Supplementary information

Title

Microevolution of *Pieris* butterfly genes involved in host-plant adaptation along a host-plant community cline

Short running title: Microevolution of host-plant adaptation genes

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Table S1 19 climate variables used for Maxent

Variables	Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (×100)
BIO4	Temperature Seasonality (standard deviation ×100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Table S2 Sampling sites and observed number of Brassicaceae

Sampling Sites	Yubari	Rusutsu	Oshamanbe	Fukushima	Nagano	Chiba	Kanagawa	Nara	Tokushima	Miyazaki	Yonaguni
latitude	43.0	42.7	42.6	37.8	36.3	35.5	35.5	34.3	33.9	32.4	24.5
longitude	142.1	140.8	140.3	140.6	137.8	140.2	139.2	136.0	134.1	131.2	123.0
Plant diversity	1.0224	0.7115	0.6374	1.6662	2.1451	1.7968	1.3765	1.8850	1.5404	0.8650	0.3125
Plant Species											
Arabidopsis thaliana	0	0	0	0	60	20	0	0	0	0	0
Arabidopsis halleri	0	0	0	0	0	0	0	83	0	0	0
Arabidopsis kamchatica	0	0	0	0	46	0	0	0	0	0	0
Arabis flagellosa	0	0	0	0	0	0	0	150	0	245	0
Arabis hirsuta	0	0	0	78	0	0	72	12	12	0	0
Barbarea orthoceras	6	0	15	7	72	0	0	0	4	0	0
Brassica napus	0	0	0	0	51	45	0	10	3	0	0
Cardamine impatiens	0	0	0	0	12	4	7	0	5	0	0
Cardamine kiusiana	0	0	0	0	0	0	0	0	14	0	0
Cardamine leucantha	438	20	130	42	140	0	0	0	0	0	0
Cardamine regeliana	0	0	0	0	81	125	0	0	0	0	0
Cardamine occulta	30	0	12	45	210	60	18	31	0	13	25
Draba nemorosa	0	0	0	0	140	0	0	0	0	0	0
Eutrema japonicum	0	0	0	130	15	0	30	0	0	0	0
Eutrema tenue	47	0	0	0	0	0	0	0	0	0	0
Lepidium virginicum	0	0	0	0	0	0	0	10	0	0	240
Nasturtium officinale	0	0	0	23	0	200	0	174	60	0	0
Orychophragmus violaceus	0	0	0	0	0	140	0	0	0	0	0
Raphanus sativus	0	0	0	0	8	0	0	0	0	0	0
Rorippa dubia	0	0	0	0	0	0	0	62	0	58	0
Rorippa indica	0	0	2	25	0	40	58	75	70	27	0
Rorippa palustris	0	101	0	0	21	10	0	10	0	0	0
Rorippa sylvestris	262	317	0	0	0	0	0	0	0	0	0
Turritis glabra	0	0	0	0	0	0	0	0	30	0	0

