TITLE:

Variation in physiological function across source populations of a New Zealand

freshwater snail[1]

AUTHORS:

Emma Sharp Greimann[1], Samuel Fahrner[2], James Daniel Woodell[1], Samantha

Hennessey[1], Michael Robert Kline[1], Jorge Armando Moreno[1], Madeline Peters[1],

Jennifer Lynn Cruise[3], Maurine Neiman[1], Joel Sharbrough*[1,4]

[1] University of Iowa, Iowa City, IA, USA

[2] University of Minnesota, Saint Paul, MN, USA

[3] University of St. Thomas, Saint Paul, MN, USA

[4] Colorado State University, Fort Collins, CO, USA

*CORRESPONDENCE TO:

Joel Sharbrough

Colorado State University

440 Biology Building

Fort Collins, CO 80523

jsharbro@rams.colostate.edu

(970) 495-2301

TOTAL NUMBER OF FIGURES:

2

RUNNING HEADLINE:

Mitochondrial function in New Zealand snails

1   **ABSTRACT**

2   The mitochondrial and nuclear-encoded genes responsible for cellular respiration are

3   expected to experience relatively intense purifying selection, meaning that variation in

4   these genes will often decrease fitness. Still, extensive variation for mitochondrial

5   function persists in natural populations. We integrated physiological, cellular, and

6   behavioral approaches to quantify phenotypes relevant to mitochondrial function across

7   a diverse sample of *Potamopyrgus antipodarum*, a New Zealand snail characterized by

8   frequent coexistence between otherwise similar sexual and asexual individuals. We

9   found extensive across-lake variation in organismal oxygen consumption and behavioral

10 response to heat stress coupled with elevated mitochondrial membrane potential in

11 males *vs.* females. These data set the stage for applying this important model system

12 for sex, host-parasite interactions, invasion biology, and ecotoxicology to novel tests of

13 the relationships between mitochondrial variation and performance in natural

14 populations.

15

19 **INTRODUCTION**

20 The production of ATP *via* cellular respiration is a critical component of eukaryotic

21 function and fitness (Chen *et al.*, 2007; Pike *et al.*, 2007; Dowling *et al.*, 2008; Barreto

22 and Burton, 2013; Dowling, 2014), which likely explains why the components of the

23 oxidative phosphorylation (OXPHOS) pathway generally appear to be evolving under

24 purifying selection (Blier *et al.*, 2001; Montooth *et al.*, 2009; Neiman *et al.*, 2010;

25 Castellana *et al.*, 2011; Zhang and Broughton, 2013; Havird and Sloan, 2016;

26 Sharbrough *et al.*, 2018). By this logic, one would expect that the OXPHOS genes

27 would tend to harbor relatively low intraspecific variation. Instead, these genes are often

28 polymorphic within species (Ballard and Whitlock, 2004; Dowling *et al.*, 2008; Galtier *et*

29 *al.*, 2009; Bock *et al.*, 2014; Dobler *et al.*, 2014; Sharbrough *et al.*, 2018), with important

30 implications for phenomena from mitonuclear incompatibilities (*e.g.,* Ellison and Burton,

31 2006) to DNA barcoding (Hebert *et al.*, 2003). The effects of this genetic variation on

32 phenotypic variation for metabolic function - and indeed, how mitochondrial function is

33 maintained over evolutionary time despite mutational pressure (Gabriel *et al.*, 1993) -

34 remain unclear despite extensive variation for metabolic and mitochondrial function in

35 natural populations of a diverse array of species (see *e.g.,* Ursi *et al.*, 2003; Sadowska

36 *et al.*, 2005; Dowling *et al.*, 2008; Nilsson *et al.*, 2009; Arnqvist *et al.*, 2010; Oellermann

37 *et al.*, 2012; Chung *et al.*, 2017; Nespolo *et al.*, 2017; Sharbrough *et al.*, 2017). Although

38 some variation in metabolic and mitochondrial traits has been linked to specific

39 environmental correlates (*e.g.,* altitude – Simonson *et al.*, 2010; Storz *et al.*, 2010,

40 temperature – Clarke and Johnston, 1999, energy source – Montooth *et al.*, 2003), we

41 lack a systematic understanding of the distribution of this variation across biogeographic

42 space. We can address this important knowledge gap by evaluating mitochondrial

43 function across species ranges, which will provide insight into how phenotypic variation

44 is partitioned across populations and environments.

45 *Potamopyrgus antipodarum,* a New Zealand freshwater snail (Winterbourn,

46 1970), is ideally suited for investigating spatial patterns in mitochondrial functional

47 variation. There is both extensive mtDNA population structure in their native range

48 (Neiman and Lively, 2004; Neiman *et al.*, 2010; Paczesniak *et al.*, 2013) and evidence

49 for local adaptation to biotic (Lively and Jokela, 1996; Krist *et al.*, 2000; Dybdahl and

50 Krist, 2004; Jokela *et al.*, 2009; King *et al.*, 2009; Koskella and Lively, 2009; Bankers *et*

51 *al.*, 2017) and abiotic (Lively and Jokela, 1996; Krist *et al.*, 2000; Krist *et al.*, 2014; Krist

52 *et al.*, 2017) environmental conditions of their source lakes. Temperature in particular

53 appears to be a primary determinant of the geographical distribution of *P. antipodarum*

54 within New Zealand (Winterbourn, 1969). Because sexual and asexual *P. antipodarum*

55 frequently coexist in nature (Lively, 1987) and because asexuality has arisen multiple

56 times within *P. antipodarum* (Neiman and Lively, 2004; Neiman *et al.*, 2011), distinct

57 asexual lineages can be treated as repeated "natural experiments" into the

58 consequences of asexuality for mitochondrial function. Asexual production of male

59 offspring by obligately asexual female *P. antipodarum* (Neiman *et al.*, 2012) also makes

60 it possible to assay sex-specific mitochondrial function, even in asexual lineages whose

61 mitochondrial genomes have been "trapped" in females for generations. These features,

62 along with extensive and heritable variation for mitochondrial performance among

63 asexual lineages (Sharbrough *et al.*, 2017), allow *P. antipodarum* to be used for

64   powerful tests of the contribution of sexual reproduction to the processes underlying

65   mitonuclear coevolution.

