**SUPPLEMENTAL FIGURES**



**Figure S1. Molecular evolution and population genetics of synonymous, nonsynonymous, conservative, and radical mutations in mitochondrial genomes of sexual *P. antipodarum*.** a) Ratios of polymorphism to divergence for synonymous, all nonsynonymous, conservative nonsynonymous, and radical nonsynonymous changes in mitochondrial genomes of sexual *P. antipodarum*. Lower-case letters reflect statistical groupings based on pairwise Fisher’s Exact Tests (*p* < 0.013). b) Nucleotide diversity estimated for sexual lineages only using *π* (circles) and *θ* (triangles) for all nonsynonymous (black), conservative nonsynonymous (blue), and radical nonsynonymous (orange) sites. Error bars reflect variance calculated as in Durrett (2008). Radical nonsynonymous sites exhibit significantly lower levels of nucleotide diversity than conservative sites (*p <* 0.0002) for both measures of polymorphism. c) Site-frequency spectra in mitochondrial genomes for synonymous (white), nonsynonymous (black), conservative nonsynonymous (blue), and radical nonsynonymous (orange) polymorphisms in sexual lineages of *P. antipodarum*.



**Figure S2. Bootstrap distributions of population genetic statistics in mitochondrial genomes of *P. antipodarum*.** Violin plot depicting distributions of 10,000bootstrap replicates for synonymous (white), nonsynonymous (grey), conservative (blue), and radical (orange) nucleotide diversity across all seven amino acid classification schemes and the scheme-averaged CRI method using a) *π*, b) *θ*, and c) *θU*. For each bootstrap replicate, 3,738 codon positions were randomly selected with replacement, the multiple sequence alignment was reconstructed using these codon positions, and population genetic statistics were calculated.



**Figure S3. Molecular evolution and population genetic comparisons of synonymous changes in sexual (blue) vs. asexual (red) *P. antipodarum*.** a) Branch-specific estimates of synonymous substitution rates were significantly higher in asexual vs. sexual lineages of *P. antipodarum* (*W* = 142, *p* = 0.025). b) Nucleotide diversity at synonymous sites did not differ across reproductive modes for *π* (circles, *p* = 0.063), *θ* (triangles, *p* = 0.15) or *θU* (diamonds, *p* = 0.057).

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**Figure S4. Null distributions of population genetic parameters in sexual vs. asexual *P. antipodarum*.** *P. antipodarum* lineages were randomly sampled without replacement to form groups 1 (n = 23) and 2 (n = 8). Next, *D12*, where *D12* =  *1* – *2*,  *1* represents a population genetic statistic for group 1, and *2* represents a population genetic statistic for group 2, was calculated for each population genetic statistic – a) *πC/πS*, b) *πR/πS*, c) *θC/θS*, and d) *θR/θS*. We used 10,000 replicates to construct the null distributions depicted here (solid black curve). We next used a subsample containing only clade C lineages of *P. antipodarum*, and sampled lineages without replacement to form groups 1 (n = 16) and 2 (n = 6) and repeated the steps taken above for e) *πC/πS*, f) *πR/πS*, g) *θC/θS*, and h) *θR/θS*. Dashed grey lines represent the significance threshold 0.05, in which 95% of the null distribution falls to the left of the line. The red line indicates the empirically determined difference between sexual and asexual lineages (*DAS*). Asexual lineages exhibited significantly higher levels of nucleotide diversity at radical sites for both *π* and *θ*, using both the entire dataset and the clade C dataset, but never exhibited significantly higher nucleotide diversity at conservative sites for *π* or *θ* in either the full dataset or the clade C dataset.

