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Appendix 1

Genetic analysis

Genomic DNA extraction and microsatellite amplification

Genomic DNA was extracted from live samples of the initial nine candidate clones to determine genotypic differences prior to mesocosm inoculation, and from bulk samples collected following the conclusion of the experiment. Bulk samples were obtained from 2 replicates out of 6 for the single colonist/low diversity mesocosms, 2 replicates out of 5 for the many colonists/low diversity mesocosms, and from all 5 replicates in the many colonists/high diversity mesocosms. Bulk samples consisted of 20 total individuals for the single colonist/low diversity and many colonists/low diversity treatments, and 50 total individuals for the many colonists/high diversity treatment. All replicates and a greater number of individuals were sampled from the many colonists/high diversity mesocosms due to the potentially higher variation in genotypic composition between replicates. Extractions followed the cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1987). DNA extracts were analyzed at 12 previously mapped microsatellite loci (Cristescu et al. 2006; Appendix 3: Table A3). The 12.5 µl polymerase chain reaction (PCR) and thermocycling regimes were conducted independently for each locus and followed that of Cristescu et al. (2006). Amplified product was diluted 20-fold and combined into groups of four according to their fluorescent labels (FAM, NED, PET, VIC). Two microliters of the diluted PCR product was mixed with 8.35 µl of HiDi formamide (Life Technologies) and 0.15 µl of GeneScan-500 LIZ size standard (Applied Biosystems, Foster City, CA, USA). Two independent PCRs for each locus on all

extracted samples were performed to confirm the genotypes of clones and repeatability of alleles identified in the mesocosms. Electrophoresis was performed using an ABI-3730XL Analyzer and chromatographs were evaluated manually using GeneMapper Software ver. 3.0 (Applied Biosystems).

Genetic verification of experimental clones species

A genetic distance-based principal coordinate analysis (PCoA) based on six microsatellite loci was conducted in GenAlEx 6.5 (Peakall and Smouse 2012) to verify the species identity and phylogenetic status of the experimental clones. We included 24 cyclically parthenogenetic (CP) *Daphnia pulex*, 24 obligately parthenogenetic (OP) *Daphnia pulex* and 54 *Daphnia pulicaria* reference individuals (including clones used in Cristescu et al. 2012), whose mode of reproduction was confirmed previously in laboratory conducted sexuality tests following the procedure in Innes et al. (1986). The experimental clones clustered with *Daphnia pulicaria* and OP *Daphnia pulex* reference individuals (Appendix 1: Fig. A1) reflecting the mixed genetic composition of many habitats and the close evolutionary relationship of these lineages. Thus, we use the term *D. pulex* sensu lato across the manuscript.

Microsatellite allele scoring procedure of mesocosm samples

Microsatellite allele bins were set up in GeneMapper based on the fragment sizes of the individually amplified clones. These allele bins were grouped into mesocosm binsets according to the size of the expected alleles in each mesocosm. Through this method we were able to easily identify novel and unexpected alleles, and rule out the possibility of experimental contamination by noting the presence of alleles falling outside the mesocosm binset. Alleles were called manually and blind to the level of genetic diversity present in mesocosm samples. Alleles were considered present regardless of fluorescent peak intensity relative to the LIZ size standard (on average 2600) since a preliminary test indicates that this approach is likely to correctly identify alleles present at low

frequency. Eight of the 9 inoculating clones used in the experiment possessed one or multiple unique alleles that were used to differentiate them from their mesocosm co-habitants. Genotype F had no unique alleles, but had a distinct genotype of shared alleles.

The identity of the persisting clones was estimated by noting the presence and absence of unique alleles. We implemented a scoring procedure based on whether a unique allele was observed in the two PCR replicates of subsample extracts. A genotype with a unique allele detected within a fragment size bin was determined to be present in the replicate (1), while those with alleles showing no sign of amplification despite being expected in a particular bin (i.e., in mixture treatment) were determined to be absent (0)(Appendix 1: Table A1).

