THE EFFECT OF VARIABLE FREQUENCY OF SEXUAL REPRODUCTION ON THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF A CYCLICAL PARTHENOGEN

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Cyclical parthenogens are a valuable system in which to empirically test theoretical predictions as to the genetic consequences of sexual reproduction in natural populations, particularly if the frequency of sexual relative to asexual reproduction can be quantified. In this study, we used a series of lake populations of the cyclical parthenogen, Daphnia pulicaria, that vary consistently in their investment in sexual reproduction, to address the questions of whether the ecological variation in investment in sex is detectable at the genetic level, and if so, whether the genetic patterns seen are consistent with theoretical predictions. We show that there is variation in the genetic structure of these populations in a manner consistent with their investment in sexual reproduction. Populations engaging in a high frequency of sex were in Hardy–Weinberg and gametic phase equilibrium, and showed little genotypic differentiation across sampled years. In contrast, populations with a lower frequency of sex deviated widely from equilibrium, had reduced multilocus clonal diversity, and showed significant temporal genotypic deviation.

KEY WORDS: Asexual, cyclical parthenogen, Daphnia, sexual.

Although there are strong theoretical predictions as to the genetic benefits of sexual reproduction (Felsenstein 1974; Kondrashov 1988; Barton 1995), understanding them on an empirical level has proven more difficult. Identifying patterns of genetic variation in populations or species that differ in their mode of reproduction has the potential to provide insight into the effects of recombination and segregation on species’ genetic architecture, and may help elucidate the evolutionary advantage of sex (Bell 1982; Ceplitis 2001). However, the key problem in experimental approaches is isolating the effects of reproductive mode from other processes such as mutation, selection, and drift, which can all affect the genetic structure of populations in potentially confounding ways. In addition, finding ecologically relevant units, such as populations, that vary their reproductive mode in such a way as to allow the impacts of sexual reproduction to be quantified is challenging, as most species tend to reproduce either wholly sexually or asexually.

The common experimental approach of comparing closely related asexual and sexual species or populations has provided an important yardstick of predicted patterns of population-genetic variation (Innes et al. 1986b; Hebert et al. 1988; Delmotte et al. 2002; Johnson and Howard 2007; Kanbe and Akimoto 2009). Asexual populations often have higher allelic divergence within loci but less genotypic variation among individuals relative to sexual populations. Additionally, sexual populations are more often in agreement with Hardy–Weinberg expectations and less likely to show linkage disequilibrium (LD) among loci (Lynch 1983; Innes et al. 1986a; Simon et al. 1996). Although these findings generally fit theoretical expectations, if the assumption that two groups differ solely in their reproductive mode is incorrect, and as...
is likely there are actually other factors inherent to a specific reproductive mode affecting genetic variation in unaccounted ways, interpretation of the data may be incorrect.

An alternative experimental approach has been to look at patterns of neutral genetic variation across chromosomal regions of variable recombination (Begun and Aquadro 1992; Hudson 1994; Payseur and Nachman 2000), with the general finding being that levels of within-species variability are positively correlated with the rate of recombination. The two major hypotheses for this correlation, background selection and selective sweeps (Maynard Smith and Haigh 1974; Charlesworth et al. 1993, 1995; Gillespie 1997), are based on the idea that there can be a build-up of LD between loci in regions of reduced recombination. In general, less recombination is seen to result in areas of reduced genetic variation and more low-frequency polymorphisms than in regions of higher recombination (Begun and Aquadro 1992; Andolfatto and Przeworski 2001).

