

CONTRASTING PATTERNS OF SYNONYMOUS AND NONSYNONYMOUS SEQUENCE EVOLUTION IN ASEXUAL AND SEXUAL FRESHWATER SNAIL LINEAGES

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In asexual lineages, both synonymous and nonsynonymous sequence polymorphism may be reduced due to severe founder effects when asexual lineages originate. However, mildly deleterious (nonsynonymous) mutations may accumulate after asexual lineages are formed, because the efficiency of purifying selection is reduced even in the nonrecombining mitochondrial genome. Here we examine patterns of synonymous and nonsynonymous mitochondrial sequence polymorphism in asexual and sexual lineages of the freshwater snail *Campeloma*. Using clade-specific estimates, we found that synonymous sequence polymorphism was significantly reduced by 75% in asexuals relative to sexuals, whereas nonsynonymous sequence polymorphism did not differ significantly between sexuals and asexuals. Two asexual clades had high negative values for Tajima's *D* statistic. Coalescent simulations confirmed that various bottleneck scenarios can account for this result. We also used branch-specific estimates of the ratio of amino acid to silent substitutions, K_a/K_s . Our study revealed that K_a/K_s ratios are six times higher in terminal branches of independent asexual lineages compared to sexuals. Coalescent-based reconstruction of gene networks for all sexual and asexual clades indicated that nonsynonymous mutations occurred at a higher frequency in recently derived asexual haplotypes. These findings suggest that patterns of synonymous and nonsynonymous nucleotide polymorphism in asexual snail lineages may be shaped by both severe founder effect and relaxed purifying selection.

KEY WORDS: Asexual reproduction, *Campeloma*, deleterious mutations, sexual reproduction, synonymous sequence polymorphism.

Selfing and asexual reproduction can have important consequences for observed levels of sequence polymorphism (Liu et al. 1999; Baudry et al. 2001; Charlesworth and Wright 2001; Graustein et al. 2002; Charlesworth 2003; Sweigart and Willis 2003) and the rates at which deleterious mutations accumulate in the genome (Paland and Lynch 2006). Reduced levels of polymorphism in selfing and asexual populations may result from selective and demographic processes, including selective sweeps (Maynard Smith and Haigh 1974), purifying selection (Charlesworth et al. 1993), and population bottlenecks (Simonsen et al. 1995). Ad-

ditionally, permanent linkage between organellar and nuclear genomes should lead to the accumulation of slightly deleterious amino acid substitutions in asexuals (Paland and Lynch 2006).

To address whether transitions to asexuality affect levels of sequence polymorphism requires that we examine how demographic and selective processes might differentially affect synonymous versus nonsynonymous nucleotide substitutions in asexual lineages. For example, a severe founder effect must occur within an asexual lineage founded from a single, parthenogenetic female from a sexual ancestral population, which reduces both

synonymous and nonsynonymous polymorphism. In addition, selective sweeps and background selection can also reduce sequence polymorphism in asexuals because of genome-wide linkage. Furthermore, due to the complete linkage of both nuclear and mitochondrial genomes, the efficiency of selection to remove mildly deleterious mutations is reduced even in the nonrecombining mitochondrial genome, and mildly deleterious mutations should accumulate at a higher rate in asexuals after the initial founding of these asexual lineages (Muller 1964; Kondrashov 1988; Charlesworth 1994; Paland and Lynch 2006). In the current study we examine patterns of synonymous and nonsynonymous nucleotide polymorphism in sexual and asexual lineages of the freshwater snail *Campeloma*.