Table S3 Primers used in this study

Primers	Description	Sequence
Primers for cloning and RFLP		
M13_F	M13 Forward	GTTTTCCCAGTCACGAC
M13_R	M13 Reverse	CAGGAAACAGCTATGAC
ND5_F	*ND5* Forward	CCTGTTTCTGCTTTAGTTCA
ND5_R	*ND5* Reverse	AATATDAGGTATAAATCATAT
NSP_RFLP_F	*NSP* Forward primer for RFLP (NSPmn_F)	ATGAAAGCTGTTGTAGTCTTATTAGC
NSP_RFLP_R	*NSP* Reverse primer for RFLP	GTCTTGTACTTCGGACTCCTTTT
Primers for sequencing		
NSPmn_F	*NSP* Forward for *P. melete* and *P. napi*	ATGAAAGCTGTTGTAGTCTTATTAGC
NSPmn_R	*NSP* Reverse for *P. melete* and *P. napi*	CTGTCCGTAAAGAGCAGGTAC
NSPmn_int1	*NSP* internal1 for *P. melete* and *P. napi*	GCTTAGATGCCTTGTCAAAGACT
NSPmn_int2	*NSP* internal2 for *P. melete* and *P. napi*	AATAGCGTGGTCGTTCTTAGC
NSPr_F	*NSP* Forward for *P. rapae*	ATGAAAGGTGTTGTAGTCTTCTTAG
NSPr_R	*NSP* Reverse for *P. rapae*	TTACTGTCCGTAAAGGGCA
NSPr_int1	*NSP* internal1 for *P. rapae*	CTCTGGAAGAACGAAGCATT
NSPr_int2	*NSP* internal2 for *P. rapae*	AACTCGGCTAGTCCTGCTTTC-
MAmn_F	*MA* Forward for *P. melete* and *P. napi*	ATGAAGACAACAATAGTGCTCCTAAG
MAr_F	*MA* Forward for *P. rapae*	ATGAAGACAACAATAGTGCTCCTC
MAmnr_R	*MA* Reverse for all the three species	TTATTGTCCCCAGAGGGTTG
MAmn_int1	*MA* internal1 for *P. melete* and *P. napi*	CCTGTTTGAGGAATTACTTCCA
MAmn_int2	*MA* internal2 for *P. melete* and *P. napi*	ATAGGCGTGTTTTGTCCGAAA
MAr_int1	*MA* internal1 for *P. rapae*	CTTCCAGCATTCTTCGGAC
MAr_int1	*MA* internal2 for *P. rapae*	TCTGATTGCACGATGATGTCC
Primers for RT-qPCR		
NSPm_qPCR_F	Forward qPCR primer for *P. melete NSP*	AATTGGCGGCTTTATACACG
NSPm_qPCR_R	Reverse qPCR primer for *P. melete NSP*	TTCTTTCCTTCGGCACTTGT
MAm_qPCR_F	Forward qPCR primer for *P. melete MA*	TGTTGCTAACGCACTGGAAG
MAm_qPCR_R	Reverse qPCR primer for *P. melete MA*	CCCTCCAACGCAGTAATGAT
Ef1am_qPCR_F	Forward qPCR primer for *P. melete Ef1a*	AGGAATTGCGTCGTGGTTAC
Ef1am_qPCR_R	Reverse qPCR primer for *P. melete Ef1a*	GCAAGCAATGTGAGCTGTGT

Table S4 Host plant usage patterns of three *Pieris* species at different sampling sites

Columns show ‘observed number of larvae’ and ‘checked number of plants’. Hyphens (-) indicate absence of a plant species at the sampling site.

P. melete

Plant sp./Sampling Sites	Yubari	Rusutsu	Oshamanbe	Fukushima	Nagano	Chiba	Kanagawa	Nara	Tokushima	Miyazaki	Yonaguni	**SUM**
Arabidopsis halleri	-	-	-	-	-	-	-	0/83	-	-	-	0/83
Arabidopsis kamchatica	-	-	-	-	0/46	-	-	-	-	-	-	0/46
Arabidopsis thaliana	-	-	-	-	0/60	0/20	-	-	-	-	-	0/80
Arabis flagellosa	-	-	-	-	-	-	-	0/150	-	0/245	-	0/395
Arabis hirsuta	-	-	-	27/78	-	-	10/72	0/12	28/12	-	-	65/174
Barbarea orthoceras	0/6	-	0/15	0/7	0/72	-	-	-	0/4	-	-	0/104
Brassica napus	-	-	-	-	0/51	0/45	-	0/10	0/3	-	-	0/109
Cardamine impatiens	-	-	-	-	0/12	0/4	9/7	-	6/5	-	-	15/28
Cardamine kiusiana	-	-	-	-	-	-	-	-	10/14	-	-	10/14
Cardamine leucantha	19/438	0/20	24/130	16/42	35/140	-	-	-	-	-	-	94/770
Cardamine occulta	0/30	-	0/12	4/45	0/210	0/60	0/18	2/31	-	0/13	0/25	6/444
Cardamine regeliana	-	-	-	-	0/81	0/125	-	-	-	-	-	0/206
Draba nemorosa	-	-	-	-	0/140	-	-	-	-	-	-	0/140
Eutrema japonicum	-	-	-	0/130	0/15	-	0/30	-	-	-	-	0/175
Eutrema tenue	0/47	-	-	-	-	-	-	-	-	-	-	0/47
Lepidium virginicum	-	-	-	-	-	-	-	0/10	-	-	0/240	0/250
Nasturtium officinale	-	-	-	0/23	-	6/200	-	0/174	0/6	-	-	6/403
Orychophragmus violaceus	-	-	-	-	-	88/140	-	-	-	-	-	88/140
Raphanus sativus	-	-	-	-	0/8	-	-	-	-	-	-	0/8
Rorippa dubia	-	-	-	-	-	-	-	1/62	-	0/58	-	1/120
Rorippa indica	-	-	0/2	0/25	-	18/40	25/58	48/75	85/70	0/27	-	176/297
Rorippa palustris	-	0/101	-	-	0/21	0/10	-	0/10	-	-	-	0/142
Rorippa sylvestris	1/262	0/317	-	-	-	-	-	-	-	-	-	1/579
Turritis glabra	-	-	-	-	-	-	-	-	21/30	-	-	21/30

