

NOTE

Reduced Mitochondrial Respiration in Hybrid Asexual Lizards

Randy L. Klabacka,^{1,*} Hailey A. Parry,¹ Kang Nian Yap,¹ Ryan A. Cook,^{1,2} Victoria A. Herron,^{1,3} L. Miles Horne,^{1,4} Matthew E. Wolak,¹ Jose A. Maldonado,⁵ Matthew K. Fujita,⁵ Andreas N. Kavazis,¹ Jamie R. Oaks,^{1,†} and Tonia S. Schwartz^{1,†}

1. Auburn University and Auburn University Museum of Natural History, Auburn, Alabama 36849; 2. Villanova University, Villanova, Pennsylvania 19085; 3. University of Missouri College of Veterinary Medicine, Columbia, Missouri 65211; 4. University of Texas at El Paso, El Paso, Texas 79968; 5. Amphibian and Reptile Diversity Research Center, University of Texas at Arlington, Arlington, Texas 76019

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ABSTRACT: The scarcity of asexual reproduction in vertebrates alludes to an inherent cost. Several groups of asexual vertebrates exhibit lower endurance capacity (a trait predominantly sourced by mitochondrial respiration) compared with congeneric sexual species. Here we measure endurance capacity in five species of *Aspidoscelis* lizards and examine mitochondrial respiration between sexual and asexual species using mitochondrial respirometry. Our results show reduced endurance capacity, reduced mitochondrial respiration, and reduced phenotypic variability in asexual species compared with parental sexual species, along with a positive relationship between endurance capacity and mitochondrial respiration. Results of lower endurance capacity and lower mitochondrial respiration in asexual *Aspidoscelis* are consistent with hypotheses involving mitonuclear incompatibility.

Keywords: parthenogenesis, asexuality, hybridization, mitochondria, endurance, variability.

Introduction

The fitness advantages of asexual reproduction predict an abundance of asexual species (Maynard Smith 1958, 1978). However, the prevalence of sexual reproduction in animals suggests that the evolutionary costs of asexual reproduction

outweigh the benefits (Speijer et al. 2015). Although theoretical and empirical studies over the past century have proposed and tested hypotheses regarding these costs, much remains to be understood regarding the direct consequences of asexual reproduction in vertebrates (Fujita et al. 2020).

For the purposes of this article, we use the term “asexuality” to refer to a reproductive strategy where all progeny are produced without male genetic contribution (as opposed to facultative asexuality). Asexual vertebrates, virtually all of which are of hybrid origin (Dawley and Bogart 1989; Avise 2008, 2015; Fujita et al. 2020; but see Sinclair et al. 2010), reproduce by premeiotically doubling their ploidy (Lutes et al. 2010). The subsequent pairing of conspecific homologous chromosomes in meiosis I results in the perpetual preservation of genome-wide heterozygosity (Vrijenhoek and Pfeiler 2008; Warren et al. 2018). With ploidy restored after the completion of meiosis, the cells are ready to develop without variation introduced via fertilization or recombination, thus maintaining the genome in a frozen hybrid state (Vrijenhoek and Pfeiler 2008; Warren et al. 2018; but for evidence of some gene conversion, see Hillis et al. 1991; Warren et al. 2018).

The effect of this unique evolutionary strategy on intracellular bioenergetics is unclear, but examining the effect of heterozygosity on mitochondrial function and overall fitness can inform predictions. Higher rates of coupled mitochondrial respiration and increased fitness (interpreted as heterosis) have been observed in F_1 hybrids from inbred *Drosophila melanogaster* lines (McDaniel and Grimwood 1971; Martinez and McDaniel 1979) and natural *Tigriopus*

* Corresponding author; email: klabacka.randy@gmail.com.

† These authors contributed equally to this article.