In the southeastern United States, there have been multiple independent origins of parthenogens from sexual ancestors in the freshwater snail *Campeloma* (Johnson and Bragg 1999; Johnson 2000, 2006). Diploid populations of parthenogenetic *Campeloma* co-occur with sexuals in the Atlantic coastal plain and the Florida peninsula. Another group of sexual and parthenogenetic *Campeloma* (Gastropoda: Viviparidae) occurs in Florida Panhandle freshwater streams and rivers: *Campeloma geniculum* is an obligate sexual and *Campeloma parthenum* are highly heterozygous triploids formed by hybridization between *C. geniculum* and Atlantic coast *Campeloma*. Recent work indicates at least six independent origins of asexuals from sexual ancestors (Johnson 2006). The ages of these asexual lineages vary considerably. Most asexual mtDNA clades are widely distributed, often at high population densities, whereas sexual clades show very restricted geographic distributions (Johnson 2006). Mitochondrial and nuclear intron sequence differentiation occurs at much smaller spatial scales in sexuals compared to parthenogens. Within the geographic regions, geographically restricted sexual mtDNA clades show considerable sequence divergence, ranging from 4% to 8% sequence divergence between clades.

In the current study, we use multiple approaches to examine the effects of demography and selection on patterns of synonymous and nonsynonymous nucleotide polymorphism in asexual and sexual *Campeloma*. First, using clade-specific estimates, we examine whether synonymous and nonsynonymous nucleotide polymorphism differs significantly between asexual and sexual clades. If severe bottlenecks occur when asexual lineages are founded from one or few sexual females, both synonymous and nonsynonymous nucleotide polymorphism should be significantly reduced in asexual snail clades relative to sexual clades. We recognize that, although severe founder effect may be a plausible explanation for reduced polymorphism in these asexual snails, both selective sweeps and background selection would produce similar outcomes. Using Tajima's D (Tajima 1989), we also examine whether deviations from neutrality occur more often in asexual clades, and then employ coalescent simulations to ex-

amine whether severe bottlenecks at particular times in the past can produce the observed deviations from neutrality. If asexual lineages persist after their initial founding, they should accumulate slightly deleterious nonsynonymous substitutions at a higher rate compared to sexual lineages (Paland and Lynch 2006). To test this prediction we used three methods: first, we compared nonsynonymous nucleotide diversity of asexual and sexual clades; second, using the maximum-likelihood branch method developed by Yang (1998), we estimated the ratio of replacement to silent substitutions ($K_a:K_s$ ratios) along internal and external branches for sexual and asexual *Campeloma*; and lastly, we used a coalescent-based reconstruction of gene networks for all sexual and asexual clades, and then tested whether recent nonsynonymous mutations occurred at a higher frequency in asexuals compared to sexuals.

Materials and Methods

Fifty-eight sites containing *Campeloma* sp. from Atlantic and Florida Gulf coast populations were sampled (see Johnson 2006 for maps and collecting localities). Individuals from these populations were originally sequenced for cytochrome *b* as part of a companion study (for sequencing details see Johnson 2006). Sequence data have been previously deposited in GenBank (DQ131229-DQ131402). We used DnaSP (Nei and Gojobori 1986; Rozas et al. 2003) to estimate three measures of mtDNA nucleotide diversity (π): total, synonymous, and nonsynonymous diversity. We determined clade-specific estimates for six asexual and 12 sexual clades. These clades have greater than 0.95 posterior probabilities from Bayesian estimation (see figure 2 in Johnson 2006). By employing clade-specific measures in which coalescence to a common ancestor was used to define groups rather than population-level estimates, we minimize the likelihood of elevated synonymous polymorphism if asexual populations are derived from diverse sexual ancestors. Using these clade-specific estimates of nucleotide diversity, we used a one-way Analysis of Variance to assess whether asexuals exhibit reduced or greater nucleotide diversity relative to sexuals (SPSS, Inc 2003). To assess neutrality, we estimated Tajima's D for each sexual and parthenogenetic clade. We used neutral coalescent simulations in DnaSP with 1000 replications under the assumption of no recombination to test for significant deviations of D . We also adjusted alpha for multiple comparisons using the Bonferroni adjustment.