**SUPPLEMENTARY TABLES**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table S1. Summary of source populations of *Potamopyrgus antipodarum.*** | | | | | |
| **Lake of Origin** | **Lineage ID** | **Lat, Long** | **RM** | **Sequencing Platform** | **Reference** |
| Alexandrina | AM | -35.440603, 139.083438 | Sexual | ABI 3730 | Neiman et al. 2010 |
| Alexandrina | AS | -35.440603, 139.083438 | Sexual | ABI 3730 | Neiman et al. 2010 |
| Alexandrina | AY | -35.440603, 139.083438 | Sexual | Illumina | This study |
| Alexandrina | AC 51 | -35.440603, 139.083438 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Alexandrina | AC 1 | -35.440603, 139.083438 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Alexandrina | AC 7 | -35.440603, 139.083438 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Brunner | Br 6 | -42.607385, 171.439636 | Asexual | ABI 3730 | This study |
| Brunner | Br 2 | -42.607385, 171.439636 | Asexual | ABI 3730 | This study |
| Denmark | Den A | 56.001158, 9.207968 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Grasmere | Gr 6 | -43.061036, 171.774569 | Asexual | ABI 3730 | This study |
| Grasmere | Gr 1 | -43.061036, 171.774569 | Asexual | ABI 3730 | This study |
| Gunn | Gn | -44.874524, 168.090031 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Heron | Hr | -43.481012, 171.169173 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Heron | Hr 2 | -43.481012, 171.169173 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Ianthe | Ia | -43.481012, 171.169173 | Sexual | ABI 3730 | Neiman et al. 2010 |
| Ianthe | Ia 2 | -43.481012, 171.169173 | Sexual | Illumina | This study |
| Kaniere | Kn 1 | -43.481012, 171.169173 | Sexual | ABI 3730 | This study |
| Kaniere | Kn 3x | -43.481012, 171.169173 | Asexual | Illumina | This study |
| Lady | Ly | -42.599558, 171.573173 | Sexual | ABI 3730 | Neiman et al. 2010 |
| Lake Superior | Duluth | 47.917620, -86.953400 | Asexual | ABI 3730 | Neiman et al. 2010 |
| McGregor | McG | -43.936019, 170.470227 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Poerua | Po 3x | -42.702716, 171.495638 | Asexual | Illumina | This study |
| Poerua | Po72 | -42.702716, 171.495638 | Asexual | ABI 3730, Illumina\* | This study |
| Rotoiti | Ri 1 | -38.037084, 176.345628 | Asexual | ABI 3730 | This study |
| Rotoroa | Rr 1 | -41.851912, 172.642727 | Sexual | ABI 3730 | This study |
| Tarawera | Tw | -38.186357, 176.429540 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Waikaremoana | Wk 36 | -38.771011, 177.109841 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Waikaremoana | Wk 37 | -38.771011, 177.109841 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Waikaremoana | Wk 372 | -38.771011, 177.109841 | Asexual | ABI 3730 | Neiman et al. 2010 |
| Waikaremoana | Wk 4 | -38.771011, 177.109841 | Asexual | Illumina | This study |
| Wales | Wales C | 52.321471, -3.703571 | Asexual | ABI 3730 | Neiman et al. 2010 |
| \* – Same DNA extraction was sequenced on both platforms | | | | | |

|  |  |  |
| --- | --- | --- |
| **Table S2. Primers used to amplify and sequence whole mitochondrial genomes in *P. antipodarum*.** | | |
| *PCR* | | |
| **Fragment** | **Forward** | **Reverse** |
| 1 | 5’-GAGGTAGGAGACTGTAGT-3’ | 5’-GAGTCCTAAGCCCAATGCA-3’ |
| 2 | 5’-GCTAGTATGAATGGTTTGACG-3’ | 5’-GTTATGGCAGCAATAGTAATTG-3’ |
| 3 | 5’-TCAGCTTGTGGATCTGA-3’ | 5’-GCCTAATCAGTATGAGGAAG-3’ |
| 4 | 5’-GGAGTGAACGGAAATCA-3’ | 5’-CTCTTGAGTATGCTGAGTACA-3’ |
| *Sequencing* | | |
| **Fragment** | **Forward** | **Reverse** |
| 1.1 | 5’TATGAATATTCAGATTTTTTAAATA-3’ | 5’-CTGCCACCTTTATTATAAAG-3’ |
| 1.2 | 5’-GGCTCATAGTTTACTTAACTT-3’ | 5’-CGTCAAACCATTCATACTAGC-3’ |
| 1.3 | 5’-GCGGTTAGACCACGAAG-3’ | 5’-AACTAATAGATGTTTCTATG-3’ |
