Evolution, 2025, **XX(XX)**, 1–13
https://doi.org/10.1093/evolut/qpaf016
Advance access publication 28 January 2025
Original Article

OXFORD

Comparing the hermaphroditic mating system of a parasitic flatworm between populations with an ancestral, three-host life cycle and a derived, facultative precocious life cycle

Jenna M. Hulke[1,2] and Charles D. Criscione[2]

[1]Host-Pathogen Interactions, Texas Biomedical Research Institute, San Antonio, TX, United States
[2]Department of Biology, Texas A&M University, College Station, TX, United States
Corresponding author: Texas Biomedical Research Institute, 8715 Military Drive, San Antonio, TX 78245-0549, United States. Email: jenna.m.hulke@gmail.com

Abstract

Evolutionary changes in development and/or host number of parasite life cycles can have subsequent ecological and evolutionary consequences for parasites. One theoretical model based on the mating systems of hermaphroditic parasites assumes a life cycle with fewer hosts will result in more inbreeding, and predicts a truncated life cycle most likely evolves in the absence of inbreeding depression. Many populations of the hermaphroditic trematode *Alloglossidium progeneticum* maintain an ancestral obligate three-host life cycle where obligate sexual reproduction occurs among adults in catfish third hosts. However, some populations have evolved a facultative precocious life cycle, where sexual development can occur while encysted within crayfish second hosts, likely leading to high inbreeding as individuals are forced to self-mate while encysted. Whether selfing represents a derived state remains untested. We compared selfing rates of 5 precocious populations to that of 4 populations with an ancestral obligate three-host life cycle. We also compared demographic estimates to genetic estimates of selfing to test the prediction of no inbreeding depression in precocious populations. Results showed that while the ancestral obligate three-host life cycle is associated with high outcrossing rates, the facultative precocious populations are highly selfing and show little evidence for inbreeding depression.

Keywords: life cycle evolution, selfing rate, Trematoda, inbreeding depression

Introduction

Parasitic life cycles exhibit a great amount of diversity in the number of developmental stages and/or the number of hosts required to complete a life cycle (Schmidt & Roberts, 2009). Many parasitic flatworms maintain complex life cycles, where two or more hosts are required for the parasite to reach sexual maturity. While there are proposed benefits of evolving or maintaining complex life cycles such as increasing parasite growth, facilitating transmission (Benesh et al., 2013, 2014, 2022; Parker et al., 2015a, 2015b), or aggregating parasites for mating opportunities (Brown et al., 2001), the trade-offs for having more hosts within a life cycle is that sexual reproduction is delayed in earlier hosts (i.e., intermediate hosts) at the risk of decreased survivorship, or a cost for generalism, which is defined as the ability to exploit different host species at different life stages (Ball et al., 2008; Iwasa & Wada, 2006; Parker et al., 2003, 2015a). Mechanisms such as low host specificity at a given life stage (Benesh et al., 2021) and host manipulation (Benesh, 2011; Cézilly et al., 2010; Hurd et al., 2001) are thought to mitigate the effects of decreased survivorship. Among trematodes, however, the evolution of precociousness, i.e., sexual reproduction occurs in what was previously a juvenile state in an intermediate host, has resulted in either the complete loss or facultative truncation of a previous definitive host, i.e., host where sexual reproduction occurs (Lefebvre & Poulin, 2005). In the case of facultative truncation, individual parasites may be able to reproduce sexually in both the intermediate and the definitive host. As this impacts the timing and localization of sexual reproduction, the evolution of precociousness may have downstream consequences for the mating systems of hermaphroditic trematodes (Brown et al., 2001).

Brown et al. (2001) proposed a model for the evolution of complex life cycles focusing on inbreeding and inbreeding depression. Their model is based upon the premise that additional hosts within a life cycle allow for parasites to be aggregated into a single host further up the food chain. By increasing the infection intensity (i.e., number of parasites in an infected host; Bush et al., 1997) within a host, parasites have increased outcrossing opportunities that would reduce the potential or need for self-mating (e.g., Detwiler et al., 2017; Hulke & Criscione, 2024). Another potential aspect of a complex life cycle that may reduce inbreeding is that unrelated individuals can be mixed at each stage during the transmission process, reducing the potential for biparental inbreeding as kin parasites are not co-transmitted to their definitive host (Criscione & Blouin, 2006; Criscione et al., 2022). As such, the Brown et al. (2001) model assumes that a shorter life cycle will lead to more inbreeding compared to the mating system of a longer life cycle. The Brown et al. (2001)

Received October 3, 2024; revisions received January 3, 2025; accepted January 24, 2025
Associate Editor: Scott Burgess; Handling Editor: Jason Wolf

model also predicts that a simpler life cycle is more likely to evolve when the fitness of an inbred offspring is greater than or equal to the fitness of an outcrossed offspring (i.e., no inbreeding depression). To date, Hulke and Criscione (2024) is the only study that has explicitly tested the assumption and the prediction of the Brown et al. (2001) model with additional support for the model from studies on the facultative precocious species *Coitoceacum parvum* (Lagrue et al., 2009; Villa & Lagrue, 2019). However, in neither of these systems was a population or species with an ancestral life cycle compared to ascertain if the high selfing rates were derived themselves. Thus, a key question remains as to whether the high amount of selfing is a result of truncating a life cycle or if high selfing was already occurring within the ancestral, obligate three-host state.

A three-host life cycle where obligate asexual reproduction occurs in a mollusk first host, cercariae larvae leave, penetrate, and encyst as juvenile metacercariae in a second host, and sexual reproduction occurs in a third host that consumed the second, is the most common among trematodes, and evolved early in the trematode phylogeny (Cribb et al., 2003; Olson et al., 2003). Nonetheless, species with precocious life cycles (facultative or obligate) occur throughout the trematode phylogeny with at least 79 species from 50 genera from 24 families (reviewed in Lefebvre & Poulin, 2005). The precocious life cycles of trematodes are presumed to be derived; however, phylogenetic trait reconstruction analysis in the trematode genus *Alloglossidium* (Kasl et al., 2018) is the only formal study to date. Within the genus, the evolution of a precocious life cycle occurred at least three times, providing evidence that precociousness is a derived state (Kasl et al., 2018). Of particular interest is the node leading to *A. progeneticum* and its sister species *A. renale*. Two trait reconstruction methods, a maximum likelihood estimate and the average frequency across trees, both indicated the most likely ancestral state at this node was an obligate three-host life cycle (Kasl et al., 2018). There are populations of *A. progeneticum* that maintain the ancestral obligate, three-host life cycle (aquatic snail to crayfishes to catfishes), while other populations have facultative precocious life cycles (Figure 1). In the populations with the ancestral life cycle, individual parasites remain in a juvenile state when encysted in crayfish antennal glands (Figure 1A). Upon infection of a catfish host, flukes excyst, sexually mature, and can outcross with other individuals in the catfish intestine. In contrast, in the facultative precocious populations, flukes commonly sexually mature while still encysted in crayfishes; hence, there is forced self-mating (Figure 1B). *Alloglossidium progeneticum* has a broad distribution throughout the southern United States including Alabama, Arkansas, Georgia, Louisiana, Oklahoma, and Mississippi (Font & Corkum, 1975; Kasl et al., 2015; McAllister et al., 2016; Sullivan & Heard, 1969). In two river systems in Georgia (Oconee and Flint Rivers), precocious populations have been identified while all other reported locations are known as obligate three-host populations (Kasl et al., 2015).

As the evolutionary history of the genus *Alloglossidium* is known, in particular, phylogenetic trait analysis supports an ancestral three-host life cycle in the lineage of *A. progeneticum* (Kasl et al., 2018), and because there is variation among populations in life cycle patterns, the system of *A. progeneticum* is ideal to ask if a trematode ancestral three-host life cycle already had high selfing or if self-mating was derived as a consequence of a life cycle change. In addition, *A. progeneticum* provides a second and independent system to test the assumption and prediction of the Brown et al. (2001) model. Along with testing the ancestral and derived life cycles for a change in the hermaphroditic mating system, we also tested for inbreeding depression from field-collected samples by comparing demographic estimates of selfing to genetic estimates of selfing in the precocious populations of *A. progeneticum* (Hulke & Criscione, 2024).

