A tale of two genomes: What drives mitonuclear discordance in asexual lineages of a freshwater snail?

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Abstract

We use genomic information to tell us stories of evolutionary origins. But what does it mean when different genomes report wildly different accounts of lineage history? This "discordance" can be a consequence of a fascinating suite of natural history and evolutionary phenomena, from the different inheritance mechanisms of nuclear vs. cytoplasmic genomes to hybridization and introgression to horizontal transfer. Here, we explore how we can use these distinct genomic stories to provide new insights into the maintenance of sexual reproduction, one of the most important unanswered questions in biology. We focus on the strikingly distinct nuclear vs. mitochondrial versions of the story surrounding the origin and maintenance of asexual lineages in *Potamopyrgus antipodarum*, a New Zealand freshwater snail. While key questions remain unresolved, these data inspire multiple testable hypotheses that can be powerfully applied to understand the maintenance of sex and origin of new asexual lineages in this unique natural animal system.

Sexuals are from Mars and asexuals are from Venus? Distinct tales from different genomes

Nuclear and cytoplasmic genomes often tell different evolutionary stories. An excellent example of this phenomenon is provided by the first-ever analysis of the Neanderthal nuclear genome (Green et al. 2010): while the earlier studies of mitochondrial genomes did not reveal any evidence for human-Neanderthal hybridization (e.g., Serre et al. 2004), the nuclear data demonstrated clear and striking evidence to the contrary. Recent sequencing of 13 Neanderthal samples revealed extensive cytonuclear discordance, with differences between mitochondrial and Y-chromosome variation providing evidence for patrilocal family structure (Skov et al. 2022). In the human-Neanderthal example, and as is typical for sexually reproducing lineages, nuclear genomes are inherited biparentally, whereas cytoplasmic (organelle) genomes are typically inherited uniparentally, often through the macrogamete (Camus et al. 2022). This separate inheritance can generate differences in nuclear vs. cytoplasmic genealogies (i.e., cytonuclear discordance).

Two primary evolutionary processes can give rise to cytonuclear discordance: differential rates of incomplete lineage sorting in nuclear vs. cytoplasmic genomes, with cytoplasmic genomes tending to sort faster than the nuclear genome (Degnan & Rosenberg 2009; Good et al. 2015), or introgression between lineages/species (reviewed by Sloan et al. 2017). In particular, sex-biased dispersal (Currat et al. 2009), sex-based asymmetry in hybrid fitness (Turelli and Moyle 2007), interspecific variation in female choosiness (Hendrick 2010), hybridization-induced loss of strict maternal inheritance (Barnard-Kubow et al. 2017), adaptive gene flow (Llopart et al. 2014; Melo-Ferreira et al. 2014; Morales et al. 2015), cytoplasmic capture (Tsitrone et al. 2003; Forsythe et al. 2020), or even horizontal transfer (Stegemann et

al. 2012) can all lead to biased introgression (e.g., Bonnet et al. 2017) or non-introgression (e.g., Sharbrough et al. 2017a) of mitochondrial DNA (mtDNA) vs. nuclear DNA (Chan and Levin 2005). Altogether, it is thus not surprising that many sexually reproducing species exhibit signatures of cytonuclear discordance (Rieseberg and Soltis 1991; Toews and Brelsford 2012).

It is more difficult to understand why cytonuclear discordance is also found in asexually reproducing lineages (e.g., Paczesniak et al. 2013; Bourret et al. 2018; Obertegger et al. 2018; Kueler et al. 2020; Meng et al. 2021), despite nuclear and cytoplasmic genome co-transmission as a single genetic unit from mother to daughter. Interspecific hybridization is strongly associated with the origin of asexuality in both parthenogenetic animals and apomictic plants ("apomixis") and is an obvious and frequent source of cytonuclear discordance in asexuals (Avise 1994; Hojsgaard and Hörandl 2019). In such lineages, cytoplasmic and nuclear genomes are "frozen" in the resulting asexual hybrid, with ~50% of nuclear alleles exhibiting discordance relative to the captured organellar genome(s). Furthermore, the evolution of cytonuclear discordance in an asexual lineage is not static, but rather can be influenced by various phenomena (e.g., ploidy, parent of origin effects, occasional sex/genetic exchange, repeated derivation, etc.) through time.

Here, we provide a novel synthetic perspective, drawing insights from natural history and mechanisms of transmission and inheritance, on how patterns of cytonuclear discordance in asexual lineages, and the mechanisms that drive them, can help illuminate one of the most important open questions in biology – the nature of the mechanisms driving the maintenance of sexual reproduction ("sex"). Our particular focus is on how the fundamentally different

transmission dynamics between the nuclear and cytoplasmic genomes in sexual taxa can provide insights into transitions to asexuality and fitness in sexual vs. asexual lineages. We provide an example from a classic animal model system for the evolution and maintenance of sexual reproduction, using its mitochondrial and nuclear genomic "stories" to glean insight into the origin(s) of asexuality.

Understanding genetic diversity in sexuals vs. asexuals: a critical tool for solving a huge evolutionary "problem"

Potamopyrgus antipodarum, a tiny freshwater snail native to New Zealand, provides a powerful means to study sex because natural populations of *P. antipodarum* often harbor both obligately sexual and obligately asexual individuals (Lively 1987). The frequent coexistence of otherwise similar organisms that differ in reproductive mode allows the direct comparisons of sexual and asexual individuals and populations that are needed to understand why sex persists in nature (Neiman et al. 2018), one of the most pressing open questions in evolutionary biology. Such systems also enable direct study of mitonuclear interactions and discordance by affording a unique opportunity to interrogate the interplay between reproductive mode and nuclear vs. cytoplasmic genome evolution

Evolutionary theory predicts that sexual populations that are prone to invasion by asexual lineages should be quickly driven to extinction (Lively 1996). This expectation is based largely on the substantial "cost of males" imposed on sexual females that devote half of their reproductive investment to sons relative to asexual females that produce only daughters. This cost of males - which will be two-fold if sexuals and asexuals are otherwise equal - translates

into a much higher growth rate of asexual vs. sexual populations that should rapidly result in the loss of the sexuals (Maynard Smith 1978).