SUM	20/783	0/438	24/159	47/350	35/856	112/644	44/185	51/617	150/144	0/343	0/265	483/4784

P. napi

Plant sp./Sampling Sites	Yubari	Rusutsu	Oshamanbe	Fukushima	Nagano	Chiba	Kanagawa	Nara	Tokushima	Miyazaki	Yonaguni	**SUM**
Arabidopsis halleri	-	-	-	-	-	-	-	11/83	-	-	-	11/83
Arabidopsis kamchatica	-	-	-	-	7/46	-	-	-	-	-	-	7/46
Arabidopsis thaliana	-	-	-	-	0/60	0/20	-	-	-	-	-	0/80
Arabis flagellosa	-	-	-	-	-	-	-	10/150	-	34/245	-	44/395
Arabis hirsuta	-	-	-	19/78	-	-	7/72	5/12	0/12	-	-	31/174
Barbarea orthoceras	0/6	-	0/15	0/7	29/72	-	-	-	0/4	-	-	29/104
Brassica napus	-	-	-	-	0/51	0/45	-	0/10	0/3	-	-	0/109
Cardamine impatiens	-	-	-	-	0/12	0/4	2/7	-	0/5	-	-	2/28
Cardamine kiusiana	-	-	-	-	-	-	-	-	0/14	-	-	0/14
Cardamine leucantha	5/438	0/20	0/130	4/42	0/140	-	-	-	-	-	-	9/770
Cardamine occulta	1/30	-	0/12	0/45	39/210	0/60	0/18	4/31	-	0/13	0/25	44/444
Cardamine regeliana	-	-	-	-	2/81	0/125	-	-	-	-	-	2/206
Draba nemorosa	-	-	-	-	0/140	-	-	-	-	-	-	0/140
Eutrema japonicum	-	-	-	0/130	0/15	-	0/30	-	-	-	-	0/175
Eutrema tenue	1/47	-	-	-	-	-	-	-	-	-	-	1/47
Lepidium virginicum	-	-	-	-	-	-	-	0/10	-	-	0/240	0/250
Nasturtium officinale	-	-	-	0/23	-	0/200	-	0/174	0/6	-	-	0/403
Orychophragmus violaceus	-	-	-	-	-	0/140	-	-	-	-	-	0/140
Raphanus sativus	-	-	-	-	0/8	-	-	-	-	-	-	0/8
Rorippa dubia	-	-	-	-	-	-	-	3/62	-	0/58	-	3/120
Rorippa indica	-	-	0/2	1/25	-	0/40	5/58	0/75	0/70	0/27	-	6/297
Rorippa palustris	-	6/101	-	-	0/21	0/10	-	0/10	-	-	-	6/142
Rorippa sylvestris	21/262	37/317	-	-	-	-	-	-	-	-	-	58/579
Turritis glabra	-	-	-	-	-	-	-	-	0/30	-	-	0/30
SUM	28/783	43/438	0/159	24/350	77/856	0/644	14/185	33/617	0/144	34/343	0/265	253/4784