66   Here, we tested whether source population, reproductive mode, or sex (male vs.

67   female) affect mitochondrial and physiological function at organismal and organellar

68   levels under laboratory conditions in field-collected *P. antipodarum*. We found evidence

69   that metabolic rate and behavioral response to heat stress vary across *P. antipodarum*

70   from different New Zealand lakes. The importance of lake-of-origin as a determinant of

71   mitochondrial phenotype demonstrates that direct and rigorous comparisons of

72   phenotypic function between sexual and asexual lineages and between males and

73   females in *P. antipodarum* will require extensive within-lake sampling. Altogether, our

74   results confirm previous reports of extensive variation for mitochondrial function in *P.*

75   *antipodarum* (Sharbrough *et al.*, 2017), extend these results to natural populations, and

76   set the stage for powerful comparisons between fitness-relevant traits in sexual and

77   asexual snails.

78

79   **MATERIALS AND METHODS**

80   **Field collections of *P. antipodarum***

81   While the phenotypic and ecological similarity of sexual *vs.* asexual *P. antipodarum*

82   enable direct comparisons across reproductive modes, it also means that definitive

83   determination of reproductive mode requires snail sacrifice. We therefore sampled field-

84   collected snails from New Zealand lakes known to harbor sexual and asexual

85   individuals, with populations at least ~10% male (Neiman *et al.*, 2011; Paczesniak *et al.*,

86   2013; Bankers *et al.*, 2017). Upon arrival at the University of Iowa, snails were housed

87   at 16°C on a 18hr light/6 hr dark schedule, and fed *Spirulina* algae 3x per week, as

88   described in (Zachar and Neiman, 2013). We arbitrarily selected adult snails from lake

89   collections (each of which consisted of 100s-1000s of individuals) and isolated each

90   snail in a 0.5 L glass container with 300ml carbon-filtered H_2O. Water was changed

91   weekly. Two distinct samples were used in this study: (1) we assayed oxygen

92   consumption under heat stress in 57 wild-caught females, and (2) we assayed

93   behavioral function and mitochondrial membrane potential in 46 wild-caught male and

94   female snails (Table 1). All functional and behavioral assays began immediately

95   following isolation, and all assays were completed within six months of arrival at the

96   University of Iowa.

97

98   **Oxygen consumption under heat stress conditions**

99   Because oxygen becomes limiting to ectotherms under elevated temperatures (Abele *et*

100   *al.*, 2007) and because *P. antipodarum* demonstrates signs of stress at elevated

101   (~30°C) temperatures (*e.g.,* reduced fecundity – Dybdahl and Kane, 2005, elevated

102   oxygen consumption and decreased righting ability – Sharbrough *et al.*, 2017), we

103   measured oxygen consumption using an aquatic respirometer as described in

104   (Sharbrough *et al.*, 2017) for 57 wild-caught female *P. antipodarum* from each of six

105   lakes. Oxygen consumption was assayed at three different water temperatures: 16°C

106   (not stressful, and similar to New Zealand lake temperatures), 22°C (moderately

107   stressful), and 30°C (stressful) (Sharbrough *et al.*, 2017). Each snail was assayed at

108   each temperature in a randomly determined order, and only snails that survived across

109   all three temperature treatments were included in our analyses. Wet mass for each

110   individual was calculated after each trial, and mean mass across all three trials was

111   used in our final analyses.

112

Behavioral response to heat stress

114   Righting time (Sharbrough *et al.*, 2017) and time to emergence following a startling

115   stimulus – hereafter "shyness" – (M Neiman pers. obs.) increase with temperature in *P.*

116   *antipodarum,* indicating that both assays can be used to assess heat-induced stress.

117   We quantified righting times and shyness under each of the same three temperature

118   treatments as for oxygen consumption in 46 wild-caught *P. antipodarum* and compared

119   behavior reaction norms across temperatures, lakes, reproductive modes, and sexes.

120   Snails were each assayed once at each temperature, and only snails surviving all three

121   temperature treatments were included in our final analyses.

122

Mitochondrial membrane potential

124   JC-1 is a small positively charged molecule that diffuses down the electrochemical

125   gradient across the inner mitochondrial membrane (Garner and Thomas, 1999). Under

126   UV illumination, JC-1 fluoresces green if dispersed and red if aggregated (e.g., when

127   inside the mitochondrial matrix) (Garner and Thomas, 1999). As a result, the median

128   ratio of red: green fluorescence in freshly isolated, live mitochondria stained with JC-1

129   can serve as a proxy for mitochondrial membrane potential. We isolated mitochondria

130   from 46 wild-caught *P. antipodarum* and measured red: green ratios in JC-1-treated

131   mitochondrial extracts as described in Sharbrough *et al.* (2017) using a Becton

132   Dickenson LSR II flow cytometer. Using only the set of 46 snails that completed

133 behavioral assays at all three temperatures, we compared median red: green ratios

134 across lakes, reproductive modes, and sexes.

135

136 **Determination of reproductive mode**

137 Sexual *P. antipodarum* are diploid and asexual *P. antipodarum* are polyploid (triploid or

138 tetraploid – Wallace, 1992; Neiman *et al.*, 2011), allowing us to use flow cytometry to

139 determine DNA content and, thus, reproductive mode of individual *P. antipodarum*. After

140 completing all physiological and/or behavioral assays, we dissected head tissue from

141 each individual snail, flash froze this tissue in liquid nitrogen, and stored the frozen

142 tissue at -80ºC until flow cytometry. We then homogenized head tissue in DAPI solution,

143 filtered this solution through a 30 µm filter, and ran the solution on a Becton-Dickinson

144 FACS Aria II following the protocol (including a chicken red blood cell standard) outlined

145 in Krist *et al.* (2014).