Whether asexual invasion actually poses a threat to real sexual populations depends on the extent to which the sexual and asexual individuals and populations are otherwise similar (Meirmans et al. 2012). Critical steps towards quantifying this threat requires using genetic markers to establish two key pieces of information that tie directly back to this question of similarity. First, are the coexisting sexual and asexual populations closely related? This information is important because relatively closely related sexuals and asexuals are likely to be phenotypically and ecologically similar and, thus, compete directly (Neiman et al. 2018). Second, how much genetic diversity does the asexual population harbor? Estimating asexual genetic diversity is important because many hypothesized mechanisms for the maintenance of sex (e.g., the Red Queen) can only maintain sex if there is high genetic diversity in the sexual population relative to asexual population (e.g., Howard and Lively 1994). These two criteria are also interrelated with respect to a third critical factor for the maintenance of sex, the rate of origin of new asexual lineages from sexual ancestors (Burt 2000). In particular, a high rate of origin poses a major threat to sex both in generating relatively high relatedness between sexuals and asexuals (because many asexual lineages will have very recent sexual ancestors) and because the repeated origin of these new asexual lineages will tend to maintain high asexual diversity relative to a situation where this rate is relatively low.

Asexual origin stories as told by two different genomes

The nuclear point of view

Dybdahl and Lively (1995) used allozyme electrophoresis of six nuclear markers to demonstrate that for each of four different lake populations, asexual *P. antipodarum* were more closely related to sympatric sexual *P. antipodarum* than to allopatric asexual counterparts. The allelic diversity of the asexual subpopulations was high, often close to that of coexisting sexual subpopulations, but was nevertheless a subset of the sexual allele pool. Heterozygosity of the asexual individuals was also not appreciably higher than that of the sexuals, despite higher ploidy levels in the former (Neiman et al. 2011). Together, these data indicated that asexual *P. antipodarum* are the product of many recent and separate transitions to asexuality from local sexual *P. antipodarum*. Fox et al. (1996) used the same nuclear-encoded markers to genotype a relatively large sample of snails collected from one of the four lakes featured in Dybdahl and Lively (1996). This study revealed virtually the same results: asexual *P. antipodarum* appeared to be recently and repeatedly derived from coexisting sexuals, translating into substantial genotypic diversity in the asexuals.

The first genomics era survey of nuclear variation in sexual vs. asexual *P. antipodarum* is reported in Paczesniak et al. (2013), who used 23 single-nucleotide polymorphism (SNP) markers to genotype over 500 *P. antipodarum* from 16 distinct New Zealand lake populations. Similar to the earlier allozyme-based studies, the results of Paczesniak et al. (2013) again pointed to recent and repeated derivation of most asexual *P. antipodarum* lineages from sympatric sexuals along with marked population genetic structure. However, this newer, more extensive survey did depart markedly from those earlier studies in revealing that asexuals in some populations harbored nuclear genotypes not found in coexisting sexuals and even sharing some of these non-local genotypes across lakes.

We can use these surveys of population-level variation in the *P. antipodarum* nuclear genome to provide an answer to the central questions about the origins and diversity of asexuals: most - but not all - new asexual *P. antipodarum* lineages are repeatedly and frequently derived from still-extant sexual lineages, maintaining high asexual diversity, and posing a fundamental challenge to the maintenance of sex.

The mitochondrial perspective

Neiman and Lively (2004) were the first to compare sequence variation and population structure in sexual and asexual *P. antipodarum* from the perspective of the mitochondrial genome. This study revealed high mitochondrial haplotypic and nucleotide diversity in which both population and biogeographic region were associated with genetic structure. The analyses also provided a distinct line of evidence for polyphyletic and often local origins of asexual lineages from coexisting sexual *P. antipodarum*. Perhaps the most striking outcome was the discovery of an overwhelmingly common haplotype, "1A", that defied the otherwise predominant pattern of strong population structure. This mitochondrial haplotype was found in nearly 1/3 of all of the *P. antipodarum* included in the study and in 15 of the 20 lakes surveyed. Neiman et al. (2005) used this same dataset to estimate the time since derivation of a representative sample of asexual *P. antipodarum* from sexual conspecifics. While most asexual lineages harbored mitochondrial haplotypes shared with sexual snails, indicating divergence from sexual *P. antipodarum* within the last 150,000 years (and likely much more recently), a handful of asexual lineages had mitochondrial haplotypes that were as much as 2% diverged

from the closest sexual relative. These data suggest that these asexual lineages, termed "old asexual" clades, could be as much as a few million years old.

Neiman et al. (2011) used a similar New Zealand-wide sample, this time combined with flow cytometric determination of sexual vs. asexual status, to again report evidence for the polyphyletic origin of mostly recently derived but some older asexual lineages. This study also replicated Neiman and Lively (2004) in finding that haplotype 1A was markedly more common than any other haplotype and took these results a step further by showing that sexual snails, and even those sympatric with haplotype 1A-bearing snails, almost never harbored haplotype 1A. Paczesniak et al. (2013) employed a similar approach to generating and evaluating mitochondrial sequence variation in sexual and asexual *P. antipodarum* as Neiman et al. (2011), reporting virtually identical results regarding population structure, polyphyletic asexual lineage derivation, including the existence of mostly young but a few distinctly old-appearing asexual lineages, and the predominance of the previously reported "common" haplotype 1A. Like Neiman et al. (2011), Paczesniak et al. (2013) also demonstrated that haplotype 1A was much more likely to be found in asexual vs. sexual snails.

With respect to our focal questions about asexual lineage origins and similarities between sexual and asexual snails, these mitochondrial data do indicate that there have been multiple separate transitions to asexuality in *P. antipodarum* and that most - but perhaps not all - of these transitions have been recent. This picture is complicated by the fact that a large fraction, and perhaps a majority, of asexual snails harbor a single mitochondrial haplotype that is extremely rare in sexual counterparts. This last point hints at a more complex reality for asexual *P. antipodarum*.

Reconciling distinct genomic stories to reveal the origins of asexuality

The outcome of the comparisons of mitochondrial and nuclear genomic variation in sexual vs. asexual *P. antipodarum* defy the expectations laid out above: while the mitochondrial and nuclear data from sexual *P. antipodarum* are largely concordant, painting a picture of extensive population structure that is shaped in large part by biogeography, the asexual story is quite different (Paczesniak et al. 2013). Here, the nuclear data generally indicate a simple origin story of recent asexual lineage capture of a high diversity of nuclear genotypes from still-extant sexual lineages and, like the sexuals, reflect marked (though distinctly less) population structure and an important role of biogeography in defining this structure.