There is much evidence that genetic disequilibria accumulate in asexual genomes and chromosomal regions in the absence of sex or recombination (Nachman 2002; Andolfatto and Wall 2003; Kim et al. 2007; Janes et al. 2009), resulting in deviations in patterns of genetic variation from that expected under free recombination (Hill and Robertson 1966). However, there is still little known about the consequences of a variable frequency of sex at the population level in sexual organisms. Cyclical parthenogens, such as the cladoceran *Daphnia pulicaria*, are the ideal study system for addressing this question. Populations may vary widely in their investment in sex (Hebert et al. 1989; Lynch et al. 1989; Cerny and Hebert 1993) and thus have the potential to provide more information on the effects of variable reproductive strategies than can be garnered by only looking at the extremes of all-or-nothing sexual–asexual reproduction. Despite this, and presumably due to the difficulty in estimating the frequency of sex in natural populations, many studies tend to group facultatively parthenogenetic populations into the category of wholly sexual, and compare them directly to closely related asexuas (Hebert et al. 1988; Delmotte et al. 2002). Alternatively, in organisms such as *Daphnia*, habitat permanency is used as an indicator of the population frequency of sex. Temporary pond populations have a high frequency of sex because they must reproduce sexually every season to produce desiccation resistant eggs (ephippia) that hatch to become the next generation when pond conditions again become favorable (Hebert 1974b). In contrast, permanent lake populations are often categorized as having a low frequency of sex because, theoretically, they are able to maintain individuals in the water column year-round and thus do not need to rely on ephippial production (Hebert 1974a). Although genetic (allozyme) patterns detected in some populations fit with expectations based on this ecological designation (Hebert 1974a,b), work on other *Daphnia* populations has produced inconsistent results (Korpelainen 1986; Mort and Wolf 1986). This discrepancy suggests that populations are not necessarily reproducing in the manner predicted by the nature of their habitat (Lynch and Spitze 1994). Clearly, the frequency of sex needs to be quantifiable to causally relate the frequency of sex to the genetic structure of populations.

In this study, we measured the effect of different frequencies of sexual reproduction on the genetic structure of six permanent lake populations of the cyclical parthenogen *D. pulicaria*. All populations reproduce sexually, but the population-level investment in sex, estimated as the production of ephippia and males over a four-year period, varies approximately 30-fold across lakes (Caceres and Tessier 2004). We used microsatellite markers to examine the patterns of genetic variation in these six populations to address the questions of whether the population investment in males and ephippia reflects the actual frequency of genetic recombination and segregation occurring in the population, and whether the genetic patterns produced in populations with varying frequency of sex fit with theoretical predictions and previous empirical approaches. Note that throughout this article we use the term recombination to specifically refer to genetic recombination occurring during meiosis, as a consequence of sexual reproduction.

**Methods**

**POPULATIONS AND SAMPLING**

*Daphnia pulicaria* are small cyclically parthenogenetic microcrustaceans that inhabit permanent freshwater lakes. They reproduce asexually for extended periods that can vary from weeks to years, before engaging in a bout of sexual reproduction, generally in response to cues of a deteriorating environment. Rather than the large clutches produced during asexual reproduction, sex results in the production of a single desiccation resistant capsule (ephippium) that generally contains one to two sexually produced eggs. These sexual eggs can lay dormant until conditions are again favorable and then hatch to either establish new populations or add new genotypes to an already existing one.

The study populations are from six lakes in Barry County in southwest Michigan (U.S.A.), and are within 30 km of each other. These lakes were chosen because they show large variation in the frequency of investment in sexual reproduction across lakes, but relatively consistent frequency within lakes across sample years (Caceres and Tessier 2004), making them the ideal natural system for studying the genetic consequences of sex at the population level. These lakes have similar levels of predation and competition, and the opportunity for migration from numerous other lake populations is equivalent across all six sampled lakes. Fst values averaged 0.3 for all pairwise comparisons of the six lakes (unpubl. data), similar to measures among other previously studied *D. pulicaria* populations (Morgan et al. 2001). *Daphnia pulicaria* prefer low temperatures, and are able to persist during cooler
periods when predation levels are low. Peak ephippial hatching occurs in early spring in all studied populations (Caceres and Tessier 2004). In summer, individuals move to deeper water to avoid increasing water temperatures and to minimize predation and intraspecific competition. It is the size and quality of these deep water refuge areas that is thought to play a role in determining the frequency of sex in these populations (Caceres and Tessier 2004). Large well-oxygenated refugia can maintain larger numbers of individuals throughout the summer period, reducing the population reliance on the dormant sexual stage as individuals are able to persist in the water column year-round.