References

Cristescu, M. E. A. et al. 2006. A microsatellite-based genetic linkage map of the waterflea, *Daphnia pulex*: on the prospect of crustacean genomics. - Genomics 88: 415–430.

Cristescu, M. E. et al. 2012. Speciation with gene flow and the genetics of habitat transitions. - Mol. Ecol. 21: 1411–1422.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. - Phytochem. Bull. 19: 11–15.

Innes, D. J. et al. 1986. Genotypic diversity and variation in mode of reproduction among populations in the daphnia pulex group. – Heredity 57: 345–355.

Peakall, R. and Smouse, P. E. 2012. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. – Bioinformatics 28: 2537–2539.

Table A1. Presence/absence (1/0) of unique alleles for each *Daphnia pulex* genotype determined across 12 microsatellite loci (see Appendix S3: Table S3) in each high diversity mesocosm (replicates 1-5 with 50 *D. pulex* analysed per replicate), along with the proportion of all replicates in which each genotype was determined to be present.

Genotype	Unique alleles	Presence/absence of unique alleles (1/0) in each 'many colonists/high diversity' replicate (#1-5)	Proportion present

		1	2	3	4	5	
C	1	0	0	0	1	0	0.2
D	1	1	1	1	1	0	0.8
E	1	1	1	1	1	1	1.0
F	0	-	-	-	-	-	-
G	1	1	1	1	1	1	1.0
L	1	1	0	1	0	1	0.6
M	9	1	1	1	1	1	1.0
O	1	0	1	1	1	1	0.8
R	2	0	0	1	1	1	0.6

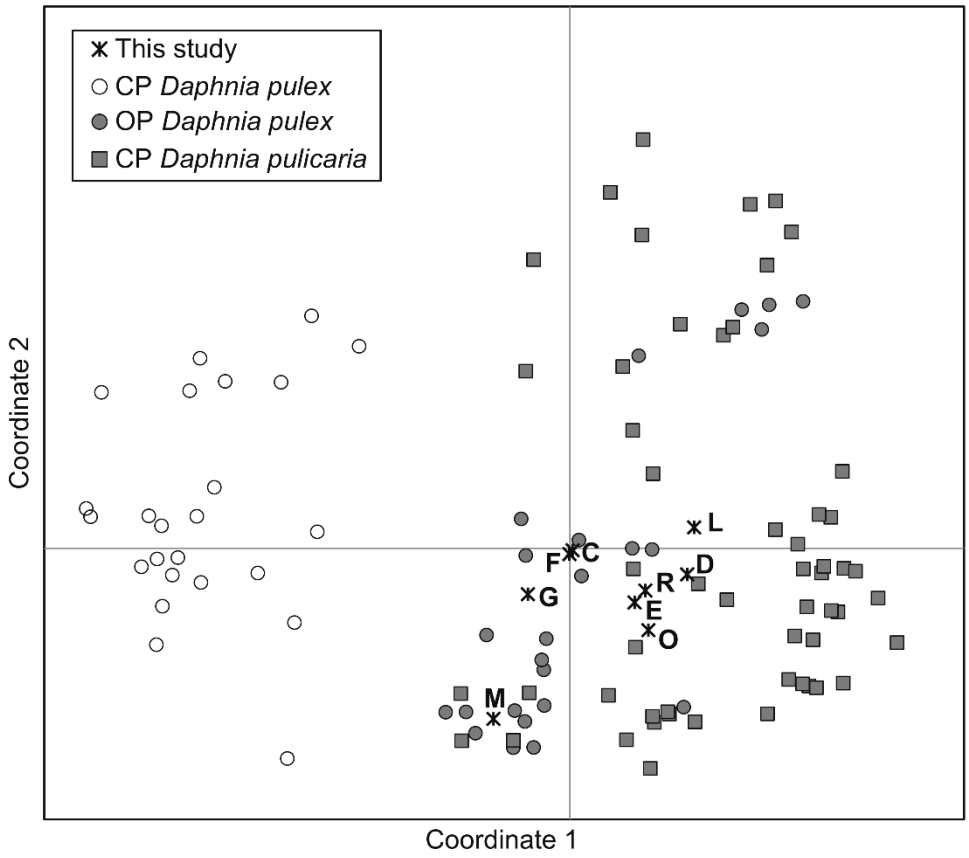

Figure A1. Genetic distance-based principal coordinate analysis (PCoA) of 24 cyclically parthenogenetic (CP) *Daphnia pulex* (empty circles), 24 obligately parthenogenetic (OP) *Daphnia pulex* (filled circles), 54 *Daphnia pulicaria* reference individuals (filled squares), and the 9 experimental genotypes used in this study (crosses).