146

147 **Statistical analyses**

148 We used a mixed-effects model framework to quantify the relationships between oxygen

149 consumption and behavioral metrics (righting time, shyness) with categorical variables

150 for temperature (16º C, 22º C, 30º C), lake of origin (n = 3-6 depending on the analysis),

151 reproductive mode (asexual, sexual), sex (male, female; only fit in models pertaining to

152 behavior assays), and a continuous variable for mass (g; only fit in model pertaining to

153 oxygen consumption). We modeled a term for snail identity as a random intercept to

154 account for repeated measures on individuals across temperatures. Finally, we modeled

155 mitochondrial membrane potential, measured as the ratio of red: green fluorescence, as

156   a function of lake, reproductive mode, and sex, using analysis of variance (ANOVA),

157   which does not assume balanced or large sample sizes in its inferences. Only main

158   effects were considered, as the study was not designed to investigate interactions.

159   We developed final models using backwards selection until only predictors with

160   *p*-values less than 0.05 remained. To test assumptions of normality and

161   heteroscedasticity of errors, we graphically inspected residuals and log- or square-root-

162   transformed response variables when necessary. We performed all statistical analyses

163   in R (R Core Team, 2017), fitting fixed-effect models with the *lm* function, fitting mixed-

164   effects models using the lme4 package (Bates *et al.*, 2007), and estimating degrees of

165   freedom for mixed-effect models using Satterthwaite's approximation *via* the lmerTest

166   package (Kuznetsova *et al.*, 2015).

167

168   **RESULTS**

169   All model-fitting results are detailed in Table 2.

170   We measured oxygen consumption in 57 female *P. antipodarum* collected from

171   six New Zealand lakes (see Table 1) under three different temperature regimes (*i.e.,*

172   16ºC, 22ºC, and 30ºC). We found that temperature ($p < 0.0001$), mass ($p = 0.00154$),

173   and lake of origin ($p = 0.0072$), but not reproductive mode, moderated the rate of

174   oxygen consumption (Fig. 1a, Table 2). This result indicates that snails collected from

175   different lakes exhibit distinct respiratory responses to laboratory-controlled heat stress

176   treatments.

177   Using a second sample of 46 male and female *P. antipodarum* from six New

178   Zealand lakes (see Table 1), we tested whether lake-of-origin, reproductive mode, sex,

179   and temperature were associated with righting time and shyness under heat stress

180   conditions. We found that temperature was a significant predictor of both our behavioral

181   assays of righting ability ($p < 0.0001$, Fig. 1b) and of shyness ($p < 0.0001$, Fig. 1c). Lake

182   of origin was a significant predictor of righting time ($p = 0.0155$) but not of shyness.

183   Neither reproductive mode nor sex were significantly associated with either behavior in

184   response to heat stress. These data indicate that elevated temperatures stress *P.*

185   *antipodarum* sufficiently to alter behavior, and that righting ability differs across New

186   Zealand lake populations of *P. antipodarum.*

187   We next assayed mitochondrial membrane potential in live mitochondrial

188   extracts using the fluorescent dye JC-1 in this same set of 46 field-collected snails. We

189   found that sex, but not lake of origin or reproductive mode, was a significant predictor of

190   mitochondrial membrane potential ($p = 0.0070$, Fig. 2), with higher mitochondrial

191   membrane potential in males *vs.* females. We had relatively low power to detect

192   differences in mitochondrial membrane potential across lakes (power = 0.427) or sexes

193   (power = 0.365). Together these data indicate that while mitochondrial membrane

194   potential differs across sexes in *P. antipodarum*, larger sample sizes are necessary to

195   compare mitochondrial membrane potential across lakes and reproductive modes.

196

197   **DISCUSSION**

198   Extensive work in the *P. antipodarum* system has documented lake-specific phenotypes

199   and local adaptation for resistance to infection by the trematode parasite *Microphallus*

200   *livelyi* (Lively and Jokela, 1996; Krist *et al.*, 2000; Dybdahl and Krist, 2004; Jokela *et al.*,

201   2009; King *et al.*, 2009; Koskella and Lively, 2009; Bankers *et al.*, 2017), life history

11

202   traits such as growth rate and size (Larkin *et al.*, 2016), and response to nutrient

203   limitation (Krist *et al.*, 2014; Krist *et al.*, 2017). Here, we report the first evidence of

204   population-structured variation for mitochondrial and behavioral function in *P.*

205   *antipodarum*. Combined with population structure for mitochondrial genetic variation

206   (Neiman and Lively, 2004; Neiman *et al.*, 2010; Neiman *et al.*, 2011; Paczesniak *et al.*,

207   2013), this result suggests the intriguing possibility that mitochondrial function is locally

208   tuned in *P. antipodarum*.

209   These data set the stage for downstream studies addressing multiple important

210   evolutionary questions. Chief among these questions is whether and how asexuality

211   might influence mitonuclear coevolution. While the elevated linkage disequilibrium

212   expected to result from asexuality should reduce the efficacy of natural selection in both

213   nuclear (Fisher, 1930; Muller, 1964; Hill and Robertson, 1966; Birky and Walsh, 1988;

214   Kondrashov, 1993; Charlesworth, 2012) and mitochondrial genomes (Gabriel *et al.*,

215   1993; Normark and Moran, 2000; Neiman and Taylor, 2009), stable transmission of

216   mitonuclear genotypes may also facilitate rapid mitonuclear coadaptation and thereby

217   local adaptation (Neiman and Linksvayer, 2006). There are few empirical tests of

218   whether and how asexuality affects mitochondrial performance, leaving an important

219   gap in our understanding of the evolutionary consequences of changes in reproductive

220   mode. Surveys of mitochondrial genomes of asexual lineages (Paland and Lynch, 2006;

221   Johnson and Howard, 2007; Neiman *et al.*, 2010; Henry *et al.*, 2012; Sharbrough *et al.*,

222   2018) have revealed elevated rates of putatively harmful mutations in mitochondrial

223   genomes compared to sexual lineages. Absent nuclear compensation for mitochondrial

224   function (*e.g.,* Harrison and Burton, 2006; Osada and Akashi, 2012), we predict

225 mitochondrial genomes carried by asexual lineages will therefore be associated with

226 reduced mitochondrial performance. Evaluating whether these mutations actually result

227 in reduced function will have profound implications for our understanding of the

228 maintenance of sex. Importantly, the strong lake effect observed here means that the

229 effects of reproductive mode and sex on mitochondrial and behavioral function can only

230 be rigorously evaluated with extensive within-lake sampling.

