Online supporting material for: "On the genetic control and origin of apomixis in *Boechera*".

Contents:

Methods Summary

Sample collection: Seeds from single were collected from various *Boechera* species sampled from throughout North America (Table S1; See Fig. 1 in Sharbel et al. (2005)). Species identification was conducted using the new *Boechera* nomenclature and descriptions (Al-Shehbaz 2003). In addition, collections were compared to known herbarium specimens. Five to 10 seeds from each collection site were placed on moist filter paper in petri plates, and vernalized at 5°C for 2 to 3 weeks. Upon germination, 3 to 5 seedlings were transferred to pots (11 x 11 x 13 cm) containing sterilized soil and grown in a controlled environmental growth room. Plants were grown in a 12 hour light/dark cycle under fluorescent lighting (cool white and GrowLux©), with a daily temperature variation from 22º to 28ºC.

Ploidy analyses of somatic and seed tissues: Ploidy levels of somatic tissue were measured using flow cytometry. Using the updated taxon names presented here, the genome size criteria of Sharbel et al. (2005) was used to group samples into 2x (diploid) and 3x (triploid) ranges. One morphological retrofracta-stricta hybrid gave a signature of apomixis near the diploid range. This line was removed from the analysis as it may contain an anueploid chromosome or may simply be a diploid hybrid.

In order to determine the reproductive mode of each plant, the flow cytometric seed screen analysis was performed on from 1 to 3 bulked samples (5 seeds per bulk) per individual using a PARTEC PA-I flow cytometer and their CyStain® UV Precise P staining kit. The FCSS analysis was performed on a subset of the individuals used in the genetic analyses, since seeds were not available for all accessions. Plants which showed flow cytometr profiles in which all seeds had a diploid embryo and triploid endosperm were labelled as sexual, while plants showing any deviation from this 2:3 ratio were recorded as being apomictic.

Microsatellite analysis: DNA was isolated from the leaf tissue of 231 individuals using a Nucleon (Amersham Pharmacia Biotech Europe GMbH) extraction kit. All individuals were genotyped at 10 SSR markers (table S2). A separate 96 well plate amplification (10 µl per reaction) for each microsatellite locus was performed using the protocol outlined by Clauss et al. (2002). An ABI 3700 genetic analyzer was used for the fragment analysis. For most runs the PCR products of 3 microsatellites (with different labels) were mixed with an internal size standard (ROX-500) and analyzed in a single run. The three labels used in this study differed in fluorescence intensity, and thus to correct for signal intensity each ABI 3700 genetic analyzer run was mixed as follows: (0.5 µl FAM-product) + (1.2 µl NED-product) + (1.2 µl HEX-product) + 7 µl loading mixture (see ABI documentation for loading mixture). A multi step process was used to score microsatellite genotypes. First, the internal size standards of all individual runs were aligned (and occasionally corrected) using the ABI GeneScan (3.x) software.

The internally-aligned samples were then imported into the ABI Genotyper (3.5) software, where locus amplification was assessed in addition to a qualitative verification of peak morphology. Fragment (i.e. allele) size and peak height (i.e. PCR product dosage) were subsequently identified for each locus using the Genotyper software, followed by manual verification of each call in order to insure proper peak designation. The checked data were then exported into SPSS for Windows (release 11.5.0, SPSS Inc.), where fragment size distributions were generated using the complete data set for each locus. This final statistical step (i.e. binning) enables fragments which differ slightly in size (a result of genetic analyzer error) to be assigned to specific distribution groups, each of which represents a particular allele. All samples were processed and analyzed blindly, and in random order.

Statistical Analysis: All population genetic analyses were conducted in GENODIVE; this program allow for the simultaneous analysis of different ploidy. Heterozygosity and genetic distance data was output into JMP Genomics 5.1 (SAS Institute, Cary, NC, USA). Ploidy and mating system were analyzed in a fixed-effect ANOVA, where a new variable of the ploidy-sexuality combination (2xSex, 2xApo, 3xApo) was the factor. Specific contrasts were made between groups using customized F-test contrasts. Simulated data distributions were compared to the real data by two-sample t-tests.

Table S1: Summary data for the 10 SSR loci used in this study.