ORCID: Klabacka, <https://orcid.org/0000-0003-3924-0143>; Yap, <https://orcid.org/0000-0001-8805-1520>; Cook, <https://orcid.org/0000-0001-9551-591X>; Herron, <https://orcid.org/0000-0001-8901-6954>; Horne, <https://orcid.org/0000-0002-9136-4170>; Wolak, <https://orcid.org/0000-0002-7962-0071>; Oaks, <https://orcid.org/0000-0002-3757-3836>; Schwartz, <https://orcid.org/0000-0002-7712-2810>.

californicus populations (Ellison and Burton 2008) compared with their parental lineages, whereas lower values for these traits have been observed when backcrosses lead to mismatched mitochondrial and introgressed nuclear genomes in natural populations of *T. californicus* (Ellison and Burton 2008) and *Urosaurus* (Haenel and Moore 2018). High heterozygosity of asexual vertebrates led numerous researchers to predict an increase in performance compared with sexual parental species (White 1970; Schultz 1971; Cole 1975; Mitton and Grant 1984; Bullini 1994; Cullum 1997), yet the results from several studies have contradicted these predictions by showing reduced aerobic performance in asexual lineages (Cullum 1997; Mee et al. 2011; Denton et al. 2017).

Aerobic activities requiring endurance (continuous exertion) are powered by oxidative phosphorylation. This catalytic conversion process occurs in the mitochondrion, where a proton gradient powered by nutrient-donated electrons facilitates the phosphorylation of ADP. Production of ATP via this electron transport system (ETS) produces the vast majority of energy used for cellular functions. While the link between mitochondrial function and endurance may seem intuitive, studies examining the association of endurance capacity with mitochondrial respiration have been primarily in the context of biomedical and exercise physiology rather than evolution (e.g., Davies et al. 1981; Gollnick and Saltin 1982; Mercier et al. 1995; Bouchard et al. 1999; Eynon et al. 2011; Jacobs and Lundby 2013; Scott et al. 2018).

Reductions in asexual aerobic performance may be explained in part by mitonuclear incompatibility—the result of interactions between poorly coadapted gene products from mitochondrial and nuclear genomes that can result in reduced mitochondrial function, reduced organismal performance, and reduced fitness (Ryan and Hoogenraad 2007; Meiklejohn et al. 2013; Hill et al. 2019; Healy and Burton 2020; Rand and Mossman 2020; Moran et al. 2021). We test the hypothesis that the reduced aerobic performance previously observed in several groups of hybrid asexual vertebrates is due to decreased mitochondrial function, as would be predicted with mitonuclear incompatibility. The evolutionary mechanisms leading to incompatibility in these F_1 hybrids could result from mismatched genomes with dominance effects as a result of Darwin's corollary (Turelli and Moyle 2007) and/or the reduced efficiency of selection on nuclear mutations imposed by the lack of recombination in asexual species (Fisher 1930; Muller 1932). Because asexual vertebrates are of hybrid origin, rather than seeking to disentangle the effects of these traits (asexual reproduction and hybrid origin), we strictly examine hypothesized contributions of an intracellular process (mitochondrial respiration) to an organismal phenomenon (reduced endurance capacity). Squamata (snakes and lizards) is the

only vertebrate clade with lineages that reproduce primarily through parthenogenesis, a mode of asexual reproduction with no male input. Using the whiptail lizard genus *Aspidoscelis* as a model system (in which roughly one-third of species reproduce parthenogenetically), we quantify endurance capacity and mitochondrial respiration to contrast a sample of sexual and asexual species with two independent origins of parthenogenesis (Densmore et al. 1989; Reeder et al. 2002).

Methods

Animal Capture

We collected individuals of three sexual species ($n = 6$ *Aspidoscelis inornatus*, $n = 6$ *A. marmoratus*, $n = 7$ *A. septemvittatus*) and two asexual species ($n = 4$ *A. neomexicanus*, $n = 7$ *A. tessellatus*) along the Rio Grande basin between Las Cruces, New Mexico, and Big Bend National Park, Texas (table S1; tables S1–S5 are available online). The estimated evolutionary relationships of these species (from Reeder et al. 2002) are depicted in figure 1A (note: although asexual lineages are not species in the typical sense [originating via cladogenesis], we join others in referring to them as such given their independent evolutionary trajectory). We caught lizards via lasso or by hand and transported all individuals to Auburn University for temporary housing. All collection and animal care procedures were approved by the US Department of the Interior, state departments, and the Auburn University Institutional Animal Care and Use Committee (2018-3286). Additional sampling information is included in the “Supplementary Methods” section of the supplemental PDF (available online).