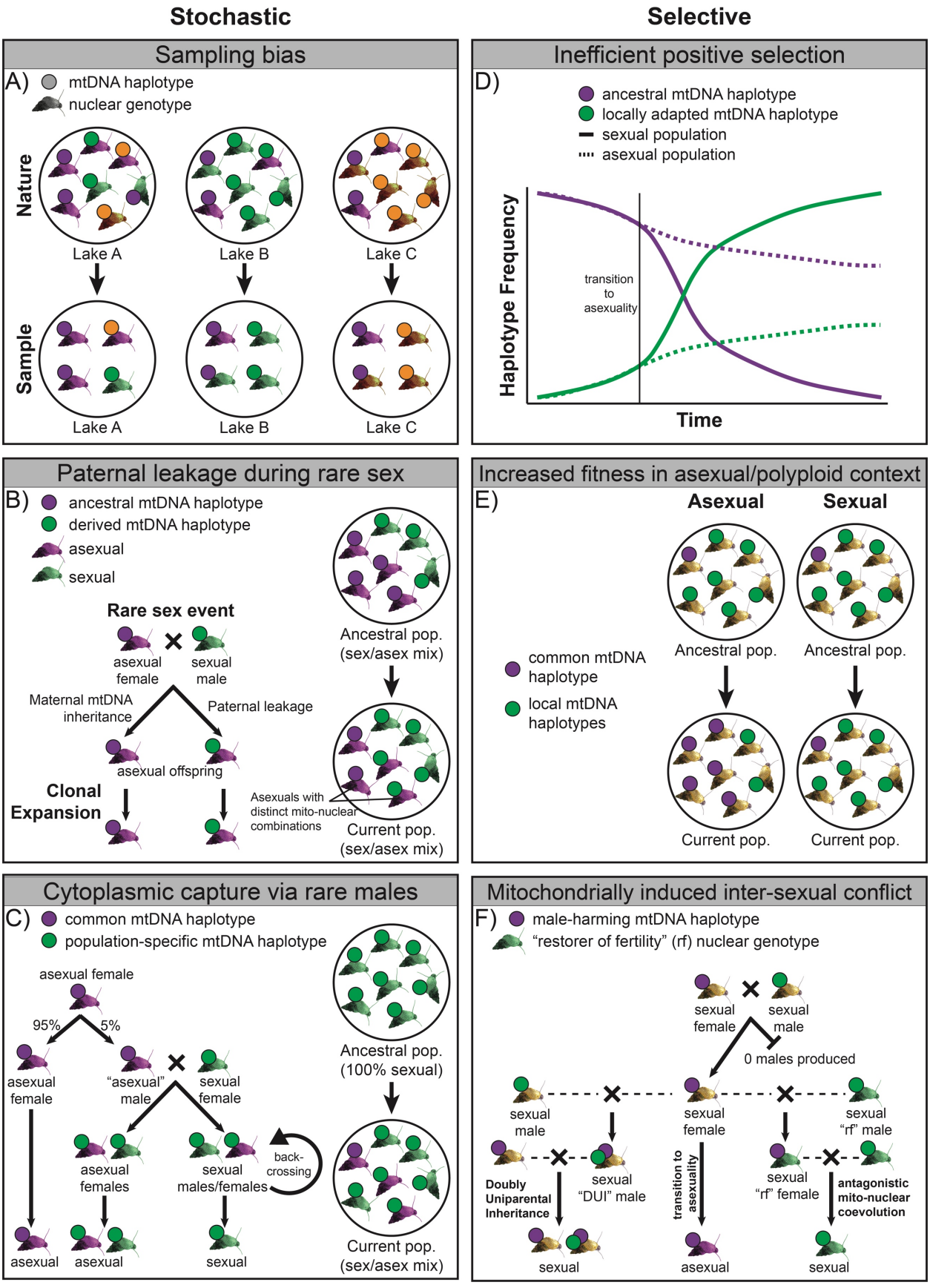
By contrast, the mitochondrial data hint at many fewer and perhaps geographically restricted origins of asexual *P. antipodarum*, especially with respect to the near-omnipresent haplotype 1A. By combining analysis of both nuclear and mitochondrial markers in the same snails, Paczesniak et al. (2013) was also able to definitively demonstrate a clear pattern of mitonuclear discordance in the asexual snails. The best example of this discordance comes from the discovery that haplotype 1A exists across many different endemic nuclear genotypes in many different lake populations of asexual *P. antipodarum*. The critical question that remains is how this haplotype, in the absence of departures from canonical sexual reproduction, can spread to such high frequency across so many nuclear genotypes in so many lakes, while simultaneously being extremely rare in coexisting sexual snails.

We address this question by laying out a series of hypotheses and predictions, each with in the text below and as illustrated in Figure 1, that delineate the various possible scenarios that

could give rise to such patterns of mitonuclear discordance in asexuals. We also discuss the fit of each hypothesis in the context of currently available data and suggest avenues for future study.

Explaining patterns of asexual mitonuclear discordance:

Both stochastic and selection-based mechanisms provide potential explanations for the existence of mitonuclear discordance in asexual *P. antipodarum* (and other asexual taxa, Figure 1). There are two selective dynamics that are most likely to produce differences in mitonuclear discordance across reproductive modes: (1) direct selection on mitochondrial function, and (2), selfish evolution of mitochondrial haplotypes. By contrast, stochastic processes that might explain these observations are diverse and varied in their mechanisms. Determining the nature of the processes contributing to mitonuclear discordance in asexual taxa is important because it may point towards – or away from – a role for mitochondria in the evolutionary maintenance of sex.


