

Supplemental Tables and Figures

The basis for haplotype complexity in VCBP, an immune-type receptor in amphioxus

L. J. Dishaw et al. 2010

Supplement table 1. Nucleotide substitution patterns among the VCBP2/5 gene loci.

Ps

	Within a locus			Between loci		
	5a	2b	5b	5a-2b	2b-5b	5b-5a
Entire region	0.218(0.019)	0.110(0.015)	0.127(0.017)	0.454(0.027)	0.419(0.022)	0.298(0.022)
V1 (420)	0.269(0.036)	0.111(0.025)	0.162(0.028)	0.347(0.041)	0.209(0.030)	0.344(0.036)
V2 (492)	0.186(0.030)	0.089(0.023)	0.119(0.028)	0.530(0.042)	0.513(0.044)	0.285(0.035)
Exon1 (58)	0.308(0.098)	0.206(0.086)	0.172(0.093)	0.274(0.071)	0.738(0.093)	0.749(0.077)
Exon2 (170)	0.199(0.047)	0.102(0.040)	0.185(0.047)	0.217(0.041)	0.216(0.040)	0.229(0.047)
Exon3 (250)	0.322(0.051)	0.116(0.034)	0.146(0.039)	0.440(0.057)	0.195(0.042)	0.440(0.053)
Exon4 (171)	0.148(0.045)	0.142(0.046)	0.192(0.052)	0.467(0.072)	0.459(0.063)	0.419(0.075)
Exon5 (135)	0.228(0.065)	0.051(0.035)	0.029(0.029)	0.662(0.092)	0.640(0.102)	0.204(0.065)
Exon6 (186)	0.202(0.050)	0.059(0.031)	0.098(0.040)	0.483(0.071)	0.464(0.084)	0.200(0.044)
Exon7 (182)	0.135(0.045)	0.134(0.043)	0.060(0.028)	0.548(0.071)	0.565(0.076)	0.091(0.025)

Ds

	Within a locus			Between loci		
	5a	2b	5b	5a-2b	2b-5b	5b-5a
Entire region	0.260(0.030)	0.121(0.019)	0.139(0.020)	0.698(0.069)	0.615(0.065)	0.380(0.035)
V1	0.336(0.063)	0.122(0.033)	0.182(0.036)	0.466(0.078)	0.245(0.042)	0.463(0.073)
V2	0.217(0.041)	0.096(0.027)	0.130(0.031)	0.926(0.182)	0.865(0.171)	0.360(0.065)
Exon1	0.425(0.237)	0.253(0.136)	0.197(0.131)	0.360(0.141)	2.038(0.638)	1.950(0.499)
Exon2	0.234(0.067)	0.114(0.046)	0.214(0.065)	0.258(0.066)	0.276(0.072)	0.263(0.064)
Exon3	0.435(0.100)	0.127(0.039)	0.163(0.046)	0.671(0.155)	0.227(0.057)	0.672(0.147)
Exon4	0.168(0.060)	0.161(0.063)	0.221(0.073)	0.733(0.221)	0.716(0.190)	0.617(0.179)
Exon5	0.280(0.109)	0.054(0.040)	0.030(0.031)	1.693(0.559)	1.510(0.566)	0.239(0.100)
Exon6	0.237(0.078)	0.062(0.038)	0.106(0.051)	0.780(0.261)	0.726(0.298)	0.234(0.062)
Exon7	0.149(0.055)	0.149(0.057)	0.064(0.032)	0.991(0.316)	1.062(0.366)	0.098(0.031)

