Supplemental Information for:

Utilizing field collected insects for next generation sequencing: effects of sampling, storage, and DNA extraction methods

Kimberly M. Ballare[1,2,7], Nathaniel S. Pope[1,3], Antonio R. Castilla[1,4], Sarah Cusser[1,5], Richard P. Metz[6], Shalene Jha[1]

[1] Department of Integrative Biology, The University of Texas at Austin, 205 W 24th Street, 401 Biological Laboratories, Austin, TX 78712, USA
[2] Current Affiliation: Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, CA 95060, USA
[3] Current Affiliation: Department of Entomology, Pennsylvania State University, University Park, PA 16802, USA
[4] Current Affiliation: Centre for Applied Ecology "Prof. Baeta Neves"/INBIO, Instituto, Superior of Agronomy, University of Lisbon. Tapada da Ajuda, 1349-017, Lisbon, Portugal.
[5] Current Affiliation: Kellogg Biological Station, Michigan State University, 3700 East Gull Rd., Hickory Corners, MI 49060, USA
[6] Genomics and Bioinformatics Service, Texas A&M AgriLife Research, College Station, TX 77845, USA
[7] Author for correspondence

Table of Contents

Sequence similarity for one- and six-sample filters (Qiagen extracted)

B. pensylvanicus

There was no significant difference in levels of sequence similarity between treatments of *B. pensylvanicus* in any binning group, although there was a marginally significant effect of treatment on sequence similarity when comparing all samples (binned by one sample, H=5.13, p=0.075). Post-hoc Dunn's tests with Holm correction for multiple comparisons revealed that Net-EtOH had significantly higher levels of sequence similarity than Net-Dry specimens in the one-sample bin (p=0.048), but no other differences between treatments were significant. In general, Vane-Dry showed the highest level of similarity for all sample filters, including the most conservative filter of six samples per treatment (Fig. S2b).

M. tepaneca

Treatment had a significant effect on sequence similarity when binned by one sample (H=12.779, p=0.010), but not when binned by six samples (H=88.14, p=0.241). Post-hoc Dunn's tests did not show any significant differences between treatments in *M. tepaneca* after Holm correction for multiple comparisons. Vane-Gly treatment showed the lowest numbers of similar loci than all other treatments, with Net-Dry, Pan, and Vane-Dry showing the highest levels of similarity in *M. tepaneca* for all sample filters including the most conservative filter (Fig. S2c).

L. bardum

There was a significant effect of treatment on sequence similarity when binned by one sample (H=21.63, p<0.001) and six samples (H=256.91, p=0.005). Post-hoc Dunn's tests with Holm correction for multiple comparisons indicate that Pan specimens had significantly more similar loci than both Net-Dry and Vane-Gly in the one-sample filter (p < 0.001), but not in the six-sample filter (Pan vs Net-Dry: p=0.276, vs Vane-Gly: p=0.350). Dunn's tests also showed that Vane-Dry specimens had significantly more sequence similarity than Vane-Gly and Net-Dry specimens in the one-sample filter (p<0.001), but not in the six-sample filter (p=0.350). In general, Pan specimens showed the highest level of sequence similarity among all treatments and in all sample filters (Fig. S2d).

Standardized Depth Models

As in the main text, here we report results for the 60% sample filter. See Tables S3-S5 for results of all models and sample filters. For the most part, we found that standardizing depth by millions of raw reads evened out differences among treatments seen in models of average (overall) locus depth. Indeed, there were no significant effects of treatment on standardized depth in either *B. pensylvanicus* (Fig S3e, f) or *M. tepaneca* (Fig S5e) after correcting for multiple comparisons. However, for *L. bardum* we did see that Net-Dry and Vane-Gly specimens had significantly higher standardized depth than both Vane-Dry (Tukey HSD, p <0.001) and Pan (Tukey HSD, p=0.004 and p <0.001 respectively, see Fig S5f).

DNAzol extracted *B. pensylvanicus* specimens

Sequence similarity

Kruskal-Wallace tests indicated that treatment had an effect on sequence similarity in the DNAzol extracted *B. pensylvanicus* specimens when comparing all samples (binned by one sample, H=9.765, p=0.009) and binned by three samples (H=189.87, p=0.029), unlike the Qiagen extracted samples which did not show a significant difference in sequence similarity between treatments. Dunn's tests revealed that Net-Dry specimens had significantly less recovered loci than Net-EtOH specimens in the one-sample filter (p=0.006). Additionally, when comparing different extraction methods within treatments, there were significant differences between the Qiagen extracted and the DNAzol extracted Net-Dry specimens at the one- and three-sample filters, where Qiagen extracted Net-Dry specimens showed slightly but significantly more matching loci than the DNAzol extracted Net-Dry specimens (Sign test, 1 sample: p=0.018; 3 samples: p=0.022). There were no other significant differences between extractions for the other two treatments. Notably, Qiagen extracted Net-EtOH specimens were more variable in the levels of sequence similarity than DNAzol extracted Net-EtOH specimens at the six-sample filter (Fig. S2).

Loci and Depth

As in the main text, here we only report the results of the 60% sample filter. See Table S1 for results of all models. We found no significant effect of extraction type on number of loci, average locus depth or standardized depth. Within DNAzol extracted specimens, Net-EtOH had a marginally significantly higher number of loci than Net-Dry specimens (z=2.62, p=0.065). Both Net-EtOH and Vane-Dry specimens had significantly higher levels of average depth than Net-Dry specimens (Net-EtOH: z=4.738, p<0.001; Vane-Dry: z=4.055, p<0.001, Fig S2), with no significant difference in average depth between Net-EtOH and Vane-Dry. We found no significant differences between treatments in standardized depth in DNAzol specimens (Fig. S2e).

DNA quality

Of the two DNA quality metrics that significantly affected locus recovery and depth in *B. pensylvanicus* (lower DNA concentration and lower Nanodrop 260/280), only the 260/280 metric was significantly affected by treatment in DNAzol extracted bumblebees (F=8.627, p=0.001). Post-hoc tests revealed that Net-Dry specimens had significantly lower 260/280 ratios than Net-EtOH specimens (p<0.001). DNAzol extracted specimens also had substantially lower 260/230 ratios than Qiagen extracted specimens, indicating presence of salt contaminants. However, this did not significantly affect the amount of recovered loci or depth.