Stochastic
Selective
Sampling bias
A)
mtDNA haplotype
nuclear genotype
Nature
Lake A
Lake B
Lake C
Sample
Lake A
Lake B
Lake C
Inefficient positive selection
D)
ancestral mtDNA haplotype
locally adapted mtDNA haplotype
sexual population
asexual population
Haplotype Frequency
transition to asexuality
Time
Paternal leakage during rare sex
B)
ancestral mtDNA haplotype
derived mtDNA haplotype
asexual
sexual
Rare sex event
asexual female
sexual male
Maternal mtDNA inheritance
Paternal leakage
asexual offspring
Clonal Expansion
Asexuals with distinct mito-nuclear combinations
Ancestral pop. (sex/asex mix)
Current pop. (sex/asex mix)
Increased fitness in asexual/polyploid context
E)
Asexual
Sexual
common mtDNA haplotype
local mtDNA haplotypes
Ancestral pop.
Ancestral pop.
Current pop.
Current pop.
Cytoplasmic capture via rare males
C)
common mtDNA haplotype
population-specific mtDNA haplotype
asexual female
95%
5%
asexual female
"asexual" male
sexual female
asexual females
sexual males/females
back-crossing
asexual
asexual
sexual
Ancestral pop. (100% sexual)
Current pop. (sex/asex mix)
Mitochondrially induced inter-sexual conflict
F)
male-harming mtDNA haplotype
"restorer of fertility" (rf) nuclear genotype
sexual female
sexual male
0 males produced
sexual male
sexual female
sexual "rf" male
Doubly Uniparental Inheritance
sexual "DUI" male
transition to asexuality
sexual "rf" female
antagonistic mito-nuclear coevolution
sexual
asexual
sexual

225

Figure 1. Mechanisms capable of generating mitonuclear discordance in asexual lineages. A) Biased sampling and/or biased transitions to asexuality from a diverse sexual population could result in observations of different mitochondrial haplotype frequencies across reproductive modes. B) During rare sex events, asexual lineages may capture new mitochondrial haplotypes via paternal leakage, resulting in diverse mitochondrial haplotypes on the same nuclear backgrounds. C) Rare males produced by asexual females may be capable of spreading asexuality, resulting in cytoplasmic capture of new mitochondrial haplotypes. D) Reduced efficacy of natural selection in asexuals may allow for persistence of multiple mitochondrial haplotypes within populations. E) Mitochondrial haplotypes that perform better in an asexual and/or polyploid context may result in distinct patterns of mitochondrial haplotype frequencies in asexual lineages compared to sympatric sexual populations. F) Male-harming mitochondrial mutations are expected to result in inter-sexual conflict. Potential outcomes that would be expected to resolve this conflict include antagonistic mito-nuclear coevolution, biparental inheritance of mitochondria in males (e.g., doubly uniparental inheritance), or uniparental inheritance of the nuclear genome (i.e., asexuality).

Stochastic mechanisms:

Hypothesis 1: Apparent mitonuclear discordance in asexuals reflects sampling bias.

If genetic diversity is partitioned differently in sexual vs. asexual lineages (e.g., for some heavily parasitized populations of *P. antipodarum*; Fox et al. 1996), it is possible that previous sampling of asexual *P. antipodarum* is incomplete and not accurately capturing mitochondrial haplotypic

frequencies (Figure 1A). An apparent "sampling bias" may instead reflect an artifact of the population bottlenecks associated with transitions to asexuality from some subset of diverse sexual lineages. Regardless, under this hypothesis, we expect standing mitochondrial genetic variation in asexual lineages to approach that of sympatric sexual populations, and intra-population structure for mitochondrial haplotype frequencies.

Do the predictions hold?

A rigorous test of this hypothesis requires truly comprehensive sampling. While a part of the mitochondrial *cytochrome b* gene has been sequenced in thousands of *P. antipodarum* (e.g., Neiman and Lively 2004; Neiman et al. 2011; Paczesniak et al. 2013; Verhaegen et al. 2018), whether this sampling is "comprehensive enough" is not clear. What we do know is that to date, the documented empirical pattern of mitonuclear discordance in *P. antipodarum* does not match the prediction of similar standing genetic variation in sympatric sexuals and asexuals, particularly with respect to the overwhelming dominance (and corresponding lack of variation with respect to other haplotypes) of haplotype 1A across asexual *P. antipodarum* on the South Island of New Zealand (Neiman and Lively 2004; Neiman et al. 2011; Paczesniak et al. 2013). Within-population sampling with respect to mitochondrial DNA sequencing is also almost completely nonexistent in this system (but see Neiman et al. 2011 for one exception, shallow and deep regions of lake Alexandrina, that nevertheless does not demonstrate obvious structure).

Hypothesis 2: Paternal leakage during rare sex facilitates the spread of mitochondrial haplotypes.

Following the loss of sex, the machinery responsible for cellular processes related to sexual reproduction are expected to degrade (e.g., Jalinsky et al. 2020), which would include the molecular machinery responsible for elimination of the paternally derived mitochondrial genome. As a result, asexual lineages may be especially ineffective at enforcing uniparental inheritance of the mitochondrial genome, perhaps to an even greater extent than the nuclear genome. Thus, barriers to gene flow between sexual and asexual lineages may be more "leaky" for mitochondrial genomes than for nuclear genomes (Figure 1B). Under this hypothesis, asexual lineages coexisting with sexuals (and, thus, a relatively high frequency of males) should exhibit greater mitonuclear discordance than asexual lineages that do not coexist with sexual counterparts. In addition, because ploidy elevation in asexual *P. antipodarum* is thought to arise from rare sex events between asexual females and males (Neiman et al. 2011, 2012), all else being equal, there should exist greater degrees of mitonuclear discordance in tetraploid than triploid asexual *P. antipodarum*. Another expectation for which absence of evidence is not necessarily meaningful but for which concrete evidence is quite suggestive would be the existence within a single asexual lineage (defined via nuclear markers) of multiple distinct mtDNA haplotypes from different clades.

Do the predictions hold?

Datasets featuring the necessary extensive sampling across New Zealand that includes both wholly asexual populations as well as sympatric sexual and asexual *P. antipodarum* are fairly

rare, with a handful of examples to be found in Neiman et al. (2011) and Paczesniak et al. (2013). The same limitation applies to the triploid vs. tetraploid comparisons, which are also only available in these two studies. Figure 4 in Paczesniak et al. (2013) permits a preliminary visual comparison, with no obvious patterns emerging. Extensive sampling in the appropriate populations will be needed to provide a rigorous assessment of whether these predictions are met.

