Supplementary Materials for

Multiple mutations in polyketide synthase led to disruption of Psittacofulvin production across diverse parrot species

Shatadru Ghosh Roy *et al.*

*Corresponding author. Email: abdu@bgu.ac.il

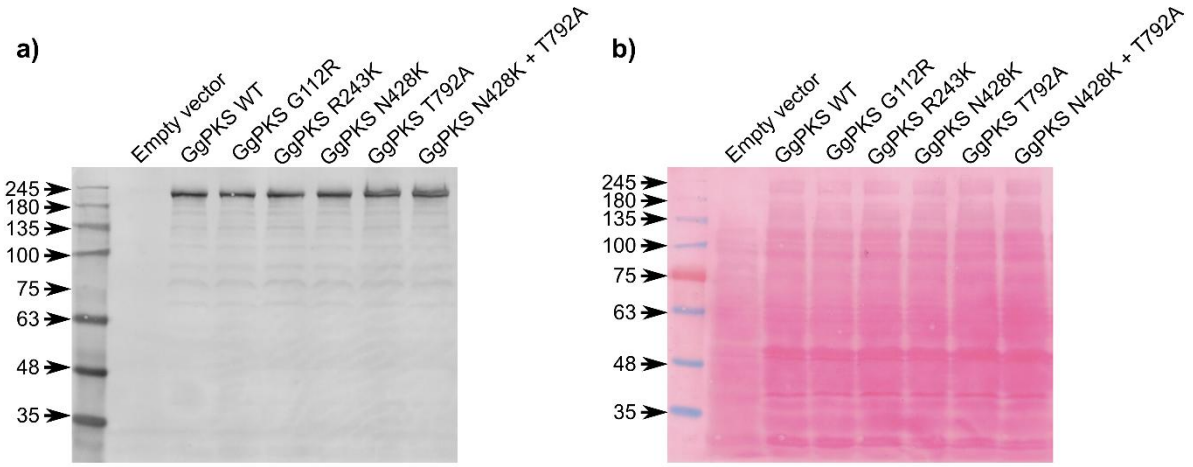

Fig. S1. Confirmation of HA-tagged GgPKS expression in BJ5464-NpgA yeast strain. (**A**) western blot against HA-tagged GgPKS expressed in yeast (expanded view of bottom row of Figure 3d). The input materials were total soluble protein extracts from the GgPKS-expressing yeast strains. (**B**) The Ponceau S-stained membrane is shown as a loading control.

Common name	**Scientific name**	**Family**	**Male**	**Female**
Rose-ringed	*Psittacula krameri*	Psittaculidae	predominantly green body with a pink and black neck ring	predominantly green body with no neck rings, or shadow-like pale to dark grey neck rings
Alexandrine	*Psittacula eupatria*	Psittaculidae	predominantly green body with red patch on the shoulders; black stripe across their lower cheeks and a pink band on their nape	Same green appearance as males with red patch on the shoulders; no black cheek stripe or pink nape band
Eclectus	*Eclectus roratus*	Psittaculidae	mostly bright green: tail feathers are green centrally and bluer as they get towards the edges.	mostly bright red with a darker purple hue on the back and wings
Galah	*Eolophus roseicapilla*	Cacatuidae	No sexual dimorphism; pale silver to grey back, pale grey rump, pink face and breast, and light pink crest	

Table S1: Species details included in this study with key features of their plumage coloration.