Table S1. Complete list of specimens including geographic information, sample year, number of raw reads, number of loci, average locus depth, fragmentation score and DNA weight score. *B.p.= Bombus pensylvanicus, M.t.=Melissodes tepaneca, L.b. = Lasioglossum bardum*

Species	Sample Treatment	Sample Number	Sample Year	Latitude	Longitude	Raw Reads	Loci	Average Locus Depth	Frag-mentation Score	DNA Weight Score
B.p.	Net-EtOH	Q004	2012	-96.2391	33.1684	3819504	4010	741.43	low	high
B.p.	Net-EtOH	Q005	2012	-96.9936	32.6078	2322205	3958	539.92	low	high
B.p.	Net-EtOH	Q006	2012	-96.9936	32.6078	3830297	3923	773.81	low	high
B.p.	Net-EtOH	Q007	2012	-96.2391	33.1684	1965781	3952	481.49	high	high
B.p.	Net-EtOH	Q008	2012	-96.2391	33.1684	2119833	3729	508.01	high	high
B.p.	Net-EtOH	Q009	2012	-96.2427	33.3139	3656199	3847	732.48	low	low
B.p.	Net-EtOH	Q010	2012	-96.2391	33.1684	2538885	3911	588.04	high	high
B.p.	Net-EtOH	Q011	2012	-96.2427	33.3139	1057320	3862	278.40	high	high
B.p.	Net-EtOH	Q189	2012	-96.2427	33.3139	3181687	9274	298.36	NA	NA
B.p.	Net-EtOH	Q190	2012	-96.2427	33.3139	2018871	8986	189.96	NA	NA
B.p.	Net-EtOH	Q191	2012	-96.2427	33.3139	2794192	9165	249.41	NA	NA
B.p.	Net-EtOH	Q192	2012	-96.2427	33.3139	202161	2253	24.01	NA	NA
B.p.	Net-EtOH	Q193	2012	-96.2428	33.3079	6575178	10209	491.21	NA	NA
B.p.	Net-Dry	Q012	2014	-97.3801	28.3021	1598698	4068	377.47	low	high
B.p.	Net-Dry	Q013	2014	-96.9203	28.7114	1594666	3909	369.98	low	high
B.p.	Net-Dry	Q014	2013	-97.5934	30.3090	518726	3480	133.68	high	low
B.p.	Net-Dry	Q015	2013	-97.5934	30.3090	762893	3711	194.71	high	low
B.p.	Net-Dry	Q016	2013	-97.5934	30.3090	454471	3260	128.17	high	low
B.p.	Net-Dry	Q017	2013	-97.7813	30.2843	608152	3527	156.27	high	low
B.p.	Net-Dry	Q018	2013	-96.6189	33.1603	475683	3405	124.73	high	low
B.p.	Net-Dry	Q019	2013	-96.6189	33.1603	781802	3741	184.95	high	low
B.p.	Net-Dry	Q194	2013	-97.9573	30.0601	239261	3005	28.40	NA	NA
B.p.	Net-Dry	Q195	2013	-96.2391	33.1684	478442	5377	51.56	NA	NA
B.p.	Vane-Dry	Q020	2013	-97.5210	30.1913	1413663	4042	329.59	low	high
B.p.	Vane-Dry	Q021	2013	-97.6649	30.2359	877835	3631	219.19	low	low
B.p.	Vane-Dry	Q022	2013	-97.6649	30.2359	609986	3364	174.87	high	high
B.p.	Vane-Dry	Q023	2013	-97.6649	30.2359	1344537	3989	321.88	high	low
B.p.	Vane-Dry	Q024	2013	-97.6351	30.2862	1275172	3912	309.70	high	low
B.p.	Vane-Dry	Q025	2013	-97.2941	32.9873	1572284	3855	285.03	low	high
B.p.	Vane-Dry	Q026	2013	-97.5210	30.1913	1352943	3913	316.94	high	high
B.p.	Vane-Dry	Q027	2013	-97.5210	30.1913	1369062	3935	332.19	high	high
M.t.	Net-EtOH	Q031	2013	-96.8674	28.3532	2882806	13010	125.49	low	high
M.t.	Net-EtOH	Q032	2013	-96.8674	28.3532	2037408	11366	113.15	low	high
M.t.	Net-EtOH	Q033	2013	-96.8252	28.7512	1831761	11571	109.20	low	high
M.t.	Net-EtOH	Q034	2013	-97.2505	28.1747	1392618	11558	84.02	low	high
M.t.	Net-EtOH	Q035	2013	-96.8607	28.4009	1711537	11148	99.71	low	high
M.t.	Net-EtOH	Q036	2013	-96.8607	28.4009	1795418	11387	98.23	low	high
M.t.	Net-EtOH	Q037	2013	-96.8607	28.4009	1553667	11484	92.24	low	high
M.t.	Net-EtOH	Q038	2013	-96.8607	28.4009	2596162	11647	115.38	low	high
M.t.	Net-Dry	Q039	2013	-98.2590	30.3321	794142	11786	41.00	high	low
M.t.	Net-Dry	Q040	2013	-98.2590	30.3321	6564960	13988	39.15	low	high
M.t.	Net-Dry	Q041	2013	-97.6137	30.1978	608179	10862	30.68	low	low
M.t.	Net-Dry	Q042	2013	-97.7813	30.2843	785623	11756	38.98	low	low
M.t.	Net-Dry	Q043	2013	-97.2548	28.1898	794257	10562	46.32	low	high
M.t.	Net-Dry	Q044	2013	-97.2548	28.1898	1161278	11979	61.02	low	high
M.t.	Net-Dry	Q045	2013	-97.2360	28.1574	967106	11348	51.91	low	high
M.t.	Net-Dry	Q046	2013	-96.9101	28.3882	1221683	12729	53.69	low	high
M.t.	Vane-Gly	Q070	2014	-98.2379	30.8896	1359432	11303	87.92	high	high
M.t.	Vane-Gly	Q071	2014	-98.2379	30.8896	1423516	10861	91.22	high	high
M.t.	Vane-Gly	Q072	2014	-98.2379	30.8896	1079308	9900	78.25	low	low
M.t.	Vane-Gly	Q073	2014	-97.9672	30.2126	1451509	10931	94.06	low	high
M.t.	Vane-Gly	Q074	2014	-97.9672	30.2126	1356315	10162	96.25	low	high