We used Hudson's (2002) ms program to simulate the population history of two asexual clades (A and E) for which we obtained highly negative values of Tajima's D . For each case we first generated output under the basic neutral model, which assumes constant population size, no recombination, panmixis, and an infinite-sites model (Hudson 2002). Under the first set of simulations, we assumed an initial population size of a single individual, the bottleneck persisted for five years, followed by an instantaneous increase in population size to N_e ; thereafter the population size

remained constant until present time. We determined N_e from empirical estimates of θ ($2N_e\mu$), assuming a neutral mutation rate (μ) on the order of 2×10^{-8} /bp/generation. We assumed one generation per year for *Campeloma* (Brown and Richardson 1992). For example, according to this procedure, N_e for asexual clade A was calculated as the ratio $\theta/u = 232,250$, where θ was estimated as 0.0093. For each set of simulations, we varied the time before the present (100,000 to 10,000 years ago) during which the bottleneck was expected to have occurred. Under the second set of simulations, we simulated less severe bottlenecks. Under these simulations, subsequent bottlenecks to 5000 individuals occurred at 100,000 or 50,000 years ago and the bottleneck was allowed to persist for 10,000 years. Ten thousand replicates were run for each situation, allowing us to generate summary statistics for Tajima's D .

Because clade-specific estimates of nonsynonymous nucleotide polymorphism do not consider the temporal history of these lineages, maximum-likelihood analysis of $K_a:K_s$ ratios allows us to test the explicit prediction that deleterious mutations accumulate in recent asexual lineages compared to recent sexual lineages (see Paland and Lynch 2006). Phylogenetic relationships among cytochrome *b* haplotypes were reconstructed using maximum likelihood (Johnson 2006). We used a maximum-likelihood approach as implemented in the PAML software package to estimate branch-specific $K_a:K_s$ ratios under various hierarchical models (Yang 1997, 1998). We mapped reproductive mode (sexual or asexual) onto the gene tree using parsimony, as implemented in the software package Mesquite (Maddison and Maddison 2006). Internal branches in which assignment to sexual or asexual reproduction was uncertain under parsimony were assigned as sexual branches because there is a much lower likelihood of reversion to sexual reproduction from asexual ancestors. We obtained identical results when uncertain internal branches were assigned as asexual branches. The maximum-likelihood gene tree also showed reversals to sex from asexuals in the external branches of Clade A. We estimated the $K_a:K_s$ ratio models under two different conditions: allowing reversal to sex in these external branches or no reversal to sex. Given that reversal to sex is unlikely, we suspect that these haplotypes may represent asexual individuals occurring with sexual individuals in mixed populations (see Johnson 2006). We examined four different maximum-likelihood models to estimate $K_a:K_s$ ratios along branches. Although numerous other models could be tested, we focus on these four because they explicitly test the predictions that nonsynonymous mutations accumulate in asexuals. We used likelihood-ratio tests to assess whether more complex models provided a significantly improved fit compared to simpler models.

The one-ratio model constrains all branches of the phylogeny to have the same $K_a:K_s$ ratio, and this model tests whether this mitochondrial protein-coding gene is mostly subject to purifying

selection. If the $K_a:K_s$ ratio is less than 1, this would indicate that purifying selection acts against amino acid altering mutations. The two-ratio model tests for the occurrence of mildly deleterious mutations by allowing different $K_a:K_s$ ratios for internal and external branches of the phylogeny. Higher $K_a:K_s$ ratios for external branches relative to internal branches would support the hypothesis that mildly deleterious nonsynonymous mutations persist in the short term but are eliminated by purifying selection in the long term. We also tested another two-ratio model assigning branches as sexual or asexual. Although not part of the hierarchical modeling, this model allows us to examine whether asexuals have higher $K_a:K_s$ ratios. The three-ratio model allows different $K_a:K_s$ ratios for the following branches: external asexual, external sexual, and internal branches. If the three-ratio model provides a significantly better fit compared to the two-ratio model, this would indicate significant differences in the $K_a:K_s$ ratio for external sexual and asexual branches. Higher $K_a:K_s$ ratios in external asexual branches relative to external sexual branches is consistent with weaker purifying selection against asexuals, leading to the accumulation of mildly deleterious mutations in asexuals. The four-ratio model allows for different $K_a:K_s$ ratios for four branches: external sexual, external asexual, internal sexual, and internal asexual. If the four-ratio model is not a significant improvement over the three-ratio model, this would suggest that purging of slightly deleterious mutations occurs for both sexuals and asexuals over longer time scales.