| 1.4 | 5’-CATAGAAACATCTATTAGTT-3’ | 5’-CTAATCCCAGTTTCCCTC-3’ |
| 2.1 | 5’-GTCTCTCTCTAATTTTATAG-3’ | 5’-GCTGATAGATGAAAGTCTC-3’ |
| 2.2 | 5’-CTCTCCTTATTTTTTAGCC-3’ | 5’-ATATTTGCAGGAATTCAGTG-3’ |
| 2.3 | 5’-GCATTGGAAGCTAAAGAC-3’ | 5’-GTTAACAGCTTCTGTTCG-3’ |
| 2.4 | 5’-CAGCACACTTGAAACATTG-3’ | 5’-CTCATATCTTGCTGCAAC-3’ |
| 3.1 | 5’-CTTCATCAATTAGCGCTTTATTT-3’ | 5’-GTGAAAGAAATCTTAGCCTA-3’ |
| 3.2 | 5’-CTTTCTACCTTAAGCCAGCTAG-3’ | 5’-GTACTAAGCCCCTAAAGGCAA-3’ |
| 3.3 | 5’-CAGCACAGCCTTTAACTAAG-3’ | 5’CTAAATGAAAGGGGTTACG-3’ |
| 3.4 | 5’-CCGCTAAATCCATTTGAAG-3’ | 5’-GTCATCCCTGTAGCTAG-3’ |
| 4.1 | 5’-CTATTGTAGTTATATTGTTGG-3’ | 5’-GAGATATTACAAGCGGTG-3’ |
| 4.2 | 5’-CAATTTCTTCCTCATACTGATT-3’ | 5’-CCTTAACTCCTAATCTTGGTAC-3’ |
| 4.3 | 5’-TTAGGGTGGATGCTATTTGC-3' | 5’-GATACAAGAGCCTCTCATAC-3’ |
| 4.4 | 5’-GATTTAGCTATTTTTTCATTAC-3’ | 5’-CTGTTGTAATAAAGTTTACTG-3’ |
|  | | |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S3. Amino acid classification schemes used to calculate CRI score.** | | | | | | | | |
|  | **AA Classification Scheme** | | | | | | | |
| **AA Change** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **CRI** |
| A <--> R | R | R | R | R | R | R | R | 1.00 |
| A <--> N | C | R | R | C | C | R | R | 0.57 |
| A <--> D | R | R | R | R | R | R | R | 1.00 |
| A <--> C | C | R | R | C | C | R | R | 0.57 |
| A <--> Q | C | R | R | R | C | R | R | 0.71 |
| A <--> E | R | R | R | R | R | R | R | 1.00 |
| A <--> G | C | R | C | C | C | C | R | 0.29 |
| A <--> H | R | R | R | R | R | R | R | 1.00 |
| A <--> I | C | C | R | R | C | C | C | 0.29 |
| A <--> L | C | C | R | R | C | C | C | 0.29 |
| A <--> K | R | R | R | R | R | R | R | 1.00 |
| A <--> M | C | C | R | R | C | C | C | 0.29 |
| A <--> F | C | C | R | R | R | C | C | 0.43 |
| A <--> P | C | C | C | C | C | C | C | 0.00 |
| A <--> S | C | R | C | C | C | R | R | 0.43 |
| A <--> T | C | R | C | C | C | R | R | 0.43 |
| A <--> W | C | C | R | R | R | C | C | 0.43 |
| A <--> Y | C | R | R | R | R | R | R | 0.86 |
| A <--> V | C | C | R | R | C | C | C | 0.29 |
| A <--> \* | R | R | R | R | R | R | R | 1.00 |
| R <--> N | R | C | R | R | R | R | R | 0.86 |
| R <--> D | R | C | R | R | R | R | R | 0.86 |
| R <--> C | R | C | R | R | R | R | R | 0.86 |
| R <--> Q | R | C | R | C | R | R | R | 0.71 |
| R <--> E | R | C | R | R | R | R | R | 0.86 |
| R <--> G | R | C | R | R | R | R | R | 0.86 |
| R <--> H | C | C | C | C | C | C | C | 0.00 |
| R <--> I | R | R | R | R | R | R | R | 1.00 |
| R <--> L | R | R | R | R | R | R | R | 1.00 |
| R <--> K | C | C | C | C | C | C | C | 0.00 |
| R <--> M | R | R | R | R | R | R | R | 1.00 |
| R <--> F | R | R | R | C | R | R | R | 0.86 |
| R <--> P | R | R | R | R | R | R | R | 1.00 |
| R <--> S | R | C | R | R | R | R | R | 0.86 |
| R <--> T | R | C | R | R | R | R | R | 0.86 |
| R <--> W | R | R | R | C | R | R | R | 0.86 |
| R <--> Y | R | C | R | C | R | R | R | 0.71 |
| R <--> V | R | R | R | R | R | R | R | 1.00 |
| R <--> \* | R | R | R | R | R | R | R | 1.00 |
| N <--> D | R | C | C | R | R | R | R | 0.71 |
| N <--> C | C | C | R | C | C | C | C | 0.14 |
| N <--> Q | C | C | C | R | C | C | C | 0.14 |
| N <--> E | R | C | C | R | R | R | R | 0.71 |
| N <--> G | C | C | R | C | C | R | C | 0.29 |
| N <--> H | R | C | R | R | R | R | R | 0.86 |
| N <--> I | C | R | R | R | C | R | R | 0.71 |
| N <--> L | C | R | R | R | C | R | R | 0.71 |
| N <--> K | R | C | R | R | R | R | R | 0.86 |
| N <--> M | C | R | R | R | C | R | R | 0.71 |
| N <--> F | C | R | R | R | R | R | R | 0.86 |
| N <--> P | C | R | R | C | C | R | R | 0.57 |
| N <--> S | C | C | R | C | C | C | C | 0.14 |
| N <--> T | C | C | R | C | C | C | C | 0.14 |