Endurance Capacity and Mitochondrial Respirometry

We quantified endurance by measuring the time that a lizard maintained forward progression at 1 km/h (on a treadmill), following previously established protocols (Garland 1994; Cullum 1997; for more details, see the “Supplementary Methods” section of the supplemental PDF). One week later, we measured mitochondrial respiration following previously established protocols (Palmer et al. 1977; Hood et al. 2019; for more details, see the “Supplementary Methods” section of the supplemental PDF). To measure oxygen consumption through the electron transport chain, we added isolated mitochondria with electron-donating substrates to electrode chamber A (for starting electron transport from complex I [CI] and continuing through complex III [CIII], complex IV [CIV], and complex V [CV]) and electrode chamber B (for starting electron transport from complex II [CII] and continuing through CIII, CIV, and CV).

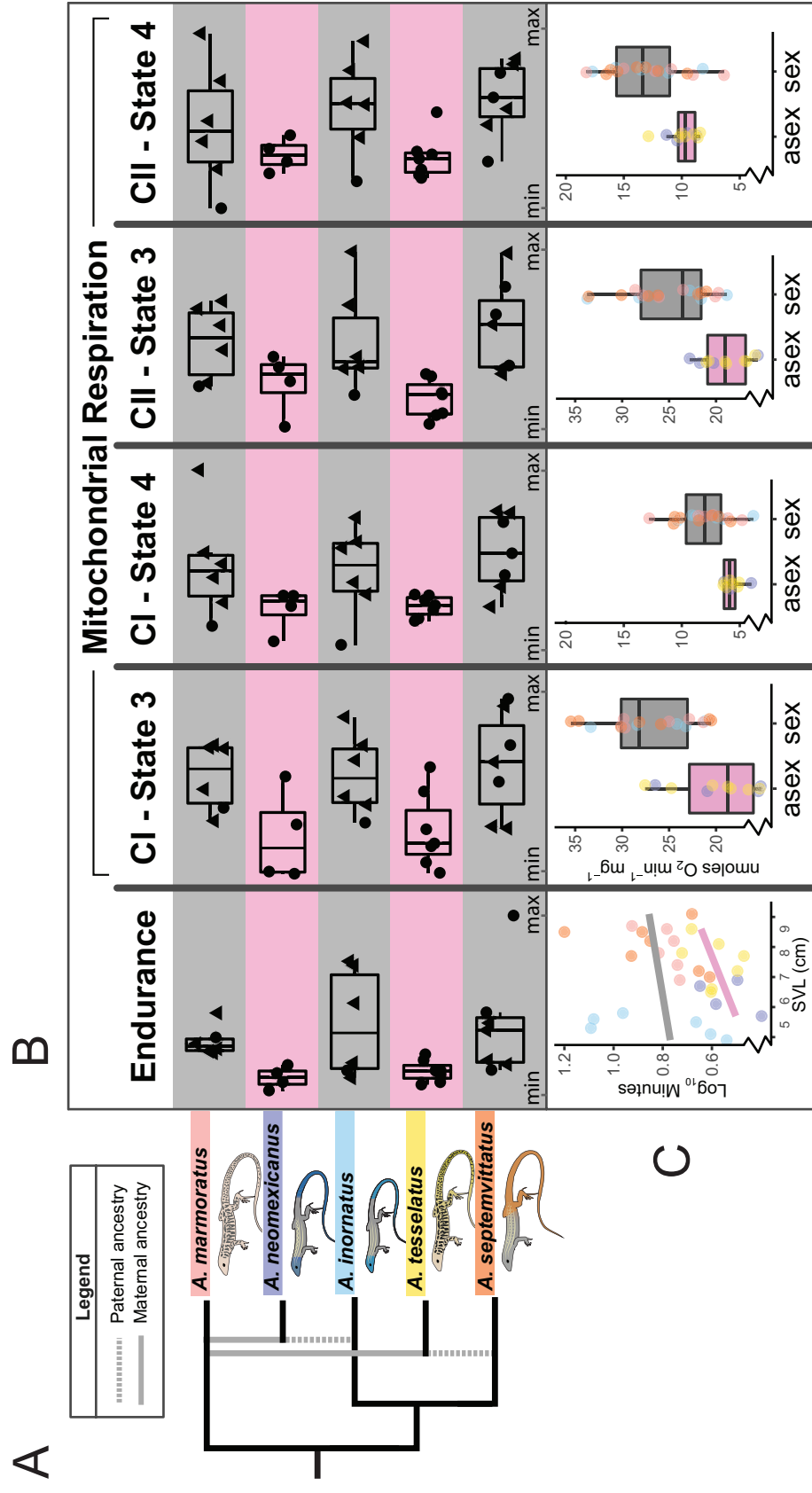


Figure 1: Reduced endurance and mitochondrial respiration in hybrid parthenogens. **A**, Evolutionary network showing relationships of all species in the study (from Reeder et al. 2002; branch lengths and divergence times are not informative). Reticulations (representing hybridization events) leading to asexual hybrids are shown by gray bars, with solid bars showing maternal ancestry and dashed bars showing paternal ancestry. **B**, Table of boxplots showing the variation and scaled response variable values for endurance, state 3 and 4 respiration starting at mitochondrial complex I (CI), and state 3 and 4 respiration starting at mitochondrial complex II (CII). Hybrid asexual species are indicated by pink-filled rows, whereas sexual species are indicated by gray-filled rows. Females are indicated by circles and males by triangles. Except for CI state 3, all shown response variables show significant differences in variation between sexual and hybrid asexual species (see table 1). **C**, Comparisons of all shown response variables were significant between reproductive modes (sexual vs. asexual) for each of the response variables shown (with $P < .05$). Plots in C correspond to the columns in B. The endurance data are shown with a scatterplot because we used body size (snout-vent length [SVL]) as a covariate in the model, and the lines of best fit represent hybrid asexual species (pink) and sexual species (gray). The y-axes for each of the mitochondrial respiration response variables are the same. Values for effect sizes, variance, and P can be found in table 1. Plots with full y-axes (along with respiratory control ratio) are shown in figure S2.

Measuring mitochondrial respiration via the electron transport chain using these two ports of entry provides independent avenues with different starting substrates to quantify respiration. Because both avenues (starting with CI or CII) comprise interacting mitochondrial and nuclear gene products (CIII, CIV, and CV), it is inappropriate to use these measures to draw conclusions regarding mitonuclear compatibility.

To initiate coupled, ADP-stimulated respiration (state 3), we added ADP to each chamber. After the phosphorylation of ADP was complete and any oxygen being consumed was driven by protons moving across the inner membrane without facilitation from ATP synthase, we recorded basal respiration (state 4). We normalized respiration rates to mitochondrial protein concentration. To calculate the respiratory control ratio (RCR), we divided state 3 respiration by state 4 respiration.

Predictions. To test our hypothesis of reduced mitochondrial respiration in hybrid asexual species compared with their sexual congeneric progenitors, we used the six mitochondrial respiration response variables (state 3, state 4, and RCR initiated from either CI or CII). State 3 respiration measures the rate of oxygen consumption when ATP is being produced (i.e., oxygen consumption is coupled with proton movement through ATP synthase [CV]). If coupled electron transport and ATP synthesis is associated with endurance capacity, we predict that state 3 respiration would be lower in hybrid asexual species. State 4 respiration measures the rate of oxygen consumption when ATP is not being produced (i.e., oxygen consumption is coupled with proton leak across the inner membrane). In this context, we predict no differences in state 4 respiration. Because RCR is an indicator of respiration efficiency (coupled respiration controlling for leak), we predict to see lower RCR in hybrid asexual species if they have lower endurance capacity.

