

Supporting information Table S1-S5

Invasions of an obligate asexual daphnid species support the nearly neutral theory

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Table S1. Information on *Daphnia pulex* specimens analyzed in the present study.

| Species | Lineage | Genotype | Lake | Area | Nation | Latitude (°N) | Longitude (°E) | DDBJ Accession number | |
|---------------------|---------|----------|-----------------|-----------|--------|---------------|----------------|-----------------------|----------------------------|
| | | | | | | | | Whole nuclear genome | Whole mitochondrial genome |
| <i>D. pulex</i> | JPN1 | AR01 | Arigatani Ike | Shizuoka | Japan | 34.691 | 138.126 | SAMD00322344 | LC632382 |
| <i>D. pulex</i> | JPN1 | DA04 | Daizahoshi Ike | Nagano | Japan | 36.706 | 138.145 | SAMD00322345 | LC632383 |
| <i>D. pulex</i> | JPN1 | DA05 | Daizahoshi Ike | Nagano | Japan | 36.706 | 138.145 | SAMD00322346 | LC632384 |
| <i>D. pulex</i> | JPN1 | FU01 | Furuichi Oike | Tottori | Japan | 35.391 | 133.339 | SAMD00322347 | LC632385 |
| <i>D. pulex</i> | JPN1 | HO03 | Hataya Onuma | Yamagata | Japan | 38.245 | 140.204 | SAMD00322348 | LC632386 |
| <i>D. pulex</i> | JPN1 | KK01 | Kokenuma | Yamagata | Japan | 38.231 | 140.195 | SAMD00322349 | LC632387 |
| <i>D. pulex</i> | JPN1 | OS02 | Osawa Tameike | Miyagi | Japan | 38.439 | 140.919 | SAMD00322350 | LC632388 |
| <i>D. pulex</i> | JPN2 | AR05 | Arigatani Ike | Shizuoka | Japan | 34.691 | 138.126 | SAMD00322351 | LC632389 |
| <i>D. pulex</i> | JPN2 | HO01 | Hataya Onuma | Yamagata | Japan | 38.245 | 140.204 | SAMD00322352 | LC632390 |
| <i>D. pulex</i> | JPN2 | PL2 | Fukami Ike | Nagano | Japan | 35.326 | 137.821 | SAMD00322353 | LC632391 |
| <i>D. pulex</i> | JPN2 | PL4 | Fukami Ike | Nagano | Japan | 35.326 | 137.821 | SAMD00322354 | LC632392 |
| <i>D. pulex</i> | JPN2 | PL7 | Fukami Ike | Nagano | Japan | 35.326 | 137.821 | SAMD00322355 | LC632393 |
| <i>D. pulex</i> | JPN3 | AWA | Awaji Nariai-ji | Hyogo | Japan | 34.283 | 134.809 | SAMD00322356 | LC632394 |
| <i>D. pulex</i> | JPN4 | SUM | Sumiyoshi Ike | Kagoshima | Japan | 31.772 | 130.592 | SAMD00322357 | LC632395 |
| <i>D. pulicaria</i> | | PUC01 | Lake Biwa | Shiga | Japan | 35.191 | 135.994 | SAMD00322358 | - |
| <i>D. pulicaria</i> | | E5 | a small lake | NY | USA | | | SAMD00322359 | - |
| <i>D. pulex</i> | | NoE14 | a small lake | NY | USA | | | SAMD00322360 | - |
| <i>D. pulex</i> | | LL05 | a small lake | Manitoba | Canada | | | SAMD00322361 | - |

Table S2. Substitution rates and estimated divergence times for each lineage

| Lineage | Reference | Avg. subst. rate | Div.time (3gen/yr) | Div.time (5en/yr) | Div.time (10gen/yr) |
|---------|-----------|---------------------|-----------------------|----------------------|------------------------|
| JPN1 | TCO | 1.07E-05 | 848 | 509 | 254 |
| | PA42 | 1.82E-05 | 499 | 299 | 150 |
| JPN2 | TCO | 9.00E-06 | 621 | 373 | 186 |
| | PA42 | 1.34E-05 | 416 | 250 | 125 |