| N <--> W | C | R | R | R | R | R | R | 0.86 |
| N <--> Y | C | C | R | R | R | C | C | 0.43 |
| N <--> V | C | R | R | R | C | R | R | 0.71 |
| N <--> \* | R | R | R | R | R | R | R | 1.00 |
| D <--> C | R | C | R | R | R | R | R | 0.86 |
| D <--> Q | R | C | C | R | R | R | R | 0.71 |
| D <--> E | C | C | C | C | C | C | C | 0.00 |
| D <--> G | R | C | R | R | R | R | R | 0.86 |
| D <--> H | R | C | R | R | R | R | R | 0.86 |
| D <--> I | R | R | R | R | R | R | R | 1.00 |
| D <--> L | R | R | R | R | R | R | R | 1.00 |
| D <--> K | R | C | R | R | R | R | R | 0.86 |
| D <--> M | R | R | R | R | R | R | R | 1.00 |
| D <--> F | R | R | R | R | R | R | R | 1.00 |
| D <--> P | R | R | R | R | R | R | R | 1.00 |
| D <--> S | R | C | R | R | R | R | R | 0.86 |
| D <--> T | R | C | R | R | R | R | R | 0.86 |
| D <--> W | R | R | R | R | R | R | R | 1.00 |
| D <--> Y | R | C | R | R | R | R | R | 0.86 |
| D <--> V | R | R | R | R | R | R | R | 1.00 |
| D <--> \* | R | R | R | R | R | R | R | 1.00 |
| C <--> Q | C | C | R | R | C | C | C | 0.29 |
| C <--> E | R | C | R | R | R | R | R | 0.86 |
| C <--> G | C | C | R | C | C | R | C | 0.29 |
| C <--> H | R | C | R | R | R | R | R | 0.86 |
| C <--> I | C | R | R | R | C | R | R | 0.71 |
| C <--> L | C | R | R | R | C | R | R | 0.71 |
| C <--> K | R | C | R | R | R | R | R | 0.86 |
| C <--> M | C | R | R | R | C | R | R | 0.71 |
| C <--> F | C | R | R | R | R | R | R | 0.86 |
| C <--> P | C | R | R | C | C | R | R | 0.57 |
| C <--> S | C | C | R | C | C | C | C | 0.14 |
| C <--> T | C | C | R | C | C | C | C | 0.14 |
| C <--> W | C | R | R | R | R | R | R | 0.86 |
| C <--> Y | C | C | R | R | R | C | C | 0.43 |
| C <--> V | C | R | R | R | C | R | R | 0.71 |
| C <--> \* | R | R | R | R | R | R | R | 1.00 |
| Q <--> E | R | C | C | R | R | R | R | 0.71 |
| Q <--> G | C | C | R | R | C | R | C | 0.43 |
| Q <--> H | R | C | R | C | R | R | R | 0.71 |
| Q <--> I | C | R | R | R | C | R | R | 0.71 |
| Q <--> L | C | R | R | R | C | R | R | 0.71 |
| Q <--> K | R | C | R | C | R | R | R | 0.71 |
| Q <--> M | C | R | R | R | C | R | R | 0.71 |
| Q <--> F | C | R | R | C | R | R | R | 0.71 |
| Q <--> P | C | R | R | R | C | R | R | 0.71 |
| Q <--> S | C | C | R | R | C | C | C | 0.29 |
| Q <--> T | C | C | R | R | C | C | C | 0.29 |
| Q <--> W | C | R | R | C | R | R | R | 0.71 |
| Q <--> Y | C | C | R | C | R | C | C | 0.29 |
| Q <--> V | C | R | R | R | C | R | R | 0.71 |
| Q <--> \* | R | R | R | R | R | R | R | 1.00 |
| E <--> G | R | C | R | R | R | R | R | 0.86 |
| E <--> H | R | C | R | R | R | R | R | 0.86 |
| E <--> I | R | R | R | R | R | R | R | 1.00 |
| E <--> L | R | R | R | R | R | R | R | 1.00 |
| E <--> K | R | C | R | R | R | R | R | 0.86 |
| E <--> M | R | R | R | R | R | R | R | 1.00 |
| E <--> F | R | R | R | R | R | R | R | 1.00 |
| E <--> P | R | R | R | R | R | R | R | 1.00 |
| E <--> S | R | C | R | R | R | R | R | 0.86 |
| E <--> T | R | C | R | R | R | R | R | 0.86 |
| E <--> W | R | R | R | R | R | R | R | 1.00 |
| E <--> Y | R | C | R | R | R | R | R | 0.86 |
| E <--> V | R | R | R | R | R | R | R | 1.00 |
| E <--> \* | R | R | R | R | R | R | R | 1.00 |
| G <--> H | R | C | R | R | R | R | R | 0.86 |
| G <--> I | C | R | R | R | C | C | R | 0.57 |
| G <--> L | C | R | R | R | C | C | R | 0.57 |
| G <--> K | R | C | R | R | R | R | R | 0.86 |
| G <--> M | C | R | R | R | C | C | R | 0.57 |
| G <--> F | C | R | R | R | R | C | R | 0.71 |
| G <--> P | C | R | C | C | C | C | R | 0.29 |
| G <--> S | C | C | C | C | C | R | C | 0.14 |
| G <--> T | C | C | C | C | C | R | C | 0.14 |
| G <--> W | C | R | R | R | R | C | R | 0.71 |
| G <--> Y | C | C | R | R | R | R | C | 0.57 |
| G <--> V | C | R | R | R | C | C | R | 0.57 |