Phylogenetic Network Estimation

Accounting for evolutionary history is critical for accurate comparative methods when multiple lineages are present in a sample set. However, in study systems where lineage history is reticulate rather than bifurcate, models with a phylogenetic network (rather than a tree) more appropriately account for evolutionary history. To estimate the history of diversification and hybridization of the five species of *Aspidoscelis*, we sequenced mitochondrial genomes (following Roelke et al. 2018) and downloaded available mitochondrial sequence data from GenBank (table S2). We used several software packages to estimate the

phylogenetic network (Than and Nakhleh 2008; Nguyen et al. 2015; Solís-Lemus et al. 2017); we provide details in the “Supplementary Methods” section of the supplemental PDF.

Statistical Analyses

We analyze the data in three ways. First, we use phylogenetic network linear models that include reticulate evolutionary relationships within the model to estimate (1) the effect of hybrid asexuality on each response variable (endurance and mitochondrial respiration [state 3, state 4, and RCR initiated from either CI or CII]) and (2) the effect of mitochondrial respiration on endurance capacity. Second, we use linear mixed effects models with species random effects to test for (1) the effect of hybrid asexuality on each response variable, (2) the effect of mitochondrial respiration on endurance capacity, and (3) differences in variability between hybrid asexual and sexual species for each response variable. Third, we use linear models for subgroups without needing to account for ancestry to test the effect of hybrid asexuality on each response variable (we made subgroup assignments based on mitochondrial history and parentage). More details for each of these approaches are provided in the “Supplementary Methods” section of the supplemental PDF. Data and code are available from GitHub (<https://doi.org/10.5281/zenodo.5784646>; Klabacka 2021) and the Dryad Digital Repository (<https://doi.org/10.5061/dryad.zs7h44j8n>; Klabacka et al. 2021).

Results

Effect of Hybrid Asexuality on Endurance and Mitochondrial Respiration

We found reduced endurance capacity and mitochondrial respiration in hybrid asexual species when using either the phylogenetic network or mixed effects linear models (fig. 1; table 1; summary statistics in table S3). We observed that hybrid asexual species had reduced endurance capacity and rates of oxygen consumption when starting from either CI or CII for state 3 and state 4 respiration. We see no support for differences in RCR (for either complex) between sexual and asexual species (fig. S2; figs. S1–S4 are available online). This is not surprising given that both state 3 and state 4 changed in the same direction, resulting in no changes in the ratio between the two measures (RCR). The effect sizes for each response variable are similar between phylogenetic network and mixed effects linear models (table 1), providing evidence for little phylogenetic signal for response variables. Within-group comparisons show the same general pattern without a statistically significant

Table 1: Results from PhyloNetwork and mixed effects linear models

	Hybrid parthenogens				Log endurance			
	β	SE	<i>P</i>	σ_s, σ_a	β	SE	<i>P</i>	<i>r</i> ²
PhyloNetwork model:								
Log endurance	-.22	.040	.031
CI state 3	-6.88	.44	6.03E-04033	.0036	.0028	.97
CI state 4	-2.33	.23	.0020095	.016	.011	.92
CI RCR	.066	.13	.66	...	-.13	.55	.84	.017
CII state 3	-6.12	1.13	.013031	.11	.060	.74
CII state 4	-2.90	.47	.0087072	.017	.023	.86
CII RCR	-.13	.09	.2652	.59	.44	.20
Mixed effects model:								
Log endurance	-.24	.050	.017	.18, .090 ^a
CI state 3	-7.10	1.77	.028	4.77, 4.51	.021	.0050	2.00E-04	.40
CI state 4	-2.31	.57	.026	2.26, .75 ^a	.049	.014	.0023	.29
CI RCR	.021	.29	.95	.69, .88	-.042	.042	.33	.23
CII state 3	-5.91	1.28	.019	4.53, 2.49 ^a	.021	.0060	4.0E-04	.37
CII state 4	-3.17	.85	.034	3.29, 1.32 ^a	.023	.011	.042	.14
CII RCR	.036	.13	.81	.36, .21	.039	.11	.71	.0035

Note: Within columns, we show the deviation of hybrid asexual species from sexual species for all response variables (left) and the effect of mitochondrial respiration states on endurance (right). The table is broken into two horizontal sections showing results from the PhyloNetwork linear model (top) and the mixed effects linear model (bottom). Effect sizes (β), standard errors, *P* values, standard deviations for reproductive modes (σ_s and σ_a indicate the standard deviation for sexual and hybrid asexual species, respectively; confidence intervals, results from the likelihood ratio test, and coefficients of variation are shown in table S5), and the coefficient of determination (*r*_s) are shown for the models. CI = complex I; CII = complex II; RCR = respiratory control ratio.