Locus Name[1]	n_a	H_E[2]	F_{ST}	R_{ST}	Chr.[3]	Dye	Primer 1 Sequence (5' to 3')	Primer 2 Sequence (5' to 3')
ATTS0392	7	0.576	0.487	0.073	I	HEX	TTTGGAGTTAGACACGGATCTG	GTTGATCGCAGCTTGATAAGC
ADH1	9	0.389	0.275	0.208	I	NED	ACCACCGGACAGATTATCG	CCCAGAAGTAAACATCGGTGTG
ICE14	9	0.725	0.371	0.377	II	HEX	TCGAGGTGCTTTCTGAGGTT	TACCTCACCCTTTGACCCA
MDC16	12	0.671	0.114	0.186	III	FAM	GAGTGGCCTCGTGTAGAGAAAG	TGTCACTCTTTCCTCTGGTTTG
AthGAPAb	9	0.773	0.166	0.159	III	HEX	CACCATGGCTCGGTTACTT	TCCTGAGAATCAGTGAAACCC
ICE4	4	0.108	0.218	0.121	V	NED	CACGAGGAATCTGGCATGGTCG	AGCGATGCAAGCGGCTCAAG
ICE8	7	0.418	0.605	0.485	V	HEX	GTGTTACCGATCTGGCTCTG	TCAGCTTGAGCATTTCACAG
ICE3	16	0.86	0.165	0.601	V	FAM	GACTAATCATCACCGACTCAGCCAC	ATTCTCTTCACTTTCTTGATCCCG
T4P13A3	5	0.224	0.022	0.029	III	HEX	CGGGTGCTTCTATCATGTC	CGCTGTCATATCCCAGAACA
MBK21B3	13	0.804	0.501	0.591	III	NED	ATTGCTTCCGTTTTGCTCAT	TTTCAATTCTCTCTGCCTCTCA

[1]Taken from Clauss et al. (2002)

[2]Gene diversity corrected for sample size (Nei, 1978)

[3]Arabidopsis thaliana chromosome location of microsatellite from http:/arabidopsis.org/home.html

Table S2: Sampling location, ploidy and sexuality of all lines analyzed.