Species	**Sample Treatment**	**Sample Number**	**Sample Year**	**Latitude**	**Longitude**	**Raw Reads**	**Loci**	**Average Locus Depth**	**Frag-mentation Score**	**DNA Weight Score**
M.t.	Vane-Gly	Q075	2014	-97.9227	30.4508	1390130	10501	88.52	low	high
M.t.	Vane-Gly	Q076	2014	-97.9227	30.4508	911183	8932	78.99	low	high
M.t.	Vane-Dry	Q047	2013	-97.6991	30.2571	1948895	13552	56.90	high	low
M.t.	Vane-Dry	Q048	2013	-97.7251	30.2821	792908	11202	43.22	high	high
M.t.	Vane-Dry	Q049	2013	-97.1730	30.0852	520044	9338	36.57	high	high
M.t.	Vane-Dry	Q050	2013	-97.6462	30.2358	960307	12294	46.43	low	high
M.t.	Vane-Dry	Q051	2013	-97.6462	30.2358	1076373	12127	54.92	low	high
M.t.	Vane-Dry	Q052	2013	-97.6137	30.1978	1340261	12745	62.31	low	low
M.t.	Vane-Dry	Q053	2013	-97.6137	30.1978	1013875	12508	44.14	low	high
M.t.	Vane-Dry	Q054	2013	-97.8267	30.2063	1456210	13247	63.41	low	high
M.t.	Pan	Q062	2013	-96.8748	28.3360	1249172	12711	62.48	high	high
M.t.	Pan	Q063	2013	-97.3767	28.2973	861252	11275	45.41	high	high
M.t.	Pan	Q064	2013	-97.2548	28.1898	978753	11735	50.63	low	high
M.t.	Pan	Q065	2013	-97.2548	28.1898	1178500	13074	53.36	low	high
M.t.	Pan	Q066	2013	-97.6462	30.2358	732326	10595	41.63	high	high
M.t.	Pan	Q067	2013	-97.6137	30.1978	593381	10597	31.14	high	low
M.t.	Pan	Q068	2013	-97.7813	30.2843	932763	10740	53.79	high	low
M.t.	Pan	Q069	2013	-97.6462	30.2358	839116	11081	47.07	low	high
L.b.	Net-Dry	Q181	2013	-98.0732	30.6226	471548	14857	21.58	NA	NA
L.b.	Net-Dry	Q182	2013	-98.0718	30.6279	335587	12594	16.09	NA	NA
L.b.	Net-Dry	Q183	2013	-98.0764	30.6179	261216	10686	14.06	NA	NA
L.b.	Net-Dry	Q184	2013	-98.0718	30.6279	455343	15172	19.66	NA	NA
L.b.	Net-Dry	Q185	2013	-98.0732	30.6226	384326	13406	18.90	NA	NA
L.b.	Net-Dry	Q186	2013	-98.0718	30.6279	548322	16734	22.99	NA	NA
L.b.	Net-Dry	Q187	2013	-98.0732	30.6226	457762	14636	20.74	NA	NA
L.b.	Net-Dry	Q188	2013	-98.0723	30.6252	234488	8823	12.92	NA	NA
L.b.	Net-Dry	Q198	2013	-98.0732	30.6226	378054	14308	19.34	NA	NA
L.b.	Vane-Gly	Q157	2014	-98.0638	30.0863	1390420	12904	84.33	NA	NA
L.b.	Vane-Gly	Q158	2014	-98.0638	30.0863	1899997	12015	137.23	NA	NA
L.b.	Vane-Gly	Q159	2014	-98.0638	30.0863	1104924	12391	48.32	NA	NA
L.b.	Vane-Gly	Q160	2014	-97.9227	30.4508	804757	12259	56.72	NA	NA
L.b.	Vane-Gly	Q161	2014	-98.0638	30.0863	1040346	12667	74.78	NA	NA
L.b.	Vane-Gly	Q162	2014	-97.9227	30.4508	918827	12242	64.55	NA	NA
L.b.	Vane-Gly	Q163	2014	-97.9227	30.4508	785779	11466	60.69	NA	NA
L.b.	Vane-Gly	Q164	2014	-97.9227	30.4508	709237	11347	55.07	NA	NA
L.b.	Vane-Dry	Q173	2013	-97.9149	30.3055	1397933	22182	54.48	NA	NA
L.b.	Vane-Dry	Q174	2013	-97.9149	30.3055	2096407	22676	41.34	NA	NA
L.b.	Vane-Dry	Q175	2013	-97.9149	30.3055	1883424	23300	37.85	NA	NA
L.b.	Vane-Dry	Q176	2013	-98.0732	30.6226	399824	11622	15.25	NA	NA
L.b.	Vane-Dry	Q177	2013	-98.0732	30.6226	1241133	15685	25.01	NA	NA
L.b.	Vane-Dry	Q178	2013	-97.9149	30.3055	1805004	23989	53.91	NA	NA
L.b.	Vane-Dry	Q179	2013	-98.0732	30.6226	797406	17215	22.24	NA	NA
L.b.	Vane-Dry	Q180	2013	-98.0732	30.6226	3027831	17603	30.23	NA	NA
L.b.	Vane-Dry	Q196	2013	-98.0732	30.6226	698731	18649	28.36	NA	NA
L.b.	Vane-Dry	Q197	2013	-98.0732	30.6226	958822	20931	39.32	NA	NA
L.b.	Pan	Q165	2013	-98.3311	30.4570	1423605	21220	60.81	NA	NA
L.b.	Pan	Q166	2013	-98.3311	30.4570	1120641	19936	50.88	NA	NA
L.b.	Pan	Q167	2013	-98.3311	30.4570	1206758	20644	54.14	NA	NA
L.b.	Pan	Q168	2013	-98.3311	30.4570	1401409	21513	58.73	NA	NA
L.b.	Pan	Q169	2013	-97.9572	30.0662	1135126	20905	48.33	NA	NA
L.b.	Pan	Q170	2013	-97.9572	30.0662	1028688	20415	45.50	NA	NA
L.b.	Pan	Q171	2013	-97.9572	30.0662	494027	16593	23.51	NA	NA
L.b.	Pan	Q172	2013	-97.9572	30.0662	1190399	21264	50.19	NA	NA

Table S2. Mean (± SE) DNA quality and ddRAD assembly quality metrics for DNAzol extracted *B. pensylvanicus*.

Treatment	Nanodrop 260/280	Nanodrop 260/230	DNA Concentration (ng/µL)	Mean Polymorphic Loci	Mean Locus Depth
Net-Dry	1.93± 0.02	0.59± 0.09	9.43± 1.05	3296.4± 157.2	163.9± 43.5
Net-EtOH	2.06± 0.01	0.95± 0.12	8.04± 1.15	5329.7± 663.5	341.8± 49.6
Vane-Dry	1.99± 0.03	0.95± 0.01	9.65± 0.76	4035.8± 181.2	297.5± 20.1

Table S3. Results from General Linear Mixed Models for *B. pensylvanicus* including sample filters a) one sample, and b-d) 40%, 60%, 80% of *B. pensylvanicus* specimens.