^a Models where two residual variances were selected.

effect for each response variable (potentially because of lower sample size; table S4). Details on the within-group comparisons are included in the supplemental PDF.

Positive Relationship between Endurance and Mitochondrial Respiration

We observed a positive relationship between endurance and rate of oxygen consumption when starting from either CI or CII for state 3 and state 4 respiration with either the phylogenetic network or the mixed effects linear models; each of these relationships is statistically significant except for the phylogenetic network model for CII state 3 (table 1; fig. 2). We see no support for a relationship between endurance and RCR.

Greater Variation in Sexual Species

We found that models incorporating different residual variation parameters for sexual and hybrid asexual groups were preferred for endurance, CII state 3 respiration, and CI and CII state 4 respiration (tables 1, S5; fig. S3; also see pink rows vs. gray rows in fig. 1B). The approximate posterior probability that sexual species have a greater mean-corrected variance than asexual species was 75%, 96%, 83%, and 94% for endurance, CI state 4, CII state 3, and CII state 4, respectively (fig. S4).

Discussion

We present novel findings of reduced mitochondrial respiration in hybrid asexual species. We also reproduce the findings of previous studies that indicate reduced endurance capacity in these asexual species relative to parental sexual species (Cullum 1997; Mee et al. 2011; Denton et al. 2017). A positive relationship between mitochondrial respiration and endurance capacity is evident in our results, which matches our prediction given that aerobic activities require a large amount of ATP. This reflects a similar correlation between endurance and mitochondrial genotype in *Drosophila* (Sujkowski et al. 2019). The lower variability in endurance, CII state 3, and CI/CII state 4 in the hybrid asexual species supports the hypothesis that asexual species have lower phenotypic variability because of decreased genetic variation (Ghiselin 1974; Williams 1975; Maynard Smith 1978). While lower phenotypic variability in locomotor performance has been previously documented in asexual *Aspidoscelis* species (Cullum 2000), our study is the first to report decreased variability in mitochondrial respiration of hybrid asexual species relative to respective parental sexual species.

Despite the high heterozygosity of hybrid asexual species, which led numerous researchers to predict an increase in performance of hybrid asexual species compared with parental sexual species (hybrid vigor; see White 1970; Schultz 1971; Cole 1975; Mitton and Grant