| G <--> \* | R | R | R | R | R | R | R | 1.00 |
| H <--> I | R | R | R | R | R | R | R | 1.00 |
| H <--> L | R | R | R | R | R | R | R | 1.00 |
| H <--> K | C | C | C | C | C | C | C | 0.00 |
| H <--> M | R | R | R | R | R | R | R | 1.00 |
| H <--> F | R | R | R | C | R | R | R | 0.86 |
| H <--> P | R | R | R | R | R | R | R | 1.00 |
| H <--> S | R | C | R | R | R | R | R | 0.86 |
| H <--> T | R | C | R | R | R | R | R | 0.86 |
| H <--> W | R | R | R | C | R | R | R | 0.86 |
| H <--> Y | R | C | R | C | R | R | R | 0.71 |
| H <--> V | R | R | R | R | R | R | R | 1.00 |
| H <--> \* | R | R | R | R | R | R | R | 1.00 |
| I <--> L | C | C | C | C | C | C | C | 0.00 |
| I <--> K | R | R | R | R | R | R | R | 1.00 |
| I <--> M | C | C | C | C | C | C | C | 0.00 |
| I <--> F | C | C | R | R | R | C | C | 0.43 |
| I <--> P | C | C | R | R | C | C | C | 0.29 |
| I <--> S | C | R | R | R | C | R | R | 0.71 |
| I <--> T | C | R | R | R | C | R | R | 0.71 |
| I <--> W | C | C | R | R | R | C | C | 0.43 |
| I <--> Y | C | R | R | R | R | R | R | 0.86 |
| I <--> V | C | C | C | C | C | C | C | 0.00 |
| I <--> \* | R | R | R | R | R | R | R | 1.00 |
| L <--> K | R | R | R | R | R | R | R | 1.00 |
| L <--> M | C | C | C | C | C | C | C | 0.00 |
| L <--> F | C | C | R | R | R | C | C | 0.43 |
| L <--> P | C | C | R | R | C | C | C | 0.29 |
| L <--> S | C | R | R | R | C | R | R | 0.71 |
| L <--> T | C | R | R | R | C | R | R | 0.71 |
| L <--> W | C | C | R | R | R | C | C | 0.43 |
| L <--> Y | C | R | R | R | R | R | R | 0.86 |
| L <--> V | C | C | C | C | C | C | C | 0.00 |
| L <--> \* | R | R | R | R | R | R | R | 1.00 |
| K <--> M | R | R | R | R | R | R | R | 1.00 |
| K <--> F | R | R | R | C | R | R | R | 0.86 |
| K <--> P | R | R | R | R | R | R | R | 1.00 |
| K <--> S | R | C | R | R | R | R | R | 0.86 |
| K <--> T | R | C | R | R | R | R | R | 0.86 |
| K <--> W | R | R | R | C | R | R | R | 0.86 |
| K <--> Y | R | C | R | C | R | R | R | 0.71 |
| K <--> V | R | R | R | R | R | R | R | 1.00 |
| K <--> \* | R | R | R | R | R | R | R | 1.00 |
| M <--> F | C | C | R | R | R | C | C | 0.43 |
| M <--> P | C | C | R | R | C | C | C | 0.29 |
| M <--> S | C | R | R | R | C | R | R | 0.71 |
| M <--> T | C | R | R | R | C | R | R | 0.71 |
| M <--> W | C | C | R | R | R | C | C | 0.43 |
| M <--> Y | C | R | R | R | R | R | R | 0.86 |
| M <--> V | C | C | C | C | C | C | C | 0.00 |
| M <--> \* | R | R | R | R | R | R | R | 1.00 |
| F <--> P | C | C | R | R | R | C | C | 0.43 |
| F <--> S | C | R | C | R | R | R | R | 0.71 |
| F <--> T | C | R | C | R | R | R | R | 0.71 |
| F <--> W | C | C | R | C | C | C | C | 0.14 |
| F <--> Y | C | R | R | C | C | R | R | 0.57 |
| F <--> V | C | C | R | R | R | C | C | 0.43 |
| F <--> \* | R | R | R | R | R | R | R | 1.00 |
| P <--> S | C | R | C | C | C | R | R | 0.43 |
| P <--> T | C | R | C | C | C | R | R | 0.43 |
| P <--> W | C | C | R | R | R | C | C | 0.43 |
| P <--> Y | C | R | R | R | R | R | R | 0.86 |
| P <--> V | C | C | R | R | C | C | C | 0.29 |
| P <--> \* | R | R | R | R | R | R | R | 1.00 |
| S <--> T | C | C | C | C | C | C | C | 0.00 |
| S <--> W | C | R | R | R | R | R | R | 0.86 |
| S <--> Y | C | C | R | R | R | C | C | 0.43 |
| S <--> V | C | R | R | R | R | R | R | 0.86 |
| S <--> \* | R | R | R | R | R | R | R | 1.00 |
| T <--> W | C | R | R | R | R | R | R | 0.86 |
| T <--> Y | C | C | R | R | R | C | C | 0.43 |
| T <--> V | C | R | R | R | C | R | R | 0.71 |
| T <--> \* | R | R | R | R | R | R | R | 1.00 |
| W <--> Y | C | R | C | C | C | R | R | 0.43 |
| W <--> V | C | C | R | R | R | C | C | 0.43 |
| W <--> \* | R | R | R | R | R | R | R | 1.00 |
| Y <--> V | C | R | R | R | R | R | R | 0.86 |