ID	Ploidy	Species	repro1	pop#	lat	long
445	3	stricta X polyantha	apo	1	45.37	-116.48
463	3	stricta X polyantha	apo	1	45.37	-116.48
495	3	stricta X polyantha	apo	1	45.37	-116.48
476	2	stricta		2	44.53	-112.62
465	2	stricta		3	44.43	-112.62
474	2	stricta		3	44.43	-112.62
484	2	stricta		3	44.43	-112.62
196	2	polyantha	apo	4	47.45	-110.58
215	2	retrofracta	sex	4	47.45	-110.58
23	3	retrofracta X polyantha	apo	4	47.45	-110.58
32	3	retrofracta X polyantha	apo	4	47.45	-110.58
119	3	retrofracta X polyantha	apo	4	47.45	-110.58
122	3	retrofracta X polyantha	apo	4	47.45	-110.58
143	3	retrofracta X polyantha	apo	4	47.45	-110.58
195	3	retrofracta X polyantha	apo	4	47.45	-110.58
216	3	retrofracta X polyantha	apo	4	47.45	-110.58
217	3	retrofracta X polyantha	apo	4	47.45	-110.58
218	3	retrofracta X polyantha	apo	4	47.45	-110.58
236	3	retrofracta X polyantha	apo	4	47.45	-110.58
275	3	retrofracta X polyantha	apo	4	47.45	-110.58
309	3	retrofracta X polyantha	apo	4	47.45	-110.58
24	2	polyantha		5	48.26	-112.62
30	2	polyantha		5	48.26	-112.62
38	2	polyantha		5	48.26	-112.62
44	2	polyantha		5	48.26	-112.62
106	2	polyantha		5	48.26	-112.62
107	2	polyantha		5	48.26	-112.62
111	2	polyantha		5	48.26	-112.62
114	2	polyantha		5	48.26	-112.62
121	2	polyantha		5	48.26	-112.62
252	2	polyantha		5	48.26	-112.62
256	2	polyantha		5	48.26	-112.62
257	2	polyantha		5	48.26	-112.62
258	2	polyantha		5	48.26	-112.62
259	2	polyantha		5	48.26	-112.62
260	2	polyantha		5	48.26	-112.62
261	2	polyantha		5	48.26	-112.62
264	2	polyantha		5	48.26	-112.62
265	2	polyantha		5	48.26	-112.62
28	2	polyantha	apo	5	48.26	-112.62
42	2	polyantha	apo	5	48.26	-112.62
45	2	polyantha	apo	5	48.26	-112.62
108	2	polyantha	apo	5	48.26	-112.62
112	2	polyantha	apo	5	48.26	-112.62
27	2	retrofracta		5	48.26	-112.62
37	2	retrofracta		5	48.26	-112.62
109	2	retrofracta		5	48.26	-112.62
262	2	retrofracta		5	48.26	-112.62
263	2	retrofracta		5	48.26	-112.62
266	2	retrofracta		5	48.26	-112.62
267	2	retrofracta		5	48.26	-112.62
322	2	retrofracta		5	48.26	-112.62
115	2	retrofracta	apo	5	48.26	-112.62
116	2	retrofracta	apo	5	48.26	-112.62
300	2	retrofracta	apo	5	48.26	-112.62
35	2	polyantha		6	46.21	-113.17
36	2	polyantha		6	46.21	-113.17
40	2	polyantha		6	46.21	-113.17
206	2	polyantha		6	46.21	-113.17
207	2	polyantha		6	46.21	-113.17
210	2	polyantha		6	46.21	-113.17
254	2	polyantha		6	46.21	-113.17
272	2	polyantha		6	46.21	-113.17
34	2	polyantha	apo	6	46.21	-113.17
205	2	polyantha	apo	6	46.21	-113.17
209	2	polyantha	apo	6	46.21	-113.17
31	2	polyantha	sex	6	46.21	-113.17
208	2	polyantha	sex	6	46.21	-113.17
213	2	polyantha	sex	6	46.21	-113.17
15	2	polyantha		7	47.02	-113.35
72	2	polyantha		7	47.02	-113.35
86	2	polyantha		7	47.02	-113.35
93	2	polyantha		7	47.02	-113.35
99	2	polyantha		7	47.02	-113.35
100	2	polyantha		7	47.02	-113.35
101	2	polyantha		7	47.02	-113.35
194	2	polyantha		7	47.02	-113.35
84	2	polyantha	apo	7	47.02	-113.35
95	2	polyantha	apo	7	47.02	-113.35
102	2	polyantha	apo	7	47.02	-113.35
104	2	polyantha	apo	7	47.02	-113.35
190	2	polyantha	apo	7	47.02	-113.35
192	2	polyantha	apo	7	47.02	-113.35
85	2	polyantha	sex	7	47.02	-113.35
87	2	polyantha	sex	7	47.02	-113.35
88	2	polyantha	sex	7	47.02	-113.35
89	2	polyantha	sex	7	47.02	-113.35
90	2	polyantha	sex	7	47.02	-113.35
92	2	polyantha	sex	7	47.02	-113.35
94	2	polyantha	sex	7	47.02	-113.35
97	2	polyantha	sex	7	47.02	-113.35
137	2	polyantha		8	45.55	-113.82
145	2	polyantha		8	45.55	-113.82
268	2	polyantha		8	45.55	-113.82
269	2	polyantha		8	45.55	-113.82
29	2	polyantha	sex	8	45.55	-113.82
129	2	stricta		8	45.55	-113.82
446	3	stricta X pendulocarpa	apo	9	45.48	-114.37
449	3	stricta X pendulocarpa	apo	9	45.48	-114.37
132	2	stricta		10	45.67	-112.88
303	3	stricta X pendulocarpa	apo	10	45.67	-112.88
301	3	stricta X pendulocarpa	apo	12	45.37	-114.55
441	3	stricta X pendulocarpa	apo	12	45.37	-114.55
452	3	stricta X pendulocarpa	apo	12	45.37	-114.55
453	3	stricta X pendulocarpa	apo	12	45.37	-114.55
455	3	stricta X pendulocarpa	apo	12	45.37	-114.55
457	3	stricta X pendulocarpa	apo	12	45.37	-114.55
494	3	stricta X pendulocarpa	apo	12	45.37	-114.55
125	2	retrofracta		13	45.77	-112.86
249	2	retrofracta		13	45.77	-112.86
250	2	retrofracta		13	45.77	-112.86
251	2	retrofracta		13	45.77	-112.86
273	2	retrofracta		13	45.77	-112.86
211	2	retrofracta	sex	13	45.77	-112.86
212	2	retrofracta	sex	13	45.77	-112.86
305	2	stricta		14	45.53	-113.82