There are two potential limitations of the above approach. First, phylogenetic methods do not use the relative frequencies of haplotypes within clades to determine the ancestral-descendent relationships among haplotypes, and thus do not incorporate data from the entire sequence dataset. Second, because K_s estimates for external branches were approximately two times higher in sexuals than in asexuals (0.0052 and 0.0027, respectively), elevated $K_a:K_s$ ratio along external asexual branches may not reflect the consequences of asexuality but instead the more recent origin of asexuals. If purifying selection takes time to remove deleterious mutations, and deleterious mutations contribute more significantly to polymorphism when more recent evolutionary history is considered, that is, along external branches, selection had only half as much time to remove deleterious mutations in asexuals than in sexuals. To address these issues, we used a coalescent-based approach to determine gene networks for each sexual and asexual clade. We constructed haplotype networks using TCS 1.13 (Clement et al. 2000). For each clade listed in Table 1, we determined the number of synonymous and nonsynonymous mutations occurring in external branches (haplotypes connected by only one branch and thus recent derivatives; Crandall et al. 1994) and internal branches (haplotypes connected by more than one branch and thus ancestral haplotypes). We used log-linear modeling (Fienberg 1983) to specifically examine whether external

Table 1. Total (π_{all}), synonymous (K_s), and nonsynonymous (K_a) nucleotide diversity, and test for neutrality (D ; Tajima's D based on π_{all} ; * $P < 0.05$ not adjusted for Bonferroni) for sexual and asexual clades. Number of synonymous and nonsynonymous mutations for each clade is given in parentheses.

Clade-reproductive mode	N	π_{all}	K_s	K_a	D
A-asex	102	0.00475	0.01249 (9)	0.0022 (6)	-1.37*
A-sex	10	0.00428	0.00726 (3)	0.01043 (3)	-0.57
B-asex	16	0.00189	0.00605 (1)	0.00051 (1)	0.09
B-sex	31	0.01227	0.04687 (19)	0.00077 (2)	-0.79
C-asex	3	0.00201	0 (0)	0.00271 (1)	
C-sex	32	0.00602	0.02306 (8)	0.00025 (1)	-0.33
D-sex	6	0.00101	0.00391 (1)	0	-0.93
E-asex	32	0.00783	0.02563 (15)	0.00167 (2)	-1.42*
E-sex	3	0.00201	0.00791 (1)	0	
F-asex	4	0.00604	0.01775 (3)	0.00204 (1)	
F-sex	9	0.00319	0.00988 (3)	0.0009 (1)	-1.14
I-asex	17	0.00382	0.0014 (1)	0.00467 (4)	-0.47
I-sex	43	0.02187	0.07456 (26)	0.00342 (6)	-0.17
J-sex	53	0.01689	0.06146 (24)	0.0018 (3)	-0.20
K-sex	56	0.02687	0.09522 (32)	0.00279 (4)	-0.01
L-sex	24	0.01806	0.06734 (22)	0.00135 (4)	-0.53
M-sex	38	0.00636	0.01992 (7)	0.0018 (1)	0.30
N-sex	43	0.02315	0.09067 (19)	0.00019 (1)	1.9*

asexual branches have a higher frequency of nonsynonymous mutations compared to external sexual branches. We used forward likelihood-ratio tests to determine which model best fit the observed data, and then examined the residuals to assess whether the observed frequency of nonsynonymous mutations was significantly higher in asexual external branches than expected under the best log-linear model.

Results

CYTOCHROME B NUCLEOTIDE POLYMORPHISM, NEUTRALITY, AND COALESCENT SIMULATIONS

Mitochondrial cytochrome *b* sequences were obtained from 521 individuals from 58 *Campeloma* populations throughout the southeastern United States. Using the mtDNA genetic code of echinoderms in DnaSP (Rozas et al. 2003), there were no stop codons, suggesting that these are mtDNA sequences instead of nuclear translocations. There were 123 variable sites from the 331 bp fragment, and synonymous substitutions were more frequent than nonsynonymous substitutions (86 vs. 24).