Table S3. The numbers of nonsynonymous and synonymous substitutions and that in non-coding regions estimated by the pairwise comparisons between genotypes within JPN1 and JPN2 lineages, and results of t-test examining the difference between these lineages

| Lineage | Pair | Nonsynonymous | Synonymous | Non-coding |
|------------------------------------------------------------------------|--------------|---------------------|---------------------|--------------------|
| | | (ΔN_{sy}) | (ΔS_{yn}) | ($\Delta non-C$) |
| JPN1 | AR01 vs DA04 | 292 | 569 | 4275 |
| | AR01 vs DA05 | 309 | 593 | 4757 |
| | AR01 vs FU01 | 305 | 574 | 4900 |
| | AR01 vs HO03 | 283 | 541 | 4168 |
| | AR01 vs KK01 | 297 | 604 | 4403 |
| | AR01 vs OS02 | 280 | 544 | 4153 |
| | DA04 vs DA05 | 295 | 514 | 4016 |
| | DA04 vs FU01 | 289 | 491 | 3901 |
| | DA04 vs HO03 | 289 | 492 | 4115 |
| | DA04 vs KK01 | 293 | 561 | 4176 |
| | DA04 vs OS02 | 276 | 533 | 4062 |
| | DA05 vs FU01 | 240 | 413 | 3253 |
| | DA05 vs HO03 | 276 | 536 | 4305 |
| | DA05 vs KK01 | 294 | 535 | 4144 |
| | DA05 vs OS02 | 287 | 557 | 4358 |
| | FU01 vs HO03 | 258 | 507 | 4338 |
| | FU01 vs KK01 | 260 | 510 | 4145 |
| | FU01 vs OS02 | 281 | 512 | 4349 |
| | HO03 vs KK01 | 274 | 531 | 4211 |
| | HO03 vs OS02 | 253 | 479 | 4067 |
| KK01 vs OS02 | 287 | 536 | 4154 | |
| JPN2 | AR05 vs HO01 | 254 | 484 | 3074 |
| | AR05 vs PL2 | 229 | 445 | 2867 |
| | AR05 vs PL4 | 243 | 433 | 2770 |
| | AR05 vs PL7 | 292 | 493 | 3234 |
| | HO01 vs PL2 | 239 | 417 | 2619 |
| | HO01 vs PL4 | 235 | 433 | 2736 |
| | HO01 vs PL7 | 304 | 515 | 3466 |
| | PL2 vs PL4 | 202 | 382 | 2465 |
| | PL2 vs PL7 | 271 | 480 | 3243 |
| | PL4 vs PL7 | 283 | 478 | 3162 |
| t-test examining statistical difference between JPN1 and JPN2 lineages | | | | |
| <i>t</i> -value | | 3.582 | 6.445 | 29.540 |
| <i>p</i> -value | | > 0.05 | < 0.001 | < 0.001 |

Table S4. List of genes with unique nonsynonymous substitutions in each genotype.

Data are listed in the order corresponding to gene stable IDs of TCO (Colbourne et al. 2012). Filled and open circles denote homozygous and heterozygous mutations on the gene, respectively.

