKS10	Tricinctum complex	F. scirpi (or recent ancestor)	+	+	100	+	+
PKS10	Sambucinum complex	FIESC (Incarnatum clade)	+	+	100	+	-
PKS22	F. torreyae relative	FIESC (Equiseti clade)	-	+	100	+	-
PKS23	Fujikuroi complex	FIESC (Incarnatum clade)	+	+	100 ^d	+	+
PKS48	Tricinctum complex (F. avenaceum relative)	FIESC (Incarnatum clade)	+	+	100	+	-
PKS62 ^e	Fujikuroi complex	FIESC	NA	NA	-	NA	+
PKS69°	Fujikuroi complex	FIESC	+f	NA	90	+	+
		HGT Events with	FIESC Donor				
NRPS19	FIESC	Sambucinum complex	+	+	74	+	±
NRPS34	FIESC (Equiseti clade)	F. longipes	-	+	-	-	±
PKS22	FIESC (Equiseti clade)	Tricinctum complex	+	+	100	+	-
		Ancestor of					
PKS42	FIESC (Equiseti clade)	Sambucinum and Chlamydosporum complexes	-	+	-	-	±
		Sambucinum complex					
PKS43	FIESC (Incarnatum clade)	(F. sporotrichioides ancestor)	-	+	-	-	±
PKS43	FIESC (Equiseti clade)	F. avenaceum (or recent ancestor)	-	+	-	-	±
PKS43	FIESC5 (or close relative)	F. aywerte (or recent ancestor)	-	+	-	-	±

Evidence for HGT event:

- Manual tree comparison: **10** HGT events
- NOTUNG analysis: 17 HGT events
- SH-AU test: 11 HGT events
- ds analysis: 6 HGT events

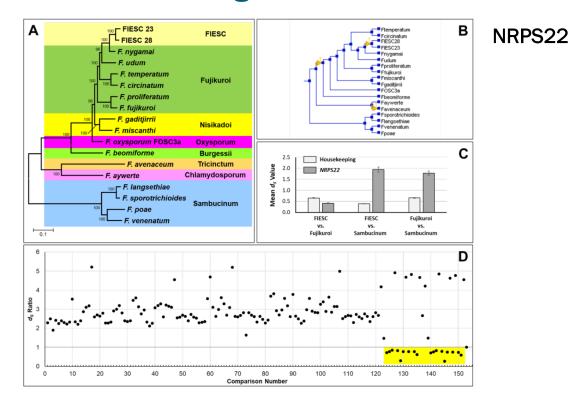
HGT has contributed to distribution of four clusters:

- 1. PKS10 (fusarin)
- 2. NRPS22 (beauvericin)
- 3. PKS23
- 4. NRPS4

Evolutionary forces acting upon distribution of SM gene clusters within FIESC Horizontal gene transfer

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Conclusion

- vertical inheritance has contributed to the distribution of almost all FIESC clusters
- gene duplication events have not contributed to distribution of NRPS/PKS genes in FIESC
- gene loss and HGT have contributed to the diversity of SM clusters in FIESC
- HGT has contributed to distribution of four clusters

understand the underlying process responsible for variability in SMs distribution has the power to prevent spread of virulence, resistance, and loss or exchange of selectable DNA among fungi







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