For this study, we chose two high-, two intermediate-, and two low-sex lakes (Caceres and Tessier 2004). From highest to lowest investment in sexual reproduction, these lakes are: Cloverdale (CD), Little Long (LL), Bristol (BR), Warner (WR) and Pine (PN) and Baker (BK). For a complete description of how the frequency of sex is calculated in these populations, see Caceres and Tessier (2004). Briefly, based on the average frequency of males and ephippial females found in each population over a three-year sampling period (1999, 2000, and 2001), the investment in sex per population was estimated as: Cloverdale 18%, Little Long 15%, Bristol 3.5%, Warner 1.5%, Pine 0.6%, and Baker 0.5%. The consistency of these frequency of sex estimates is supported anecdotally by our own sampling during 2002–2005 (Allen and Lynch 2008). We sampled these lakes repeatedly during the peak periods of sexual reproduction (late May/June) to collect ephippial females (individuals that had already reproduced sexually). We found that the number of samples (vertical net tows) required to collect sufficient individuals with ephippia increased directly and substantially as the predicted population frequency of sex decreased.

Samples were collected from these six populations three times each over a period of four years, for a total of 17 samples. Cloverdale, Little Long, Bristol, Warner, and Pine were all sampled over two consecutive days in the spring of 2002, 2004, and 2005, whereas Baker was sampled in the spring of 2003 and 2004. Baker was also sampled in 2005, but there were no detectable D. pulex. Sampling took place shortly after spring hatching to ensure our sample represented both new sexually produced individuals, and genotypes that had successfully persisted from previous years. Sampling consisted of multiple (20–30) vertical net tows from locations widely distributed across each lake, including repeated tows from the deepest point in each lake. In most of these lakes, a single tow collects hundreds of individuals. All samples were sorted in the laboratory, and approximately 100 individuals per population were randomly chosen from the entire population sample. These animals were isolated in individual 250-mL beakers and maintained via clonal reproduction in the laboratory until tissue could be collected for DNA extraction. This process was repeated in each of the collection years.

MICROSATELLITE GENOTYPING
DNA was extracted from all clones using a standard cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle 1987). All individuals were genotyped at 10 microsatellite loci (Fig. 1), chosen because they were each informative in at least four of the six populations and because these loci are distributed across nine of the 12 chromosomes in the closely related species, D. pulex (Cristescu et al. 2006). All microsatellites were amplified using a three-primer polymerase chain reaction (PCR) protocol where the locus-specific forward primers had an M13 sequence attached to them, and the third primer was color labeled and specific to that same M13 sequence (Schuelke 2000).

PCR reactions were prepared in a 12.5 μl volume, containing 5 ng template DNA, 1xPCR buffer with 15 mM MgCl₂, 2.5 nmol each dNTP, 0.5 U Taq polymerase (Eppendorf), 1 pmol forward primer, and 2 pmol each of the reverse primer and the fluorescent-labeled M13 primer. PCR amplification was conducted as follows: 94°C (5 min), then 10 cycles at 94°C (30 sec)/58–48°C (30 sec)/72°C (45 sec) where the annealing temperature was reduced by 1°C every cycle, 30 cycles at 94°C (30 sec)/48°C (30 sec)/72°C (45 sec), and a final extension at 72°C for 10 min. The amplified product was diluted 40-fold, and 2 μl added to 8.9 μl H₂O and 0.1 μl Genescan-500 Liz size standard (Applied Biosystems, Carlsbad, CA). Samples were denatured at 95°C for 5 min, and immediately cooled on ice. Microsatellite products were resolved on an ABI 3730 and individual genotypes visualized using Genotyper and Genescan software (Applied Biosystems). All genotypes were manually checked, and plate-wide adjustments made to any allele differences that were a result of variation in ABI 3730 runs.

DATA ANALYSIS
Allele frequencies and expected and observed heterozygosity per locus (Nei 1978) were calculated for each sample in GENEPOP Version 3.4 (Raymond and Rousset 1995). Deviations from Hardy–Weinberg expectations at individual loci were assessed using an exact test (Guo and Thompson 1992) in GENEPOP, which calculates the probability of the observed sample relative to all other possible combinations based on the observed allele frequencies. Pairwise genotypic LD between all pairs of loci in each sample was tested in FSTAT version 2.9.3 (Goudet 1995) using a log-likelihood ratio G-statistic. Bonferroni corrections for multiple testing were applied prior to determining significance.