Appendix 2

Pilot study and life-history trial methods

The pilot experiment occurred from 14-May-2016 until 30-May-2016, and was used to identify *D. pulex* clone lines that were unique in both genotype and phenotype. Twenty-two candidate adult female *D. pulex* were collected from lakes from the Muskoka region of central Ontario, Canada and 36 from lakes in the region surrounding QUBS in southern Ontario, Canada, to provide a total of 54 candidate individuals. For the Muskoka genotypes, candidate *D. pulex* individuals (collected from lakes in 2015) were obtained from cultures maintained at FLAMES (Field Laboratory for the Assessment of Multiple Ecological Stressors) in Dorset, ON, Canada on 6-Apr-2016. Candidate *D. pulex* from the QUBS region were initially collected on 13-Apr-2016 from the pelagic region of Elbow, Lindsay and Round lakes (Appendix 3: Table A1) using vertical tows with an 80 µm, 15 cm diameter net. Individual *D. pulex* were isolated into separate 120 ml glass containers filled with 100 ml of Elbow Lake water (filtered through a G4 glass fibre filter with 1.2 µm pore size; Fisher Scientific, Waltham, Massachusetts, USA). Opinicon Lake water was not used as our media because we did not want to expose the genotypes to their intended experimental environment prior to introduction. All individuals were kept in environmental chambers at Queen's University, ON, Canada and acclimated to a 16:8 h day-night photoperiod with 20°C daytime and 15°C nighttime temperatures. These environmental conditions were chosen to be similar to conditions their offspring would eventually encounter at the time of experimental introduction (mid-summer) at our intended field site (SE Arnott, unpublished data). For each *D. pulex* individual, upon production of their first brood, five female neonates were collected and isolated to create the first generation, with all mothers discarded once the first generation had subsequently produced their own initial brood. This process was repeated to create the subsequent second and third generations. All isolated *D. pulex* had their water changed weekly, with 50% retained from the previous week and 50% replaced with fresh, filtered Elbow lake water. They were also fed an amount of *Chlamydomonas reinhardtii* equivalent to 30 µg of carbon per individual per day. This amount produced high lipid stores in a previous experiment that used a member of the *D. pulex* species complex (*D. pulicaria*, Sinclair and Arnott 2017) and so ensured food was not limited.

On 14-May-2016, five newly released female neonates from the 3rd generation for each candidate clone line (54 clone lines with five replicates each totaling 270 individuals) were individually isolated in 120 ml glass containers filled with 100 ml of filtered Elbow lake water, which was replaced every day during life-history trials and checked daily for 14 days. Data were collected on body size (measured from the top to the base of the carapace) and tail size (measured from the base of the carapace to the tip of the tail) of individuals upon initial release, upon deposition of eggs into the brood pouch, upon release of the first brood from the pouch, and upon

any subsequent eggs deposited and broods released over the 14-day duration of the trails. Additionally, the total number of neonates released over the course of the 14-day trials was recorded, along with the days on which eggs were deposited into the brood pouch, days on which neonates were released, and adult mortality.