		Recovered Loci				Locus Depth				Standardized Depth			
Minimum Sample Filter	Variable	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)
a) One Sample	Vane-Dry (Intercept)	-2.159	0.118	-18.346	<0.001	4.053	0.141	28.690	<0.001	2.259	0.051	44.408	< 0.001
	Net-EtOH	0.789	0.170	4.642	<0.001	0.237	0.165	1.435	0.151	-0.001	0.063	-0.021	0.983
	Net-Dry	-1.055	0.146	-7.216	<0.001	-0.795	0.172	-4.612	<0.001	0.067	0.066	1.007	0.314
	Qiagen Extracted	0.717	0.138	5.178	<0.001	-0.374	0.234	-1.599	0.110	0.173	0.054	3.199	0.001
	DNA concentration	0.167	0.090	1.847	0.065	0.213	0.074	2.888	0.004	-0.037	0.014	-2.688	0.007
	Nanodrop 260/280	0.054	0.116	0.463	0.643	0.246	0.102	2.418	0.016	0.047	0.018	2.659	0.008
	Nanodrop 260/230	-0.572	0.092	-6.240	<0.001	0.013	0.122	0.109	0.913	-0.028	0.023	-1.209	0.227
	Net-EtOH x Qiagen	-0.119	0.257	-0.462	0.644	0.375	0.174	2.156	0.031	-0.139	0.041	-3.397	0.001
	Net-Dry x Qiagen	0.481	0.156	3.086	0.002	0.529	0.210	2.516	0.012	-0.069	0.043	-1.595	0.111
b) 40%	Vane-Dry (Intercept)	1.548	0.146	10.574	<0.001	5.034	0.161	31.267	<0.001	3.231	0.057	56.668	< 0.001
	Net-EtOH	0.398	0.172	2.306	0.021	0.255	0.195	1.306	0.191	0.013	0.068	0.186	0.852
	Net-Dry	-0.405	0.182	-2.227	0.026	-0.794	0.235	-3.384	0.001	0.062	0.072	0.858	0.391
	Qiagen Extracted	0.148	0.250	0.590	0.555	-0.374	0.256	-1.460	0.144	0.203	0.072	2.817	0.005
	DNA concentration	0.202	0.080	2.514	0.012	0.230	0.081	2.831	0.005	-0.045	0.017	-2.568	0.010

		Recovered Loci				Locus Depth				Standardized Depth			
Minimum Sample Filter	Variable	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)
	Nanodrop 260/280	0.351	0.129	2.724	0.006	0.276	0.113	2.442	0.015	0.071	0.025	2.840	0.005
	Nanodrop 260/230	-0.042	0.133	-0.313	0.755	0.009	0.138	0.065	0.948	-0.040	0.029	-1.371	0.170
	Net-EtOH x Qiagen	-0.388	0.233	-1.664	0.096	0.344	0.246	1.398	0.162	-0.177	0.055	-3.227	0.001
	Net-Dry x Qiagen	0.348	0.221	1.575	0.115	0.528	0.262	2.013	0.044	-0.076	0.058	-1.312	0.189
c) 60%	Vane-Dry (Intercept)	2.249	0.156	14.444	<0.001	5.136	0.149	34.510	<0.001	3.331	0.061	54.711	< 0.001
	Net-EtOH	0.243	0.176	1.377	0.169	0.255	0.194	1.320	0.187	0.010	0.071	0.140	0.889
	Net-Dry	-0.310	0.195	-1.592	0.111	-0.801	0.197	-4.060	<0.001	0.054	0.075	0.717	0.473
	Qiagen Extracted	-0.110	0.276	-0.399	0.690	-0.369	0.257	-1.430	0.151	0.216	0.083	2.618	0.009
	DNA concentration	0.186	0.082	2.258	0.024	0.232	0.082	2.820	0.005	-0.047	0.019	-2.453	0.014
	Nanodrop 260/280	0.418	0.131	3.180	0.001	0.286	0.115	2.490	0.013	0.085	0.029	2.910	0.004
	Nanodrop 260/230	0.173	0.139	1.244	0.213	0.007	0.128	0.050	0.957	-0.046	0.033	-1.382	0.167
	Net-EtOH x Qiagen	-0.453	0.237	-1.911	0.056	0.330	0.213	1.550	0.121	-0.194	0.064	-3.026	0.002
	Net-Dry x Qiagen	0.361	0.295	1.225	0.221	0.527	0.230	2.290	0.022	-0.077	0.068	-1.129	0.259
d) 80%	Vane-Dry (Intercept)	2.558	0.147	17.388	<0.001	5.224	0.176	29.757	<0.001	3.419	0.064	53.737	< 0.001
	Net-EtOH	0.157	0.180	0.876	0.381	0.258	0.210	1.228	0.219	0.013	0.075	0.178	0.858
	Net-Dry	-0.316	0.188	-1.680	0.093	-0.798	0.213	-3.750	<0.001	0.053	0.081	0.651	0.515

		Recovered Loci				Locus Depth				Standardized Depth			
Minimum Sample Filter	Variable	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)
	Qiagen Extracted	-0.115	0.257	-0.448	0.654	-0.374	0.308	-1.212	0.225	0.219	0.086	2.548	0.011
	DNA concentration	0.164	0.083	1.979	0.048	0.233	0.087	2.674	0.007	-0.046	0.019	-2.389	0.017
	Nanodrop 260/280	0.433	0.127	3.413	0.001	0.288	0.123	2.331	0.020	0.084	0.030	2.841	0.005
	Nanodrop 260/230	0.201	0.138	1.462	0.144	0.014	0.146	0.093	0.926	-0.043	0.034	-1.254	0.210
	Net-EtOH x Qiagen	-0.511	0.257	-1.988	0.047	0.330	0.251	1.317	0.188	-0.197	0.066	-2.985	0.003
	Net-Dry x Qiagen	0.370	0.283	1.306	0.191	0.525	0.269	1.952	0.051	-0.081	0.070	-1.166	0.244

Table S4. Results from General Linear Mixed Models for *M. tepaneca* including sample filters a) one sample, and b-d) 40%, 60%, 80% of *M. tepaneca* specimens.

		Recovered Loci				Locus Depth				Standardized Depth			
Minimum Sample Filter	Variable	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)
a) One Sample	Vane-Dry (Intercept)	-0.389	0.040	-9.830	<0.001	3.001	0.080	37.630	<0.001	1.572	0.075	20.953	< 0.001
	Vane-Gly	-0.531	0.056	-9.474	<0.001	0.179	0.134	1.340	0.180	0.161	0.125	1.293	0.196
	Net-EtOH	-0.049	0.059	-0.830	0.407	0.899	0.125	7.200	<0.001	0.172	0.120	1.433	0.152
	Net-Dry	0.030	0.058	0.510	0.610	-0.041	0.128	-0.320	0.746	-0.298	0.110	-2.722	0.007
	Pan	-0.206	0.056	-3.688	<0.001	-0.164	0.118	-1.390	0.165	0.102	0.119	0.855	0.393
	DNA concentration	-0.067	0.036	-1.871	0.061	-0.125	0.052	-2.390	0.017	0.015	0.051	0.294	0.769
	NanoDrop 260/280	-0.062	0.032	-1.919	0.055	-0.034	0.046	-0.740	0.457	0.117	0.045	2.590	0.010
	NanoDrop 260/230	0.055	0.037	1.501	0.133	0.030	0.055	0.540	0.589	-0.087	0.053	-1.651	0.099
b) 40%	Vane-Dry (Intercept)	1.691	0.057	29.442	<0.001	3.340	0.091	36.550	<0.001	1.913	0.080	23.929	<0.001
	Vane-Gly	-0.653	0.094	-6.965	<0.001	0.087	0.144	0.600	0.545	0.069	0.123	0.562	0.574
	Net-EtOH	-0.131	0.081	-1.622	0.105	0.831	0.144	5.760	<0.001	0.104	0.123	0.844	0.398
	Net-Dry	0.048	0.088	0.550	0.583	-0.037	0.141	-0.270	0.790	-0.294	0.128	-2.289	0.022
	Pan	-0.146	0.094	-1.561	0.119	-0.180	0.133	-1.360	0.175	0.086	0.120	0.720	0.471
	DNA concentration	-0.053	0.041	-1.300	0.194	-0.129	0.057	-2.280	0.022	0.011	0.051	0.227	0.821
	NanoDrop 260/280	-0.051	0.036	-1.429	0.153	-0.040	0.049	-0.820	0.415	0.111	0.044	2.498	0.013

		Recovered Loci				Locus Depth				Standardized Depth			
Minimum Sample Filter	Variable	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)
	NanoDrop 260/230	0.037	0.043	0.859	0.390	0.025	0.059	0.420	0.673	-0.092	0.053	-1.729	0.084
c) 60%	Vane-Dry (Intercept)	2.395	0.063	38.050	<0.001	3.513	0.079	44.420	<0.001	2.086	0.083	25.214	<0.001
	Vane-Gly	-0.700	0.110	-6.350	<0.001	0.063	0.133	0.470	0.638	0.045	0.139	0.323	0.747
	Net-EtOH	-0.223	0.093	-2.400	0.017	0.803	0.121	6.650	<0.001	0.075	0.127	0.593	0.553
	Net-Dry	0.053	0.103	0.520	0.603	-0.014	0.125	-0.110	0.909	-0.271	0.132	-2.060	0.039
	Pan	-0.137	0.095	-1.450	0.148	-0.168	0.118	-1.430	0.154	0.098	0.118	0.831	0.406
	DNA concentration	-0.043	0.044	-0.980	0.326	-0.124	0.053	-2.350	0.019	0.018	0.051	0.346	0.730
	NanoDrop 260/280	-0.041	0.038	-1.070	0.282	-0.044	0.047	-0.940	0.349	0.107	0.044	2.449	0.014
	NanoDrop 260/230	0.023	0.046	0.490	0.621	0.030	0.055	0.540	0.588	-0.088	0.054	-1.620	0.105
d) 80%	Vane-Dry (Intercept)	3.222	0.083	38.830	<0.001	3.740	0.086	43.660	<0.001	2.312	0.088	26.256	<0.001
	Vane-Gly	-0.663	0.134	-4.960	<0.001	0.075	0.134	0.560	0.573	0.058	0.136	0.422	0.673
	Net-EtOH	-0.422	0.128	-3.300	0.001	0.791	0.129	6.150	<0.001	0.064	0.133	0.479	0.632
	Net-Dry	0.038	0.130	0.290	0.769	0.034	0.131	0.260	0.793	-0.222	0.134	-1.654	0.098
	Pan	-0.072	0.121	-0.600	0.550	-0.146	0.122	-1.200	0.230	0.120	0.124	0.971	0.331
	DNA concentration	0.018	0.052	0.350	0.727	-0.111	0.049	-2.270	0.023	0.031	0.050	0.619	0.536
	NanoDrop 260/280	-0.019	0.045	-0.420	0.676	-0.048	0.041	-1.180	0.240	0.102	0.042	2.409	0.016

		Recovered Loci				Locus Depth				Standardized Depth			
Minimum Sample Filter	Variable	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)
	NanoDrop 260/230	0.018	0.055	0.330	0.741	0.051	0.051	1.000	0.317	-0.066	0.052	-1.256	0.209

Table S5. Results from General Linear Mixed Models for *L. bardum* including sample filters a) one sample, and b-d) 40%, 60%, 80% of *L. bardum* specimens.

		Recovered Loci				Locus Depth				Standardized Depth			
Minimum Sample Filter	Variable	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)
a) One Sample	Vane-Dry (Intercept)	-0.218	0.039	-5.630	<0.001	2.945	0.115	25.509	<0.001	1.211	0.058	20.723	<0.001
	Vane-Gly	-1.665	0.038	-43.620	<0.001	0.211	0.186	1.138	0.255	0.668	0.093	7.173	<0.001
	Net-Dry	-0.945	0.039	-24.030	<0.001	-0.590	0.187	-3.152	0.002	0.835	0.105	7.926	<0.001
	Pan	-0.244	0.043	-5.710	<0.001	0.057	0.216	0.265	0.791	0.289	0.106	2.729	0.006
	DNA Concentration	0.268	0.033	8.000	<0.001	0.083	0.060	1.377	0.169	-0.176	0.035	-5.061	<0.001
	NanoDrop 260/280	-0.063	0.036	-1.740	0.081	-0.038	0.092	-0.413	0.679	-0.170	0.051	-3.311	0.001
	NanoDrop 260/230	0.166	0.038	4.320	<0.001	0.171	0.137	1.247	0.212	0.367	0.072	5.118	<0.001
b) 40%	Vane-Dry (Intercept)	2.102	0.066	31.980	<0.001	3.375	0.080	42.320	<0.001	1.639	0.059	27.661	<0.001
	Vane-Gly	-2.089	0.114	-18.290	<0.001	0.252	0.125	2.010	0.045	0.698	0.097	7.179	<0.001
	Net-Dry	-0.729	0.100	-7.270	<0.001	-0.566	0.126	-4.470	<0.001	0.863	0.105	8.232	<0.001
	Pan	-0.049	0.104	-0.470	0.636	0.075	0.146	0.510	0.607	0.298	0.112	2.650	0.008
	DNA Concentration	0.320	0.068	4.690	<0.001	0.084	0.058	1.450	0.148	-0.184	0.038	-4.818	<0.001
	260/280	-0.075	0.067	-1.130	0.260	-0.050	0.073	-0.690	0.492	-0.179	0.052	-3.421	<0.001
	260/230	0.278	0.085	3.280	0.001	0.193	0.100	1.930	0.053	0.394	0.079	4.974	<0.001
c) 60%	Vane-Dry (Intercept)	2.783	0.077	36.320	<0.001	3.566	0.092	38.610	<0.001	1.828	0.070	26.085	<0.001