The data that underlie Paczesniak et al. (2013; DOI:10.5061/dryad.j18pv) do demonstrate that individual asexual lineages (represented by particular multilocus nuclear SNP genotypes) can harbor multiple mtDNA haplotypes. It is difficult to explain this pattern without resorting to paternal leakage as described above, although nuclear-encoded mitochondrial sequences (numts) could contribute to confusing intra-individual patterns (Hazkani-Cova et al. 2010). Experimental studies that pair sexual males with asexual females (which readily mate; Neiman and Lively 2005; Stork et al. 2022) and then track mitochondrial inheritance will provide an important means of detecting direct evidence for presence and frequency of paternal leakage.

Hypothesis 3: Production of rare males by asexual females and contagious asexuality results in cytoplasmic capture by asexual lineages.

Asexual *P. antipodarum* occasionally produce males (Neiman et al. 2012) that produce sperm (Soper et al. 2013) and copulate (Soper et al. 2015). If these males are able to successfully fertilize sexual females, and if asexuality can be "contagiously" transmitted by males (e.g., Maccari et al. 2014) such that some of these offspring are asexual females, new mitochondrial

haplotypes from sexual populations can be captured in asexual lineages (Figure 1C). Under this hypothesis, we would expect haplotype sharing across sexual and asexual lineages in these sympatric populations. Additionally, mitochondrial haplotype networks would be expected to provide a higher estimate of the number of separate transitions to asexuality compared to nuclear genealogies, reflecting the more recent coalescence with sexual lineages of the newly acquired mitochondrial genomes.

Do the predictions hold?

The data presented in Neiman and Lively (2004), Neiman et al. (2011), and in Paczesniak et al. (2013) do demonstrate some haplotype sharing across sympatric sexual and asexual individuals. Analogous to described above, experiments that pair asexual males with sexual females (copulation does occur in this setting; Soper et al. 2015) and then track offspring production and reproductive mode will be needed to directly demonstrate asexual contagion.

Selective mechanisms:

Hypothesis 4: Inefficient positive selection in asexual lineages may prevent fixation of locally adapted mitochondrial haplotypes.

Mitochondrial haplotypes can experience strong selection to match local environmental conditions (e.g., Xu et al. 2017). The efficacy of natural selection is expected to be reduced for asexual lineages (Hill and Robertson 1966), which should affect both the nuclear and mitochondrial genomes (Neiman and Taylor 2009). The implications are that if transitions to asexuality occur during mitochondrial selective sweeps, and if positive selection on

mitochondrial haplotypes is an important local selective force, then mitochondrial diversity and mitonuclear discordance would be expected to persist much longer in asexual lineages than in coexisting sexuals (Sharbrough et al. 2018) (Figure 1D). Under this hypothesis, mitochondrial substitution rates within lakes are expected to be higher in sexual vs. asexual lineages and sexual populations might exhibit starkly different (and less diverse) mitochondrial haplotype distributions compared to asexual lineages derived from those very same populations.

Do the predictions hold?

The data that we would need to perform a rigorous within-lake comparison of substitution rates do not exist. The second prediction, regarding different mtDNA haplotype distributions across sexual and asexual lineages, is reflected in the many New Zealand lakes where nearly all asexual individuals harbor haplotype 1A while sympatric sexual counterparts instead have different haplotypes, and more unique haplotypes per snail sampled (Neiman et al. 2011, Paczesniak et al. 2013).

Hypothesis 5: Elevated fitness of particular mitochondrial haplotypes in asexual and/or polyploid context.

Energetic demand could differ between asexuals and sexual counterparts for a variety of reasons, ranging from hybrid origin (reviewed in Hill et al. 2018, but no evidence for such to date in asexual *P. antipodarum*) to different life histories (e.g., more rapid maturation in asexual vs. sexual *P. antipodarum*, Larkin et al. 2016) or markedly different traits (e.g., resting egg production in some sexually vs. asexually reproducing monogonont rotifers (Pourroit and

Snell 1983). These phenomena could result in different mitochondrial haplotype frequencies in asexuals compared to sexuals (Figure 1E).

From this perspective, the most relevant way in which sexual and asexual *P. antipodarum* are known to differ is in ploidy; sexual *P. antipodarum* are diploid, and asexuals are typically triploid or tetraploid (Wallace 1992, Neiman et al. 2011). There is growing evidence that cytonuclear interactions are perturbed by polyploidy (Sharbrough et al., 2017b), such that polyploids exhibit elevated cytoplasmic genome copy numbers compared to diploid relatives (Fernandes-Gyorfy et al. 2021). As a consequence, polyploids may experience substantially different selection dynamics than sexuals, particularly with respect to cytoplasmic genome replication rate. Under this hypothesis, we might expect that haplotype 1A has a replication advantage over population-specific mitochondrial haplotypes, resulting in distinct mitonuclear combinations in sexual vs. asexual lineages.

Do the predictions hold?

We currently have no data on mitochondrial genome copy numbers per cell in sexual vs. asexual *P. antipodarum* lineages. Whole-genome sequencing data from sympatric sexual and asexual snails can be used to evaluate whether this prediction holds by comparing mitochondrial (relative to nuclear) read depth across reproductive modes. Evaluating replication rate advantages is particularly difficult to assess, but if heteroplasmy can be introduced into sexual lineages (see next hypothesis), relative comparisons of intra-individual mitochondrial genome proportions may be possible.

Hypothesis 6: Frequent inter-sexual conflict arising from male-harming mitochondrial mutations.

Because mitochondria are predominantly maternally transmitted, selfish mitochondria can bias their own transmission if they harbor mutations that have sex-specific fitness effects (e.g., male-harming mutations, Hurst 1991). The evolution of sex-specific fitness effects in mitochondria (i.e., inter-sexual conflict) is in turn expected to result in inter-genomic conflict between the nuclear and mitochondrial genomes (Camus et al. 2022). Resolution of inter-genomic conflict can occur through three potential pathways: 1) antagonistic co-evolution in which "restorers of fertility" mutations that arise in the nuclear genome restore male function (e.g., cytoplasmic male sterility, Hornett et al. 2006), 2) loss of strict maternal mitochondrial inheritance (e.g., Doubly Uniparental Inheritance, "DUI"; Zouros 2013), or acquisition of uniparental nuclear inheritance via transitions to asexuality (Figure 1F). If mitochondrial mutations with sex-specific effects are common, then the occurrence of the above mechanisms of resolution should also be common. Notably, DUI has only been documented in mollusks (Ghiselli et al. 2021), and cytoplasmic male sterility has recently been demonstrated in a gastropod (David et al. 2022), raising the intriguing possibility that male-harming mutations in molluscan mitochondrial genomes are relatively common (Breton et al. 2022).