P. rapae

Plant sp./Sampling Sites	Yubari	Rusutsu	Oshamanbe	Fukushima	Nagano	Chiba	Kanagawa	Nara	Tokushima	Miyazaki	Yonaguni	**SUM**
Arabidopsis halleri	-	-	-	-	-	-	-	0/83	-	-	-	0 /83
Arabidopsis kamchatica	-	-	-	-	0/46	-	-	-	-	-	-	0 /46
Arabidopsis thaliana	-	-	-	-	0/60	0/20	-	-	-	-	-	0 /80
Arabis flagellosa	-	-	-	-	-	-	-	0/150	-	0/245	-	0 /395
Arabis hirsuta	-	-	-	0/78	-	-	0/72	0/12	0/12	-	-	0 /174
Barbarea orthoceras	2/6	-	0/15	0/7	0/72	-	-	-	0/4	-	-	2 /104
Brassica napus	-	-	-	-	35/51	34/45	-	0/10	0/3	-	-	69 /109
Cardamine impatiens	-	-	-	-	0/12	0/4	0/7	-	0/5	-	-	0 /28
Cardamine kiusiana	-	-	-	-	-	-	-	-	25/14	-	-	25 /14
Cardamine leucantha	0/438	0/20	0/130	0/42	0/140	-	-	-	-	-	-	0 /770
Cardamine occulta	0/30	-	0/12	0/45	0/210	0/60	0/18	0/31	-	0/13	0/25	0 /444
Cardamine regeliana	-	-	-	-	0/81	0/125	-	-	-	-	-	0 /206
Draba nemorosa	-	-	-	-	0/140	-	-	-	-	-	-	0 /140
Eutrema japonicum	-	-	-	0/130	0/15	-	0/30	-	-	-	-	0 /175
Eutrema tenue	0/47	-	-	-	-	-	-	-	-	-	-	0 /47
Lepidium virginicum	-	-	-	-	-	-	-	0/10	-	-	8/240	8 /250
Nasturtium officinale	-	-	-	1/23	-	0/200	-	13/174	6/6	-	-	20 /403
Orychophragmus violaceus	-	-	-	-	-	0/140	-	-	-	-	-	0 /140
Raphanus sativus	-	-	-	-	4/8	-	-	-	-	-	-	4 /8
Rorippa dubia	-	-	-	-	-	-	-	0/62	-	0/58	-	0 /120
Rorippa indica	-	-	0/2	11/25	-	0/40	0/58	7/75	13/70	0/27	-	31 /297
Rorippa palustris	-	20/101	-	-	0/21	0/10	-	0/10	-	-	-	20 /142
Rorippa sylvestris	29/262	1/317	-	-	-	-	-	-	-	-	-	30 /579
Turritis glabra	-	-	-	-	-	-	-	-	0/30	-	-	0 /30
SUM	31 /783	21 /438	0 /159	12 /350	39 /856	34 /644	0 /185	20 /617	44 /144	0 /343	8 /265	209 /4784