Pn

	Within a locus			Between loci		
	5a	2b	5b	5a-2b	2b-5b	5b-5a
Entire region	0.114(0.011)	0.032(0.005)	0.026(0.005)	0.227(0.015)	0.211(0.017)	0.141(0.013)
V1	0.132(0.020)	0.031(0.009)	0.022(0.009)	0.160(0.020)	0.085(0.018)	0.149(0.021)
V2	0.148(0.020)	0.036(0.009)	0.021(0.007)	0.297(0.031)	0.282(0.034)	0.156(0.020)
Exon1	0.021(0.020)	0.042(0.028)	0.170(0.050)	0.031(0.017)	0.476(0.103)	0.481(0.102)
Exon2	0.149(0.037)	0.030(0.013)	0.018(0.014)	0.179(0.036)	0.117(0.035)	0.154(0.033)
Exon3	0.121(0.025)	0.032(0.014)	0.024(0.011)	0.148(0.027)	0.063(0.022)	0.145(0.028)
Exon4	0.198(0.039)	0.063(0.020)	0.037(0.017)	0.272(0.048)	0.242(0.053)	0.203(0.035)
Exon5	0.099(0.030)	0.018(0.012)	0.026(0.014)	0.316(0.057)	0.335(0.061)	0.117(0.030)
Exon6	0.131(0.031)	0.021(0.012)	0.000(N/5a)	0.309(0.050)	0.283(0.051)	0.135(0.031)
Exon7	0.032(0.012)	0.025(0.011)	0.010(0.007)	0.263(0.036)	0.253(0.036)	0.022(0.008)

Dn

	Within a locus			Between loci		
	5a	2b	5b	5a-2b	2b-5b	5b-5a
Entire region	0.123(0.013)	0.033(0.006)	0.027(0.005)	0.271(0.023)	0.248(0.024)	0.157(0.015)
V1	0.145(0.025)	0.032(0.010)	0.022(0.009)	0.181(0.028)	0.090(0.022)	0.166(0.027)
V2	0.167(0.028)	0.037(0.010)	0.022(0.007)	0.379(0.048)	0.354(0.051)	0.174(0.025)
Exon1	0.021(0.021)	0.043(0.027)	0.200(0.069)	0.032(0.018)	0.758(0.369)	0.773(0.373)
Exon2	0.167(0.046)	0.031(0.013)	0.018(0.013)	0.205(0.048)	0.127(0.043)	0.174(0.043)
Exon3	0.132(0.030)	0.033(0.014)	0.024(0.011)	0.165(0.034)	0.066(0.023)	0.161(0.034)
Exon4	0.233(0.054)	0.066(0.023)	0.038(0.018)	0.339(0.078)	0.293(0.078)	0.236(0.050)
Exon5	0.108(0.035)	0.019(0.012)	0.027(0.016)	0.411(0.094)	0.443(0.111)	0.128(0.040)
Exon6	0.146(0.038)	0.021(0.012)	0.000(N/5a)	0.398(0.097)	0.355(0.092)	0.151(0.040)
Exon7	0.033(0.012)	0.025(0.011)	0.010(0.007)	0.324(0.066)	0.308(0.064)	0.022(0.008)

Reference

MSDC2	Ps 0.078(0.016), Pn 0.011(0.003), Ds 0.083(0.018), Dn 0.011(0.003)
Arfaptin2	Ps 0.116(0.021), Pn 0.000(N/A), Ds 0.126(0.025), Dn 0.000(N/A)

Abbreviations: Arfaptin2, ADP-ribosylation factor interacting protein 2b ; MSDC2, male sterility domain containing-2 (Rossmann-fold NAD(P)(+)-binding proteins). Data compares reference BACs and PACs.

Supplement table 2a. Nucleotide substitution; genetic regions unlinked to VCBP2/5 cluster.

Genes	#alleles	Ps	Pn	Ds	Dn
VCBP3	7	0.193(0.015)	0.008(0.002)	0.224(0.021)	0.008(0.002)
	V1	0.219(0.025)	0.008(0.004)	0.261(0.038)	0.008(0.004)
	V2	0.162(0.024)	0.010(0.004)	0.185(0.033)	0.010(0.004)
Big Dfsn	8	0.082(0.021)	0.011(0.004)	0.088(0.024)	0.011(0.005)
GAPDH	6	0.061(0.018)	0.004(0.003)	0.064(0.019)	0.004(0.003)

Supplement table 2b. Additional alleles compared; estimates of average codon-based evolutionary divergence between sequences.