| Y <--> \* | R | R | R | R | R | R | R | 1.00 |
|  | | | | | | | | |

**Table S3. Amino acid classification schemes used to calculate CRI score.** Pairwise amino acid change classifications for all seven amino acid classification schemes were used to calculate the conservative/radical index (CRI). Amino acid changes with CRI values ≤ 0.5 were treated as conservative amino acid changes, while those amino acid changes with CRI values > 0.5 were treated as radical amino acid changes in the scheme-averaged amino acid classifier. Asterisks indicate stop codons, which were always treated as radical amino acid changes.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S4. Mutational target sites in *P. antipodarum* lineages.** | **Rad7** | 4744.33 | 4745 | 4749.04 | 4744.67 | 4744.67 | 4744.67 | 4787.54 | 4795.25 | 4749.25 | 4748.71 | 4744 | 4950 | 4753.92 | 4744.67 | 4745.33 | 4744.67 | 4745.33 | 4744 | 4751.5 | 4746.33 | 4745 | 4744.33 | 4806.62 | 4786.71 | 4782.83 | 4752.83 | 4748.5 | 4747.17 | 4753.17 | 4748.83 | 4749.92 | 4749.33 | **4760.93** | CRI - Model-averaged amino acid classification scheme  1-7 - Numbers refer to amino acid classification scheme from Table 1 |
| **Con7** | 3871.67 | 3870.67 | 3872.1 | 3877 | 3877 | 3877 | 3832.85 | 3825.79 | 3871.63 | 3873.77 | 3872.33 | 3635.33 | 3860.46 | 3870.33 | 3870.67 | 3872.33 | 3873.67 | 3871.67 | 3875.92 | 3869 | 3870 | 3872.67 | 3801.9 | 3832.94 | 3832.58 | 3874.58 | 3880.58 | 3879.92 | 3874.25 | 3875.92 | 3870.29 | 3886.67 | **3857.64** |
| **Rad6** | 4490.33 | 4491 | 4494.25 | 4494.33 | 4494.33 | 4494.33 | 4541.92 | 4545.83 | 4493.83 | 4493.92 | 4490 | 4737 | 4501.83 | 4490 | 4491.33 | 4493.33 | 4494 | 4489.33 | 4501.67 | 4494.33 | 4490.67 | 4491 | 4558.08 | 4535.25 | 4530.33 | 4503.33 | 4499.33 | 4497 | 4504 | 4498.67 | 4494.17 | 4496 | **4510.28** |
| **Con6** | 4125.67 | 4124.67 | 4126.9 | 4127.33 | 4127.33 | 4127.33 | 4078.48 | 4075.21 | 4127.04 | 4128.56 | 4126.33 | 3848.33 | 4112.54 | 4125 | 4124.67 | 4123.67 | 4125 | 4126.33 | 4125.75 | 4121 | 4124.33 | 4126 | 4050.44 | 4084.4 | 4085.08 | 4124.08 | 4129.75 | 4130.08 | 4123.42 | 4126.08 | 4126.04 | 4140 | **4108.29** |
| **Rad5** | 4233.67 | 4233.67 | 4235.96 | 4240 | 4240 | 4240 | 4281.46 | 4266.08 | 4235.75 | 4237.63 | 4233.67 | 4347.33 | 4238.42 | 4232.67 | 4233.67 | 4232.33 | 4235 | 4232.33 | 4241.17 | 4230 | 4233 | 4233.67 | 4254.37 | 4256.29 | 4260.5 | 4241.83 | 4243.5 | 4243.5 | 4241.83 | 4234.17 | 4236.42 | 4272.33 | **4244.51** |
| **Con5** | 4382.33 | 4382 | 4385.19 | 4381.67 | 4381.67 | 4381.67 | 4338.94 | 4354.96 | 4385.13 | 4384.85 | 4382.67 | 4238 | 4375.96 | 4382.33 | 4382.33 | 4384.67 | 4384 | 4383.33 | 4386.25 | 4385.33 | 4382 | 4383.33 | 4354.15 | 4363.35 | 4354.92 | 4385.58 | 4385.58 | 4383.58 | 4385.58 | 4390.58 | 4383.79 | 4363.67 | **4374.06** |
| **Rad4** | 5196.67 | 5196.67 | 5198.46 | 5201 | 5201 | 5201 | 5215.29 | 5199.08 | 5198.42 | 5200.46 | 5196.33 | 5202.33 | 5197.42 | 5196 | 5196.33 | 5197.67 | 5199 | 5195.33 | 5203.83 | 5192.33 | 5195 | 5196 | 5185.54 | 5196.79 | 5200.5 | 5202.5 | 5205.83 | 5206.83 | 5202.83 | 5196.5 | 5198.42 | 5226.67 | **5199.08** |
| **Con4** | 3419.33 | 3419 | 3422.69 | 3420.67 | 3420.67 | 3420.67 | 3405.1 | 3421.96 | 3422.46 | 3422.02 | 3420 | 3383 | 3416.96 | 3419 | 3419.67 | 3419.33 | 3420 | 3420.33 | 3423.58 | 3423 | 3420 | 3421 | 3422.98 | 3422.85 | 3414.92 | 3424.92 | 3423.25 | 3420.25 | 3424.58 | 3428.25 | 3421.79 | 3409.33 | **3419.49** |
| **Rad3** | 5671.33 | 5670.67 | 5671.35 | 5675 | 5675 | 5675 | 5703.1 | 5695.63 | 5671.13 | 5673.35 | 5672.33 | 5753 | 5672.63 | 5670.33 | 5671 | 5671.67 | 5673.67 | 5670 | 5676.58 | 5668 | 5670 | 5671.33 | 5682.31 | 5687.52 | 5687.25 | 5676.92 | 5678.25 | 5679.92 | 5676.92 | 5671.92 | 5671.46 | 5697.67 | **5678.53** |