Populations with periods of asexual reproduction can accumulate multiple copies of the same individual genotype either due to selection favoring a particular phenotype or due to random genetic drift. This can lead to a reduction in the number of unique multilocus genotypes (MLGs) found in the population relative to that expected in a randomly mating sexual population with the same allele frequencies. To test for this reduction in genotypic
diversity, we compared the observed number of unique MLGs in each sample with the number expected in a sample of the same size from a freely recombining sexual population with the same allele frequencies. Monte Carlo simulations with 1000 iterations were used to construct null distributions of the expected number of genotypes for each of the 17 samples, against which the observed numbers were tested for significant deviation from expected values. The measure, reported as genotypic diversity ratio (GDR, Table 1), is the ratio of the observed to the expected number of MLGs, where the expected numbers are the means from the simulated distributions.

To assess the level of within-population structuring, we calculated $F_{is}$ (Wright 1921) for each sample in each year according to Weir (Weir and Cockerham 1984) in FSTAT (Goudet 1995). This statistic measures any within-sample heterozygote deviation. Significant deviation within populations was assessed by permuting alleles among individuals within samples 1000 times, where $n$ is sample size and $l$ is the number of the loci, and comparing the observed value to the randomized dataset.

Comparisons of genotype frequencies across years, within each of the six populations, were carried out using a log-likelihood based exact test ($G$) (Goudet et al. 1996) in GENEPOP (Raymond and Rouset 1995). This program calculates the unbiased estimate of the probability of the statistic $G$, testing the null hypothesis that the genotypic distribution is identical across populations. We were only interested in how the frequency of sex affected the genotypic distribution across time within populations, rather than differentiation among populations, thus only probability values for temporal comparisons within populations were used.

Results

There was an average of two alleles per locus across all populations. All populations except Bristol were monomorphic at one or
Table 1. Summary statistics for each population and sample year. The population frequencies of sex (FS) are listed as high (H), medium (M), and low (L), from the highest frequency of sex population (Cloverdale) at the top, to the lowest frequency of sex population (Baker) at the bottom.

<table>
<thead>
<tr>
<th>FS Population</th>
<th>N</th>
<th>H_e (H_o)</th>
<th>F_is</th>
<th>GDR</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloverdale 02</td>
<td>38</td>
<td>0.14 (0.13)</td>
<td>0.06</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>H Cloverdale 04</td>
<td>47</td>
<td>0.12 (0.10)</td>
<td>0.18</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Clove0rdale 05</td>
<td>51</td>
<td>0.12 (0.11)</td>
<td>0.05</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>Little Long 02</td>
<td>60</td>
<td>0.27 (0.26)</td>
<td>0.02</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>H Little Long 04</td>
<td>50</td>
<td>0.27 (0.25)</td>
<td>0.08</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Little Long 05</td>
<td>44</td>
<td>0.24 (0.21)</td>
<td>0.10</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>Bristol 02</td>
<td>60</td>
<td>0.32 (0.32)</td>
<td>0.02</td>
<td><strong>0.5</strong></td>
<td>7</td>
</tr>
<tr>
<td>M Bristol 04</td>
<td>49</td>
<td>0.33 (0.37)</td>
<td><strong>-0.13</strong></td>
<td><strong>0.5</strong></td>
<td>6</td>
</tr>
<tr>
<td>Bristol 05</td>
<td>60</td>
<td><strong>0.38 (0.30)</strong></td>
<td>0.21</td>
<td>0.5</td>
<td>12</td>
</tr>
<tr>
<td>Warner 02</td>
<td>60</td>
<td>0.34 (0.41)</td>
<td><strong>-0.20</strong></td>
<td>0.4</td>
<td>11</td>
</tr>
<tr>
<td>M Warner 04</td>
<td>54</td>
<td>0.37 (0.44)</td>
<td><strong>-0.21</strong></td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Warner 05</td>
<td>58</td>
<td>0.38 (0.44)</td>
<td><strong>-0.16</strong></td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>Pine 02</td>
<td>60</td>
<td>0.23 (0.29)</td>
<td><strong>-0.30</strong></td>
<td>0.3</td>
<td>5</td>
</tr>
<tr>
<td>L Pine 04</td>
<td>47</td>
<td>0.22 (0.25)</td>
<td><strong>-0.14</strong></td>
<td>0.4</td>
<td>4</td>
</tr>
<tr>
<td>Pine 05</td>
<td>27</td>
<td><strong>0.27 (0.22)</strong></td>
<td>0.19</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>Baker 03</td>
<td>60</td>
<td>0.39 (0.39)</td>
<td>0.02</td>
<td>0.6</td>
<td>14</td>
</tr>
<tr>
<td>L Baker 04</td>
<td>56</td>
<td><strong>0.33 (0.37)</strong></td>
<td><strong>-0.14</strong></td>
<td><strong>0.14</strong></td>
<td>10</td>
</tr>
</tbody>
</table>

Statistics listed are: N = sample size; $H_e$ ($H_o$) = expected and observed heterozygosity; $F_{is}$ = Wright's inbreeding coefficient; GDR = genotypic diversity ratio (the ratio of observed to expected number of MLGs); and LD = the number of locus pairs in each sample that deviate significantly from linkage equilibrium (relative to that expected under random mating). Values that significantly deviate from expectations under random mating are shown in bold ($P < 0.05$).