Genes	# alleles	Domain	Ps	s.e.	Pn	s.e.
5a VCBP5	4	V1	0.22	0.03	0.11	0.02
		V2	0.18	0.03	0.14	0.02
2b VCBP2	8	V1	0.15	0.02	0.07	0.01
		V2	0.14	0.02	0.08	0.01
5b VCBP5	6	V1	0.13	0.02	0.02	0.01
		V2	0.11	0.02	0.03	0.01

Supplement table 1 and 2

The proportion of synonymous (Ps) and non-synonymous substitutions (Pn) and the number of synonymous (Ds) and nonsynonymous (Dn) differences per site is shown with standard errors estimated by a bootstrap procedure (100 replicates; as implemented in MEGA4). For each gene, BAC/PAC reference alleles were included. Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Big Dfsn, Big Defensin.

Supplement table 3. Accession numbers of referenced sequences.

Gene (locus)	Allele	Genbank accession
VCBP5S1 (5b)	VCBP5S1*01-04	AF532183, BK006758, BK006763, BK006769
	VCBP5S1*05-07	HM048952-54
VCBP5b	VCBP5b-01	HM048966
VCBP5	VCBP5-01-04	HM048967-70
VCBP2/5-HV	VCBP2/5HV-NS04M	HM048971
VCBP5S2 (5a)	VCBP5S2*01-03	BK006759, BK006761, BK006755
	VCBP5S2*04	HM048946
VCBP5a	VCBP5a-01-04	HM048962-65
VCBP2S1 (2b)	VCBP2S1*01-09	AF520473, BK006760, BK006762, BK006768, HM048947-51
VCBP2-like	VCBP2*01-07	HM048955-61
VCBP2b	VCBP2b-01-05	HM048972-76
VCBP3S1	VCBP3S1*01-08	AF520474, BK006764-66, HM048936-38
Big Defensin	Bf-BD*01-08	HM048928-35
GAPDH	GAPDH*01-06	HM048940-45
Arfaptin2	JGI-ProteinID Brafl1_96986 Brafl1_79183	derived from EU875589-90
MSDC2	JGI-ProteinID Brafl1_79185 Brafl1_96985	

Reference Haplotype	Genbank accession
BAC contig 63n5-43b24	EU875590
BAC 62d19	EU875589
PAC 37d15	AC135603.5

Supplement figure 1. Point mutations that lead to single amino acid changes in EX2 can be consistent with distinct allelic lineages. (A.) Shown here are four distinct allelic lineages (i.e., subtype variants) of the -LAAD- type from gene 5a. Representative alleles have been recovered from multiple animals. Shown for illustration purposes is a portion of the intron between EX1 and EX2. Shading is only used to specify allelic groups or lineages; additional variation is not shaded. (B.) Single example from each group in (A) shown to illustrate EX2 peptide sequence subtype variation. Sequence numbers refer to internal identification system.

	*	20	*	40	*	60	*	80	*	100	*	120	*	14
Z1313		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z1097		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z1099		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z1312		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z1103		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z1307		GYATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z1105		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z1305		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z3933		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z4479		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z4481		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z3945		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z3939		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2155		AAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2215		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2211		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2209		GAACCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2675		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2677		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2135		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2137		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z5423		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
63n5		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2701		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2089		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								
Z2697		GAATCAAGTTACGCGAGTAAAGGGTGTCAAGTTTGCTGCCA	TACTCAGTTCCTCTAAGGCTCGAGCTTAGGCTTGCATGTA	ACTATTAGGACTAGGTATCGGTACGTGTCAGCGTACAAA	:	139								

	0	*	160	*	180	*	200	*	220	*	240	*	260	*	2
Z1313		ACCGCTTTTTTTTTTFTGTTGGACTGATCTA	AAAAAACCGGCCCTGAA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Z1097		ACCGCTTTTTTTTTTFTGTTGGACTGATCTA	AAAAAACCGGCCCTGAA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Z1099		ACCGCTTTTTTTTTTFTGTTGGACTGATCTA	AAAAAACCGGCCCTGAA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Z1312		ACCGCTTTTTTTTTTFTGTTGGACTGATCTA	AAAAAACCGGCCCTGAA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Z1103		ACCGCTTTTTTTTTTFTGTTGGACTGATCTA	AAAAAACCGGCCCTGAA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Z1307		ACCGCTTTTTTTTTTFTGTTGGACTGATCTA	AAAAAACCGGCCCTGAA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Z1105		ACCGCTTTTTTTTTTFTGTTGGACTGATCTA	AAAAAACCGGCCCTGAA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Z1305		ACCGCTTTTTTTTTTFTGTTGGACTGATCTA	AAAAAACCGGCCCTGAA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
Z3933		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z4479		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z4481		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z3945		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z3939		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2155		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2215		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2211		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2209		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2675		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2677		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2135		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2137		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2133		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z5423		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
63n5		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2701		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2089		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								
Z2697		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG								