| **Con3** | 2944.67 | 2945 | 2949.79 | 2946.67 | 2946.67 | 2946.67 | 2917.29 | 2925.42 | 2949.75 | 2949.13 | 2944 | 2832.33 | 2941.75 | 2944.67 | 2945 | 2945.33 | 2945.33 | 2945.67 | 2950.83 | 2947.33 | 2945 | 2945.67 | 2926.21 | 2932.12 | 2928.17 | 2950.5 | 2950.83 | 2947.17 | 2950.5 | 2952.83 | 2948.75 | 2938.33 | **2940.03** |
| **Rad2** | 3524.67 | 3526.33 | 3529 | 3524.33 | 3524.33 | 3524.33 | 3538 | 3544.67 | 3529 | 3529 | 3526 | 3586 | 3529.67 | 3526.33 | 3525.67 | 3524 | 3523.67 | 3525.67 | 3530.67 | 3526.33 | 3525.33 | 3525 | 3549 | 3533.67 | 3537 | 3530.33 | 3527.33 | 3526.33 | 3530.67 | 3527.33 | 3529 | 3525.67 | **3530.92** |
| **Con2** | 5091.33 | 5089.33 | 5092.15 | 5097.33 | 5097.33 | 5097.33 | 5082.4 | 5076.37 | 5091.88 | 5093.48 | 5090.33 | 4999.33 | 5084.71 | 5088.67 | 5090.33 | 5093 | 5095.33 | 5090 | 5096.75 | 5089 | 5089.67 | 5092 | 5059.52 | 5085.98 | 5078.42 | 5097.08 | 5101.75 | 5100.75 | 5096.75 | 5097.42 | 5091.21 | 5110.33 | **5087.64** |
| **Rad1** | 2296.33 | 2297 | 2298.63 | 2295.33 | 2295.33 | 2295.33 | 2361.13 | 2358.08 | 2298.42 | 2298.63 | 2292 | 2594.33 | 2306.75 | 2295.67 | 2295.67 | 2295.67 | 2298 | 2295.33 | 2296.83 | 2297.33 | 2296 | 2295.33 | 2375.71 | 2354.96 | 2343.83 | 2298.17 | 2295.83 | 2296.83 | 2300.17 | 2294.17 | 2300.08 | 2304.67 | **2316.54** |
| **Con1** | 6319.67 | 6318.67 | 6322.52 | 6326.33 | 6326.33 | 6326.33 | 6259.27 | 6262.96 | 6322.46 | 6323.85 | 6324.33 | 5991 | 6307.62 | 6319.33 | 6320.33 | 6321.33 | 6321 | 6320.33 | 6330.58 | 6318 | 6319 | 6321.67 | 6232.81 | 6264.69 | 6271.58 | 6329.25 | 6333.25 | 6330.25 | 6327.25 | 6330.58 | 6320.13 | 6331.33 | **6302.02** |
| **RadCRI** | 4017.67 | 4016.67 | 4021.81 | 4021.67 | 4021.67 | 4021.67 | 4069.4 | 4071.37 | 4021.88 | 4022.81 | 4016 | 4254.33 | 4024.37 | 4016 | 4017.67 | 4021.67 | 4023.33 | 4015.33 | 4025.75 | 4017.67 | 4016.67 | 4016.33 | 4077.85 | 4063.31 | 4057.08 | 4026.75 | 4025.75 | 4026.75 | 4027.08 | 4021.08 | 4022.54 | 4027 | **4036.13** |
| **ConCRI** | 4598.33 | 4599 | 4599.33 | 4600 | 4600 | 4600 | 4551 | 4549.67 | 4599 | 4599.67 | 4600.33 | 4331 | 4590 | 4599 | 4598.33 | 4595.33 | 4595.67 | 4600.33 | 4601.67 | 4597.67 | 4598.33 | 4600.67 | 4530.67 | 4556.33 | 4558.33 | 4600.67 | 4603.33 | 4600.33 | 4600.33 | 4603.67 | 4597.67 | 4609 | **4582.44** |
| **Nsyn** | 8616 | 8615.67 | 8621.15 | 8621.67 | 8621.67 | 8621.67 | 8620.4 | 8621.04 | 8620.88 | 8622.48 | 8616.33 | 8585.33 | 8614.37 | 8615 | 8616 | 8617 | 8619 | 8615.67 | 8627.42 | 8615.33 | 8615 | 8617 | 8608.52 | 8619.65 | 8615.42 | 8627.42 | 8629.08 | 8627.08 | 8627.42 | 8624.75 | 8620.21 | 8636 | **8618.57** |
| **Syn** | 2598 | 2598.33 | 2592.85 | 2592.33 | 2592.33 | 2592.33 | 2593.6 | 2592.96 | 2593.13 | 2591.52 | 2597.67 | 2628.67 | 2599.63 | 2599 | 2598 | 2597 | 2595 | 2598.33 | 2586.58 | 2598.67 | 2599 | 2597 | 2605.48 | 2594.35 | 2598.58 | 2586.58 | 2584.92 | 2586.92 | 2586.58 | 2589.25 | 2593.79 | 2578 | **2594.89** |
| **Lineage ID** | AC 1 | AC 51 | AC 7 | AM | AS | AY | Br 2 | Br 6 | Den A | Duluth | Gn | Gr 1 | Gr 6 | Hr | Hr 2 | Ia | Ia 2 | Kn 1 | Kn 3x | Ly | McG | Po 3x | Po 72 | Ri 1 | Rr 1 | Tw | Wales C | Wk 36 | Wk 37 | Wk 372 | Wk 4 | *P. est* | **Mean** |

**Table S4. Mutational target sites in *P. antipodarum* lineages.** The number of mutational target sites across 11,214 bp of the protein-coding region of the mitochondrial genome for all seven amino acid classification schemes, plus the scheme-averaged classifier. For any given scheme, the number of conservative sites and the number of radical sites should sum to equal the total number of nonsynonymous sites.