We determined total nucleotide diversity (π) for asexual and sexual clades (Table 1). Average π (± 2 SE) was not significantly different between asexual and sexual clades ($\pi_{\text{parthenogens}} = 0.0044 \pm 0.001$ and $\pi_{\text{sexuals}} = 0.0175 \pm 0.007$, respectively ($F = 1.64$; $df = 1, 16$; $P = 0.22$)). However, synonymous nucleotide diversity of asexual clades was significantly lower compared to sexual clades ($\pi_{\text{parthenogens}} = 0.0106 \pm 0.004$ and $\pi_{\text{sexuals}} = 0.0423 \pm 0.010$, respectively ($F = 4.79$; $df = 1, 16$; $P = 0.04$)). Synonymous nucleotide diversity of asexual clades was approx-

imately 75% lower than that of sexual clades. In contrast, nonsynonymous nucleotide diversity of parthenogenetic clades was approximately 40% higher than sexual clades, although the difference was not statistically significant ($\pi_{\text{parthenogens}} = 0.0023 \pm 0.0006$ and $\pi_{\text{sexuals}} = 0.0014 \pm 0.0004$, respectively ($F = 2.02$; $df = 1, 16$; $P = 0.17$)). To examine deviations from neutrality, we determined Tajima's D for asexual and sexual clades (Table 1). Two parthenogenetic clades had highly negative Tajima's D , but were not significant after Bonferroni adjustment. Results from our coalescent simulations suggest that various bottleneck scenarios match the observed negative Tajima's D in these asexual clades (Table 2). For both clades, severe bottlenecks occurring 50,000 years or generations in the past produced negative Tajima's D values that were significantly greater than zero and close to the observed value. Interestingly, both older (200,000) and more recent bottlenecks produced values more consistent with expectations under the neutral model (data not shown). For less-severe bottlenecks, negative D 's were not significantly different from zero. We were also able to generate negative D values under very recent (5000 years ago) and less-severe bottlenecks ($n = 100$), but these negative values were of low magnitude and not significantly different from zero.

BRANCH-SPECIFIC ESTIMATES OF K_a/K_s RATIOS AND COALESCENT-BASED ESTIMATES OF NONSYNONYMOUS MUTATIONS

We employed maximum-likelihood estimation of K_a/K_s ratios under different branch models. The one-ratio model forces all

Table 2. Comparisons of bottleneck models with observed Tajima's *D* for parthenogenetic clades. Size, population size at bottleneck; Years, time since the bottleneck based on assumptions explained in text: BN1–BN3 simulate bottleneck of a single female persisting for five years, whereas BN4–5 simulate bottlenecks of 5000 individuals at various times which then persist for 100 years; *D*, Tajima's *D* (1989); Range, 0.025 and 0.975 percentiles from ms simulations.

Model	Size	Years	<i>D</i>	Range
Clade A Observed			–1.37	
Neutral Model			–0.09	
BN1	1	100,000	–1.07	(–1.92, 0.11)
BN2	1	50,000	–1.39	(–2.03, –0.34)
BN3	1	10,000	–1.43	(–2.02, 0.00)
BN4	5000	100,000	–0.94	(–1.91, 0.93)
BN5	5000	50,000	–1.16	(–2.03, 1.10)
Clade E Observed			–1.42	
Neutral Model			–0.09	
BN1	1	100,000	–1.09	(–1.89, 0)
BN2	1	50,000	–1.41	(–2.1, –0.14)
BN3	1	10,000	–1.09	(–1.88, 0)
BN4	5000	100,000	–1.01	(–2.01, 1.05)
BN5	5000	50,000	–1.10	(–2.10, 1.44)