	80	*	300	*	320	*	340	*	360	*	380	*	400	*	*
Z1313		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCCCTCGTGTCAAGTCTGCAACGGTTGTTG	GCACTACAGGCTTGCATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									403
Z1097		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									403
Z1099		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									403
Z1312		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									403
Z1103		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									403
Z1307		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									403
Z1105		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									403
Z1305		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									402
Z3933		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									381
Z4479		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									381
Z4481		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									381
Z3945		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									381
Z3939		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									380
Z2155		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									380
Z2215		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									390
Z2211		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									390
Z2209		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									390
Z2675		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									390
Z2677		AAAATAACTAGTGTCAAGCAGAGGAGCTGATAGTC	--TTTTACTAGCTCCCTGGTCAAGTCTGCAACGGCTTGC	ATGTA	--GGTGAATTCCTCCACGCCAAATCCCAT	--A									390
Z2135		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
Z2137		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
Z2133		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
Z5423		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
63n5		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
Z2701		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
Z2089		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
Z2697		TTTTCTCTGTGTTGA	GAGATCAGAA	AAA	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG

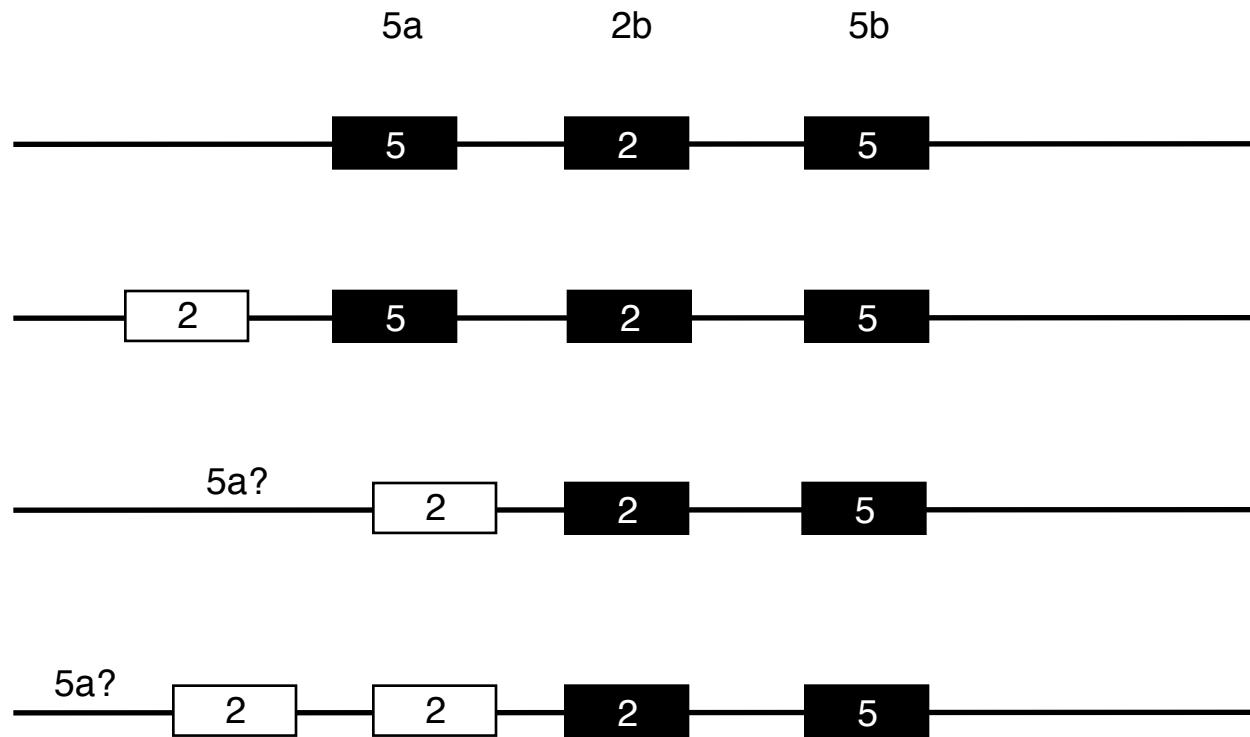
	420	*	440	*	460	*	480	*	500	*	520	*	540	*	*
Z1313		GCAAACTCCCTTCTCGCAAACCCCTTCTGTGACATGAA	--	CAACGTTGAATCTGTTGAA	AAAAAAGAATGTTTCCACTACAGAGATGGCAGTGTCA	GAACATGACGAGGATACCTGGATGG									