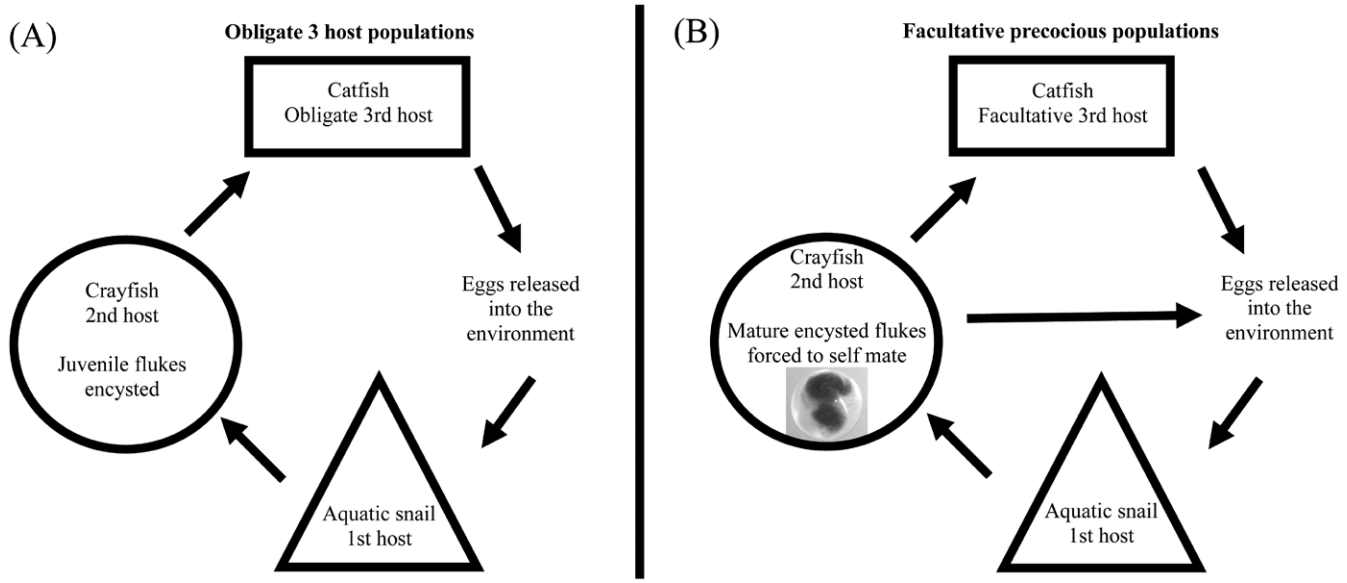

Figure 1. Life cycles of *Alloglossidium progeneticum*. In both life cycles, obligate asexual reproduction occurs within the snail first host. (A) In the obligate three-host populations, the encysted fluke remains in a juvenile state within the crayfish second host. Upon ingestion of a catfish third host, the fluke excysts, sexually matures, and can outcross with other individuals in the intestine. (B) In the facultative precocious populations, *A. progeneticum* commonly sexually matures while encysted in the crayfish host. As the fluke is encysted, all offspring will be the product of self-fertilization (pictured is an encysted gravid fluke). Catfish can still be infected with non-encysted adult flukes.

Methods

Collections

Alloglossidium progeneticum samples were collected in May 2018 from nine locations (Supplementary Figure S1; GPS coordinates are listed in Supplementary Table S1). Five locations from two river drainages in Georgia had parasite populations with facultative precocious life cycles: Big Indian Creek, Calls Creek, and Yellow River from the Oconee River drainage, and Cane Creek and Richland Creek from the Flint River drainage. The other four locations had parasite populations with obligate three-host life cycles. These locations consisted of two populations in Louisiana (Chappapeela Creek and Hays Creek), one location in Arkansas (Nix Creek), and one in Texas (Gus Engling Wildlife Management Area, GEWMA). With the exception of GEWMA, where baited fish traps were used to collect catfishes only, crayfish and catfish hosts were collected by backpack electroshocking into a seine net.