branches to have the same K_a/K_s ratios. The K_a/K_s ratio under this model is 0.094 (Table 3A), indicating that purifying selection acts against most amino acid substitutions. The two-ratio model allows different K_a/K_s ratios for internal and external branches of the gene tree. The two-ratio model provides a significantly better fit to the data than the one-ratio model (Table 3A; $2\Delta l = 9.5$, $df = 1$, $P < 0.01$). Reduced K_a/K_s ratio in internal branches relative to external branches (0.0688 and 0.166, respectively) is consistent with purifying selection removing mildly deleterious mutations in the long term and persistence of mildly deleterious mutations in the short term. The two-ratio model allowing different K_a/K_s ratios for sexual and asexual branches provides a significantly better fit to the data than the one-ratio model (Table 3A; $2\Delta l = 9.02$, $df = 1$, $P < 0.01$). Sexual branches had lower K_a/K_s ratios relative to asexual branches (0.077 and 0.213, respectively). The three-ratio model allows different ratios for the following branches: external sexual, external asexual, and internal branches. The three-ratio model provides a significantly better fit to the data than the two-ratio model (Table 3A; $2\Delta l = 14.76$, $df = 1$, $P < 0.001$). External asexual branches have more than six times higher K_a/K_s ratios than external sexual branches (0.6844 and 0.1048, respectively), indicating that mildly deleterious mutations accumulate more in recent asexual lineages. The four-ratio model allows different ratios for the following branches: internal sexual, internal asexual, external sexual, and external asexual. The four-ratio model does not provide a significantly better fit to the data than the

Table 3. ρ is the number of free parameters for each model. l is log-likelihood value, and $K_a:K_s$ is the maximum-likelihood estimate of the ratio of amino acid to silent substitutions for each model. Table 3A assumes no reversals to sex in external branches in clade A and Table 3B allows reversals to sex in external branches in clade A.

(A) No reversals to sex				
Model	ρ	l	$K_a:K_s$	
One-ratio	1	–2642.15	All branches = 0.0940	
Two-ratio	2	–2637.40	Internal branches = 0.0688	External branches = 0.1660
Two-ratio	2	–2637.64	Sexual branches = 0.077	Asexual branches = 0.213
Three-ratio	3	–2630.02	Internal branches = 0.0683	External sexual branches = 0.1048
			External asexual branches = 0.6844	
Four-ratio	4	–2630.06	Internal sexual branches = 0.0677	Internal asexual branches = 0.0743
			External sexual branches = 0.1034	
			External asexual branches = 0.6844	
(B) Reversals to sex				
Model	ρ	l	$K_a:K_s$	
One-ratio	1	–2642.15	All branches = 0.0940	
Two-ratio	2	–2637.40	Internal branches = 0.0688	External branches = 0.1660
Two-ratio	2	–2638.97	Sexual branches = 0.0801	Asexual branches = 0.1933
Three-ratio	3	–2631.78	Internal branches = 0.0686	External sexual branches = 0.1150
			External asexual branches = 0.6448	
Four-ratio	4	–2631.77	Internal sexual branches = 0.0677	Internal asexual branches = 0.0743
			External sexual branches = 0.1151	
			External asexual branches = 0.6448	

three-ratio model (Table 3A; $2\Delta l = -0.04$, $df = 1$, $P > 0.9$). Allowing reversals to sexuality in external branches does not change the major conclusion that external asexual branches have significantly higher K_a/K_s ratios than external sexual branches (Table 3B). Under the three-ratio model, external asexual branches are much longer than external sexual branches in the nonsynonymous tree (Fig. 1).

The log-linear modeling of synonymous and nonsynonymous mutations in the asexual and sexual clades showed that there were the following significant interactions: Mutation type \times reproductive mode (log-likelihood Chi-square = 15.83, $df = 1$, $P < 0.001$) and branch-type \times mutation-type (log-likelihood chi-square = 5.57, $df = 1$, $P = 0.02$). For external asexual branches, 55% of all mutations were nonsynonymous (residual = 2.0; $P < 0.05$) whereas for external sexual branches, only 16% of all mutations were nonsynonymous (residual = –2.0; $P < 0.05$). We also note that, of the 15 nonsynonymous mutations found in asexuals, there was only one site (bp 22) in which the same amino acid change