| Gene stable ID | JPN1 | | | | | | | JPN2 | | | | |
|-------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|---------|---------|---------|
| | AR0 1 | DA0 4 | DA0 5 | FU0 1 | HO0 3 | KK0 1 | OSO 2 | AR0 5 | HO0 1 | PL 2 | PL 4 | PL 7 |
| DAPPUDRAFT_100142 | | | | | | | | | | ○ | | |
| DAPPUDRAFT_100184 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_100200 | | | | | | | | | | | ● | |
| DAPPUDRAFT_101046 | | | | | | ○ | | | | | | |
| DAPPUDRAFT_101087 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_102050 | ● | | | | | | | | | | | |
| DAPPUDRAFT_102106 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_102133 | ● | | | | | | | | | | | |
| DAPPUDRAFT_102173 | | | | | | | | | | | | ● |
| DAPPUDRAFT_102448 | | | | | | | | | | | | ● |
| DAPPUDRAFT_102589 | | | | | | ○ | | | | | | |
| DAPPUDRAFT_102606 | | | | | | | ● | | | | | |
| DAPPUDRAFT_102698 | ○ | | | | | | | | | | | |
| DAPPUDRAFT_103010 | | | | | ○ | | | | | | | |
| DAPPUDRAFT_103833 | | | | ● | | | | | | | | |
| DAPPUDRAFT_104592 | | | | | | | | ○ | | | | |
| DAPPUDRAFT_104787 | ○ | | | | | | | | | | | |
| DAPPUDRAFT_104885 | | | | | | ○ | | | | | | |
| DAPPUDRAFT_105173 | | | ○ | | | | | | | | ○ | |
| DAPPUDRAFT_105768 | | | | | | | | ○ | | | | |
| DAPPUDRAFT_106149 | ○ | | | | | | | | | | | |
| DAPPUDRAFT_106477 | | | | | ● | | | | | | | |
| DAPPUDRAFT_106666 | | | | | ● | | | | | | | |
| DAPPUDRAFT_106883 | | | | | | | | ○ | | | | |
| DAPPUDRAFT_106899 | | ○ | | | | | | | ● | | | |

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|-------------------|---|---|---|---|---|---|---|---|---|---|---|---|
| DAPPUDRAFT_107340 | | ○ | | | | | | | | | | |
| DAPPUDRAFT_107700 | | | | | ● | | | | | | | |
| DAPPUDRAFT_108150 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_108326 | | ○ | | | | | | | | | | |
| DAPPUDRAFT_108478 | | | | | | | | ○ | | | | |
| DAPPUDRAFT_109031 | | | | | | | | ○ | | | | |
| DAPPUDRAFT_109282 | | ● | | | | | | | | | | |
| DAPPUDRAFT_109456 | | | ○ | | | | | | | | | ○ |
| DAPPUDRAFT_109764 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_109956 | | | | | | | | ○ | | | | |
| DAPPUDRAFT_110077 | | | | | | | | | | ○ | | |
| DAPPUDRAFT_110200 | | | | | | | ○ | | | | | |
| DAPPUDRAFT_110341 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_110847 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_111210 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_111549 | | | | | | | | | ● | | | |
| DAPPUDRAFT_113018 | | | | | | | | | | ○ | | |
| DAPPUDRAFT_113547 | | | | | | | | | ● | | | |
| DAPPUDRAFT_113947 | | | | | | | | | | ○ | | |
| DAPPUDRAFT_114229 | ● | | | | | | | | | | | |
| DAPPUDRAFT_114231 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_114273 | | | | | | | | | | | ● | |
| DAPPUDRAFT_114320 | | | | | | ○ | | | | | | |
| DAPPUDRAFT_114421 | | | | | | | ○ | | | | | |
| DAPPUDRAFT_114604 | | | | | | | | | | | | ○ |
| DAPPUDRAFT_114926 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_116883 | | | | | | | ● | | | | | |
| DAPPUDRAFT_116884 | | | | | | | | | | | | ● |
| DAPPUDRAFT_117019 | | | | | | | | | | | ● | |
| DAPPUDRAFT_117545 | | | ○ | | | | | | | | | |
| DAPPUDRAFT_117736 | | | | | | | | ○ | | | | |
| DAPPUDRAFT_117737 | | | ○ | | | | | | | | | |
| DAPPUDRAFT_117879 | | | | | | | ○ | | | | | ○ |
| DAPPUDRAFT_119275 | | | | | | | | | | ● | | |
| DAPPUDRAFT_119531 | | | | | | | | ○ | | | | |