231   Asexuality, and its implications for mitonuclear coevolution are especially

232 relevant considering the invasion success of some asexual *P. antipodarum* lineages.

233 Indeed, invasive lineages of *P. antipodarum* are entirely asexual (Wallace, 1992);

234 however, only a few lineages of asexual *P. antipodarum* have successfully colonized

235 novel environments to become invasive (Stadler *et al.*, 2005; Dybdahl and Drown, 2011;

236 Levri *et al.*, 2017). The extensive but heterogeneous colonization success around the

237 world by *P. antipodarum* therefore suggests that there exists substantial variation for

238 invasive potential among asexual lineages, making this snail model a useful system in

239 which to test hypotheses relating to traits associated with successful invasion (*e.g.,*

240 Dybdahl and Kane, 2005). Notably, the indirect evidence that a particular mitochondrial

241 haplotype is spreading among asexual lineages (haplotype 1A –Paczesniak *et al.*,

242 2013), is particularly intriguing in this context, as it may indicate an association between

243 mitochondrial haplotype and migration in asexual *P. antipodarum*. The relationship

244 between mitochondrial performance and stress documented here and in (Sharbrough *et*

245 *al.*, 2017), combined with the co-transmission of mitonuclear genotypes in asexual

246 lineages outlined above raises the possibility that invasive *P. antipodarum* may exhibit

247 particularly good mitochondrial performance in response to environmental stress.

248 Maternal transmission of mitochondrial genomes precludes inheritance of all

249 mitochondrial mutations originating in males, with two primary consequences: 1) ~50%

250 reduction in N_e relative to a scenario in which cytoplasmic genomes are inherited

251 biparentally, and 2) sexually antagonistic mutations only experience effective natural

252 selection in females (Frank and Hurst, 1996; Gemmell *et al.*, 2004). This latter

253 phenomenon, the so-called "mother's curse", is predicted to result in the accumulation

254 of mutations that are neutral or beneficial in females, but deleterious in males (Camus *et*

255 *al.*, 2012). The lack of widespread evidence for mother's curse (but see Innocenti *et al.*,

256 2011; Patel *et al.*, 2016; Camus and Dowling, 2017) may point to mechanisms that

257 prevent the spread of male-specific deleterious mutations in mitochondrial genomes

258 (*e.g.,* paternal leakage – Kuijper *et al.*, 2015, inbreeding – Wade and Brandvain, 2009,

259 kin selection – Wade and Brandvain, 2009, nuclear-encoded restorers of male function

260 – Delph *et al.*, 2007; Dowling *et al.*, 2007). We found that male *P. antipodarum* have

261 higher mitochondrial membrane potential than females, leading us to speculate that

262 male *P. antipodarum* do not suffer from mother's curse type mutations that decrease

263 their ability to generate a proton motive force. Importantly, mitochondrial membrane

264 potential depends upon protons pumped by OXPHOS complexes comprised of both

265 nuclear and mitochondrial gene products (*i.e.,* complexes I, III, and IV Hatefi, 1985),

266 meaning that we should expect to detect reduced mitochondrial membrane potentials if

267 male-harming mutations accumulate in *P. antipodarum* mitochondrial genomes. Still,

268 sex-specific optima for mitochondrial membrane potentials make this prediction difficult

269 to evaluate. Asexual *P. antipodarum* occasionally produce males (Neiman *et al.*, 2012),

270 such that nuclear and mitochondrial genomes that have been "trapped" in females for

14

271   generations are suddenly expressed in a male context. These asexual males are

272   expected to exhibit particularly poor mitochondrial performance because selection

273   against male-harming mutations is completely ineffective in asexual lineages, making

274   them uniquely well suited to evaluate the efficacy of selection against mother's curse in

275   nature.

276

277   **CONCLUSIONS**

278   Our results point toward population-specific mitochondrial and behavioral function that is

279   related to temperature, which has implications for climate change response and

280   colonization success in this globally invasive species. This lake effect necessitates

281   extensive sympatric sampling when comparing mitochondrial function in *P. antipodarum*.

282   Together, our results establish *P. antipodarum* as a model system for evaluating

283   mutational hypotheses for sex *via* comparisons of mitochondrial performance in sexual

284   *vs.* asexual snails and for evaluating the strength and efficacy of selection against

285   mother's curse in sexual populations.

DATA ACCESSIBILITY

286 Oxygen consumption, behavioral, and mitochondrial membrane potential data will be

288 made freely accessible upon acceptance.

289

AUTHOR CONTRIBUTIONS

291 ESG, JTS contributed to all aspects of study, SF to statistical analyses and manuscript

292 drafting, JLC, MN to concept, statistical design, and manuscript drafting, and JDW, SKH,

293 MRK, JAM, and MRP to data collection and manuscript editing.

294

CONFLICT OF INTEREST

296 The authors declare no conflicts of interest.

297

ACKNOWLEDGEMENTS

299 Flow cytometry was performed at UI's Flow Cytometry Facility. We thank Justin Havird

300 and two anonymous reviewers for comments on an earlier version of this manuscript,

301 and Laura Bankers, Kaitlin Hatcher, Katelyn Larkin, and Kyle McElroy for snail

302 collections.

16

303 **FIGURE LEGENDS**

304 **Figure 1. Physiological responses to heat stress for *P. antipodarum* across**

305 **source lakes**. a) Oxygen consumption/hour/gram. b) Righting time. c) Shyness.

306

307 **Figure 2. Mitochondrial membrane potential in field-collected *P. antipodarum*.**

308 Ratios of red: green fluorescence of JC-1-treated mitochondrial extracts for a) snails

309 from all six New Zealand lakes, b) male *vs.* female snails from all lakes.