Sl no.	Exon#	Fw	Rv
1.	1	GGGAAGCTCACCTTTTAAC	CTACAGAAGGGAGGAACGTG
2.	2	GTGGGAGTTCCTCCTAAC	GCTCTCCTTGTGCCTTGCAC
3.	3	CAGTTACTGCTTTGCTTTAG	CCTTAGATCTCCAGTGCTAG
4.	4	GCACTATTGATGCCATGTCG	CCTGGAGTAAAATAGAAAC
5.	5	CGGGATTGAAGAGGTGTTAG	GCAACCACTCTTATGTTAC
6.	6a	CCCCTACTATGGGAAATG	GCATCATCAGAAGCACTTTG
7.	6b	GGAAATATTGGGCACACTG	GGAAGGGTTATGGAATTCC
8.	6c	GCCTCTGGAATTAGTTCTGC	CTTTCACCTCTTGCTGGCAG
9.	6d	CGCTGAAGGAGTTCAGTGAG	CCTCAACAGGGATGTTTCC
10.	6e	CCCTGGCAGATGCAGTC	CATCATCAGAAGCAGCCAC
11.	6f	GCATGGATATTATACTTG	GCCAGTTGGGATTATATCC
12.	6g	GTTCTCTTCTTTGCAAAATG	CTTGATGCTCAAGACTTGGG
13.	6h	GTGGCTTTAGTCCCTGGTGC	CTTGTTTACCTTTATGCTTG
14.	6i	GTATGGCTCCATATTCAAGC	CTGTGAAAGAGTTATTTC
15.	6j	GCCATCACTTTCATGAAG	CTTTCAGTTGTTCTGTAG
16.	6k	CTATCGCCAAGTTGTTGTG	GTTGAGTAGCAGTGCTGGTGG
17.	6l	CAGAAATGTTTACTTTGTAC	CCTGGAATCTGAACTCTGG
18.	6m	GCAACGGGCGTTGATGTAAAG	GTCGATTGCCACACACTATC
19.	6n	CCACTGCTGATAAGTTCCG	CAGCTTCTGGGATTCATACAG
20.	6o	CCTGCAAATCTGTCCCAC	GCCCAATGAAGATTTAGGG
21.	6p	GCAGGGAGTCCAATCAAAG	GTAAGCTCTTCCTGAGATAC
22.	6q	CAAAACCATCTTCAGTCCC	CATGGTTCTGAATTGCCATAG
23.	6r	CTAACCATGAATACAGCAC	GAAGAGTACACTCAGCATC

Table S2. Intronic primers used for genotyping PKS coding sequence of *P. Krameri*, *P. eupatria*, *E. roratus*. Exon 6 has been split into 18 (6a-6r) overlapping amplicons of 500bp approximately.

	Exon#	Fw	Rv
1.	1	GGGAAGCTCACCTTTTAAC	CTACAGAAGTAAGGGAGGAATG
2.	2	GTGGGAGTTCCTCGTAACT	CAGTTCTCCCTGTGCCTTGCAC
3.	3	CAGTTACTGCTTTGCTTTTC	CCTTAGATCTCCAGTGCTAG
4.	4	GCACTACTGATGCCATGTAG	CCTGGAGTGAAATAGAAAC
5.	5	GGGACTGAAGAGATGCTAGAC	GCAACCACTCTTATGTTAC
6.	6a	CCCCTACTATGGGAAATG	GCATCATCAGAAGCACTTTG
7.	6b	GGAAATATTGGGCACACTG	GGGAGGGTTATGGAATTCC
8.	6c	GCCTCTTGAATTAGTTCTGC	CTTTCACCTCTTGCTGGCAG
9.	6d	CACTGAAGGAGTTCAGTGAGG	CTCTTCAACAGGGATGTTTCC
10.	6e	CCCTGGCAGATGCAGTC	CATTTTCAGAAGCAGCCAC
11.	6f	GCATGGATATGATACTTGG	GCCAGTTGGGATTATATCC
12.	6g	GTTCTCGTCTTTGCAAAATG	CTTGATGCTCAAGACTTGGG
13.	6h	GTGGCTTTAGTCCCTGGTGC	CCTTGTTCACCTTTATGCTTG
14.	6i	GTATGGCTCCGTATTCAAGC	CTGTGAAAGAGATATTTC
15.	6j	GTTACCATCACTTTCATGAAG	CTTTCAGTTGTTCTGTAG
16.	6k	CTATCGCCAAGTTGTTGTAGCG	GCTGAGTAGCAGTGCTGGTGG
17.	6l	CAGAAACATTTACTTTGTA	GATCTCCCATGCAATGATAAAG
18.	6m	CAGAATTCAGATTCCAGGAAC	GTCGACTGCCACACACTATC
19.	6n	CCACTCCTGATAGGTTCCGG	CAGCTTCTGGGATTCATACAG
20.	6o	CCTGCAAATCTGTCCCAC	GCCCAATGAAGATTTAGGG
21.	6p	GTGGGGAGTCCAATCAAAG	GTAAGCTCTTCCTGAGATAC
22.	6q	CAAAACCATCTTCAGTCCTTTTTG	CACGGTTCTGAATCGTCATAG
23.	6r	GAACTAACTATGAATACAGCAC	GAAGAGTACACTCAGCATC

Table S3. Intronic primers used for genotyping PKS coding sequence of *E. roseicapilla*. Exon 6 has been split into 18 (6a-6r) overlapping amplicons of 500bp approximately.