Only some of the traits calculated from these initial trials, specifically age of first reproduction (in days), body size at first reproduction, total number of offspring released over the course of the trials, and survivorship were informative of phenotypic differences between candidate clones. Using these morphological and reproductive traits, possible clone lines for use in the experiment were selected from the original 54 for genomic analysis. These clones were selected to be from different source lakes, and selection was also based on which genotypes represented full coverage of measured life-history trait space (a similar approach to that employed by Vellend et al. 2010). This was determined by analyzing all average life-history trait values (scaled to a mean of 0 and standard deviation of 1) for each genotype in a principal components analysis (PCA). A genotype was selected as a candidate if it occupied a unique location in multivariate PCA space (i.e. no other genotypes were nearby in the PCA), if it was located the furthest along a particular axis (genotypes with the highest/lowest trait values), or if it was the most central in PCA space (a genotype with average values in multiple traits). Of the original 54 clones, we selected eleven that were from unique source lakes and that covered the full range of trait space. Live individuals from each of these eleven candidate clone lines were analyzed at McGill University, QC, Canada (see Appendix 1 for genomic methods), with nine of these determined to be genetically unique based on twelve microsatellite markers (Appendix 3: Table A3). These nine unique genotypes were the clones used in our experiment.

References

Sinclair, J. S. and Arnott, S. E. 2017. Relative importance of colonist quantity, quality, and arrival frequency to the extinction of two zooplankton species. - Oecologia 184: 441–452.

Vellend, M. et al. 2010. Effects of genotype identity and diversity on the invasiveness and invasibility of plant populations. - Oecologia 162: 371–381.

Table A1. Origin and trait values from the 21-day life-history trials for each *Daphnia pulex* genotype involved in the experiment. Trait values were determined based on average body size (mm) at which each genotype released their first brood, average age class (in days) at which each genotype released their first brood, the average number of offspring produced per day, and survivorship per age class.

Genotype	Source lake	Origin region	Size at first reproduction (mm)	Age at first reproduction (days)	Net reproductive rate (R_0)	Intrinsic rate of increase (r)
C	Crozier	Muskoka	1.99	12.4	8.4	0.15
D	Dyson	Muskoka	1.99	11.8	18.0	0.20
E	Elbow	QUBS	1.82	10.3	12.1	0.18
F	Fifteen Mile	Muskoka	1.90	11.9	11.7	0.16
G	Grandview	Muskoka	2.26	11.2	31.7	0.23
L	Lindsay	QUBS	1.80	10.0	15.8	0.21
M	McKay	Muskoka	2.11	9.2	40.6	0.28
O	Round	QUBS	1.97	9.9	25.0	0.24
R	Ridout	Muskoka	1.97	10.8	20.4	0.21

Appendix 3

Tables for methods and statistical analyses

Table A1. Information for all lakes involved in the experiment. Water chemistry data for all Muskoka lakes was collected in summer 2011 by the Canadian Aquatic Invasive Species Network (CAISN). Water chemistry for all Queen's University Biology Station (QUBS) lakes was collected in summer 2016 by S.E. Arnott. Opinicon Lake was the source of all mesocosm water and Buck Lake was the source of the native zooplankton community. The 'Region' column indicates the broad geographic region in which each lake is located. 'M' represents the Muskoka region, located in central Ontario, Canada, and 'Q' represents the region surrounding the QUBS in southern Ontario, Canada. Water chemistry variables are average total phosphorus (μg l^{-1}, Av. TP), calcium (mg l^{-1}, Ca), conductivity (μS cm^{-1}, Cond.), dissolved organic carbon (mg l^{-1}, DOC), pH and sodium (mg l^{-1}, Na).

Lake	Region	Latitude Longitude	Area (ha)	Av. TP	Ca	Cond.	DOC	pH	Na
Buck	Q	44.534001 -76.438326	784.6	7.6	23.3	187.0	4.6	7.6	5.2
Crozier	M	45.334557 -78.849833	56.8	3.3	2.8	31.0	3.1	6.9	1.0
Dyson	M	45.216222 -79.652563	92.4	3.7	3.1	31.0	2.7	7.1	0.9
Elbow	Q	44.475241 -76.428946	27.8	10.2	10.5	77.5	7.0	7.3	1.0
Fifteen Mile	M	45.349633 -78.965305	81.1	4.6	1.9	19.6	3.4	6.9	0.6
Grandview	M	45.202002 -79.051840	64.0	6.9	4.5	75.8	2.7	6.4	6.0
Lindsay	Q	44.536869 -76.390861	13.2	10.7	37.1	241.0	6.6	6.4	1.0
McKay	M	45.058557 -79.172913	132.0	8.5	2.6	22.2	6.1	6.3	1.0
Opinicon	Q	44.558982 -76.328055	792.2	14.9	22.8	187.0	5.8	8.1	4.4