17

REFERENCES

Abele, E., Philip, E., Gonzalez, P.M., & Puntarulo, S. (2007). Marine invertebrate mitochondria and oxidative stress. *Frontiers in Bioscience,* 12, 933-946.

Arnqvist, G., Dowling, D.K., Eady, P., Gay, L., Tregenza, T., Tuda, M., & Hosken, D.J. (2010). Genetic architecture of metabolic rate: Environment specific epistasis between mitochondrial and nuclear genes in an insect. *Evolution,* 64, 3354-3363.

Ballard, J.W., & Whitlock, M.C. (2004). The incomplete natural history of mitochondria. *Molecular Ecology,* 13, 729-744.

Bankers, L., Fields, P., McElroy, K.E., Boore, J.L., Logsdon, J.M., & Neiman, M. (2017). Genomic evidence for population-specific responses to co-evolving parasites in a New Zealand freshwater snail. *Molecular Ecology,* 26, 3663-3675.

Barreto, F.S., & Burton, R.S. (2013). Elevated oxidative damage is correlated with reduced fitness in interpopulation hybrids of a marine copepod. *Proceedings of the Royal Society of London B: Biological Sciences,* 280, 20131521.

Bates, D., Sarkar, D., Bates, M.D., & Matrix, L. (2007). The lme4 package. R package version 2:74.

Birky Jr., C.W., & Walsh, J.B. (1988). Effects of linkage on rates of molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America,* 85, 6414-6418.

Blier, P.U., Dufresne, F., & Burton, R.S. (2001). Natural selection and the evolution of mtDNA-encoded peptides: Evidence for intergenomic co-adaptation. *Trends in Genetics,* 17, 400-406.

Bock, D.G., Andrew, R.L., & Rieseberg, L.H. (2014). On the adaptive value of cytoplasmic genomes in plants. *Molecular Ecology,* 23, 4899-4911.

Burton, R.S., Pereira, R.J., & Barreto, F.S. (2013). Cytonuclear genomic interactions and hybrid breakdown. *Annual Review of Ecology, Evolution, and Systematics,* 44, 281-302.

Camus, M.F., Clancy, D.J., & Dowling, D.K. (2012). Mitochondria, maternal inheritance, and male aging. *Current Biology,* 22, 1717-1721.

Camus, M.F., & Dowling, D.K. (2017). Mitochondrial genetic effects on reproductive success: Signatures of positive intra-sexual, but negative inter-sexual pleiotropy. *bioRxiv*, 138180.

Castellana, S., Vicario, S., & Saccone, C. (2011). Evolutionary patterns of the mitochondrial genome in metazoa: Exploring the role of mutation and selection in mitochondrial protein coding genes. *Genome Biology and Evolution*, 3, 1067-1079.

Charlesworth, B. (2012). The effects of deleterious mutations on evolution at linked sites. *Genetics,* 190, 5-22.

Chen, J.H., Hales, C.N., & Ozanne, S.E. (2007). DNA damage, cellular senescence and organismal ageing: Causal or correlative? *Nucleic Acids Research*, 35, 7417-7428.

Chung, D.J., Bryant, H.J., & Schulte, P.M. (2017). Thermal acclimation and subspecies-specific effects on heart and brain mitochondrial performance in a eurythermal teleost (*Fundulus heteroclitus*). J*ournal of Experimental Biology*, 220, 1459-1471.

Clarke, A., & Johnston, N.M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, 68, 893-905.

Delph, L.F., Touzet, P., & Bailey, M.F. (2007). Merging theory and mechanism in studies of gynodioecy. *Trends in Ecology and Evolution*, 22, 17-24.

Dobler, R., Rogell, B., Budar, F., & Dowling, D.K. (2014). A meta-analysis of the strength and nature of cytoplasmic genetic effects. *Journal of Evolutionary Biology,* 27, 2021-2034.

Dowling, D.K. (2014). Evolutionary perspectives on the links between mitochondrial genotype and disease phenotype. *Biochimica et Biophysica Acta,* 1840, 1393-1403.

Dowling, D.K., Friberg, U., & Arnqvist, G. (2007). A comparison of nuclear and cytoplasmic genetic effects on sperm competitiveness and female remating in a seed beetle. *Journal of Evolutionary Biology*, 20, 2113-2125.

Dowling, D.K., Friberg, U., & Lindell, J. (2008). Evolutionary implications of non-neutral mitochondrial genetic variation. *Trends in Ecology and Evolution*, 23, 546-554.

Dybdahl, M.F., & Drown, D.M. (2011). The absence of genotypic diversity in a successful parthenogenetic invader. *Biological Invasions*, 13, 1663-1672.

Dybdahl, M.F., & Kane, S.L. (2005). Adaptation vs. phenotypic plasticity in the success of a clonal invader. *Ecology*, 86, 1592-1601.

Dybdahl, M.F., & Krist, A.C. (2004). Genotypic vs. condition effects on parasite-driven rare advantage. *Journal of Evolutionary Biology*, 17, 967-973.

Ellison, C.K., & Burton, R.S. (2006). Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. *Evolution*, 60, 1382-1391.

19

Fisher, R.A. (1930). *The genetical theory of natural selection*. Oxford, UK: The Clarendon Press.

Frank, S.A., & Hurst, L.D. (1996). Mitochondria and male disease. *Nature*, 383, 224-224.

Gabriel, W., Lynch, M., & Burger, R. (1993). Muller's ratchet and mutational meltdowns. *Evolution*, 47, 1744-1757.

Galtier, N., Nabholz, B., Glemin, S., & Hurst, G.D. (2009). Mitochondrial DNA as a marker of molecular diversity: A reappraisal. *Molecular Ecolology*, 18, 4541-4550.

Garner, D.L., & Thomas, C.A. (1999). Organelle-specific probe JC-1 identifies membrane potential differences in the mitochondrial function of bovine sperm. *Molecular Reproduction and Development*, 53, 222-229.