Do these predictions hold?

The data do not yet exist to evaluate the extent to which this prediction is met. We do know that *Potamopyrgus* mitochondrial genomes exhibit signs of inter-haplotype recombination and apparent heteroplasmy (Sharbrough et al. 2022) and signatures of sex-specific optima of

mitochondrial function (Greimann et al. 2020), indicating that this type of sexual conflict is at least biologically possible in *P. antipodarum*. Additional tests of inheritance patterns in sexual lineages will provide a critical test of whether "leaky" mitochondrial inheritance exists in this species. Comparing mitochondrial function of mitochondrial haplotypes in male versus female contexts will be invaluable in testing for the presence of sex-specific effects of mutations, with the specific prediction that male mitochondrial function is expected to be reduced compared to female mitochondrial function.

Summary & open questions

The presence of mitonuclear discordance in naturally occurring asexual lineages is surprising, but can be generated by multiple stochastic and selective mechanisms. Determining which of these mechanisms, if any, contribute to asexual mitonuclear discordance, will both provide important information about the evolutionary history of the system as well as illuminate the broader question of whether mitonuclear discordance reveals a role for mitochondrial function in the evolutionary maintenance of sex.

Regarding stochastic hypotheses, mitochondrial genomes are most likely to suffer the harmful effects of Muller's Ratchet (Gabriel et al. 1993), but diverse mitochondrial haplotypes (hypothesis 1) will delay the onset of mutational meltdown and reduce the effects of clonal interference. Novel acquisition of new mitochondrial haplotypes by rare sex and leaky inheritance (hypothesis 2) or by cytoplasmic capture via contagious asexuality (hypothesis 3) can effectively reset the ratchet altogether. With respect to the selective mechanisms, reduced efficacy of selection in mitochondrial genomes of asexual lineages compared to sexual lineages

(hypothesis 4) could lead to reduced capacity for local adaptation of mitochondrial function in asexual vs. sexual lineages. The transition to higher ploidy levels in asexuals could also dramatically alter the energetic performance of asexual lineages (hypothesis 5), which may have both advantages (e.g., increased ATP production capacity) and disadvantages (e.g., increased energy demand) compared to diploids. Finally, if, as is posed in our sixth hypothesis, sex-specific mutations provide a scenario in which transitions to asexuality are common, then selection on mitochondria directly influences competition between sexuals and asexuals within populations, with direct implications for hypotheses such as the Red Queen, which is not expected to favor sexual reproduction when asexuals harbor similar diversity to competing sexuals (Howard and Lively 1994). Together, these possibilities raise a series of important and unanswered questions both for *P. antipodarum* and for other similar sexual/asexual systems, with direct relevance to understanding the maintenance of sex:

- What is the cause of mitonuclear discordance in asexual *P. antipodarum*?
- Does mitochondrial performance vary across sympatric sexual and asexual lineages? Across sexes?
- How "leaky" is mitochondrial inheritance in sexual *Potamopyrgus*?
- Does polyploidy alter the parameters of energy production?
- What is the mechanism underlying transitions to asexuality in *P. antipodarum*?

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Author Contributions

The paper idea came from MN. Both MN and JS contributed to paper conceptualization and drafting and editing of the paper. JS designed and created the figure.

Literature Cited

Avise, J. C. 1994. Molecular markers, Natural History, and Evolution. Chapman and Hall. New York.

Barnard-Kubow, K. B., M. A. McCoy, and L. F. Galloway. 2017. Biparental chloroplast inheritance leads to rescue from cytonuclear incompatibility. New Phytologist 213: 1466-1476.

Bonnet, T., R. Leblois, F. Rousset, and P. A. Crochet. 2017. A reassessment of explanations for discordant introgressions of mitochondrial and nuclear genomes. Evolution 71: 2140-2158.

Bourret, T. B., R. A. Choudhury, H. K. Mehl, C. L. Blomquist, N. McRoberts, and D. M. Rizzo. 2018. Multiple origins of downy mildews and mito-nuclear discordance within the paraphyletic genus *Phytophthora*. PloS ONE 13: e0192502.

Breton, S., D. T. Stewart, J. Brémaud, J. C. Havird, C. H. Smith, and W. R. Hoeh. 2022. Did doubly uniparental inheritance (DUI) of mtDNA originate as a cytoplasmic male sterility (CMS) system? BioEssays 44: 2100283.

Burt, A. 2000. Perspective: Sex, recombination, and the efficacy of selection: was Weismann right? Evolution 54: 337-351.

Camus, M. F., B. Alexander-Lawri, J. Sharbrough, and G. D. Hurst. 2022. Inheritance through the cytoplasm. Heredity 129: 31-43.

Currat, M., M. Ruedi, R. J. Petit, and L. Excoffier. 2008. The hidden side of invasions: massive introgression by local genes. Evolution 62: 1908-1920.

David, P., C. Degletagne, N. Saclier, A. Jennan, P. Jarne, S. Plénet, L. Konecny, C. François, L. Guéguen, N. Garcia, T. Lefébure, and E. Luquet. 2022. Extreme mitochondrial DNA divergence underlies genetic conflict over sex determination. Current Biology 32: 2325-2333.

Degnan, J. H., and N. A. Rosenberg. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends in Ecology & Evolution 24: 332-340.

Dybdahl, M. F., and C. M. Lively. 1995. Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail. Journal of Evolutionary Biology 8: 385-398.

Fernandes Gyorfy, M., E. R. Miller, J. L. Conover, C. E. Grover, J. F. Wendel, D. B. Sloan, and J. Sharbrough. 2021. Nuclear–cytoplasmic balance: whole genome duplications induce elevated organellar genome copy number. The Plant Journal 108: 219-230.