Ridout	M	45.176259 -78.978642	49.9	3.9	1.7	17.6	4.3	6.7	0.7
Round	Q	44.537855 -76.400036	15.0	5.1	25.2	177.0	4.5	8.1	1.5

Table A2. Results of linear regressions for all effect size models. Response variables were the log-transformed continuous values for the effect sizes of increasing quantity or genetic diversity, while predictor variables were the continuous values for genotype intrinsic rates of increase (*r*), average body size at first reproduction (mm), and habitat similarity (Euclidean distance).

Metric	n	t	df	p	R^2
Effect sizes of quantity and *r*	9	1.03	1,7	0.34	0.13
Effect sizes of quantity and body size	9	2.39	1,7	0.048	0.45
Effect sizes of quantity and similarity	9	0.042	1,7	0.97	0.00025
Effect sizes of quantity and origin	9	0.40	1,7	0.70	0.11
Effect sizes of genetic diversity and *r*	9	2.69	1,7	0.031	0.51
Effect sizes of genetic diversity and body size	9	0.57	1,7	0.58	0.044
Effect sizes of genetic diversity and similarity	9	0.82	1,7	0.44	0.088
Effect sizes of genetic diversity and origin	9	0.55	1,7	0.59	0.042

Table A3. The twelve microsatellite loci used to genotype individual clones from the low diversity and high diversity mesocosm treatments. The linkage group of each primer is documented in Cristescu et al. (2006) and allele fragment size ranges are based on sizes detected in this study.

Primer name	Linkage group	Allele size range
D005	X	302-304
D006	XI	250-270
D009	I	362-363
D010	III	190-198
D012	VI	384-387
D078	III	167-191
D087	V	296-305
D088	IX	115-125
D091	I	262-278
D105	IV	141-151
D145	IX	257-286
D148	I	220-227

Cristescu, M. E. A. et al. 2006. A microsatellite-based genetic linkage map of the waterflea, *Daphnia pulex*: on the prospect of crustacean genomics. - Genomics 88: 415–430.

Appendix 4

Buck Lake zooplankton species composition (individuals l^{-1}) from 2013–2016. Composition was assessed using samples from vertical net tows (80 μm) taken from 2 m above the bottom of the lake to the surface in the pelagic region. Species are ordered alphabetically from left to right. Columns 'Year', 'Month' and 'Day' indicate the date on which samples were collected. Note two species of *Bosmina* are combined into a single designation (*Bosmina freyi/liederi*) due to difficulty in morphologically distinguishing between these species.

			Species												
Year	**Month**	**Day**	*Bosmina freyi/liederi*	*Ceriodaphnia lacustris*	*Daphnia dubia*	*Daphnia mendotae*	*Daphnia retrocurva*	*Diacyclops thomasi*	*Epischura lacustris*	*Leptodiaptomus minutus*	*Limnocalanus macrurus*	*Mesocyclops edax*	*Onychodiaptomus birgei*	*Sida crystallina*	*Skistodiaptomus oregonensis*
2013	July	30	0.0090	0.0	0.12	0.076	0.022	0.72	0.022	0.19	0.14	0.50	0.0	0.022	0.095
2014	July	23	0.16	0.0	0.34	0.0	0.0	1.0	0.058	0.058	0.65	0.058	0.0	0.087	0.058
2015	Aug	3	0.40	0.83	0.94	0.31	0.031	2.7	0.031	0.24	2.7	0.031	0.0	0.031	0.24
2016	July	23	0.13	0.0	0.23	0.17	0.0	2.1	0.013	0.37	0.29	0.19	0.013	0.0	0.0