Table S5 Statistics for *NSP* and *MA*

(a) *NSP*

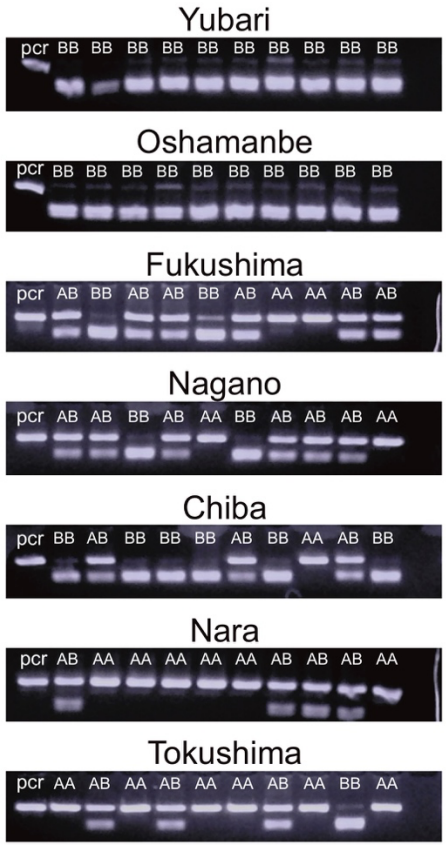
Species	Sampling sites	Sequences	Number of sites	S sites	*NSP* π	AA diversity	Tajima's	*P* value
P. melete	ALL	69	1860	34	0.0047	0.0070	0.561	-
	Yubari (Hokkaido)	10	1860	5	0.0007	0.0009	-1.035	0.188
	Oshamanbe	10	1860	4	0.0005	0.0003	-1.245	0.116
	Fukushima	9	1860	23	0.0052	0.0080	0.738	0.803
	Nagano	10	1860	18	0.0047	0.0070	1.772	0.980
	Chiba	10	1860	22	0.0045	0.0065	0.406	0.685
	Nara	10	1857-1860	24	0.0049	0.0069	0.314	0.662
	Tokushima	10	1857-1860	27	0.0059	0.0091	0.773	0.815
P. napi	ALL	69	1860	66	0.0098	0.0156	0.370	-
	Yubari (Hokkaido)	10	1860	5	0.0006	0.0015	-1.388	0.094
	Rusutsu (Hokkaido)	10	1860	2	0.0004	0.0012	0.120	0.686
	Fukushima	9	1860	27	0.0052	0.0043	-0.107	0.483
	Nagano	10	1860	29	0.0056	0.0039	0.132	0.584
	Kanagawa	10	1860	27	0.0053	0.0041	0.162	0.610
	Nara	10	1860	26	0.0051	0.0043	0.178	0.614
	Miyazaki	10	1860	19	0.0037	0.0012	0.182	0.615
P. rapae	ALL	74	1869	43	0.0039	0.0037	-0.704	-
	Yubari (Hokkaido)	10	1869	22	0.0029	0.0032	-1.485	0.062
	Rusutsu (Hokkaido)	9	1869	21	0.0033	0.0030	-1.031	0.164
	Fukushima	9	1869	23	0.0047	0.0038	0.152	0.587
	Nagano	10	1869	21	0.0029	0.0028	-1.257	0.105
	Chiba	8	1869	18	0.0033	0.0030	-0.676	0.281
	Nara	10	1869	26	0.0043	0.0038	-0.664	0.265
	Tokushima	10	1869	28	0.0053	0.0052	-0.221	0.518
	Yonaguni (Okinawa)	8	1869	18	0.0036	0.0037	-0.485	0.427

(a) *MA*

Species	Sampling sites	Sequences	Number of sites	S sites	*MA* π	AA diversity	Tajima's *D*	*P* value
P. melete	ALL	68	1896	44	0.0048	0.0056	-0.2495	-
	Yubari (Hokkaido)	10	1896	21	0.0035	0.0032	-0.5370	0.314
	Oshamanbe	10	1896	23	0.0045	0.0043	0.1832	0.617
	Fukushima	10	1896	20	0.0044	0.0065	0.8698	0.840
	Nagano	10	1896	24	0.0041	0.0049	-0.4326	0.348
	Chiba	8	1896	16	0.0039	0.0031	0.9582	0.859
	Nara	10	1896	31	0.0059	0.0076	0.0867	0.579
	Tokushima	10	1896	29	0.0052	0.0069	-0.2318	0.437
P. napi	ALL	70	1896	56	0.0061	0.0087	-1.1531	-
	Yubari (Hokkaido)	10	1896	39	0.0056	0.0088	-1.2381	0.134
	Rusutsu	10	1896	11	0.0023	0.0022	0.4869	0.711
	Fukushima	10	1896	17	0.0029	0.0022	-0.3511	0.389
	Nagano	10	1896	14	0.0021	0.0021	-0.9809	0.176
	Kanagawa	10	1896	15	0.0029	0.0029	0.2393	0.632
	Nara	10	1896	14	0.0028	0.0031	0.3970	0.687
	Miyazaki	10	1896	10	0.0021	0.0020	0.5833	0.747
P. rapae	ALL	76	1896	34	0.0038	0.0025	-0.0747	-
	Yubari (Hokkaido)	10	1896	18	0.0040	0.0033	0.8915	0.844
	Rusutsu	9	1896	20	0.0042	0.0020	0.3914	0.687
	Fukushima	10	1896	18	0.0033	0.0024	-0.0542	0.508
	Nagano	10	1896	15	0.0032	0.0025	0.6447	0.772
	Chiba	9	1896	16	0.0038	0.0025	1.0538	0.881
	Nara	10	1896	14	0.0027	0.0016	0.2119	0.625
	Tokushima	10	1896	22	0.0041	0.0012	-0.0399	0.513
	Yonaguni	8	1896	22	0.0046	0.0033	0.0754	0.548

Fig. S1(a) PCR-RFLP of *NSP* exon 1 from seven populations of *P. melete*. "pcr" refers to an undigested PCR product, corresponding to 'type A'. Note that 'type B' bands in the agarose gels consist of two restriction fragments of similar size. (b) Exon 1 sequences of type A and type B *NSP* with *Hin*cII restriction site in type B. SNPs distinguishing types A and B of *NSP* are shown in red.