Supplement figure 1 continued:

B.

>z1313_5a-01	DAVSITTVPYHNYVLAADRPWDPTWVNVEIGCEYTISPA
>z3933_5a-02	DAVSITTVPYHNY F VLAADRPWDPTWVNVEIGCEY S ISPA
>z2211_5a-03	DAVSITTVPYHNYVLAADRPWDPTWVNVE T GCEY S ISPA
>z2137_5a-04	DAVSITTVPYHNYVLAADRPWDPTWVNVE I RCEYTISPA

Supplement figure 2. Polymorphism of the VCBP2/5 cluster includes haplotype diversity with CNV. Predicted from genomic PCR haplotyping and sequencing performed on additional PAC library animals, as well as various independent animals.

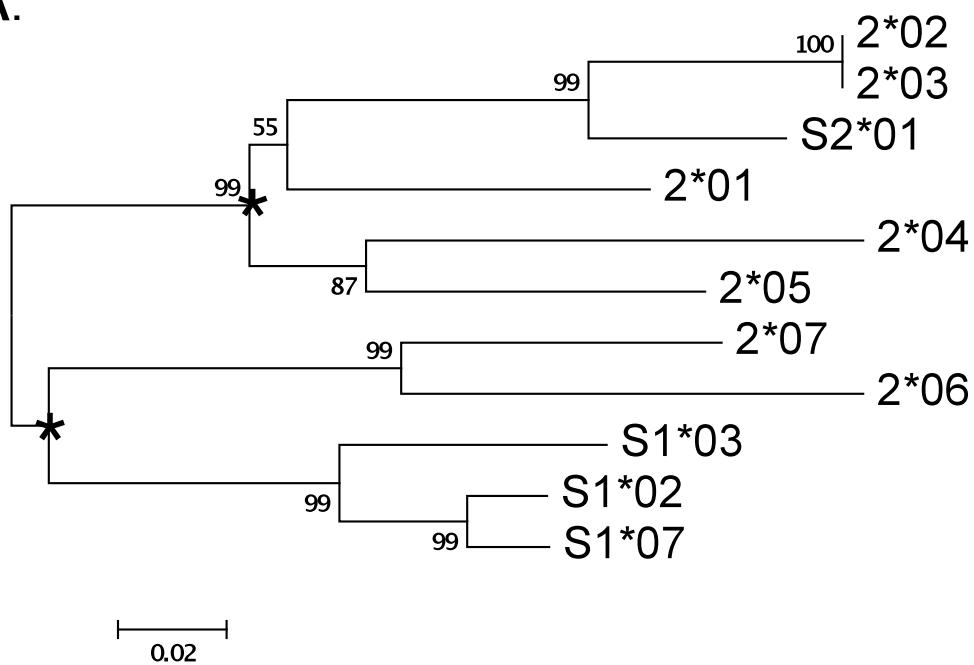


Supplement figure 3. Genetic complexity in VCBP 2 genes; alleles or paralogous copies?

VCBP2 genes (2b locus and others, i.e., CNVs) form as many as 6 major groups of sequences, each group possessing a specific hotspot type (encoded in EX2) with many subtype variants. Determining their relationships can be difficult due to varying degrees of polymorphism.