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| **Table S5. Molecular evolution and population genetic comparisons of radical and conservative amino acid changes in sexual vs. asexual *P. antipodarum.*** | ***θU/θU-S*b** | *p* | 0.025\* | 0.011\* | 0.011 | 0.022\*\* | 0.074 | 0.0010\*\* | 0.16 | 0.086 | 0.16 | 0.0051\*\* | 0.048 | 0.015\*\* | 0.11 | 0.0014\*\* | 0.12 | 0.0017\*\* | a – Branch-specific estimates of substitution rate compared with Wilcoxon Signed Rank Tests  b – *π*, *θ*, and *θU* were compared using one-sided tests of the *D* statistic  \* ­– *p*-value significant at α level = 0.05  \*\* – *p-*value significant after correcting for multiple comparisons using the Holm procedure |
| *D* | 0.28 | 0.27 | 0.29 | 0.24 | 0.2 | 0.38 | 0.2 | 0.31 | 0.17 | 0.34 | 0.3 | 0.25 | 0.2 | 0.35 | 0.2 | 0.34 |
| Asex | 0.53 | 0.33 | 0.48 | 0.29 | 0.4 | 0.46 | 0.46 | 0.41 | 0.4 | 0.45 | 0.53 | 0.32 | 0.43 | 0.43 | 0.43 | 0.43 |
| Sex | 0.25 | 0.055 | 0.19 | 0.051 | 0.20 | 0.084 | 0.26 | 0.094 | 0.22 | 0.10 | 0.23 | 0.070 | 0.23 | 0.079 | 0.23 | 0.087 |
| ***θ*/*θS*b** | *p* | 0.88 | 1.0 x 10-4 \* | > 0.99 | 0.00010\*\* | > 0.99 | 0.00070\*\* | 1.0 x 10-4 \*\* | 0.86 | 0.00090\*\* | 0.51 | 0.33 | 0.11 | 0.034 | 0.59 | 0.0022\*\* | 0.81 |
| *D* | 0.0070 | 0.039 | -0.13 | 0.035 | -0.015 | 0.049 | 0.065 | 0.0026 | 0.047 | 0.015 | 0.024 | 0.023 | 0.035 | 0.015 | 0.044 | 0.0085 |
| Asex | 0.13 | 0.068 | 0.12 | 0.046 | 0.10 | 0.092 | 0.14 | 0.078 | 0.13 | 0.080 | 0.15 | 0.049 | 0.11 | 0.09 | 0.12 | 0.083 |
| Sex | 0.12 | 0.029 | 0.25 | 0.011 | 0.12 | 0.044 | 0.073 | 0.075 | 0.080 | 0.065 | 0.12 | 0.026 | 0.073 | 0.075 | 0.073 | 0.075 |
| ***π/πSb*** | *p* | 0.88 | 0.039\* | 0.30 | 0.66 | 0.083 | 0.82 | 0.99 | 0.020 | 0.97 | 0.070 | 0.51 | 0.31 | 0.036 | 0.86 | 0.020 | 0.87 |
| *D* | -0.0075 | 0.013 | 0.0036 | -0.0026 | 0.0084 | -0.0076 | -0.022 | 0.014 | -0.014 | 0.012 | 0.00053 | 0.0033 | 0.017 | -0.012 | 0.016 | -0.0096 |
| Asex | 0.099 | 0.043 | 0.091 | 0.024 | 0.084 | 0.057 | 0.12 | 0.050 | 0.11 | 0.052 | 0.12 | 0.027 | 0.089 | 0.059 | 0.099 | 0.051 |
| Sex | 0.11 | 0.03 | 0.087 | 0.026 | 0.075 | 0.065 | 0.14 | 0.036 | 0.12 | 0.040 | 0.12 | 0.024 | 0.072 | 0.070 | 0.083 | 0.061 |
| ***K/Ksa*** | *p* | 0.91 | 0.00065\* | 0.074 | 0.40 | 0.082 | 0.0012\*\* | 0.17 | 0.0023\*\* | 0.64 | 0.067 | 0.36 | 0.84 | 0.33 | 0.77 | 0.64 | 0.0017\*\* |
| *W* | 95 | 168 | 132 | 73 | 53 | 164 | 61 | 160 | 81 | 133 | 113 | 87 | 114 | 99 | 81 | 162 |
| *D* | -0.00025 | 0.0016 | 0.00095 | -0.00018 | -0.00073 | 0.0026 | -0.0011 | 0.0016 | 0.00057 | 0.00067 | 0.0006 | 0.0007 | 0.00086 | 0.00048 | -0.00029 | 0.0014 |
| Asex | 0.026 | 0.0063 | 0.018 | 0.010 | 0.019 | 0.012 | 0.030 | 0.009 | 0.030 | 0.0069 | 0.023 | 0.0086 | 0.024 | 0.0087 | 0.028 | 0.0064 |
| Sex | 0.026 | 0.0048 | 0.017 | 0.010 | 0.020 | 0.0089 | 0.031 | 0.0074 | 0.030 | 0.0062 | 0.023 | 0.0079 | 0.023 | 0.0082 | 0.028 | 0.0049 |
|  | Mutation Type | Con | Rad | Con | Rad | Con | Rad | Con | Rad | Con | Rad | Con | Rad | Con | Rad | Con | Rad |
|  | AA Scheme | CRI | | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | |