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Insights & Perspectives

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 genomic "discordance" can be a consequence of a fascinating suite of natural history and evolutionary phenomena, from the different inheritance mechanisms of nuclear versus cytoplasmic (mitochondrial and plastid) 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 versus 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 across a broad range of taxa toward a deeper understanding of the causes and consequences of mitonuclear discordance, the maintenance of sex, and the origin of new asexual lineages.

KEYWORDS
apomixis, cytonuclear, mitochondrial, mollusk, parthenogenesis, sexual reproduction

INTRODUCTION: SEXUALS ARE FROM MARS AND ASEXUALS ARE FROM VENUS? DISTINCT TALES FROM DIFFERENT GENOMES

Nuclear and cytoplasmic (mitochondrial and plastid) genomes often tell different evolutionary stories. This cytonuclear "discordance" is found between mitochondrial or plastid genomes and their nuclear counterparts (mitonuclear and plastid-nuclear discordance, respectively) and arises as a consequence of the different biology and genetic inheritance of these different genome types. An excellent example of cytonuclear discordance is provided by the first-ever analysis of the Neanderthal nuclear genome[1]: while the earlier studies of mitochondrial genomes did not reveal any evidence for human-Neanderthal hybridization,[2] the nuclear data demonstrated clear and striking evidence to the contrary. Additional more recent sequencing of 13 Neanderthal samples revealed extensive mitonuclear discordance.[3] In the human-Neanderthal example, and as is typical for sexually reproducing lineages, nuclear genomes are inherited biparentally, whereas mitochondrial genomes are typically inherited uniparentally.[4] This separate inheritance can generate differences in nuclear versus mitochondrial genealogies (i.e., mitonuclear discordance).

Two evolutionary processes can give rise to mitochondrial discordance: differential rates of incomplete lineage sorting in nuclear versus mitochondrial genomes, with mitochondrial genomes tending to sort faster than the nuclear genome,[5,6] or introgression between lineages/species [reviewed by 7]. In particular, sex-biased dispersal,[8] sex-based asymmetry in hybrid fitness,[9] interspecific variation in female choosiness,[10] hybridization-induced loss of

strict maternal inheritance,[11] adaptive gene flow,[12–14] mitochondrial capture,[15,16] or even horizontal transfer[17] can all lead to biased introgression[18] or non-introgression[19] of nuclear versus mitochondrial DNA.[20] Altogether, it is thus not surprising that many sexually reproducing species exhibit signatures of mitonuclear discordance.[21,22]

It is more difficult to understand why mitonuclear discordance is also found in asexually reproducing lineages,[23–30] despite co-transmission of the nuclear and mitochondrial genomes as a single genetic unit. Interspecific hybridization is strongly associated with the origin of asexuality in animals and plants and is an obvious and frequent source of mitonuclear discordance in asexuals.[31,32] In such lineages, mitochondrial and nuclear genomes are "captured" in the resulting asexual hybrid, with ~50% of nuclear alleles exhibiting discordance relative to the captured mitochondrial genome.

Here, we provide a novel synthetic perspective, drawing insights from natural history and mechanisms of transmission and inheritance, on how patterns of mitonuclear 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 mitochondrial genomes in sexual taxa can provide insights into transitions to asexuality and fitness in sexual versus asexual lineages.

We provide an example from a classic animal model system for the evolution and maintenance of sexual reproduction, using its nuclear and mitochondrial genomic "stories" to generate a set of testable hypotheses that can be used to glean insight into the origin and evolutionary fate of asexuality. We also hope that these ideas will inspire evaluation from these perspectives in other naturally occurring mixed sexual/asexual taxa.[28–30]

Understanding genetic diversity in sexuals versus 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.[33] The frequent coexistence of otherwise similar organisms that differ in reproductive mode allows for the direct comparisons of sexual and asexual individuals and populations that are needed to understand why sex persists in nature,[34] 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 versus mitochondrial genome evolution.

Evolutionary theory predicts that sexual populations that are prone to invasion by asexual lineages should be quickly driven to extinction.[35] 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 versus sexual populations that should rapidly result in the loss of the sexuals.[36]

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.[37] Critical steps towards quantifying this threat require 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.[34] 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 the asexual population.[38] 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.[39,28,29] 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[40] 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.[41] 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.[42] used the same nuclear-encoded markers to genotype a relatively large sample of snails collected from one of the four lakes featured in ref. [40]. 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 versus asexual *P. antipodarum* is reported in ref.[23], 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, Paczesniak et al.[23] 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 populations.

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[43] 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.[44] 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 – including haplotype 1A – harbored mitochondrial haplotypes shared at least on occasion 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.[41] used a similar New Zealand-wide sample, this time combined with flow cytometric determination of sexual versus 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[43] 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.[23] employed a similar approach to evaluating mitochondrial sequence variation in sexual and asexual *P. antipodarum* as Neiman et al.[41], and reported 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.,[41] Paczesniak et al.[23] also demonstrated that haplotype 1A was much more likely to be found in asexual versus 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 versus 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[23]. 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. Like the sexuals, these data also reflect marked (though distinctly less) population structure and an important role for 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.[23] 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 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 populations, while simultaneously being extremely rare in coexisting sexual snails.

We address this question by laying out a series of hypotheses and predictions 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. These discussions, while explicitly focused

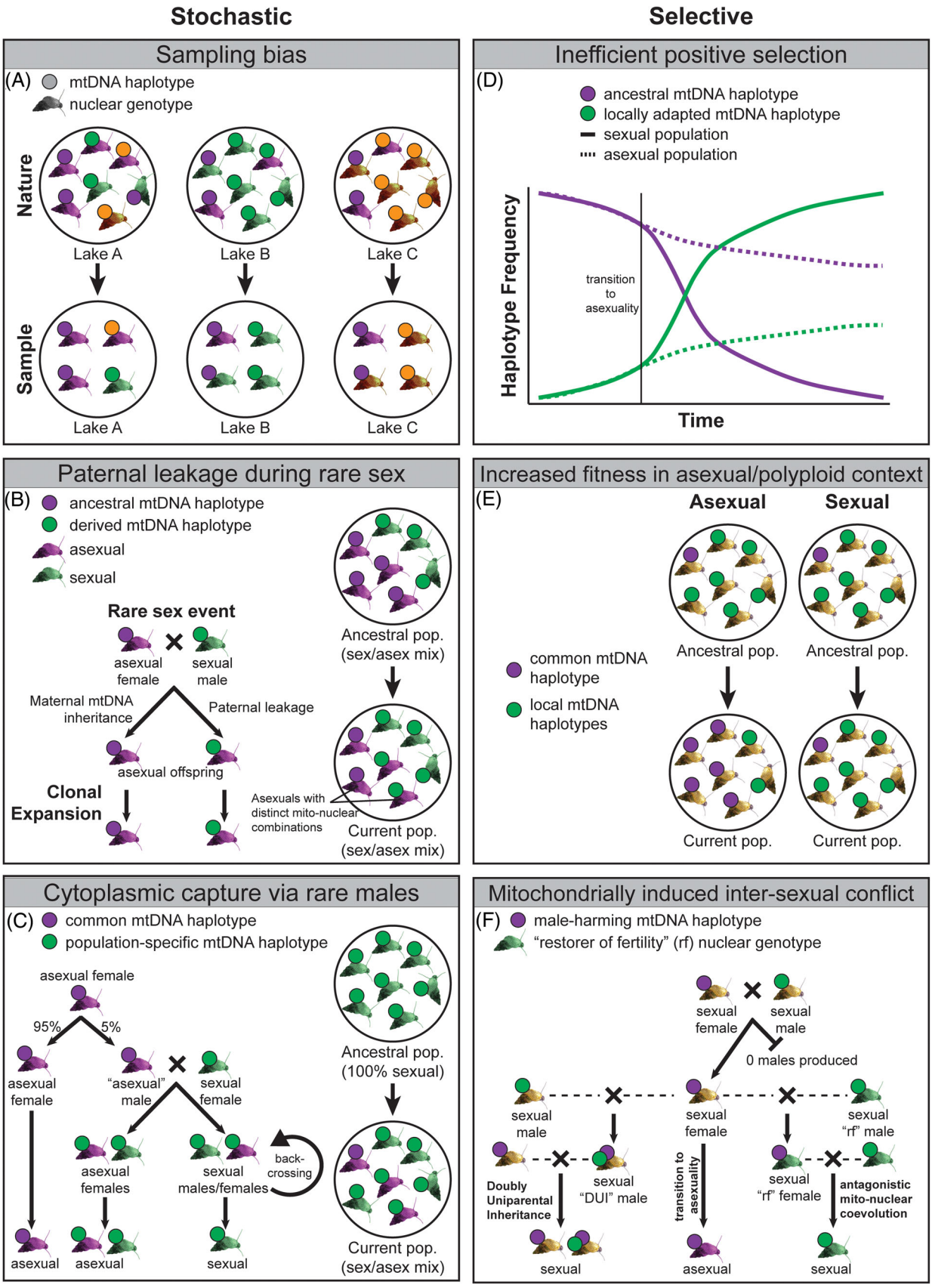

FIGURE 1 Mechanisms capable of generating mitonuclear discordance in asexual lineages. (A) Biased sampling and/or biased transitions to asexuality from a diverse sexual population could result in observations of different mitochondrial haplotype frequencies across reproductive modes. (B) During rare sex events, asexual lineages may capture new mitochondrial haplotypes via paternal leakage, resulting in diverse

on *P. antipodarum*, are generalizable to other naturally occurring mixed sexual/asexual animal systems as well as to any eukaryotic system with the potential for mitonuclear conflict. Accordingly, we hope and believe that the collection of hypotheses and ideas we have laid out here will inspire biologists from multiple fields and across a diverse array of study systems.

Explaining patterns of asexual mitonuclear discordance

Determining the nature of the processes contributing to mitonuclear discordance in asexual lineages is important because it may point toward – or away from – a role for mitochondria in the evolutionary maintenance of sex. 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). The stochastic processes that might explain these observations are diverse and varied in their mechanisms. By contrast, 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.

Stochastic mechanisms

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

If genetic diversity differs spatially or in other systematic ways across sexual versus asexual lineages (e.g., for some heavily parasitized populations of *P. antipodarum* in which asexual but not sexual subpopulations feature spatial substructure[42]), 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. This kind of spatial and/or temporal genetic structure across reproductive modes has also been observed in a variety of asexual taxa[30,45–48].

Predictions under Hypothesis 1: Standing mitochondrial genetic variation in asexual lineages will approach that of sympatric sexual populations, and there will exist intra-population structure for mitochondrial haplotype frequencies.

*Are the Predictions Met?*Collecting the data required to assess whether these predictions hold requires truly comprehensive sampling. While a part of the mitochondrial *cytochrome b* gene has been sequenced in thousands of *P. antipodarum*[23,41,43,49], 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[23,41,43]. Within-population sampling with respect to mitochondrial DNA sequencing is also almost completely nonexistent in this system (but see Neiman et al.[41] for one exception, shallow and deep regions of lake Alexandrina, that nevertheless does not demonstrate obvious structure).

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

Following the loss of sex, the machinery responsible for cellular processes related to sexual reproduction is expected to degrade[50], 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). The possibility of paternal leakage in rare sex events has not been evaluated in asexuals, but one proof-of-principle example of leaky cytoplasmic inheritance following divergence can be found in *Campanula americana* interpopulation hybrids in which subpopulation divergence is correlated with the degree of biparental chloroplast inheritance[11].

Predictions underHypothesis 2: Asexual lineages coexisting with sexuals (and, thus, with a relatively high frequency of males) should exhibit greater mitonuclear discordance than asexual lineages that do not coexist with sexual counterparts. In addition, because the polyploidy of asexual *P. antipodarum* (which are triploid or tetraploid, while sexual *P. antipodarum* are diploid[41,51]) is thought to arise from rare sex events between asexual females and males[41,52], all else being equal, there should exist greater degrees of mitonuclear discordance in tetraploid than triploid asexual *P. antipodarum*. That is, as the number of rare sex events that contribute to an asexual lineage's genome accumulates, the greater the amount of coalescence time since those nuclear and mito-

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 mitonuclear coevolution, biparental inheritance of mitochondria in males (e.g., doubly uniparental inheritance), or uniparental inheritance of the nuclear genome (i.e., asexuality).

chondrial genomes co-existed in the same individual. The existence within a single asexual lineage (defined via nuclear markers) of multiple distinct mtDNA haplotypes from different clades would also be suggestive of paternal leakage.

*Are the Predictions Met?*Datasets featuring the necessary extensive sampling across New Zealand that includeboth 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.[41] and Paczesniak et al.[23] The same limitation applies to the triploid versus tetraploid comparisons, which are also only available in these two studies. Figure 4 in Paczesniak et al.[23] 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. [23] (https://doi.org/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[53]. Experimental studies that pair sexual males with asexual females (which readily mate[54,55]) and then track mitochondrial inheritance will provide an important means of detecting direct evidence for presence and frequency of paternal leakage.

Hypothesis 3. *Contagious asexuality results in mitochondrial capture by asexual lineages.*

Asexual *P. antipodarum* occasionally produce males[52] that make sperm[56] and copulate[57]. If these males are able to successfully fertilize sexual females, and if asexuality can be "contagiously" transmitted by males[58] such that some of these offspring are asexual females, new mitochondrial haplotypes from sexual populations can be captured in asexual lineages (Figure 1C). Such mitochondrial capture events are particularly common among asexual lineages formed by hybridization[28,29].

Predictions under Hypothesis 3: We predict that we will see haplotype sharing across sexual and asexual lineages in sympatric populations. We also predict that mitochondrial haplotype networks will 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.

Are the Predictions Met?The data presented in Neiman and Lively[43] Neiman et al.[41] and Paczesniak et al.[23] 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[57]) and then track offspring production and reproductive mode will be needed to directly demonstrate asexual contagion. The prediction regarding the number of independent transitions is not supported by existing nuclear and mitochondrial data, which indicate more transitions for the former than the latter[23], with the major caveat that the comparison of different marker types with different rates of evolution and levels of resolution makes rigorous comparison a challenge. Indeed, the orders-of-magnitude more markers available and the higher rate of evolution of nuclear markers like microsatellites relative to the mitochondrial nucleotide substitutions used to characterize haplotype identity might render this type of comparison formally impossible.