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|-------------------|---|---|---|---|---|---|---|---|---|---|---|---|
| DAPPUDRAFT_300404 | | | | | | | | | | | | ● |
| DAPPUDRAFT_300508 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_301167 | | | | | | | | ○ | | | | |
| DAPPUDRAFT_301201 | | | ○ | | | | | | | | | |
| DAPPUDRAFT_301407 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_301532 | | | | | | | | | | | | ○ |
| DAPPUDRAFT_301877 | | | | | | ● | | | | | | |
| DAPPUDRAFT_301897 | | | | | ○ | | | | | | | |
| DAPPUDRAFT_302117 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_302134 | | | | | | ○ | | | | | | |
| DAPPUDRAFT_302138 | | | | | ○ | | | | | | | |
| DAPPUDRAFT_302484 | | ● | | | | | | | | | | |
| DAPPUDRAFT_302612 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_302657 | | | | | ○ | | | | | | | |
| DAPPUDRAFT_302833 | | | | | | | | | | | | |
| DAPPUDRAFT_302844 | | | | | | | ● | | | | | |
| DAPPUDRAFT_302885 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_303105 | | | | | | | | | | ○ | | |
| DAPPUDRAFT_303198 | | ● | | | | | | | | | | ● |
| DAPPUDRAFT_303205 | | | ○ | | | | | | | | | |
| DAPPUDRAFT_303246 | | | | | ○ | | | | | ● | | |
| DAPPUDRAFT_303405 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_303483 | | | | | | | | | | | | ○ |
| DAPPUDRAFT_303885 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_303904 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_303927 | ○ | | | | | | | | | | | |
| DAPPUDRAFT_304023 | | | | | | | | | | | | ● |
| DAPPUDRAFT_304124 | ○ | | | | | | | | ○ | | | |
| DAPPUDRAFT_304125 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_304131 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_304382 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_304684 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_304801 | | | ○ | | | | | | | | | |
| DAPPUDRAFT_304875 | | | | | | | | | | | | |
| DAPPUDRAFT_304939 | | | | | ○ | | | | | | | |

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|-------------------|--|---|---|---|---|--|---|--|---|--|---|---|
| DAPPUDRAFT_325795 | | | | | | | ○ | | | | | |
| DAPPUDRAFT_325898 | | | | | ● | | | | | | | |
| DAPPUDRAFT_326717 | | | | | | | | | | | | ○ |
| DAPPUDRAFT_326941 | | | ○ | | | | | | | | | |
| DAPPUDRAFT_326957 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_327022 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_327679 | | | | | | | | | ● | | | |
| DAPPUDRAFT_328037 | | ○ | | | | | | | | | | |
| DAPPUDRAFT_328318 | | | | | | | ○ | | | | | |
| DAPPUDRAFT_328712 | | | | | ● | | | | | | | |
| DAPPUDRAFT_329106 | | | ○ | | | | | | | | | |
| DAPPUDRAFT_329385 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_329664 | | | | | | | ○ | | | | | ○ |
| DAPPUDRAFT_329805 | | | | | | | ● | | | | | |
| DAPPUDRAFT_329962 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_330286 | | | | | | | | | | | ● | |
| DAPPUDRAFT_330570 | | | | | ○ | | | | | | | |
| DAPPUDRAFT_330625 | | | | | | | | | | | | ● |
| DAPPUDRAFT_330844 | | | | | | | | | ○ | | | |
| DAPPUDRAFT_330970 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_331526 | | | | | | | | | | | | ○ |
| DAPPUDRAFT_331838 | | | | | ● | | | | | | | |
| DAPPUDRAFT_332703 | | | | | ○ | | ○ | | | | | |
| DAPPUDRAFT_332853 | | | | | | | ○ | | | | | ○ |
| DAPPUDRAFT_33293 | | | | | ○ | | | | | | | |
| DAPPUDRAFT_332948 | | ○ | | | | | | | | | | |
| DAPPUDRAFT_333387 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_333581 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_333653 | | | | | | | ○ | | | | | |
| DAPPUDRAFT_333828 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_334025 | | | | | | | ○ | | | | | |
| DAPPUDRAFT_334105 | | | | ○ | | | | | | | | |
| DAPPUDRAFT_334794 | | ● | | | | | | | | | | |
| DAPPUDRAFT_335346 | | | | | | | | | | | ○ | |
| DAPPUDRAFT_335601 | | | | | | | ● | | | | | |