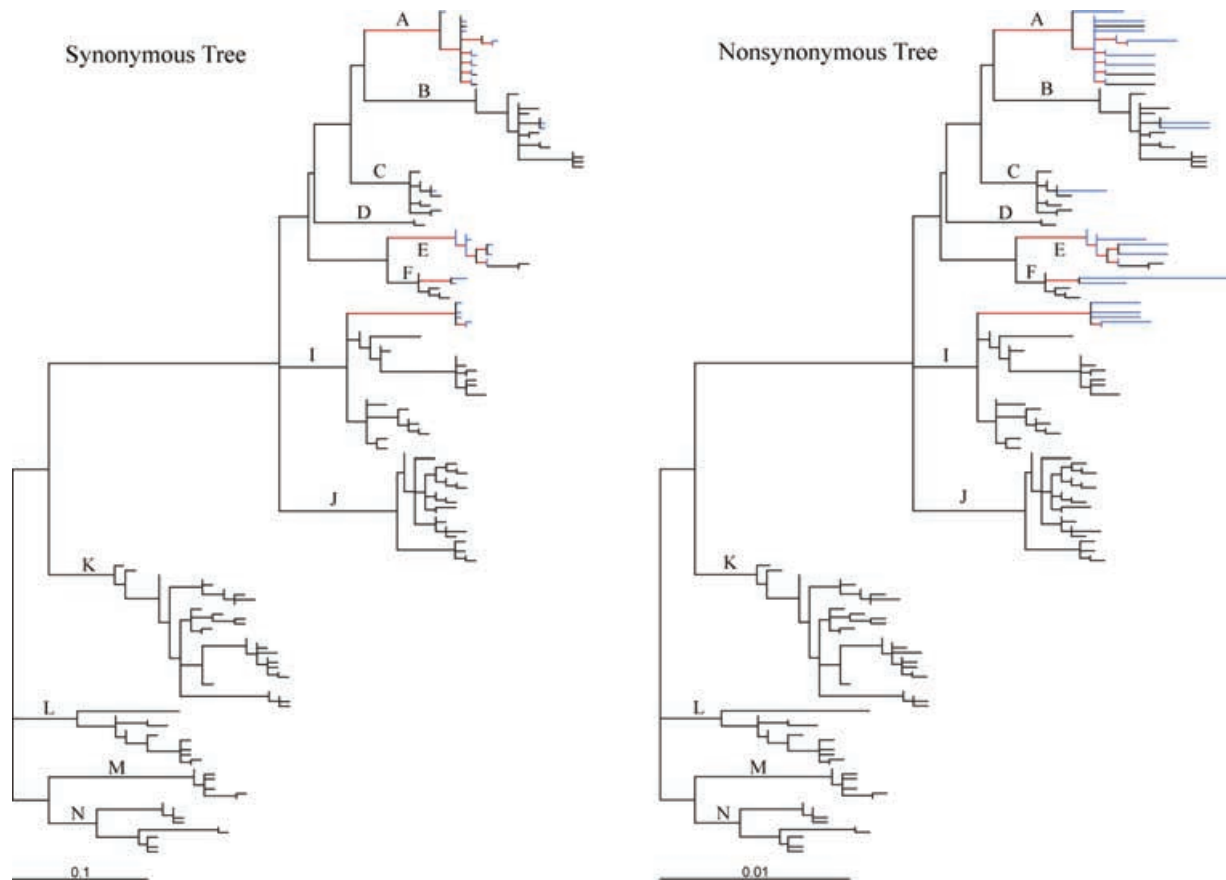


Figure 1. Maximum-likelihood gene trees of cytochrome *b* *Campeloma* haplotypes based on synonymous and nonsynonymous substitutions under the three-ratio PAML branch model. Black branches represent sexual branches and red and blue branches represent internal and external asexual branches, respectively. Well-supported clades are represented by letters (see Johnson 2006).

occurred in two different asexual clades. Thus there is no evidence that repeated, convergent amino acid changes occur in asexuals as might be expected if selection were driving the changes.

Discussion

Sequencing of a mitochondrial gene in multiple lineages of asexual and sexual *Campeloma* resulted in three important findings. First, only clade-specific synonymous sequence polymorphism was significantly reduced in asexuals relative to sexuals. Second, two parthenogenetic clades have significantly negative Tajima's *D* consistent with bottlenecks. Lastly, based on branch-specific estimates using maximum-likelihood and log-linear modeling of substitution types from gene networks, slightly deleterious mutations accumulate in asexual lineages compared to sexual lineages.