In the facultative precocious populations, we could derive a demographic-based estimate of the selfing rate using the proportion of gravid encysted trematodes in crayfish out of all total adult trematodes (i.e., those in catfish and crayfish). For this demographic selfing-rate estimate, we needed an estimate of the ratio of crayfish to catfish population sizes (derivation of estimate described below). To get an estimate of the ratio, we haphazardly sampled presumed suitable crayfish and catfish habitat (e.g., partially submerged logs, debris, or rocks) along a 0.8- to 1.6-km stretch of a creek. A sampling draw constituted a person electroshocking approximately 3–6 m upstream of the seine net and walking toward the seine till it was reached. Between 10 and 20 draws were taken per creek depending on suitability and availability of habitat in a creek. For each draw, the observed number of crayfish and catfish were recorded. We kept a subset of crayfish and/or catfish for subsequent dissection and released the remainder downstream to preclude resampling. The ratio of crayfish to catfish population sizes for each location was calculated based on the total number of crayfish and catfish across all draws, and an estimate of the ratio's error was obtained from bootstrapping over draws (described below).

Crayfish were dissected, and *A. progeneticum* were removed from the antennal glands of the host, excysted, and examined for the presence of eggs. Within the facultative precocious populations, a few parasites within the crayfishes were not gravid. As these individuals can either be juveniles that will eventually become sexually mature while encysted or they might remain in a juvenile state, thus needing the catfish host to sexually develop, we recorded the number of gravid and nongravid flukes from crayfish at each location for downstream analysis. Catfish were dissected and *A. progeneticum* were removed from the gut track of the host. All flukes from catfishes were mature as indicated by the presence of eggs except a single individual from Richland Creek. All parasites were preserved in 70% ethanol for later DNA extractions.

DNA extraction

Our goal was to microsatellite genotype approximately 20–30 individuals per location. To minimize the chance of sampling kin (e.g., clonemates arising from asexual reproduction in the first host), we attempted to extract only one trematode per host. However, in two populations, Hays Creek and Nix Creek, we had to extract two or three parasites from a few hosts with larger intensities due to a lower number of sampled hosts. For seven out of nine locations, only encysted individuals from crayfish hosts were extracted and used for genetic analysis. However, in Chappapeela Creek individuals from both crayfish and catfish hosts were extracted so that only one individual per host was used. Individuals extracted from GEWMA were only from catfish as we were unable to catch crayfish in 2018. Our goal was to microsatellite genotype and sequence a mtDNA locus at approximately 20–30 individuals per location. Due to some unexplained failures in some runs for genotyping or sequencing, sample sizes may not be the same between the microsatellite multilocus genotypes and the mtDNA sequences generated within a location. Previous sampling revealed no precocious development within the crayfish hosts in GEWMA (Kasl et al., 2015). While encysted individuals from crayfish hosts had no opportunities for outcrossing, individuals from catfish may have been exposed to sperm from other individuals. To avoid genotyping stored sperm and for consistency, only the anterior portion near the oral sucker, which lacks reproductive structures, of each fluke was used for extractions. DNA was extracted in a 25 µl, 5% chelex solution containing 0.2 mg/ml of Proteinase K and incubated for 2 hr at 56 °C before being boiled at 100 °C for 8 min. The extraction was subsequently stored at –20 °C.

Microsatellite genotyping and mitochondrial sequencing

Tissue from three *A. progeneticum* collected from Calls Creek crayfish and three *A. progeneticum* from GEWMA catfish were sent to Cornell Life Sciences Core Laboratory Center (Ithaca, NY) to produce microsatellite libraries. Construction of the microsatellite library as well as the screening of microsatellite loci followed the methods of Hulke and Criscione (2023). The final data set consisted of 22 polymorphic loci we developed from these libraries (primers and GenBank accession numbers given in Supplementary Table S2).

Whole genome amplification using the Illustra Ready-to-Go GenomiPhi V3 DNA Amplification kit, and the polymerase chain reactions (PCR) followed the protocols from Hulke and Criscione (2023). Genotyping was conducted on a 3730xl 96-Capillary Genetic Analyzer, using the 500 LIZ size standard at the Keck DNA Sequencing Core at Yale School of Medicine (New Haven, CT). Genotypes were manually scored using GENOTYPER 3.7 (Applied Biosystems).