		Recovered Loci				Locus Depth				Standardized Depth			
Minimum Sample Filter	Variable	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)	Estimate	SE	z value	Pr(>|z|)
	Vane-Gly	-2.089	0.117	-17.900	<0.001	0.257	0.149	1.730	0.084	0.703	0.111	6.303	<0.001
	Net-Dry	-0.685	0.148	-4.620	<0.001	-0.556	0.165	-3.370	0.001	0.874	0.124	7.027	<0.001
	Pan	-0.012	0.139	-0.090	0.931	0.067	0.160	0.420	0.674	0.290	0.125	2.322	0.020
	DNA Concentration	0.343	0.078	4.370	<0.001	0.090	0.064	1.400	0.163	-0.188	0.042	-4.491	<0.001
	NanoDrop 260/280	-0.052	0.083	-0.630	0.531	-0.046	0.082	-0.560	0.576	-0.173	0.056	-3.087	0.002
	NanoDrop 260/230	0.218	0.107	2.030	0.042	0.199	0.121	1.640	0.101	0.402	0.083	4.823	<0.001
d) 80%	Vane-Dry (Intercept)	3.420	0.127	26.857	<0.001	3.761	0.109	34.540	<0.001	2.022	0.074	27.187	<0.001
	Vane-Gly	-1.631	0.211	-7.731	<0.001	0.300	0.183	1.640	0.102	0.745	0.120	6.227	<0.001
	Net-Dry	-0.641	0.189	-3.400	0.001	-0.541	0.181	-3.000	0.003	0.886	0.130	6.829	<0.001
	Pan	0.169	0.223	0.758	0.448	0.046	0.211	0.220	0.826	0.270	0.140	1.925	0.054
	DNA Concentration	0.391	0.097	4.021	<0.001	0.101	0.070	1.430	0.151	-0.187	0.045	-4.115	<0.001
	NanoDrop 260/280	-0.005	0.102	-0.046	0.963	-0.028	0.088	-0.310	0.754	-0.151	0.059	-2.586	0.010
	NanoDrop 260/230	0.108	0.144	0.754	0.451	0.189	0.144	1.310	0.190	0.389	0.093	4.201	<0.001

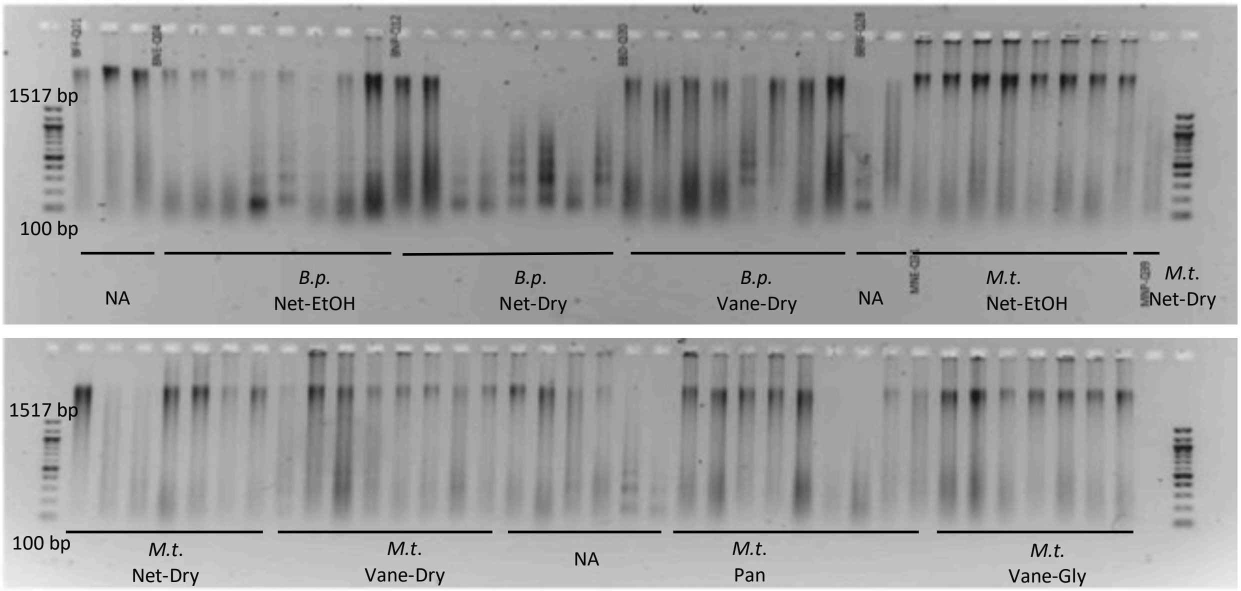

Fig S1. Visualization of DNA extractions on a 2% agarose gel showing levels of DNA fragmentation and weight of select samples of Qiagen extracted *B. pensylvanicus* (*B.p.*) and *M. tepaneca* (*M.t.*) by treatment. Samples were orginially given two qualitative DNA quality scores: 1) fragmentation (high = no visible bands or bands ~100-200 bp), and 2) weight (high= dark band above ~1500 bp). Although most samples had “high” fragmentation and “low” weight or vice versa, some samples had bands of both high and low molecular weights, and so would be categorized as both “high” fragmentation and “high” weight (ex. see first two samples in *M.t.* Pan). Ultimately, neither of these measures were significant in initial models, and so were dropped from further analysis. NA indicates samples that were not included in the present study.