Discussion

This study provided the unique opportunity to measure the genetic consequences of sex in six populations of the same species that all have the same mating system (cyclical parthenogens) but are known to vary widely and consistently in their investment in sexual reproduction. We used this to ask whether the variation in the frequency of sex across these populations was discernable at the genetic level, and whether the genetic structure of these populations fit with theoretical expectations.

A single bout of random mating is sufficient to restore a population to Hardy–Weinberg equilibrium, thus, if the sampled individuals were the product of random mating during the prior episode of sex the population should conform to H-W expectations. This was seen in the two highest sex populations, where all loci in all sampled years were in H-W equilibrium (Fig. 1). However, as the population frequency of sex decreased, the number of loci deviating from H-W expectations increased, to the extreme that Baker, the population with the lowest frequency of sex, had six of 10 loci in disequilibrium in one sampled year (Fig. 1). This increased disequilibrium in the low-sex populations is consistent with clonal selection generating high-frequency copies of particular genotypes during extended periods of asexual reproduction, and the associated amplification of random combinations of linked neutral variants. Similarly, when we measured LD among loci, we found that while there was not an actual gradient of increasing disequilibria specifically matching the decreasing population frequency of sex, the among-locus associations were only seen in the four populations with lowest frequency of sex (Table 1). The two populations with the highest frequency of sex showed no

more loci, and both Cloverdale and Little Long each had one locus that remained monomorphic across all three years. Most alleles were shared across populations, and when alleles were unique to a population they were at low frequencies of approximately 1–17%.

The GDR varied widely across populations, ranging from 0.3 in one of the lowest sex populations to 1.0 in three of the high-sex population samples (Table 1). All four of the lower sex populations showed a significant ($P < 0.05$) reduction in multilocus genotypic diversity relative to that expected in a randomly mating sexual population. The values stayed quite consistent within populations across years, except in Warner where the GDR increased from about 0.4 in 2002 to over 0.7 in 2004 and 2005 (Table 1).

The two populations with the highest frequency of sex, Cloverdale and Little Long, were consistent with Hardy–Weinberg (H-W) expectations at all loci (Fig. 1). Individual loci in remaining populations deviated from H-W expectations (Fig. 1), with both homozygote and heterozygote excess at multiple loci in Bristol, Pine, and Baker, but only excess heterozygosity in Warner. Measuring across all loci at the population level, significant deviation from random mating ($F_{is}$, $P < 0.05$) was seen in seven of the 17 samples. Bristol 2005 and Pine 2005 showed overall excess heterozygosity, and Baker 2004, Pine 2002, and all three Warner samples exhibited excess heterozygosity (Table 1). No deviation from random mating was seen in either of the two high-sex populations in any sampled year (Table 1).

The four lower sex populations, Bristol, Warner, Pine, and Baker, showed significant LD among some locus pairs, whereas all locus pairs in the two high-sex populations were in linkage equilibrium (Table 1). In Bristol, seven locus pairs were in significant disequilibrium in at least two of the sample years, whereas both Pine and Baker had four locus pairs significant across sample years.

There was significant temporal genotypic differentiation between most years in the four lowest sex populations. Baker 2003 and 2004, all three Pine sample years, Warner 2002 and 2004, and Warner 2002 and 2005 were all significantly different ($G$, $P < 0.01$), as was Bristol 2005 from both other sample years ($G$, $P < 0.05$). There was no genotypic differentiation between any sample years in Cloverdale or Little Long.
deviation from among-locus linkage equilibrium, consistent with what would be expected in a randomly mating sexual population.