41	2	pendulocarpa		15	46.87	-113.99
43	2	pendulocarpa		15	46.87	-113.99
133	2	pendulocarpa		15	46.87	-113.99
176	2	pendulocarpa		15	46.87	-113.99
178	2	pendulocarpa		15	46.87	-113.99
179	2	pendulocarpa		15	46.87	-113.99
180	2	pendulocarpa		15	46.87	-113.99
183	2	pendulocarpa		15	46.87	-113.99
185	2	pendulocarpa		15	46.87	-113.99
25	2	pendulocarpa	sex	15	46.87	-113.99
26	2	pendulocarpa	sex	15	46.87	-113.99
177	2	pendulocarpa	sex	15	46.87	-113.99
182	2	pendulocarpa	sex	15	46.87	-113.99
186	2	pendulocarpa	sex	15	46.87	-113.99
187	2	pendulocarpa	sex	15	46.87	-113.99
142	2	pendulocarpa		16	47.08	-112.37
135	2	pendulocarpa	sex	16	47.08	-112.37
141	2	pendulocarpa	sex	16	47.08	-112.37
124	2	stricta		19	46.12	-112.91
126	2	stricta		19	46.12	-112.91
128	2	stricta		19	46.12	-112.91
134	2	stricta		19	46.12	-112.91
120	2	stricta X retrofracta		19	46.12	-112.91
130	3	stricta X retrofracta	apo	19	46.12	-112.91
238	2	pendulocarpa		20	44.51	-114.23
149	2	pendulocarpa	apo	20	44.51	-114.23
245	2	pendulocarpa	apo	20	44.51	-114.23
246	2	pendulocarpa	apo	20	44.51	-114.23
247	2	pendulocarpa	apo	20	44.51	-114.23
443	2	stricta		20	44.51	-114.23
444	2	stricta		20	44.51	-114.23
447	2	stricta		20	44.51	-114.23
464	2	stricta		20	44.51	-114.23
14	3	stricta X pendulocarpa	apo	20	44.51	-114.23
17	3	stricta X pendulocarpa	apo	20	44.51	-114.23
147	3	stricta X pendulocarpa	apo	20	44.51	-114.23
148	3	stricta X pendulocarpa	apo	20	44.51	-114.23
189	3	stricta X pendulocarpa	apo	20	44.51	-114.23
234	3	stricta X pendulocarpa	apo	20	44.51	-114.23
239	3	stricta X pendulocarpa	apo	20	44.51	-114.23
240	3	stricta X pendulocarpa	apo	20	44.51	-114.23
241	3	stricta X pendulocarpa	apo	20	44.51	-114.23
242	3	stricta X pendulocarpa	apo	20	44.51	-114.23
243	3	stricta X pendulocarpa	apo	20	44.51	-114.23
76	2	polyantha	sex	21	46.55	-113.65
64	2	retrofracta		21	46.55	-113.65
65	2	retrofracta		21	46.55	-113.65
67	2	retrofracta		21	46.55	-113.65
68	2	retrofracta		21	46.55	-113.65
70	2	retrofracta		21	46.55	-113.65
71	2	retrofracta		21	46.55	-113.65
73	2	retrofracta		21	46.55	-113.65
74	2	retrofracta		21	46.55	-113.65
78	2	retrofracta		21	46.55	-113.65
144	2	retrofracta		21	46.55	-113.65
66	2	retrofracta	apo	21	46.55	-113.65
139	2	stricta		21	46.55	-113.65
276	3	stricta X retrofracta	apo	21	46.55	-113.65
302	3	stricta X pendulocarpa	apo	22	44.22	-113.83
294	2	stricta		23	44.13	-113.72
131	2	stricta		24	45.81	-112.99
270	2	stricta		24	45.81	-112.99
175	2	stricta		25	46.07	-113.27
278	2	stricta		25	46.07	-113.27
47	2	polyantha		26	45.28	-117.40
48	2	polyantha		26	45.28	-117.40
51	2	polyantha		26	45.28	-117.40
52	2	polyantha		26	45.28	-117.40
54	2	polyantha		26	45.28	-117.40
55	2	polyantha		26	45.28	-117.40
57	2	polyantha		26	45.28	-117.40
59	2	polyantha		26	45.28	-117.40
60	2	polyantha		26	45.28	-117.40
79	2	polyantha		26	45.28	-117.40
199	2	polyantha		26	45.28	-117.40
197	2	polyantha	apo	26	45.28	-117.40
49	2	polyantha	sex	26	45.28	-117.40
50	2	polyantha	sex	26	45.28	-117.40
53	2	polyantha	sex	26	45.28	-117.40
56	2	polyantha	sex	26	45.28	-117.40
58	2	polyantha	sex	26	45.28	-117.40
61	2	polyantha	sex	26	45.28	-117.40
62	2	polyantha	sex	26	45.28	-117.40
63	2	polyantha	sex	26	45.28	-117.40
193	2	polyantha	sex	26	45.28	-117.40
198	2	polyantha	sex	26	45.28	-117.40
200	2	polyantha	sex	26	45.28	-117.40
202	2	polyantha	sex	26	45.28	-117.40
203	2	polyantha	sex	26	45.28	-117.40
204	2	polyantha	sex	26	45.28	-117.40
282	3	stricta X retrofracta	apo	27	40.08	-120.61
284	3	stricta X retrofracta	apo	27	40.08	-120.61
283	3	retrofracta X polyantha	apo	28	38.95	-120.08
289	3	retrofracta X polyantha	apo	29	38.79	-119.95
285	3	retrofracta X polyantha	apo	30	38.53	-119.80
290	2	retrofracta		31	38.32	-119.60
279	2	stricta		32	36.43	-118.23
280	2	stricta		32	36.43	-118.23
291	3	stricta X retrofracta	apo	32	36.43	-118.23
286	3	stricta X retrofracta	apo	33	38.83	-120.03
287	3	retrofracta X polyantha	apo	34	39.31	-119.65
293	2	stricta		35	37.60	-112.82
292	2	stricta		36	37.80	-109.80
295	2	stricta		37	36.72	-112.10
307	2	stricta		39	39.15	-112.32
478	2	stricta		42	44.13	-113.72
479	2	stricta		42	44.13	-113.72
480	2	stricta		42	44.13	-113.72
298	3	stricta X retrofracta	apo	44		
327	2	stricta		45	44.64	-114.14
468	2	stricta		45	44.64	-114.14
469	2	stricta		45	44.64	-114.14
470	2	stricta		45	44.64	-114.14
481	2	stricta		45	44.64	-114.14
482	2	stricta		45	44.64	-114.14