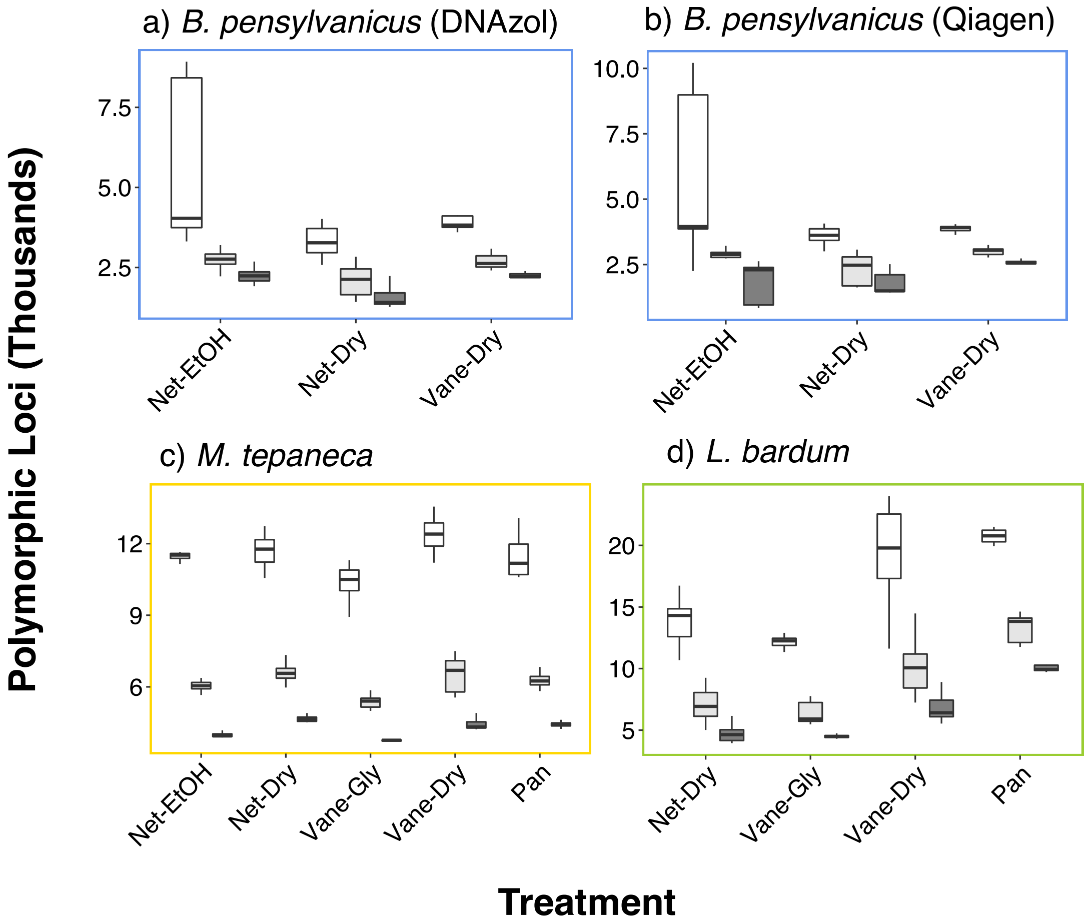

Fig S2. Box plots showing numbers of polymorphic loci retained between specimens ("sequence similarity") when grouping by different numbers of individuals. For each species and treatment, groupings are shown from left to right, showing median number of loci and quantile spread when binned by: 1) one individual (left, white), 2) three individuals (middle, light gray), and 3) six individuals (right, dark gray). Sample treatments are coded as in Table 1, plot border color corresponds to species as in Figure 1.

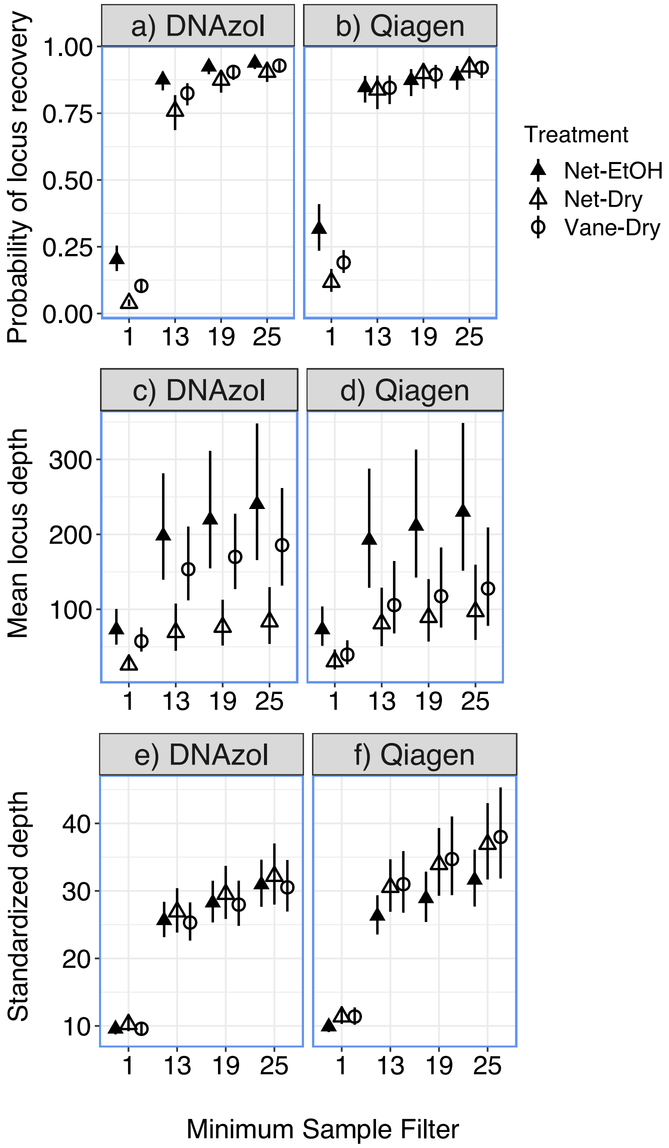

Fig S3. Treatment effects on *B. pensylvanicus* ddRAD results, split by extraction method. a,b) Number of loci, measured as scaled probability of a locus occurring in another random sample, c,d) average depth per locus, and e,f) standardized locus depth (average depth per locus per one million reads). All panels show results filtered by a minimum sample of one specimen, and 40%, 60% and 80% of specimens respectively. Bars represent 95% confidence intervals. Sample method-storage treatments are represented by different markers and coded as in Table 1: Vane-Dry (blue vane-dry, pinned; unfilled circles), Net-EtOH (netted, 100% Ethanol; filled triangles) and Net-Dry (netted, pinned; unfilled triangles).