Significant deviation from random mating ($F_{is}$, Table 1), as seen in the four lower sex populations, resulted in both heterozygote and homozygote excess, depending on the locus, population, and sample year. Under strict clonal reproduction, heterozygosity should increase indefinitely as the accumulation of independent mutations leads to within-locus allelic divergence (Birky Jr. 1996). However, if the population experiences recombination events, the variance among $F_{is}$ estimates across loci is expected to increase (Balloux et al. 2003; de Meeus and Balloux 2004). Although we did see overall population level excess heterozygosity in seven of the 11 low-sex samples (five significant), the direction of deviation from random mating clearly varied among loci within each sample in all four lower sex populations (Fig. 1). Due to the different frequency of sex in these populations, the variation in levels of excess heterozygosity is not altogether surprising. What is interesting, however, is that even with regular periods of asexual reproduction, the two high-sex populations have $F_{is}$ values consistent with a randomly mating sexual population.

In the absence of linkage among loci, or selection on MLGs, populations undergoing random mating should exhibit neutral-marker genotype frequencies that are solely a function of the allele frequencies in the population. However, in cyclically parthenogenetic species, periods of asexual reproduction can result in high-frequency copies of individual genotypes. As with the previous population-genetic measures, we found that the two high-sex populations exhibited a diversity of genotypes that was consistent with a randomly mating sexual population (Table 1). This suggests that novel recombinant genotypes are regularly added to the population from ephippial hatching, and that the periods of asexual reproduction are not sufficient to allow individual genotypes to reach a high copy number. In contrast, the remaining four lower sex populations showed a significant reduction in the number of unique genotypes relative to that of a sexually reproducing population, in all years sampled. Thus, although low-sex populations likely have some unique or low-frequency genotypes from recently hatched ephippia, they mostly consist of high copy number genotypes that have amplified during extended periods of asexual reproduction. A similar relationship has recently been identified in the parthenogenic freshwater planarian Schmidtea polychroa, where the subpopulation frequency of tetraploid individuals (a potential indicator of occasional sex) was found to be positively correlated with a measure of genotypic diversity (D’Souza and Michieles 2006). Interestingly, despite being predominantly parthenogenic, all but one of the six subpopulations in that study had seemingly high genotypic diversity.

As neutral microsatellite loci are not directly undergoing selection, allele and genotype frequencies are expected to vary little across generations in a randomly mating sexual population. However, in the absence of recombination, neutral loci will be subject to the effects of selection on neighboring loci (Gillespie 1997), or in the case of clonal reproduction, selection on the entire genome (Lynch 1987). Thus, allele and genotype frequencies may vary stochastically due to their chance association with favored genetic combinations. We found that extended periods of asexual reproduction do result in significant temporal differentiation in both allele and genotype frequencies in the low-sex populations, and that the MLGs reaching a high copy number varied across sample years. As the population frequency of sex increased, temporal differentiation decreased, to the extent that the two high-sex populations had consistent allele and genotype frequencies across the three sample years. Previous work has shown that variation among populations of Daphnia can be due to random fluctuations in selection intensity (Lynch 1987), as slight variations in environmental conditions can lead to different genotypes being favored in neighboring populations. This argument can also be applied to the within-population temporal variation seen here, as clonal reproduction can result in different genetic combinations rapidly reaching a high frequency with slight changes in selection pressure. Alternatively, different combinations of genes may produce the same favored phenotype and thus by chance association with the selected alleles, different neutral variants may be amplified in any given year.

Despite well-defined theoretical predictions as to the effects of genetic recombination on population-genetic diversity (Felsenstein 1974; Barton 1995), the inherent variability of natural populations has made it difficult to directly attribute empirically measured patterns to a variable frequency of sex. Here, we have shown that a series of populations known to have differing frequencies of sex also have patterns of genetic variation consistent with the relative investment in sex. Recently, several studies have suggested that the combination of season length, population and egg bank size, and clonal selection strength (De Meester et al. 2006); or ephippial hatching rates (Thielsch et al. 2009), can be used to predict the genetic structure of facultative parthenogenic populations. The data from the six lake populations in this study strongly support the idea that the simpler measure of the proportional investment in sex alone explains the observed genetic patterns. In addition, the patterns of genetic variation across the gradient of high to low frequency of sex in are in agreement with predicted effects of decreased recombination and segregation (Przeworski et al. 2001; Nachman 2002; Andolfatto and Wall 2003).

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LITERATURE CITED

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