Selective mechanisms

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

Local environmental conditions can impose strong selection on phenotypes associated with particular mitochondrial haplotypes[59]. The efficacy of natural selection is expected to be reduced for asexual lineages relative to sexual counterparts[60]. From the perspective of the mitochondrial genome, its co-transmission with the nuclear genome in asexual lineages will translate into selective interference that will hinder the spread of positively selected mitochondrial haplotypes[61,62]. Consistent with this prediction, a number of asexual lineages exhibit signatures of accelerated mutation accumulation in their mitochondrial genomes compared to sexual counterparts, including *P. antipodarum*[63] as well as *Tinema* stick insects.[64]

Predictions under Hypothesis 4: 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 should persist much longer in asexual lineages than in coexisting sexuals[63] (Figure 1D).

*Are the Predictions Met?*The data that we would need to perform a rigorous within-population 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 populations where nearly all asexual individuals harbor haplotype 1A while sympatric sexual counterparts instead have different haplotypes, and more unique haplotypes per snail sampled[23,41].

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 ref. [66], 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*[66]) or markedly different traits (e.g., resting egg production in some sexually vs. asexually reproducing monogonont rotifers[67]). These phenomena could result in different mitochondrial haplotype frequencies in asexuals compared to sexuals (Figure 1E).

From this perspective, the most relevant difference between sexual and asexual *P. antipodarum* differ is that asexuals are polyploid while sexuals are diploid. There is growing evidence that mitonuclear interactions are perturbed by polyploidy.[68] For example, polyploid dicots

and monocots exhibit elevated mitochondrial genome copy numbers compared to diploid relatives[69]. As a consequence, polyploids may experience substantially different selection dynamics than sexuals, particularly with respect to mitochondrial genome replication rate.

Predictions Under Hypothesis 5: We might predict that haplotype 1A would be associated with a replication advantage over population-specific mitochondrial haplotypes, resulting in distinct mitonuclear combinations in sexual versus asexual lineages. Because many asexual taxa are polyploid,[70] this hypothesis could be generalizable across many sexual/asexual animal and plant systems.

*Are the Predictions Met?*We have no data on mitochondrial genome copy numbers per cell in sexual versus asexual *P. antipodarum* lineages, but polyploid plants exhibit higher mitochondrial genome copy numbers per cell compared to their diploid relatives.[69] 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[71]). 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[4]. Such sex-specific effects of mitochondrial mutations are common across the tree of life (e.g., *Drosophila*, cytoplasmic male sterility in plants), but mollusks are particularly replete with such examples [reviewed in ref. 73], including a recent demonstration of cytoplasmic male sterility in a gastropod[73].

Predictions underHypothesis 6: 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[74]), (2) loss of strict maternal mitochondrial inheritance (e.g., Doubly Uniparental Inheritance, "DUI"[75]), 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[76], and cytoplasmic male sterility has recently been demonstrated in a gastropod[73], raising the intriguing possibility that male-harming mutations in molluscan mitochondrial genomes are relatively common[77].

Are the Predictions Met? The data needed to evaluate these predictions do not exist. We do know that *Potamopyrgus* mitochondrial genomes exhibit signs of inter-haplotype recombination and apparent heteroplasmy[78] and signatures of sex-specific optima of mitochondrial function[79], 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.

CONCLUSIONS AND PROSPECTS

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 in *P. antipodarum* and potentially beyond to other mixed sexual/asexual taxa[28,29].

Regarding stochastic hypotheses, mitochondrial genomes are most likely to suffer the harmful effects of Muller's Ratchet[80], 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 events 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 versus 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 populationswith 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[38]. Together, these possibilities raise a series of important and unanswered questions both for *P. antipodarum* and for other sexual/asexual systems, with direct relevance to understanding the maintenance of sex in nature:

- What is the cause of mitonuclear discordance in asexual lineages?
- Does mitochondrial performance vary across sympatric sexual and asexual lineages? Across sexes?
- How "leaky" is mitochondrial inheritance in sexual lineages?

- Does polyploidy alter the parameters of energy production?
- What is the mechanism underlying transitions to asexuality?

AUTHOR CONTRIBUTIONS

The paper idea came from Maurine Neiman. Both Maurine Neiman and Joel Sharbrough contributed to paper conceptualization and drafting and editing of the paper. Joel Sharbrough designed and created the figure.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

No new data were generated in the production of this manuscript. The data that we discuss in Paczesniak et al.[23] can be accessed in Dryad at https://doi.org/10.5061/dryad.j18pv

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