VCBP2 alleles can be 99% identical to as low as 70%, which in some cases have been confirmed as paralogous genes. The presence of CNVs preclude the assignment of all 2-type alleles as 2b-type genes. Genomic comparison of introns and intergenic DNA is further required. **A.** Minimum evolution analysis of translated VCBP2 cDNA sequences form five major groups (clades) sharing specific, previously characterized, hotspot types. EX2 motifs are shown, as recognized between the first two proline residues. Genomic haplotyping further confirms that some of the clades represent CNV genes (i.e., paralogs); asterisks. Poisson corrected distances were implemented; gaps were eliminated via complete deletion; branching support was estimated with 1000 bootstrap repetitions in Mega 4. **B.** Full length sequence alignment of the cDNA alleles from A.

A.



EX2 type

YRGSWVVIWHT
YRGSWVVIWHT
YRGSWVMIWNT
DREYWMLWLHR
YRSGWVVMWAR
NRASWVVMVAR
DRSASYIMLWHS
DRSASYIMLWHS
DRSAGWVYLRYS
DRSAAAGWVYLRYS

Supplement figure 3 continued

B.

	*	20	*	40	*	60	*	80	:	77	
2*04	:	DAVSITNVTVPYRS---	G	WVVMWARPGD	PTWVNRIE	FRCEYTI	SPASATPPTITWL	GSFADEDRO	VVYKWSSSGEVYVH		
2*05	:	DAVSITNVTVPNRA--	S	WVVMVARPDDPTWVNRIE	FRCEYSIS	SPASATPPTVTWL	KGSFGHAD	RQVIYKWS	SSGEVYVH	: 77	
2*02	:	DAVAITNVTAPYRG--	S	WVVIWHTWGDPTFVN	RVEIGCEYSI	SSAPATPPTITWL	KGSFT	--DRQVIYKL	LSSSGEVYVH	: 75	
2*03	:	DAVAITNVTAPYRG--	S	WVVIWHTWGDPTFVN	RVEIGCEYSI	SSAPATPPTITWL	KGSFT	--DRQVIYKL	LSSSGEVYVH	: 75	
S2*01	:	DAVSITNVTAPYRG--	S	WVMIWNTWWDP	TWVNVRVEFGCEYSI	SPAPATPPTITWL	KGSFT	--DRQVIYKL	TSSSGEVYVH	: 75	
2*01	:	DAVSITNVTPDRE--	Y	WLMWHRPDDPTWVN	RVEFGCEYSI	SPAPATPPTITWL	KGSFT	--DRQVIYKL	WSSSGEEVYVH	: 75	
S1*02	:	GAVSITNVTPDRSAAAGWVLYR	YSPD	-PTWVNRIE	FRCEYTI	SPASANPPTITWL	KGPFT	--DRQVIYKWS	SSSGEVYVH	: 77	
S1*07	:	-----AGWVLYR	YSPD	-PTWVNRIE	FRCEYTI	SPASANPPTITWL	KGPFT	--DRQVIYKWS	SSSGEVVN	: 61	
S1*03	:	GALSITNVTL	PDRS	--AGWVLYR	YSPD	-PTWVNRIE	FRCEYSI	SPAPATPPTITWL	KGPFTDADRI	VVYKWSSSGEVYVH	: 77
2*07	:	DAVSITNVTPDRS--	ASYIMLWHRPDD	-PTWENRVE	FRCEYNI	SPAPATPPTITWL	MGSFTNAARH	VIYKWS	SSSGEVYVH	: 78	
2*06	:	DAVSITNVTPDRS--	ASYIMLWHRPEG	PTWQN	RVEFRCEYD	ISPA	ATPPTITWL	MGSFTNAABQ	VIYKWS	SSSGEVYVH	: 78
	*	100	*	120	*	140	*	160	:	153	