Gemmell, N.J., Metcalf, V.J., & Allendorf, F.W. (2004). Mother's curse: The effect of mtDNA on individual fitness and population viability. *Trends in Ecology and Evolution,* 19, 238-244.

Harrison, J.S., & Burton, R.S. (2006). Tracing hybrid incompatibilities to single amino acid substitutions. *Molecular Biology and Evolution*, 23, 559-564.

Hatefi, Y. (1985). The mitochondrial electron transport and oxidative phosphorylation system. *Annual Review of Biochemistry*, 54, 1015-1069.

Havird, J.C., & Sloan, D.B. (2016). The roles of mutation, selection, and expression in determining relative rates of evolution in mitochondrial versus nuclear genomes. *Molecular Biology and Evolution*, 33, 3042-3053.

Hebert, P.D., Ratnasingham, S., & deWaard, J.R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B: Biological Sciences,* 270, S96-99.

Henry, L., Schwander, T., & Crespi, B.J. (2012). Deleterious mutation accumulation in asexual *Timema* stick insects. *Molecular Biology and Evolution*, 29, 401-408.

Hill, W.G., & Robertson, A. (1966). The effect of linkage on limits to artificial selection. *Genetical Research*, 8, 269-294.

Innocenti, P., Morrow, E.H., & Dowling, D.K. (2011). Experimental evidence supports a sex-specific selective sieve in mitochondrial genome evolution. *Science*, 332, 845-848.

Johnson, S.G., & Howard, R.S. (2007). Contrasting patterns of synonymous and nonsynonymous sequence evolution in asexual and sexual freshwater snail lineages. *Evolution*, 61, 2728-2735.

Jokela, J., Dybdahl, M.F., & Lively, C.M. (2009). The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails. *American Naturalist*, 174, S43-S53.

King, K.C., Delph, L.F., Jokela, J., & Lively, C.M. (2009). The geographic mosaic of sex and the red queen. *Current Biology,* 19, 1438-1441.

Kondrashov, A.S. (1993). Classification of hypotheses on the advantage of amphimixis. *Journal of Heredity*, 84, 372-387.

Koskella, B., & Lively, C.M. (2009). Evidence for negative frequency-dependent selection during experimental coevolution of a freshwater snail and a sterilizing trematode. *Evolution,* 63, 2213-2221.

Krist, A.C., Bankers, L., Larkin, K., Larson, M.D., Greenwood, D.J., Dyck, M.A., & Neiman, M. (2017). Phosphorus availability in the source population influences response to dietary phosphorus quantity in a New Zealand freshwater snail. *Oecologia*, 185, 595-605.

Krist, A.C., Kay, A.D., Larkin, K., & Neiman, M. (2014). Response to phosphorus limitation varies among lake populations of the freshwater snail *Potamopyrgus antipodarum*. *PLoS ONE*, 9, e85845.

Krist, A.C., Lively, C.M., Levri, E.P., & Jokela, J. (2000). Spatial variation in susceptibility to infection in a snail-trematode interaction. *Parasitology*, 121, 395-401.

Kuijper, B., Lane, N., Pomiankowski, A. (2015). Can paternal leakage maintain sexually antagonistic polymorphism in the cytoplasm? *Journal of Evolutionary Biology,* 28, 468-480.

Kuznetsova, A., Brockhoff, P.B., & Christensen, R.H.B. (2015). Package 'lmertest'. R package version 2.

Larkin, K., Tucci, C., & Neiman, M. (2016). Effects of polyploidy and reproductive mode on life history trait expression. *Ecology and Evolution*, 6, 765-778.

Levri, E.P., Landis, S., Smith, B., Colledge, E., Metz, E., & Li, X.S. (2017). Variation in predator-induced behavioral changes in introduced and native populations of the invasive New Zealand mud snail (*Potamopyrgus antipodarum,* Gray, 1843). *Aquatic Invasions*, 12, 499-508.

Lively, C.M. (1987). Evidence from a New Zealand snail for the maintenance of sex by parasitism. *Nature*, 328, 519-521.

Lively, C.M., & Jokela, J. (1996). Clinal variation for local adaptation in a host-parasite interaction. *Proceedings of the Royal Society of London B: Biological Sciences*, 263, 891-897.

Montooth, K.L., Abt, D.N., Hofmann, J.W., & Rand, D.M. (2009). Comparative genomics of *Drosophila* mtDNA: Novel features of conservation and change across functional domains and lineages. *Journal of Molecular Evolution*, 69, 94-114.

Montooth, K.L., Marden, J.H., & Clark, A.G. (2003). Mapping determinants of variation in energy metabolism, respiration and flight in *Drosophila*. *Genetics*, 165, 623-635.

Muller, H.J. (1964). The relation of recombination to mutational advance. *Mutation Research*, 106, 2-9.

Neiman, M., Hehman, G., Miller, J.T., Logsdon Jr., J.M., & Taylor, D.R. (2010). Accelerated mutation accumulation in asexual lineages of a freshwater snail. *Molecular Biology and Evolution*, 27, 954-963.

Neiman, M., Larkin, K., Thompson, A.R., & Wilton, P. (2012). Male offspring production by asexual *Potamopyrgus antipodarum*, a New Zealand snail. *Heredity*, 109, 57-62.

Neiman, M., & Linksvayer, T.A. (2006). The conversion of variance and the evolutionary potential of restricted recombination. *Heredity*, 96, 111-121.

Neiman, M., & Lively, C.M. (2004). Pleistocene glaciation is implicated in the phylogeographical structure of *Potamopyrgus antipodarum*, a New Zealand snail. *Molecular Ecology*, 13, 3085-3098.

Neiman, M., Paczesniak, D., Soper, D.M., Baldwin, A.T., & Hehman, G. (2011). Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. *Evolution*, 65, 3202-3216.

Neiman, M., & Taylor, D.R. (2009). The causes of mutation accumulation in mitochondrial genomes. *Proceedings of the Royal Society of London B: Biological Sciences*, 276, 1201-1209.