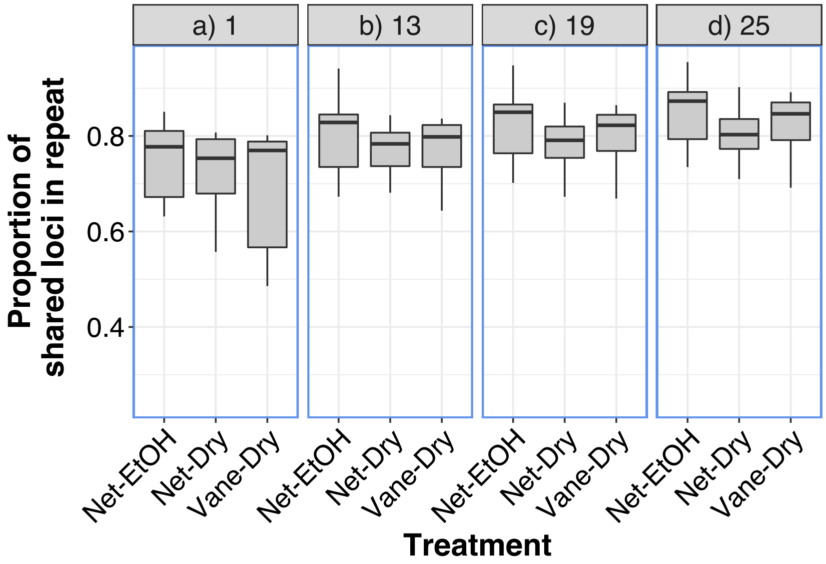

Fig S4. Boxplots showing proportion of shared loci between replicate extracted bumblebees (each bumblebee specimen was split in half and extracted using both Qiagen and DNAzol reagants respectively). Plots show proportion of shared loci between extraction types when data is filtered by a minimum of a) 1 specimen, b) 40%, c) 60%, and d) 80% of specimens. There was no significant difference in recovery of matching loci between individuals among the treatments at any sample filter level.

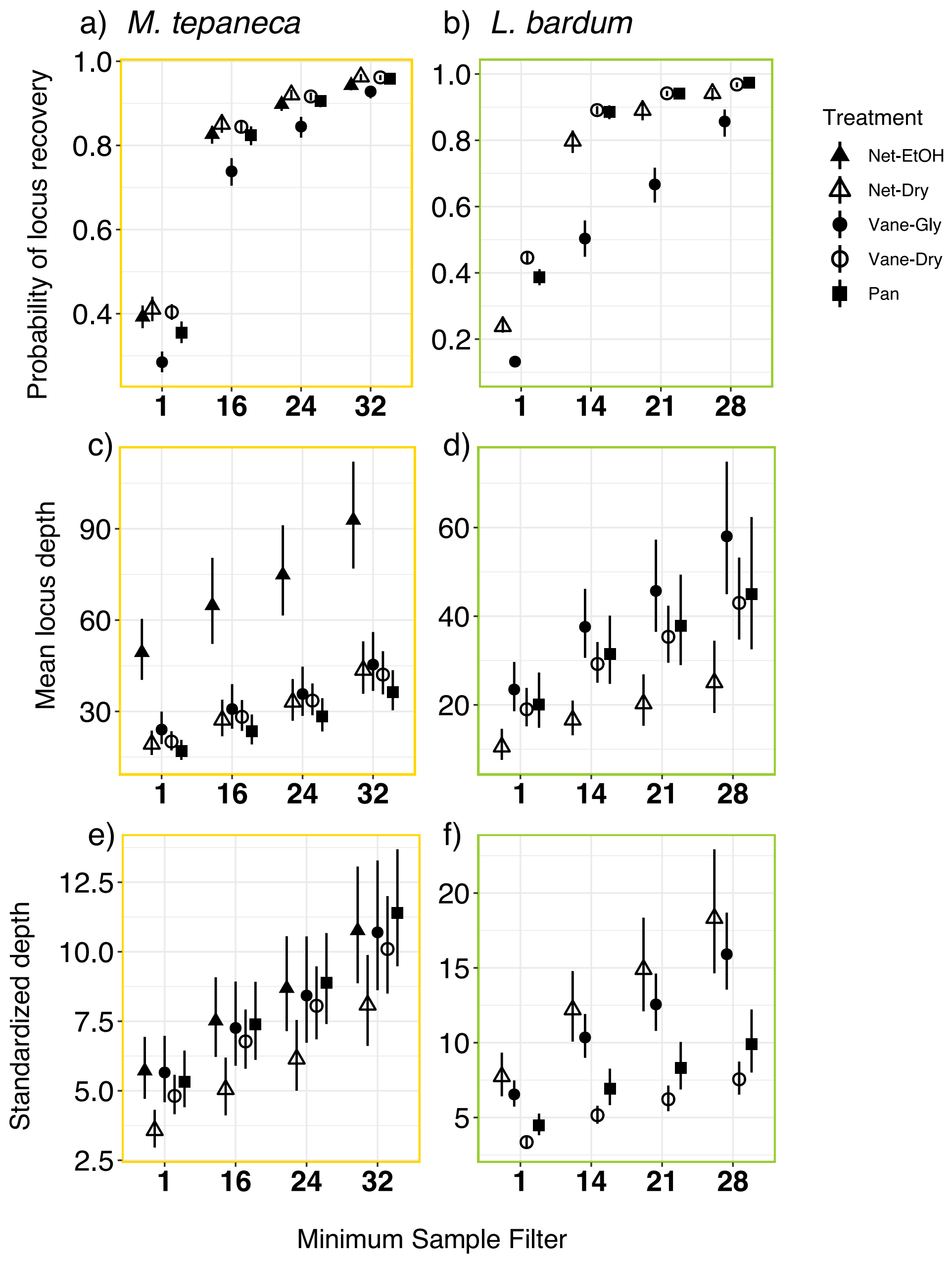

Fig S5. Treatment effects on *M. tepaneca* (a, c, e) and *L. bardum* (b, d, f) ddRAD results. a, b) Number of shared loci, measured as scaled probability of a locus occurring in another random sample. c,d) average depth per locus, and e,f) standardized locus depth (average depth per locus per one million reads). All panels show results filtered by a minimum sample of one specimen, and 40%, 60% and 80% of specimens respectively. Bars represent 95% confidence intervals. Sample method-storage treatments are represented by different markers and coded as in Table 1: Net-EtOH (netted, 100% Ethanol; filled triangles), Net-Dry (netted, pinned; unfilled triangles), Vane-Dry (blue vane-dry, pinned; unfilled circles), Vane-Gly (blue vane-glycol, pinned; solid circles), and Pan (pan trap, pinned; solid squares). Panel color corresponds to species as in Figure 1.