Table S5. List of primers for sequencing whole genome DNA. Names of primer used for amplifying each region (set 1, 2 and 3) are shown in bold.

| | name | sequence |
|--------------------|--------------------|---------------------------|
| Set 1 | Dpu_01488F1 | GCTCTTAGACTTTTAATTCGCGCCG |
| | Dpu_02163F1 | ATTATTAGCCACGAAAGAGG |
| | Dpu_02863F1 | CTTCAATCGAATGACAGCATTC |
| | Dpu_03521F1 | ACGCCGTACCTGGTCGTTTAAATC |
| | Dpu_04918R1 | GAGGCAATGAACAAGATTATTCCC |
| | Dpu_05556R1 | CAAGACCACAGATCTAATTGTC |
| | Dpu_06200R1 | TCAATGATTTGAGAACAGCT |
| | Dpu_06899R1 | GCCTTTCTTAGCGGGGTTTACTCT |
| | Set 2 | Dpu_06487F2 |
| Dpu_07124F2 | | TCTAAGGGTAGACAATGCAA |
| Dpu_07650F2 | | GATAGCCATAGAAGCAACAA |
| Dpu_07822F2 | | TTCTCCCCCTAAAAATCACTC |
| Dpu_08126F2 | | CTGTCTGTTCAATGTTGGGTAGAC |
| Dpu_08436R2 | | ATTAGTCACGGCCTCTGTTC |
| Dpu_08641F2 | | AGGGACAACCTCGAACTATTCCG |
| Dpu_09209F2 | | AGGCCTAATCCAAACCCGCTTA |
| Dpu_09729F2 | | ACACCCAAAGCTCCCAAAGCTCCA |
| Dpu_10758R2 | | GGGATAGCAGAGAGTAAATTGG |
| Dpu_11130R2 | | CTTCTACAGGCTTTGCTCCGATTC |
| Dpu_11445R2 | | TAGGTTAAACAATCTTATGC |
| Dpu_12035R2 | | TTGGATGTCTACGAGGAGTAGCCCA |
| Dpu_12336R2 | | TACATTCAGTTGCGTAAGGGGC |
| Dpu_12606R2 | | AGCCAGGTTGGTTTCTATCCTC |
| Dpu_12761R2 | | CTAGGGATAACAGCGTAATC |
| Dpu_13323R2 | | GTGTTTATCTATCTACCAGAATAC |
| Dpu_13787R2 | | CCATCTTTGTCCAAATTGTG |
| Dpu_14188R2 | | AGGCTTAAAACTCAGGTGAAGGTGG |
| Set 3 | | Dpu_13944F3 |
| | Dpu_14626F3 | AGCCAGATTCAAACCTTCCC |
| | Dpu_14685F3 | CGGAACCACTTTAGCGCAAGTA |
| | Dpu_00747R3 | CTGTCAATCAACCAGCCTACATG |
| | Dpu_01182R3 | GAAAGTAGATCATGGATAGC |
| | Dpu_01385R3 | ATGGCCGAGGAGTAGGCATTAA |
| | Dpu_02003R3 | TGCTAGGACTGGTAGGCTCAATAG |
| | | |