As another means to assess within-population genetic diversity as well as relationships among precocious and obligate three-host populations of *A. progeneticum*, we amplified and sequenced 678 base pairs of the mitochondrial NADH-dehydrogenase subunit 1 gene (ND1). The PCR amplifications were performed following the protocol of Kasl et al. (2015) with a total reaction volume of 25 µl that consisted of 3 µl of template, 16.25 µl water, 2.5 µl 10× buffer, 1.5 µl $MgCl_2$ (25 mM), 0.5 µl dNTP (10 mM/each), 0.5 µl of each primer, and 0.25 µl of Taq polymerase (Omega Bio-Tek, Inc.). The forward primer was MB352 (5′-CGTA AGGGKCCTA AYA AG-3′; Criscione & Blouin, 2004) and the reverse primer was CC28 (5′-CWTCTCAARGTTAACAGCCT-3′; anchored in the asparagine tRNA). The thermocycler profile is described in Criscione and Blouin (2004). PCR products were purified using the E.Z.N.A. Cycle-Pure Kit from Omega Bio-tek and were sequenced at Eurofin Genomics. Contiguous ND1 sequences from individuals were assembled within the

BioEdit program, version 7.1.8 (Hall, 1999) and submitted to GenBank (accession numbers PP578427–PP578663).

Within and among population structure and genetic diversity

At the microsatellite loci, deviations from Hardy–Weinberg Equilibrium (HWE) were quantified by the inbreeding coefficient F_{IS} (Weir & Cockerham, 1984). By-locus F_{IS}, allelic richness (rarefied to the smallest collection sample size), F_{ST} (Weir & Cockerham, 1984), and microsatellite gene diversities (H_S) were calculated for all nine populations using FSTAT v2.9.4 (Goudet, 1995). To assess whether the multilocus F_{IS} significantly deviated from HWE in any of the nine populations, we used SPAGEDI version 1.2 (Hardy & Vekemans, 2002) to obtain a two-tailed p-value by randomizing alleles among individuals 10,000 times. Welch two-sample t tests accounting for unequal variances were performed in base R (R Core Team, 2020) to assess whether allelic richness (averaged across loci per population) or gene diversities (averaged across loci per population) differed significantly between the facultative precocious populations and the obligate three-host populations. Genotypic disequilibrium was tested using GENEPOP v4.7.5 (Rousset, 2008) with the Markov chain set for 1,000 dememorization, 100 batches, and 1,000 iterations per batch. We used the exact binomial test ($\alpha = 0.05$) to globally determine if the number of significant pairwise comparisons was greater than what was expected by chance. Global and pairwise F_{ST} was tested with the G-based test (using FSTAT v2.9.4) with 1,000 randomizations of multilocus genotypes among populations.

To explore relationships among the sampled populations of *A. progeneticum*, a haplotype network of ND1 was constructed with TCS version 1.21 (Clement et al., 2000) and visualized using POPART (Leigh & Bryant, 2015). For heuristic purposes, we also constructed an ND1 network with our newly generated haplotypes and those previously published in Kasl et al. (2015) (GenBank accession numbers: KT455825.1–KT455707.1). We note that in Figure 3 of Kasl et al. (2015) there was an accidental miss-shading such that individuals from Big Indian Creek were represented with vertical lines and Calls Creek were shown with dots. These individuals from these two locations only had Haplotypes 13 and 14. This shading should be reversed such that individuals with Haplotype 14 were predominately from Calls Creek and individuals with Haplotype 13 were predominately from Big Indian Creek (Kasl et al., 2015). Nothing changes in their interpretations nor in our interpretations herein as the mistake occurred between individuals in two precocious populations in the Oconee River drainage (Calls Creek and Big Indian Creek) and with two haplotypes separated by a single base difference. The accession numbers in GenBank (Calls Creek: KT455758.1–KT455768.1 and Big Indian Creek: KT455748.1–KT455757.1) from Kasl et al. (2015) are correctly labeled for their location origin and the mistake was only in the shading of the key of Figure 3 in Kasl et al. (2015). Haplotype (H_d) and nucleotide diversities (π) within the nine populations were calculated in DnaSP version 6 (Librado & Rozas, 2009). Welch two-sample t tests were conducted to determine if H_d and π (nucleotide diversity; Nei, 1987, p. 512) differed between populations with facultative precocious life cycles to those with obligate three-host life cycles.