Forsythe, E. S., A. D. Nelson, and M. A. Beilstein. 2020. Biased gene retention in the face of introgression obscures species relationships. Genome Biology and Evolution 12: 1646-1663.

Fox, J. A., M. F. Dybdahl, J. Jokela, and C. M. Lively. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopyrgus antipodarum*). Evolution 50: 1541-1548.

Gabriel, W., M. Lynch and R. Bürger. 1993. Muller's ratchet and mutational meltdowns. Evolution 47: 1744-1757.

Ghiselli, F., A. Gomes-dos-Santos, C. M. Adema, M. Lopes-Lima, J. Sharbrough, and J. L. Boore. 2021. Molluscan mitochondrial genomes break the rules. Philosophical Transactions of the Royal Society B 376: 20200159.

Good, J. M., D. Vanderpool, S. Keeble, and K. Bi. 2015. Negligible nuclear introgression despite complete mitochondrial capture between two species of chipmunks. Evolution 69: 1961-1972.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H.-Y. Fritz, N. F. Hansen, E. Y. Durand, A.-S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prüfer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Höber, B. Höffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Ž. Kucan, I. Gušic, V. B. Doronichev, L. V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. F. Johnson, E. E. Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M. Lachmann, D. Reich, and S. Pääbo. 2010. A draft sequence of the Neandertal genome. Science 328: 710-722.

Greimann, E. S., S. F. Ward, J. D. Woodell, S. Hennessey, M. R. Kline, J. A. Moreno, M. Peters, J. L. Cruise, K. L. Montooth, M. Neiman, and J. Sharbrough. 2020. Phenotypic variation in mitochondria-related performance traits across New Zealand snail populations. Integrative and Comparative Biology, 60: 275-287.

Hazkani-Covo, E., R. M. Zeller, and W. Martin. 2010. Molecular poltergeists: mitochondrial DNA copies (numts) in sequenced nuclear genomes. PLoS Genetics 6: e1000834.

Hedrick, P. 2010. Cattle ancestry in bison: explanations for higher mtDNA than autosomal ancestry. Molecular Ecology 19: 3328-3335.

Hill, G. E. Mitonuclear mate choice: A missing component of sexual selection theory? BioEssays 40: 1700191.

Hill, W. G., and A. Robertson. 1966. The effect of linkage on limits to artificial selection. Genetical Research 8: 269-294.

Hojsgaard, D., and E. Hörandl. 2019. The rise of apomixis in natural plant populations. Frontiers in Plant Science 10: 358.

Hornett, E. A., S. Charlat, A. M. R. Duplouy, N. Davies, G. K. Roderick, N. Wedell, and G. D. D. Hurst. 2006. Evolution of male-killer suppression in a natural population. PLoS Biology 4: e283.

Howard, R. S., and C. M. Lively. 1994. Parasitism, mutation accumulation, and the maintenance of sex. Nature 367: 554-557.

Hurst, L. D. 1991. The incidences and evolution of cytoplasmic male killers. Proceedings of the Royal Society of London Series B: Biological Sciences 244: 91-99.

Jalinsky, J., J. M. Logsdon, Jr., and M. Neiman. 2020. Male phenotypes in a female framework: Evidence for degeneration in sperm produced by male snails from asexual lineages. Journal of Evolutionary Biology 33: 1050-1059.

553 Keuler, R., A. Garretson, T. Saunders, R. J. Erickson, N. St. Andre, F. Grewe, H. Smith, H. Thorsten
554 Lumbsch, J.-P. Huang, L. L. St. Clair, and S. D. Leavitt. 2020. Genome-scale data reveal the role of
555 hybridization in lichen-forming fungi. Scientific Reports 10: 1-14.
556
557 Larkin, K., C. Tucci, and M. Neiman. 2016. Effects of polyploidy and reproductive mode on life
558 history trait expression. Ecology and Evolution 6: 765-778.
559
560 Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism.
561 Nature 328: 519-521.
562
563 Lively, C. M. 1996. Host-parasite coevolution and sex. Bioscience, 46: 107-114.
564
565 Llopart, A., D. Herrig, E. Brud, and Z. Stecklein. 2014. Sequential adaptive introgression of the
566 mitochondrial genome in *Drosophila yakuba* and *Drosophila santomea*. Molecular Ecology 23:
567 1124-1136.
568
569 Maccari, M., F. Amat, F. Hontoria, and A. Gómez. 2014. Laboratory generation of new
570 parthenogenetic lineages supports contagious parthenogenesis in *Artemia*. PeerJ 2: e439.
571
572 Maynard Smith, J. 1978. The evolution of sex. Cambridge University Press, London, UK.
573

574 Meirmans, S., P. G. Meirmans, and L. R. Kirkendall. 2012. The costs of sex: Facing real-world
575 complexities. Quarterly Review of Biology 87: 19-40.
576
577 Melo-Ferreira, J., J. Vilela, M. M. Fonseca, R. R. da Fonseca, P. Boursot, and P. C. Alves. 2014.
578 The elusive nature of adaptive mitochondrial DNA evolution of an arctic lineage prone to
579 frequent introgression. Genome Biology and Evolution 6: 886-896.
580
581 Meng, K.-K., S.-F. Chen, K.-W. Xu, R.-C. Zhou, M.-W. Li, M. K. Dhamala, W.-B. Liao, and Q. Fan.
582 2021. Phylogenomic analyses based on genome-skimming data reveal cyto-nuclear discordance
583 in the evolutionary history of Cotoneaster (Rosaceae). Molecular Phylogenetics and Evolution
584 158: 107083.
585
586 Morales, H. E., A. Pavlova, L. Joseph, and P. Sunnucks. 2015. Positive and purifying selection in
587 mitochondrial genomes of a bird with mitonuclear discordance. Molecular Ecology 24: 2820-
588 2837.
589
590 Neiman, M., and C. M. Lively. 2004. Pleistocene glaciation is implicated in the
591 phylogeographical structure of *Potamopyrgus antipodarum*, a New Zealand snail. Molecular
592 Ecology 13: 3085-3098.
593

Neiman, M., and C. M. Lively. 2005. Male New Zealand (*Potamopyrgus antipodarum*) persist in copulating with asexual and parasitically castrated females. American Midland Naturalist 154: 88-96.