2*04	:	PEFAGRVSVESRTR	P	PTLVLTD	DTKFAD	WGRYFCRITNE	NQSDD	FGTDEGSVLFWY	KY-GYD	--PRGQSQFTSVEVD	
2*05	:	PEYAGRVSVESRTR	P	PTLVLTD	DAKLD	WGRYCRVTNE	EQSDD	FGTDEGSLLFWY	QW-GYS	--PARGHSHYTF	
2*02	:	PEYAGRVSVPSRTR	P	PTLVLTD	DSKF	WGRYCRVTNE	EQSDE	FGTDEESRLFWFRS	-SYD	--PARGDYYSFVKV	
2*03	:	PEYAGRVSVPSRTR	P	PTLVLTD	DSKF	WGRYCRVTNE	EQSDE	FGTDEESRLFWFRS	-SYD	--PARGDYYSFVKV	
S2*01	:	PEYAGRVSVPSRTH	P	PTLVLTD	DAKF	WGRYCRVTNE	EQSDD	FGTDEESRLFWFKS	-GYD	--PARGSHYSFVQ	
S1*02	:	SEFAGRVSVPSRTH	P	PTLVLTD	DAKY	DDWGRYCRVTNE	DQSDE	FGTDEESLLFY	YYKSTVY	--PARGSHYSFVE	
S1*07	:	PEFAGRVSVPSRTR	P	PTLVLTD	DAKF	DDWGRYCRVTNE	DQSDE	FGTDEESLLFY	YYKSTVY	--DYDVPARGGQYSFVE	
S1*03	:	PEFAGRVSVPSRTR	P	PTLVLTD	DAKF	DDWGRYCRVTNE	EQSDE	FGTDEESLLFWY	KSTVY	--DYDAPPREGYSSFVE	
2*07	:	PDTYTRGVSVESRTH	P	PTLILVNQ-	--GG	WGRYCRVTNE	EQSDE	FGTDEESLLFY	YYNTGAYN	--PPRGSGYSFVE	
2*06	:	PDEAGRVSVESRTH	P	PTLILVNQ-	--GG	WGRYCRVTNE	EQSDE	FGTDEESLLFY	YYTTGAYN	--PPRGSGFSFVE	
	*	180	*	200	*	220	*	240	:	231	
2*04	:	RVETGGAARLNCDGL	G	LGKEASIV	WFKGPN	CTQDGNCNVYEMVINKTA	VEHFSP	--DPGT	VNVSPK	YAGRASL	
2*05	:	RVKTGGTARLRCEGI	G	LGKEASIV	WFKGP	CTQDGNCNVYEMVINKTA	VGHWSP	--DPGT	VNVSPK	YAGRASL	
2*02	:	RVKTGGTAKLLCEG	G	WGKEASIV	WFKGP	CTQDGNCNVYEMVINKTA	EEHFSP	--DPGT	VNVSPK	YAGRASL	
2*03	:	RVKTGGTAKLLCEG	G	WGKEASIV	WFKGP	CTQDGNCNVYEMVINKTA	AEEHFSP	--DPGT	VNVSPK	YAGRASL	
S2*01	:	RVKTGGTAKLHCEG	G	WGKSASIV	WFKGP	CTQDGNCNVYEMVINKTA	EVHFSP	--DPGT	VNVSPN	YAGRASL	
S1*01	:	RVKTGATARLHCEG	G	LGKEASIAW	WFKGP	CTQDGNCNVYEMVINKTA	VGHRSP	--DPGT	VNVSPK	YAGRASL	
S1*02	:	RVKAGGTARLNCEG	G	SGGPLASIV	WFKGP	CTQDGKCNVYEMVINKTA	VPQPHPI	LGP	GT	VNVSPK	YAGRASLD
S1*07	:	RVETGGTARLHCEG	G	WGGLPLASIV	WFKGP	CTQDGNCNVYEMVINKTA	VPQPHPI	LGP	GT	VNVSPK	DFNDGGYY
S1*03	:	RVKTGGTARLNQCGA	G	GGPLASIV	WFKGP	CSQDGNCNVYEIVINKTA	VPQPHAI	LGP	GT	VNVSPK	DFNDGPYY
2*07	:	RVKAGGTARLNCEG	G	GGPLASIV	WFKGP	CTQDGKCNVYEMVINKTA	VPQPHPI	LGP	GT	VNVSPK	DFNDGGYY
2*06	:	HVKAGGTARLHCEG	G	MGKEASIAW	WFKGP	CTQDGNCNVYEIVINKTA	VGHSSP	DPGTAA	VGHSSP	VNVSPK	YAGRASL
	*	260	*	280	*	300	*	320	:	299	
2*04	:	-----LDLTITDIRPT	D	VGRY	WCTNDWP	LWF-MNEIHSRDSRSVV	VLLDDEAP	--SCDGKPDGMY	QDPGDCSRYYT		
2*05	:	-----LDLTITDIRP	D	VGRY	WCTNDWP	FMS-WNE-----				: 260	