Estimating and testing selfing rates from genetic data

Following the methods in Hulke and Criscione (2024), we obtained genetic-based estimates of the selfing rate (s_G) using four methods. The first method used F_{IS} with selfing rates (designated s_{GF}) obtained through the inbreeding equilibrium relationship $s_{GF} = 2F_{IS}/(1 + F_{IS})$ (Jarne & David, 2008). Confidence intervals (CI) of multilocus F_{IS} were obtained by 10,000 bootstraps over individuals using the program GENETIX v4.05 (Belkhir et al., 2004). The second method used identity disequilibrium as calculated by the g_2 statistic, which quantifies the relative excess of genotypes heterozygous at two loci and can be equated to a selfing rate (David et al., 2007). We used the R package InbreedR (Stoffel et al., 2016) to obtain the selfing estimate (s_{GG}), test for statistical significance of the selfing rate with 10,000 permutations of single-locus data among individuals, and produce CI by bootstrapping 10,000 times over individuals. The next two methods for estimating selfing rates were based on Bayesian model-based approaches implemented in the softwares BES (Redelings et al., 2015) and INSTRUCT (Gao et al., 2007). BES models coalescence events while accounting for identity disequilibrium to estimate the likelihood of selfing rates. With BES, the generic script parameter of "f_other" was set to zero and three independent chains of 100,000 iterations were run to infer the selfing rate (s_{GB}). Using the "statreport" command, we obtained the median selfing estimate and credible interval of s_{GB}. The Potential Scale Reduction Factor for the s_{GB} estimates was between 1.00 and 1.01 for all nine populations, indicating similar distributions among the three independent chains within each population. INSTRUCT uses information on homozygosity and allele frequencies to estimate selfing rates. With INSTRUCT, the selfing rate (s_{GI}) and credible intervals were estimated using the best run (the highest posterior median log-likelihood) of three independent chains. Parameters were set for a single population (K set to 1) and Mode 2 (infer population selfing rates) with each chain containing a total of 1,000,000 iterations (burn-in of 500,000). Median and credible intervals for the Gelman–Rubin statistic for convergences were good, ranging from 0.999 to 1.038 for all nine populations.

Welch two-sample t tests were performed to assess whether the s_G estimates differed significantly between the facultative precocious populations and the obligate three-host populations. As we conducted four tests, one for each of the genetic estimators, we used a sequential Bonferroni correction (Rice, 1989).

Testing for inbreeding depression

Building upon theory from Ritland (1990), Hulke and Criscione (2024) demonstrated how a comparison of genetic selfing-rate estimates to demographic selfing-rate estimates could be used to test for inbreeding depression from field-collected samples. The demographic selfing rate presented herein differs from that of Hulke and Criscione (2024), who used infection intensity data within a population to obtain the demographic estimates (theory developed by Detwiler et al., 2017). As *A. progeneticum* is forced to self-mate within the second host (sexually matures while encysted), intensities within crayfish have no bearing on an individual's potential for selfing (i.e., it is 100% if it sexually matures while encysted in a crayfish).

Table 1. Summary of genetic diversity from both microsatellite and mitochondrial ND1 markers

State	Location	Number of individuals genotyped	Multilocus F_{IS}	H_S[a]	A[b]	Number of ND1 haplotypes[c]	ND1 H_d[d]	ND1 π[e]
Georgia	Big Indian Creek	33	0.924 (p = 0)	0.289	3.028	2 (33)	0.436	0.00064
	Calls Creek	30	0.97 (p = 0)	0.104	1.559	1 (25)	0.000	0.00000
	Cane Creek	30	0.393 (p = 0)	0.687	8.48	9 (30)	0.825	0.00627
	Richland Creek	24	0.822 (p = 0)	0.393	3.392	4 (27)	0.393	0.00424
	Yellow River	28	0.424 (p = 0)	0.169	3.406	4 (30)	0.193	0.00186
Louisiana	Chapappela Creek	28	0.13 (p = 0)	0.836	11.181	9 (30)	0.789	0.0035
	Hays Creek	27	0.04 (p = .029)	0.803	10.342	12 (22)	0.887	0.00493
Arkansas	Nix Creek	28	0.032 (p = .046)	0.852	12.222	9 (19)	0.678	0.00252
Texas	GEWMA	30	–0.016 (p = .314)	0.849	12.381	9 (21)	0.895	0.00365