Percent Identity	GgPKS	EorPKS	MuPKS	EcrPKS	PkPKS	PePKS
GgPKS	100	79.4	79.13	79.4	79.17	79.22
EorPKS		100	90.68	91.21	90.36	90.41
MuPKS			100	93.87	93.39	93.44
EcrPKS				100	97.53	97.58
PkPKS					100	99.86
PePKS						100

Table S4. Percent Identity Matrix (created by Clustal2.1) of deduced amino acid sequence of parrot PKS homologs tested in this study along with annotated MuPKS as reference and GgPKS used for functional validation.

Species	Sample #	Phenotype	SNP found as homozygous
P. krameri	1	Green	None
P. krameri	2	Green	None
P. krameri	3	Green	None
P. krameri	4	Green	None
P. krameri	5	Green	None
P. krameri	6	Green	None
P. krameri	7	Green	None
P. krameri	8	*blue*	G334A
P. krameri	9	*blue*	G334A
P. krameri	10	*blue*	G334A
P. krameri	11	*blue*	G2005T
P. krameri	12	*blue*	G2005T
P. krameri	13	*blue*	G334A
P. krameri	14	*blue*	G334A
P. krameri	15	*blue*	G2005T
P. krameri	16	*blue*	G2005T

Table S5. Phenotypic and genotypic details of *P. krameri* specimens tested in this study.

Species	Sample #	Phenotype	SNP found as homozygous
P. eupatria	1	Green	None
P. eupatria	2	Green	None
P. eupatria	3	Green	None
P. eupatria	4	Green	None
P. eupatria	5	Green	None
P. eupatria	6	Green	None
P. eupatria	7	Green	None
P. eupatria	8	Green	None
P. eupatria	9	Green	None
P. eupatria	10	Green	None
P. eupatria	11	Green	None
P. eupatria	12	Green	None
P. eupatria	13	Green	None
P. eupatria	14	Green	None
P. eupatria	15	Green	None
P. eupatria	16	Green	None
P. eupatria	17	Green	None
P. eupatria	18	Green	None
P. eupatria	19	Green	None
P. eupatria	20	Green	None
P. eupatria	21	Green	None
P. eupatria	22	Green	None
P. eupatria	23	Green	None
P. eupatria	24	Green	None
P. eupatria	25	Green	None
P. eupatria	26	*blue*	G728A
P. eupatria	27	*blue*	G728A
P. eupatria	28	*blue*	A2647T
P. eupatria	29	*blue*	A2647T
P. eupatria	30	*blue*	G728A
P. eupatria	31	*blue*	A2647T
P. eupatria	32	*blue*	A2647T
P. eupatria	33	*blue*	G728A
P. eupatria	34	*blue*	G728A
P. eupatria	35	*blue*	A2647T
P. eupatria	36	*blue*	A2647T

Table S6. Phenotypic and genotypic details of *P. eupatria* specimens tested in this study.

Species	Sex	Sample #	Phenotype	SNP found as homozygous
E. roratus	Male	1	Green (Male)	None
E. roratus	Male	2	Green (Male)	None
E. roratus	Female	3	Red (Female)	None
E. roratus	Male	4	*blue* (Male)	C5869T
E. roratus	Male	5	*blue* (Male)	C5869T
E. roratus	Female	6	*blue* (Female)	C5869T
E. roratus	Female	7	*blue* (Female)	C5869T
E. roratus	Male	8	*blue* (Male)	C5869T

Table S7. Phenotypic and genotypic details of *E. roratus* specimens tested in this study.

Species	Sex	Sample #	Phenotype	SNP found as homozygous
E. roseicapilla		1	Pink	None
E. roseicapilla		2	Pink	None
E. roseicapilla		3	Pink	None
E. roseicapilla		4	Pink	None
E. roseicapilla		5	*blue*	T1284G; A2374G
E. roseicapilla		6	*blue*	T1284G; A2374G
E. roseicapilla		7	*blue*	T1284G; A2374G
E. roseicapilla		8	*blue*	T1284G; A2374G
E. roseicapilla		9	*blue*	T1284G; A2374G

Table S8. Phenotypic and genotypic details of *E. roseicapilla* specimens tested in this study.