EFFECT OF REPRODUCTIVE MODE ON MTDNA SEQUENCE POLYMORPHISM

In a wide range of organisms, numerous studies indicate that reduced polymorphism occurs in regions with lower recombination rates (Begun and Aquadro 1992; Andolfatto and Przeworski 2001), in selfing organisms (Liu et al. 1999; Baudry et al. 2001;

Graustein et al. 2002; Sweigart and Willis 2003), and in domesticated plants undergoing strong artificial selection and bottlenecks (Eyre-Walker et al. 1998; White and Doebley 1999; Hamblin et al. 2004; Wright et al. 2005). To our knowledge, our work represents the first quantitative study showing contrasting patterns of synonymous and nonsynonymous mtDNA sequence polymorphism in asexual versus sexual lineages. Using clade-specific measures, synonymous nucleotide diversity is reduced by 75% in asexual clades compared to sexual clades. Additionally, two parthenogenetic mtDNA *Campeloma* clades had negative Tajima's *D* values. Because organelle and nuclear genes are in linkage disequilibrium in nonrecombining organisms, neutral variants may be driven either to fixation by association with advantageous mutations, eliminated by purifying selection against deleterious mutations linked to these sites, or by bottlenecks. We believe that the most plausible mechanism for reduced synonymous nucleotide polymorphism and negative Tajima's *D* in parthenogenetic *Campeloma* is that severe bottlenecks occurred when founders (i.e., asexual females) arose from a single sexual female. Additionally, bottlenecks may occur after the origin of parthenogens because these parthenogens inhabit marginal,

resource-poor habitats in which there may be frequent reductions in population size or they have undergone widespread range expansions (Johnson 2000, 2005, 2006). If there are continued bottlenecks in parthenogens, this increases the likelihood of detecting its effect on Tajima's D because bottlenecks are only likely to be detected if they occurred recently (Simonsen et al. 1995). Our simulations indicate that a severe bottleneck approximately 50,000 years ago is consistent with the observed D value. Although most polymorphism will be lost during a severe bottleneck, most polymorphism will be the result of new mutations, which should be rare. In asexuals, most of these rare haplotypes found in the exterior of the mtDNA networks have nonsynonymous mutations.

Although we consider severe founder effects to be the most biologically realistic mechanism for reduced synonymous nucleotide diversity in asexuals, there are other possible scenarios involving selection, especially because of strong linkage of the mitochondrial and nuclear genome in asexuals. For example, lower polymorphism in asexuals could also result from genetic bottlenecks due to selective sweeps or strong background selection. Another mechanism causing reduced asexual polymorphism is that, if there are numerous origins of asexuals from sexuals, certain asexual haplotypes may competitively replace other asexual haplotypes, thus producing the signature of a genetic bottleneck. All of these potential mechanisms illustrate the difficulty of disentangling demographic and selection processes on patterns of nucleotide diversity.

Another major result is that external asexual branches, relative to sexual branches, have over six times higher K_a/K_s ratios, indicating that asexual snails accumulate slightly deleterious mutations at a higher rate in the short term. These results are consistent with elevated K_a/K_s ratios in external branches of *Daphnia* parthenogens (Paland and Lynch 2006), suggesting that mildly deleterious mutations accumulate in asexual lineages. Log-linear modeling of nonsynonymous mutations in external branches of sexual and asexual gene networks also strongly supports the idea that weaker purifying selection acts on slightly deleterious mutations in asexual lineages. These results support the notion that a major advantage of sexual reproduction is the reduced accumulation of deleterious mutations. Future work will focus on whether these patterns of nucleotide polymorphism in asexuals occur in the nuclear genome. Furthermore, recent evidence that the genomic deleterious mutation rate in *Drosophila* exceeds one (Haag-Liautard et al. 2007) provides increasing support for the hypothesis that mutation accumulation may eventually doom asexual lineages to extinction.

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