Appendix 5

Effect sizes by region of origin

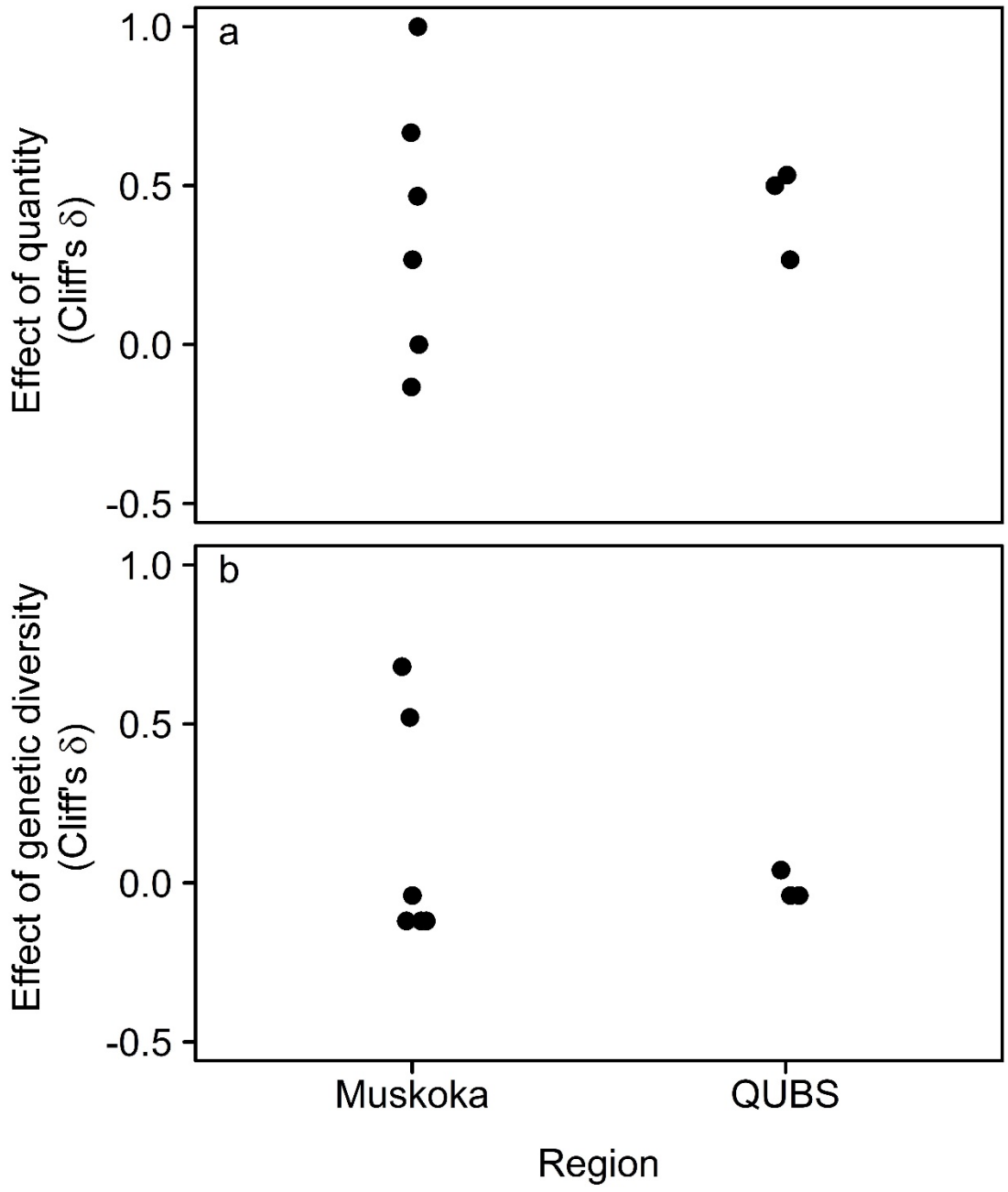

Figure A1. Effect sizes (Cliff's δ) of the (a) quantity and (b) genetic diversity treatments for genotypes from the Muskoka (n = 6) and QUBS (n = 3) regions. Note that points have been jittered on the x-axis to make them easier to distinguish.