Neiman, M., and D. R. Taylor. 2009. The causes of mutation accumulation in mitochondrial genomes. Proceedings of the Royal Society of London B 276: 1201-1209.

Neiman, M., J. Jokela, and C. M. Lively. 2005. Variation in asexual lineage age in *Potamopyrgus antipodarum*, a New Zealand snail. Evolution 59: 1945-1952.

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

Neiman, M., P. G. Meirmans, T. Schwander, and S. Meirmans. 2018. Sex in the wild: how and why field-based studies contribute to solving the problem of sex. Evolution 72: 1194-1203.

Neiman, M., D. Paczesniak, D. M. Soper, A. T. Baldwin, and G. Hehman. 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.

614 Obertegger, U., A. Cieplinski, D. Fontaneto, and S. Papakostas. 2018. Mitonuclear discordance
615 as a confounding factor in the DNA taxonomy of monogonont rotifers. Zoologica Scripta 47:
616 122-132.
617
618 Paczesniak, D., J. Jokela, K. Larkin, and M. Neiman. 2013. Discordance between nuclear and
619 mitochondrial genomes in sexual and asexual lineages of the freshwater snail *Potamopyrgus*
620 *antipodarum*. Molecular Ecology 22: 4695-4710.
621
622 Pourriot, R., and T. W. Snell. 1983. Resting eggs in rotifers. Hydrobiologia 104: 213-224.
623 Rieseberg, L. H., and D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in
624 plants. Evolutionary Trends in Plants 5: 65–84.
625
626 Serre, D., A. Langaney, M. Chech, M. Teschler-Nicola, M. Paunovic, P. Mennecier, M. Hofreiter,
627 G. Possnert, and S. Pääbo. 2004. No evidence of Neandertal mtDNA contribution to early
628 modern humans. PLoS Biology: e57.
629
630 Sharbrough, J., J. L. Conover, J. A. Tate, J. F. Wendel, and D. B. Sloan. 2017. Cytonuclear
631 responses to genome doubling. American Journal of Botany 104: 1277-1280.
632
633 Sharbrough, J., J. C. Havird, G. R. Noe, J. M. Warren, and D. B. Sloan. 2017. The mitonuclear
634 dimension of Neanderthal and Denisovan ancestry in modern human genomes. Genome
635 Biology and Evolution 9: 1567-1581.

636

637 Sharbrough, J., M. Luse, J. L. Boore, J. M. Logsdon, Jr, M. Neiman. 2018. Radical amino acid
638 mutations persist longer in the absence of sex. Evolution 72: 808-824.

639

640 Sharbrough, J., L. Bankers, E., Cook, P. D. Fields, J. Jalinsky, K. E. McElroy, M. Neiman, J. M.
641 Logsdon, Jr., and J. L. Boore. 2022. Single-molecule sequencing of animal mitochondrial
642 genomes reveals chloroplast-like architecture and repeat-mediated recombination. bioRxiv
643 https://doi.org/10.1101/2022.08.11.503648.

644

645 Skov, L., S. Peyrégne, D. Popli, L. N. M. Iasi, T. Devièse, V. Slon, E. I. Zavala, M. Hajdinjak, A. P.
646 Sümer, S. Grote, A. Bossoms Mesa, D. López-Herráez, B. Nickel, S. Nagel, J. Richter, E. Essel, M.
647 Gansauge, A. Schmidt, P. Korlević, D. Comeskey, A. P. Derevianko, A. Kharevich, S. V. Markin, S.
648 Talamo, K. Douka, M. T. Krajcarz, R. G. Roberts, T. Higham, B. Biola, A. I. Krivoshapkin, K. A.
649 Kolobova, J. Kelso, M. Meyer, S. Pääbo, and B. M. Peter. 2022. Genetic insights into the social
650 organization of Neanderthals. Nature 610: 519-525.

651

652 Sloan, D. B., J. C. Havird, and J. Sharbrough. 2017. The on-again, off-again relationship between
653 mitochondrial genomes and species boundaries. Molecular Ecology 26: 2212-2236.

654

655 Soper, D. M., K. M. Hatcher, and M. Neiman. 2015. Documentation of copulatory behaviour in
656 triploid male freshwater snails. Ethology Ecology & Evolution 28: 110-116.

657

Soper, D. M., M. Neiman, O. P. Savytskyy, M. E. Zolan, and C. M. Lively. 2013. Spermatozoa production by triploid males in the New Zealand freshwater snail *Potamopyrgus antipodarum*. Biological Journal of the Linnean Society 110: 227-234.

Stegemann, S., M. Keuthe, S. Greiner, and R. Bock. 2012. Horizontal transfer of chloroplast genomes between plant species. Proceedings of the National Academy of Sciences of the United States of America 109: 2434-2438

Stork, S., J. Jalinsky, and M. Neiman. 2022. Evidence for stronger discrimination between conspecific and heterospecific mating partners in sexual vs. asexual female snails. PeerJ, in press.

Toews, D. P., and A. Brelsford. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Molecular Ecology 21: 3907-3930.

Tsitrone, A., M. Kirkpatrick, and D. A. Levin. 2003. A model for chloroplast capture. Evolution 57: 1776-1782.

Turelli, M., and L. C. Moyle. 2007. Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. Genetics 176: 1059-1088.

679 Verhaegen, G., M. Neiman, and M. Haase. 2018. Ecomorphology of a generalist freshwater
680 gastropod: complex relations of shell morphology, habitat, and fecundity. Organisms, Diversity
681 and Evolution 18: 425-441.
682
683 Wallace, C. 1992. Parthenogenesis, sex, and chromosomes in *Potamopyrgus*. Journal of
684 Molluscan Studies 58: 93-107.
685
686 Xu, S.-Y., D.-R. Sun, N. Song, T.-X. Gao, Z.-Q. Han, and B.-N. Shui. 2017. Local adaptation shapes
687 pattern of mitochondrial population structure in *Sebastiscus marmoratus*. Environmental
688 Biology of Fishes 100: 763-774.
689
690 Zouros, E. 2013. Biparental inheritance through uniparental transmission: the doubly
691 uniparental inheritance (DUI) of mitochondrial DNA. Evolutionary Biology 40: 1-31.
692
693
694