Primer name	Sequence
G112R_F	TGCAaGAGTCCCTGTAGAAGC
G112R_R	AACAAAAACGCCCGTTTTGGTGCCACTGATGGCTTCTACAGGGAC TCtTGCATCCTCCAGTGCTTTGTATG
R243K_F	GGAAaGGGAGAAGGCTGTGGTG
R243K_R	ATTGCCTTTTCCAGTGGTTTGAGGAATACAACACCACAGCCTTCTC CCtTTCCATAGCCATCTGCC
N428K_F	CCAAgGCCCATGTTGTAGTC
N428K_R	AAGGCAGGAAGAGGCTCTGGCTGCTTAACCTGCCTGACTACAACA TGGGCcTTGGTTCCACCAAATCC
T792A_F	AGAAgCTGCAGCTAGAAACAGG
T792A_R	GAGGACTTATTTCAACAAACACTACATTTTCCCTGTTTCTAGCTGC AGcTTCTATGGCTTGAGTGAAAG

Table S9. Primers used for introducing mutations into GgPKS coding sequence.

Description of Supplementary data: DATA_HPLC_MS.zip, provided in Zenodo (https://zenodo.org/records/14184888)

All the data files from UHPLC/HRAM-QTOF analyses are in the Bruker compass native format The .d file format is native raw data format of the Bruker aquisition software for LC/MS analyses. Hence, to open the files with .d extension we recommend to use Bruker's DataAnalysis software. To download the software a user must first register at bruker.com (my bruker ->register) [https://sso.bruker.com/auth/realms/bruker/login-actions/registration?client_id=aem-bruker.com&tab_id=-rTFVcu1eA0]. After that a section to download mass-spectrometry software unlocks (bruker.com -> services & support -> software support & upgrades -> mass spectrometry [here a user needs to be signed in] -> software solutions -> DataAnalysis) [https://www.bruker.com/protected/en/services/software-downloads/mass-spectrometry/software-solutions.html]. This is the direct link to dowload the software (user needs to be signed in on bruker.com) [https://www.bruker.com/protected/en/services/software-downloads/mass-spectrometry/software-solutions/_jcr_content/root/sections/section_795158518/sectionpar/twocolumns_copy/contentpar-2/maintenancewrapper/content/externaldownload_cop_1204623677.external.zip/BDAL/LSMS/DataAnalysis/6.0/6.0.536.33/dataanalysis-sfx-6.0.536.33-ISO.zip]. Even though the software requires a paid license to offer all the functions, viewing and exporting in various formats is available in unlicensed versions of the DataAnalysis software. Alternatively, the files are possible to be opened using opensource program ProteoWizard (https://proteowizard.sourceforge.io/). However, the full compatibility using this software is not guaranteed.

Psittac11_EP-1_Ph-APCI_FA_Pos_01_RA7_1_8601.d
PDA and MS data of **psittacofulvins extracted from green feathers** of Psittacula krameria using Phenyl-hexyl column and APCI ionization in positive mode.
Psittac11_EP-2_Ph-APCI_FA_Pos_01_RA8_1_8602.d
PDA and MS data of **psittacofulvins extracted from yeast expressing empty vector** using Phenyl-hexyl column and APCI ionization in positive mode.
Psittac11_EP-3_Ph-APCI_FA_Pos_01_RB1_1_8603.d
PDA and MS data of **psittacofulvins extracted from yeast expressing GgPKS WT** using Phenyl-hexyl column and APCI ionization in positive mode.
Psittac11_EP-4_Ph-APCI_FA_Pos_01_RB2_1_8604.d
PDA and MS data of **psittacofulvins extracted from yeast expressing GgPKS G112R** using Phenyl-hexyl column and APCI ionization in positive mode.
Psittac11_EP-5_Ph-APCI_FA_Pos_01_RB3_1_8606.d
PDA and MS data of **psittacofulvins extracted from yeast expressing GgPKS R243K** using Phenyl-hexyl column and APCI ionization in positive mode.
Psittac11_EP-6_Ph-APCI_FA_Pos_01_RB4_1_8607.d
PDA and MS data of **psittacofulvins extracted from yeast expressing GgPKS N428K** using Phenyl-hexyl column and APCI ionization in positive mode.
Psittac11_EP-7_Ph-APCI_FA_Pos_01_RB5_1_8608.d
PDA and MS data of **psittacofulvins extracted from yeast expressing GgPKS T792A** using Phenyl-hexyl column and APCI ionization in positive mode.
Psittac11_EP-8_Ph-APCI_FA_Pos_01_RB6_1_8609.d

PDA and MS data of **psittacofulvins extracted from yeast expressing combination of GgPKS N428K and T792A** using Phenyl-hexyl column and APCI ionization in positive mode.
Psittac11_EP-TB_Ph-APCI_FA_Pos_01_RA6_1_8600.d
Technical **blank measurement** using Phenyl-hexyl column and APCI ionization in positive mode.