(a)



(b)

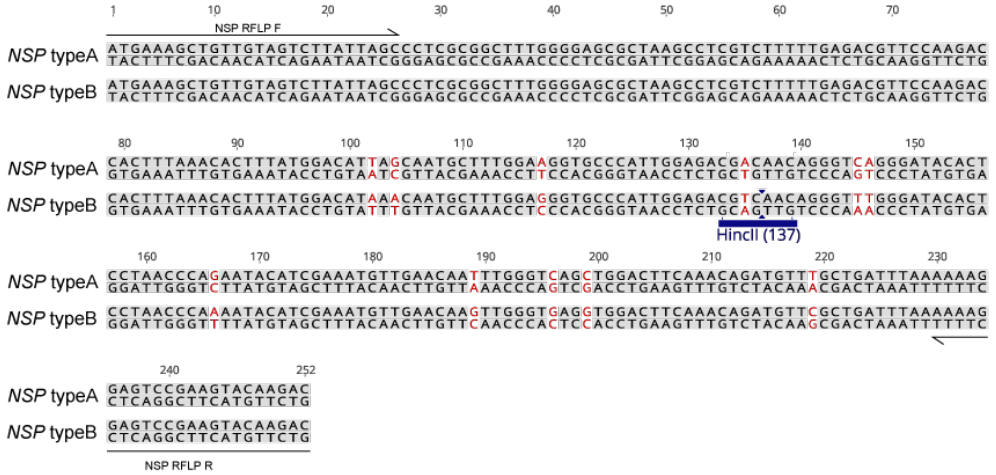


Fig. S2 *NSP* and *MA* gene copy numbers in *P. melete*. Genotypes (AA, AB and BB) are the same as in Fig. S1. RT-qPCR was done with N = 8 replicates per genotype and *Ef1α* as a reference gene. Data were standardized with mean of genotype AA set as 1. Bars shows means (± SE). Genotypes do not differ in *NSP* (yellow bars) or *MA* (blue bars) gene copy numbers.

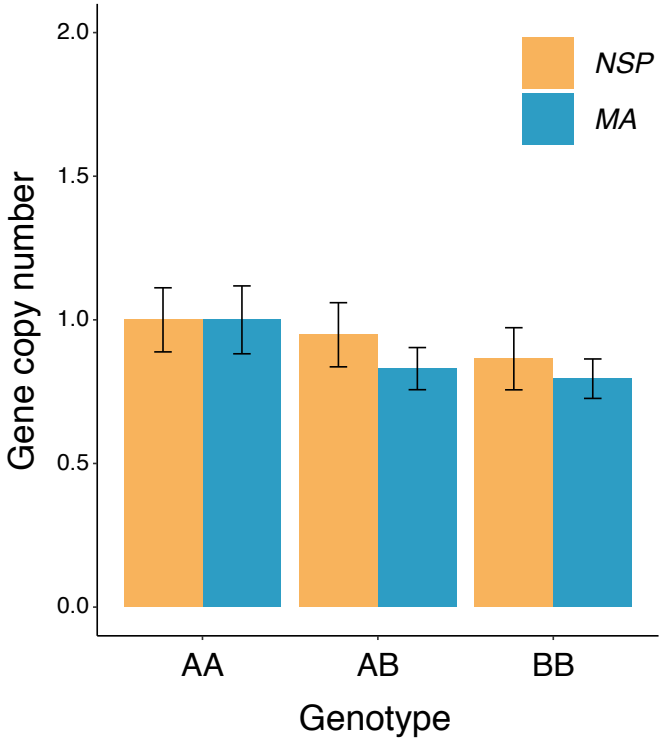


Fig. S3 Patterns of host plant use of *Pieris* species in the field. The plant species data were summarized to genus level for visualization. The pie charts show composition of plant genus and each bar show number of individuals (either plants or larvae).