Nespolo, R.F., Figueroa, J., & Solano-Iguaran, J.J. (2017). Studying the evolutionary significance of thermal adaptation in ectotherms: The diversification of amphibians' energetics. *Journal of Thermal Biology*, 68, 5-13.

Nilsson, J.A., Akesson, M., & Nilsson, J.F. (2009). Heritability of resting metabolic rate in a wild population of blue tits. *Journal of Evolution Biology*, 22, 1867-1874.

Normark, B.B., & Moran, N.A. (2000). Opinion - testing for the accumulation of deleterious mutations in asexual eukaryote genomes using molecular sequences. *Journal of Natural History*, 34, 1719-1729.

Oellermann, M., Portner, H.O., & Mark, F.C. (2012). Mitochondrial dynamics underlying thermal plasticity of cuttlefish (*Sepia officinalis*) hearts. *Journal of Experimental Biology*, 215, 2992-3000.

Osada, N., & Akashi, H. (2012). Mitochondrial-nuclear interactions and accelerated compensatory evolution: Evidence from the primate cytochrome c oxidase complex. *Molecular Biology and Evolution*, 29, 337-346.

Paczesniak, D., Jokela, J., Larkin, K., & Neiman, M. (2013). Discordance between nuclear and mitochondrial genomes in sexual and asexual lineages of the freshwater snail *Potamopyrgus antipodarum*. *Molecular Ecology,* 22, 4695-4710.

Paland, S., & Lynch, M. (2006). Transitions to asexuality result in excess amino acid substitutions. *Science,* 311, 990-992.

Patel, M.R., Miriyala, G.K., Littleton, A.J., Yang, H.K., Trinh, K., Young, J.M., Kennedy, S.R., Yamashita, Y.M., Pallanck, L.J., & Malik, H.S. (2016). A mitochondrial DNA hypomorph of cytochrome oxidase specifically impairs male fertility in *Drosophila melanogaster*. *eLife,* 5, e16923.

Pike, T.W., Blount, J.D., Bjerkeng, B., Lindstrom, J., & Metcalfe, N.B. (2007). Carotenoids, oxidative stress and female mating preference for longer lived males. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 1591-1596.

R Core Team. (2017). R: A language and environment for statistical computing. Vienna, Austria; 2014.

Rand, D.M. (2001). The units of selection on mitochondrial DNA. *Annual Review of Ecology and Systematics*, 32, 415-448.

Sadowska, E.T., Labocha, M.K., Baliga, K., Stanisz, A., Wroblewska, A.K., Jagusiak, W., & Koteja, P. (2005). Genetic correlations between basal and maximum metabolic rates in a wild rodent: Consequences for evolution of endothermy. *Evolution*, 59, 672-681.

Sharbrough, J., Cruise, J.L., Beetch, M., Enright, N.M., & Neiman, M. (2017). Genetic variation for mitochondrial function in the New Zealand freshwater snail *Potamopyrgus antipodarum*. *Journal of Heredity*, 108, 759-768.

Sharbrough, J., Luse, M., Boore, J.L., Logsdon Jr., J.M., & Neiman, M. (2018). Radical amino acid mutations persist longer in the absence of sex. *Evolution*.

Simonson, T.S., Yang, Y.Z., Huff, C.D., Yun, H.X., Qin, G., Witherspoon, D.J., Bai, Z.Z., Lorenzo, F.R., Xing, J.C., Jorde, L.B., Prchal, J.T., & Ge, R.L. (2010). Genetic evidence for high-altitude adaptation in tibet. *Science*, 329, 72-75.

Sloan, D.B., Havird, J.C., & Sharbrough, J. (2017). The on-again, off-again relationship between mitochondrial genomes and species boundaries. *Molecular Ecology,* 26, 2212-2236.

Städler, T., Frye, M., Neiman, M., & Lively, C.M. (2005). Mitochondrial haplotypes and the New Zealand origin of clonal european *Potamopyrgus*, an invasive aquatic snail. *Molecular Ecology*, 14, 2465-2473.

Storz, J.F., Scott, G.R., & Cheviron, Z.A. (2010). Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *Journal of Experimental Biology*, 213, 4125-4136.

Ursi, S., Pedersen, M., Plastino, E., & Snoeijs, P. (2003). Intraspecific variation of photosynthesis, respiration and photoprotective carotenoids in *Gracilaria birdiae* (Gracilariales: Rhodophyta). *Marine Biology*, 142, 997-1007.

Wade, M.J., & Brandvain, Y. (2009). Reversing mother's curse: Selection on male mitochondrial fitness effects. *Evolution*, 63, 1084-1089.

Wallace, C. (1992). Parthenogenesis, sex and chromosomes in *Potamopyrgus*. *Journal of Molluscan Studies*, 58, 93-107.

Winterbourn, M. (1969). Water temperature as a factor limiting the distribution of *Potamopyrgus antipodum* (Gastropoda‐Prosobranchia) in the New Zealand thermal region. *New Zealand Journal of Marine and Freshwater Research*, 3, 453-458.

Winterbourn, M. (1970). Population studies on the New Zealand freshwater gastropod, *Potamoptrgus antipodarum* (Gray). *Journal of Molluscan Studies*, 39, 139-149.

Zachar, N., Neiman, M. (2013). Profound effects of population density on fitness-related traits in an invasive freshwater snail. *PLoS ONE*, 8, e80067.