2*02	:	-----LDLTITDIRP	D	VGRY	WCTNDWP	PLYF-RNEVQSRDSQS	VVVLLDDEAP	--SCDGKADGMY	QDPGDCSRYYT	: 297	
2*03	:	-----LDLTITDIRP	D	VGRY	WCTNDWP	PLYF-RNEVQSRDSQS	VVVLLDDEAP	--SCDGKADGMY	QDPGDCSRYYT	: 297	
S2*01	:	-----LDLTITDIRP	D	VGRY	WCTNDWP	PLYF-RNEVQSRDSQS	VVVLLDDEAP	--SCDGKADGMY	QDPGDCSRYYT	: 297	
2*01	:	-----LDLTITDIRP	D	VGRY	WCTNDWP	PLYF-RNEVQSRDSQS	VVVLLDDEAP	--SCDGKADGMY	QDPGDCSRYYT	: 298	
S1*02	:	NYYYP-----	DLT	ITDIRPT	DVGRY	WCTDDAPLWYQ-	NDLRSRDSQS	VVVLLDDEAP	--SCDGKADGMY	RDPGDCSRYYT	
S1*07	:	NYYYP-----	DLT	ITDIRPT	DVGRY	WCTDDAPLWYQ-	NDLASRDSQS	VVVLLDDEAP	--SCDGKADGMY	QDPGDCSRYYS	: 311
S1*03	:	NYYYP-----	DLT	ITDIRP	DVGRY	WCTDDAPLWYQ-	NDLRSRDSQS	VVVLLDDEAP	--SCDGKADGMY	QDPGDCSRYYT	: 295
2*07	:	NYYYP-----	DLT	ITDIRP	DVGRY	WCTDDAPLWYQ-	NDLRSRDSQS	VVVLLDDEAP	--SCDGKADGMY	QDPGDCSRYYT	: 311
2*06	:	GLTFPDMRSLDLT	ITDIRP	DVGRY	WCTNDWP	LAEQFNDLHSRDSQS	VVVLLDDEAP	--SCDGKADGMY	QDPGDCSRYYT	: 307	
	*	340									
2*04	:	CSGGWLYGPVPCLT	G	LFNFNEAL	:	321					
2*05	:	-----			:	-					
2*02	:	CSGGWLYGPAPCLT	G	LFNFNEAL	:	319					
2*03	:	CSGGWLYGPAPCLT	G	LFNFNEAL	:	319					
S2*01	:	CSGGWLYGPVPCLT	G	LFNFNEAL	:	319					
2*01	:	CSGGWLYGPVPCLT	G	LFNFNEAL	:	320					
S1*02	:	CSGGWLYGPVPCLT	G	LFNFNEAL	:	333					
S1*07	:	CSGGWLYGPVSCLT	G	LFNFNEAL	:	317					
S1*03	:	CSGSWLYGPVPCLT	G	LFNFNEAL	:	333					
2*07	:	CSGGWLYGPAPCLT	G	LFNFNEP-	:	328					
2*06	:	CSGGWLYGPQPCLT	G	LFNFNEAL	:	332					

Supplemental figure 4. Putative crossover example in an allele from animal SW01 in the intron just upstream to the hotspot exon, EX2. The first 510bp of allele 5a-01 from SW01 shows sequence identity to BAC clone 62d19 and the remainder of the sequence is most similar to BAC contig clone 63n5-43b24. Potential crossover region is boxed; small indel noted (arrow). Bold dashed lines in BAC alleles represent sequence removed for illustration purposes.

Sequence alignment showing mutations and a recombination event across six DNA fragments (62d19-Ge, 3927-392, 63n5-43b, 62d19-Ge, 3927-392, 63n5-43b). The alignment is numbered from 220 to 850. A dashed line separates the first two groups from the second. A dotted line highlights a recombination event between positions 500 and 550. An arrow points to a mutation at position 730.