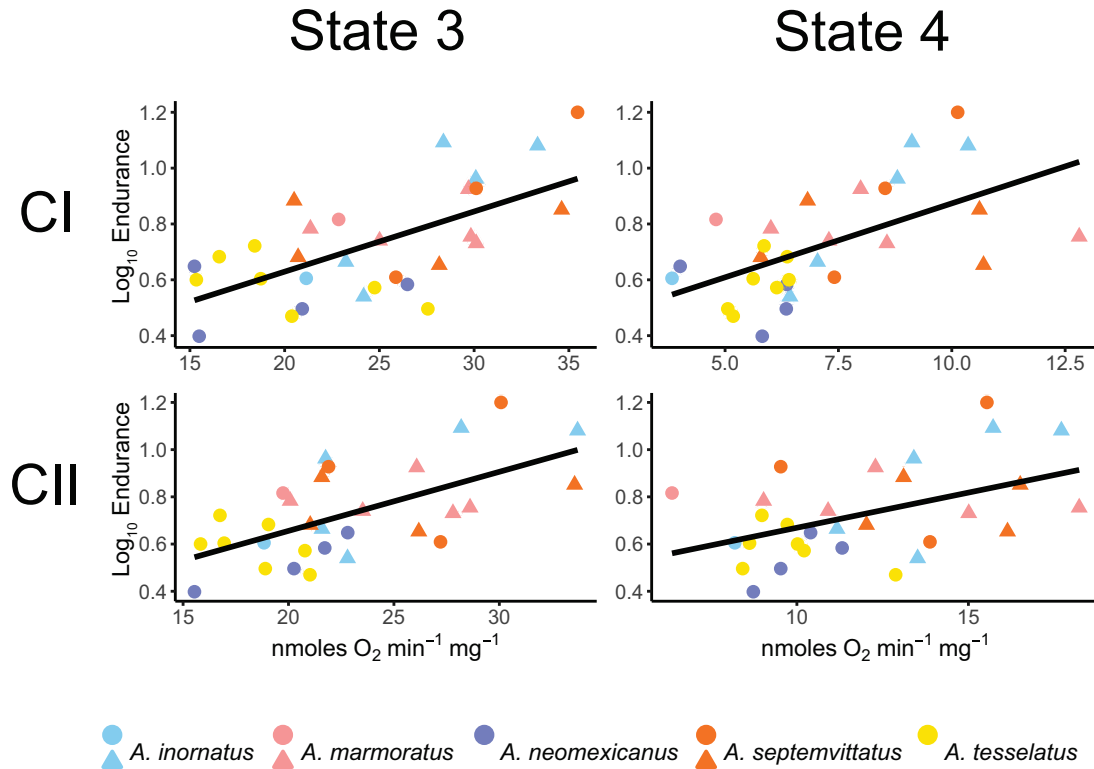


Figure 2: Positive relationship between mitochondrial respiration states and endurance. Scatterplot shows effect of mitochondrial respiration for the initiation complexes (CI and CII) and respiration states (state 3 and state 4) on \log_{10} endurance. Colors and shapes correspond to species and sex, respectively (circles = females, squares = males). Values for effect size (slope), P , and r^2 can be found in table 1.

1984; Bullini 1994; Cullum 1997), several studies have shown reduced aerobic performance in asexual vertebrate species (Cullum 1997; Mee et al. 2011; Denton et al. 2017). Historically, this decrease in performance has been attributed to (a) genomic incompatibility (consequence of hybridization via negative epistasis [Cullum 1997; Denton et al. 2017] and/or subsequent gene conversion), (b) mutational erosion (consequence of asexuality via Muller's Ratchet [Muller 1964; Leslie and Vrijenhoek 1978; Cullum 1997; Vorburger 2001]), or (c) the inability of the organism to “keep up” with the evolution of parasites because of lack of variation (consequence of asexuality via Red Queen [Valen 1973; Hamilton et al. 1990; Lively et al. 1990; Moritz et al. 1991; Mee and Rowe 2006; Mata-Silva et al. 2008]). It is also possible that the inability of asexual lineages to combine beneficial alleles that arise in a population via sexual recombination (Maynard Smith 1978) results in the failure of the nuclear genome to efficiently compensate for deleterious mutations that arise in the mitochondrial genome. This hypothesis, an extension of the Hill-Robertson effect (Fisher 1930; Muller 1932; Felsenstein 1974; Hill and Robertson 2007) in the context of accelerated compensatory evolution in nuclear-encoded

mitochondrial genes, was originally posed to explain the origin and prevalence of sexual reproduction among eukaryotes (Havird et al. 2015). Additional biological factors, such as demography, ecology, and/or life history strategies specific to *Aspidoscelis*'s sexual mode, may contribute to the differences in endurance and mitochondrial respiration, although several studies have found little to no differences in factors such as response to habitat disturbance (Cosentino et al. 2019), thermal preference (Díaz de la Vega-Pérez et al. 2013), reproductive strategies (Schall 1993), and diet (Smith 1989; Mata-Silva et al. 2013). This study is unable to identify which of the previously described non-mutually-exclusive hypotheses best explain our observation of reduced mitochondrial respiration in hybrid asexual species. We recommend that future work integrates genomic sequencing approaches with physiological and cellular measurements (e.g., RNA-seq and individual ETS complex activity) to disentangle potential contributions from these hypotheses.