Zhang, F., Broughton, R.E. (2013). Mitochondrial-nuclear interactions: Compensatory evolution or variable functional constraint among vertebrate oxidative phosphorylation genes? *Genome Biology and Evolution*, 5, 1781-1791.
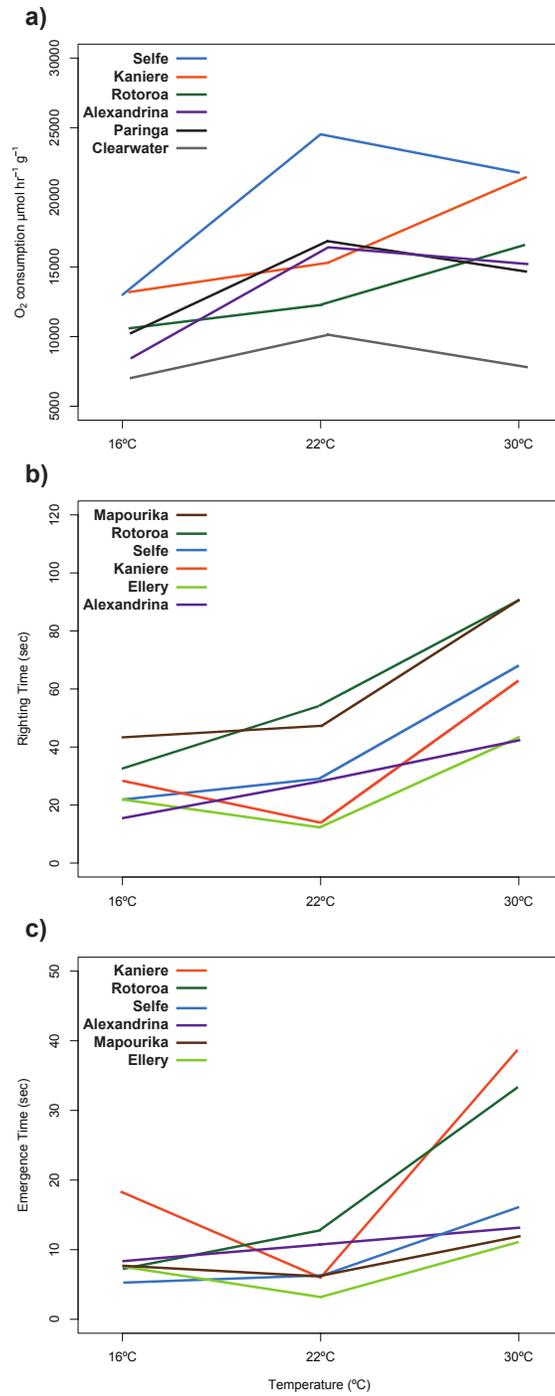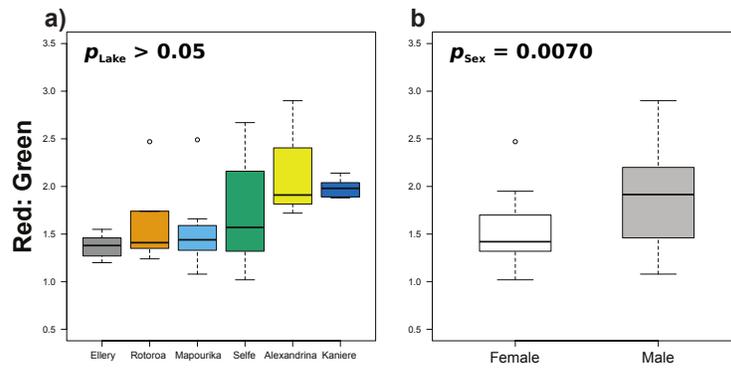
Table 1. Summary of source populations of *Potamopyrgus antipodarum* collected from New Zealand lakes.

Oxygen consumption assay

Lake	Latitude, Longitude	Sexual	Asexual	Male	Female
Alexandrina	-43.900476, 170.453978	14	2	-	16
Clearwater	-43.602131, 171.043917	-	4	-	4
Kaniere	-42.832886, 171.14759	16	-	-	16
Paringa	-43.713068, 169.411348	5	-	-	5
Rotoroa	-41.855414, 172.637882	-	17	-	17
Selfe	-43.237765, 171.520449	-	3	-	3

Behavior and mitochondrial membrane potential assays[1]

Lake	Latitude, Longitude	Sexual	Asexual	Male	Female
Alexandrina	-43.900476, 170.453978	3	-	3	-
Ellery	-44.046898, 168.654261	2	3	-	5
Kaniere	-42.832886, 171.14759	5	1	4	2
Mapourika	-43.315212, 170.204061	8	2	6	4
Rotoroa	-41.855414, 172.637882	4	1	-	5
Selfe	-43.237765, 171.520449	9	8	9	8

[1] – Same individual snails were used in behavioral and mitochondrial membrane potential assays

Table 2. Linear and mixed-effects models of select predictors on oxygen consumption, righting time, shyness, and mitochondrial membrane potential.

Oxygen consumption[1]

Factor	χ^2	df	p	Non-significant predictors[6]
Intercept[2]	2.700	1	0.1004	
Temperature	39.038	2	< 0.0001***	Reproductive Mode
Mass	11.061	1	0.0009***	
Lake of Origin	15.280	5	0.0092**	

Righting time[3]

Factor	χ^2	df	p	Non-significant predictors[6]
Intercept[2]	59.205	1	< 0.0001***	
Temperature	73.661	2	< 0.0001***	Reproductive Mode, Sex
Lake of Origin	14.020	5	0.0155*	

Shyness[4]

Factor	χ^2	df	p	Non-significant predictors[6]
Intercept[2]	448.891	1	< 0.0001***	Reproductive Mode,
Temperature	46.646	2	< 0.0001***	Sex, Lake of Origin

Mitochondrial membrane potential[5]

Factor	Sum of Squares	df	F	p	Non-significant predictors[6]
Intercept	55.876	1	305.104	< 0.0001***	Reproductive Mode,
Sex	1.466	1	8.004	0.0070**	Lake of Origin
Residuals	8.058	44			

[1] – Type III Repeated-Measures Analysis of Deviance χ2 Test of O_2 consumption per hour

[2] – Snail ID was fit as a random intercept

[3] – Type III Repeated-Measures Analysis of Deviance χ2 Test of log-transformed righting times

[4] – Type III Repeated-Measures Analysis of Deviance χ2 Test of square-root-transformed shyness

[5] – Type III Analysis of Variance F Test of log-transformed ratios of red: green in mitochondrial extracts

[6] – Non-significant predictors listed in order of elimination from the model

* – $p < 0.05$

** – $p < 0.01$

*** – $p < 0.001$