Table S3: Allele calls for all lines and loci. The call "0" indicates an unknown allelic state. These was imputed for analyses in GENODIVE.

ID	a812_1	gc16_1	gc42_1	h103_1	h23_1	h34_1	h5_1	h77_1	mbk21b_1	t4p13a_1
445	195:225:0	134:140:0	0:0:0	253:265:0	74:76:0	183:183:0	109:117:0	53:59:62	177:181:0	167:167:0
463	195:225:0	134:140:0	147:151:0	253:259:262	74:76:0	183:183:0	109:117:0	53:59:62	177:181:0	167:167:0
495	195:225:0	134:140:0	147:151:0	253:259:262	74:76:0	183:183:0	109:117:0	53:59:62	177:181:0	167:167:0
476	225:228	140:140	147:151	262:262	76:76	181:183	117:117	59:62	177:177	167:167
465	225:238	134:134	147:147	259:259	84:86	183:183	119:119	59:65	177:183	167:167
474	221:221	140:140	151:151	262:262	76:76	183:183	117:117	62:62	177:177	167:167
484	225:238	134:134	147:147	253:259	78:78	183:183	115:115	53:59	177:179	0:0
196	216:219	137:137	147:151	259:259	88:90	183:183	117:117	59:59	187:189	161:167
215	216:216	137:137	0:0	259:259	88:88	183:183	111:117	59:59	189:189	161:167
23	212:216:219	134:137:140	145:147:149	259:259:0	88:88:0	181:183:0	111:115:0	59:59:0	187:187:0	167:167:0
32	216:219:221	137:140:0	147:149:151	259:259:0	86:92:0	181:183:0	119:119:0	59:59:0	175:175:0	167:167:0
119	0:0:0	134:137:140	145:147:149	259:259:0	88:88:0	181:183:0	115:119:0	59:59:0	175:187:0	167:167:0
122	216:219:221	0:0:0	147:149:151	259:265:0	90:90:0	178:181:0	111:115:117	59:59:0	0:0:0	161:167:0
143	212:216:219	134:137:140	145:147:149	259:259:0	88:88:0	178:181:0	115:119:0	59:59:0	175:187:0	167:167:0
195	212:216:219	134:137:140	145:147:149	259:259:0	88:88:0	181:183:0	115:119:0	59:59:0	175:187:0	167:167:0
216	212:216:219	140:140:0	147:149:151	259:265:0	86:92:0	181:183:0	111:115:117	59:59:0	175:183:0	167:167:0
217	212:216:219	134:137:140	0:0:0	259:265:0	88:88:0	181:183:0	115:117:119	59:59:0	175:187:0	161:167:0
218	212:216:219	140:140:0	149:151:0	259:265:0	86:92:0	181:183:0	117:117:0	59:59:0	175:183:0	167:167:0
236	212:216:219	0:0:0	151:151:0	259:265:0	86:92:0	181:183:0	111:115:117	59:59:0	175:183:0	167:167:0
275	212:216:219	134:137:140	145:147:149	259:259:0	88:88:0	181:183:0	115:119:0	59:59:0	175:187:0	167:167:0
309	212:216:219	134:137:140	145:151:155	259:259:0	88:88:0	181:183:0	115:117:119	59:59:0	175:187:0	167:167:0
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