While reduced endurance is observed in several groups of asexual vertebrates (including in this study), examination of parthenogenetic geckos in the *Heteronotia binoei* complex has shown no difference (Roberts et al. 2012)

and increased endurance (Kearney et al. 2005) in hybrid asexual species compared with sexual progenitors in different studies. Variation in results between asexual groups may be due to (1) differences in age of asexual lineages, (2) divergence between parental species at the time of hybridization, (3) ploidy, or (4) ecology. Compared with *Heteronotia*, *Aspidoscelis* possesses (1) younger asexual lineages (Reeder et al. 2002; Kearney et al. 2006), (2) greater divergence times between parental species (Strasburg and Kearney 2005; Zheng and Wiens 2016), (3) diploid asexuals (in this study; however, for diploid and triploid asexual *Aspidoscelis* species see Cullum 1997; Kearney et al. 2005; Roberts et al. 2012), and (4) a more active foraging strategy (Milstead 1957; Bauer 2007). We do not refer to these differences between taxa as factors that wholly explain our observations; rather, we point out that complexities within these biological systems may be responsible for the seemingly contrasting results.

Decreased RCR between sexual parent and asexual hybrid species is not evident given our data, which contradicts our predictions. We attribute this to the significant differences in state 4 respiration, which we did not predict. State 4, commonly called the “leak” or “basal” state, occurs when ADP has been exhausted. Oxygen consumption occurring via the ETS is being driven by protons leaking across the mitochondrial inner membrane rather than via ATP synthase. Low state 4 respiration tends to lead to high reactive oxygen species (ROS) production (Brand 2000); therefore, a higher state 4 in sexual species compared with hybrid asexual species may be an adaptive trait to mitigate oxidative damage. Investigation into potential differences in ROS production and oxidative damage between sexual and hybrid asexual species is needed to test this hypothesis. We also recommend that future studies include additional respiration states (e.g., state 4o induced by a CV inhibitor, state 3u induced by an uncoupling agent) for examination of respiratory ratios (Gnaiger 2020).

Lower state 3 respiration in hybrid asexual species suggests decreased mitochondrial respiratory capacity and, as a result, diminished ATP production. The positive relationship we observed between mitochondrial respiration and endurance capacity affirms our predicted relationship between these traits and supports the hypothesis that efficient oxidative phosphorylation increases endurance capacity. Here we show novel evidence for costs incurred by hybrid asexual species on mitochondrial respiration and reproduce findings of their reduced endurance capacity. Determining the evolutionary underpinnings of these phenomena, thus shedding light on which hypotheses are responsible, will require integrating physiological and genomic sequencing approaches. While the benefits of asexual reproduction can explain the genesis of asexual lineages, incurred costs for this strategy may explain their

short evolutionary existence. Reduced mitochondrial respiration and variability in hybrid asexual species may be evolutionary disadvantages when performance and variation are important factors in the realm of natural selection.

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Statement of Authorship

R.L.K.: conceptualization, funding acquisition, experimental design, permit acquisition, sample collection, animal care, data collection, data processing, data analysis, data visualization, resource provision (field equipment), code scripting, writing (original draft, review and editing); H.A.P.: data collection, data processing, writing (review and editing); K.N.Y.: data collection, data processing, writing (review and editing); R.A.C.: sample collection, animal care, data collection; V.A.H.: sample collection, animal care, data collection; L.M.H.: sample collection, writing (review and editing); M.E.W.: data analysis, code scripting, data visualization, writing (review and editing); J.A.M.: sample collection, data collection; M.K.F.: conceptualization, resource provision (lab equipment, reagents), writing (review and editing); A.N.K.: resource provision (lab equipment), supervision, writing (review and editing); J.R.O.: conceptualization, experimental design, resource provision (field equipment, reagents), supervision, writing (review and editing); T.S.S.: conceptualization, experimental design, resource provision (field equipment, reagents), supervision, writing (review and editing).

Data and Code Availability

Data and code are available from GitHub (<https://doi.org/10.5281/zenodo.5784646>; Klabacka 2021) and the Dryad Digital Repository (<https://doi.org/10.5061/dryad.zs7h44j8n>; Klabacka et al. 2021).

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An *Aspidoscelis tessellatus* from Sierra County in New Mexico. Photo by Paulina Klabacka.