Note. [a]Mean gene diversity across loci.
[b]Mean allelic richness over all 22 loci, rarefied to the smallest sample size of 22 individuals due to missing genotypes at a few loci in a few individuals.
[c]number of sequenced individuals in parentheses.
[d]Haplotype diversity.
[e]Nucleotide diversity.

We first note that we only estimate the demographic selfing rate (s_D) for the five facultative precocious populations. The s_D we present for *A. progeneticum* is simply the proportion of encysted gravid individuals, which are forced to self, over the total number of reproductive individuals in the population, $s_D = T_G/(T_G + T_I)$, where T_G is the total number of gravid parasites within crayfish and T_I is the total number of gravid parasites in catfishes. This estimate has the following assumptions: (1) there is random reproductive success across all individuals, and (2) there is 100% outcrossing among individuals in catfishes. We return to these assumptions in the discussion, but we highlight the latter assumption is supported by the finding of genetic selfing-rate estimates at or near zero in the obligate three-host populations (see Results) where sexual reproduction only occurs in catfishes. We also note that some encysted, but nongravid individuals were found in crayfish in some locations. We have no way of knowing the reproductive fate of these individuals, i.e., will they later sexually mature within their cyst or will they end up in a catfish to be able to outcross. Hence, we did not include juvenile encysted individuals (i.e., metacercariae) in the counts. Likewise, we did not count the single immature fluke collected from a catfish.

The totals T_G and T_I are not directly obtainable, but mean abundances (i.e., the average number of parasites per host sampled including both infected and uninfected hosts; Bush et al., 1997) can be estimated from field-collected data. Given that the mean abundance of encysted gravid individuals in crayfish (G) times the total number of crayfish in the population (N_C) equals T_G (i.e., $GN_C = T_G$) and that the mean abundance of trematodes in catfish (I) times the total number of catfishes in the population (N_I) equals T_I (i.e., $IN_I = T_I$), then through substitution we get $s_D = GN_C/(GN_C + IN_I)$. In our sampling, N_C and N_I are not obtainable, but the ratio of caught crayfish to caught catfish, $r = N_C/N_I$ (described in our sampling above), can be estimated. Rearranging to $N_C = rN_I$, followed by substitution, we obtain $s_D = Gr/(Gr + I)$, which enables us to used field-collected variables to estimate a demographic selfing rate of *A. progeneticum*.

A test for inbreeding depression is conducted by comparing s_D to s_G (see assumptions in Hulke & Criscione, 2024). If $s_D = s_G$, there would be no evidence for inbreeding depression. If selfed offspring disproportionately die relative to the outcrossed offspring (i.e., inbreeding depression), $s_D > s_G$. If $s_D < s_G$ then one would infer outbreeding depression.

CI for s_D were estimated by obtaining bootstrap estimates of G, I, and r in each of the five facultative precocious population separately. A single bootstrap round contained an independent bootstrap estimate for each of G, I, and r followed by the calculation of s_D. In a given round, a bootstrap estimate of G was obtained by sampling with replacement among all crayfish (sample size equal to that of the given population at hand) followed by the calculation of G. A bootstrap estimate of I was obtained in the same manner among catfish hosts. A bootstrap estimate of r was obtained by sampling with replacement of the number of electroshocking draws (sample size equal to that of the given population at hand) followed by total counts of the number of crayfish and catfish to calculate r. A total of 10,000 bootstraps were conducted for each value of G, I, and r.

To assess if the s_D significantly deviated from s_G, we compared s_D to s_G 84% CI (or credible intervals for the Bayesian estimates). Two population estimates with overlapping 95% CI can still be significantly different a p = .05 (Krzywinski &

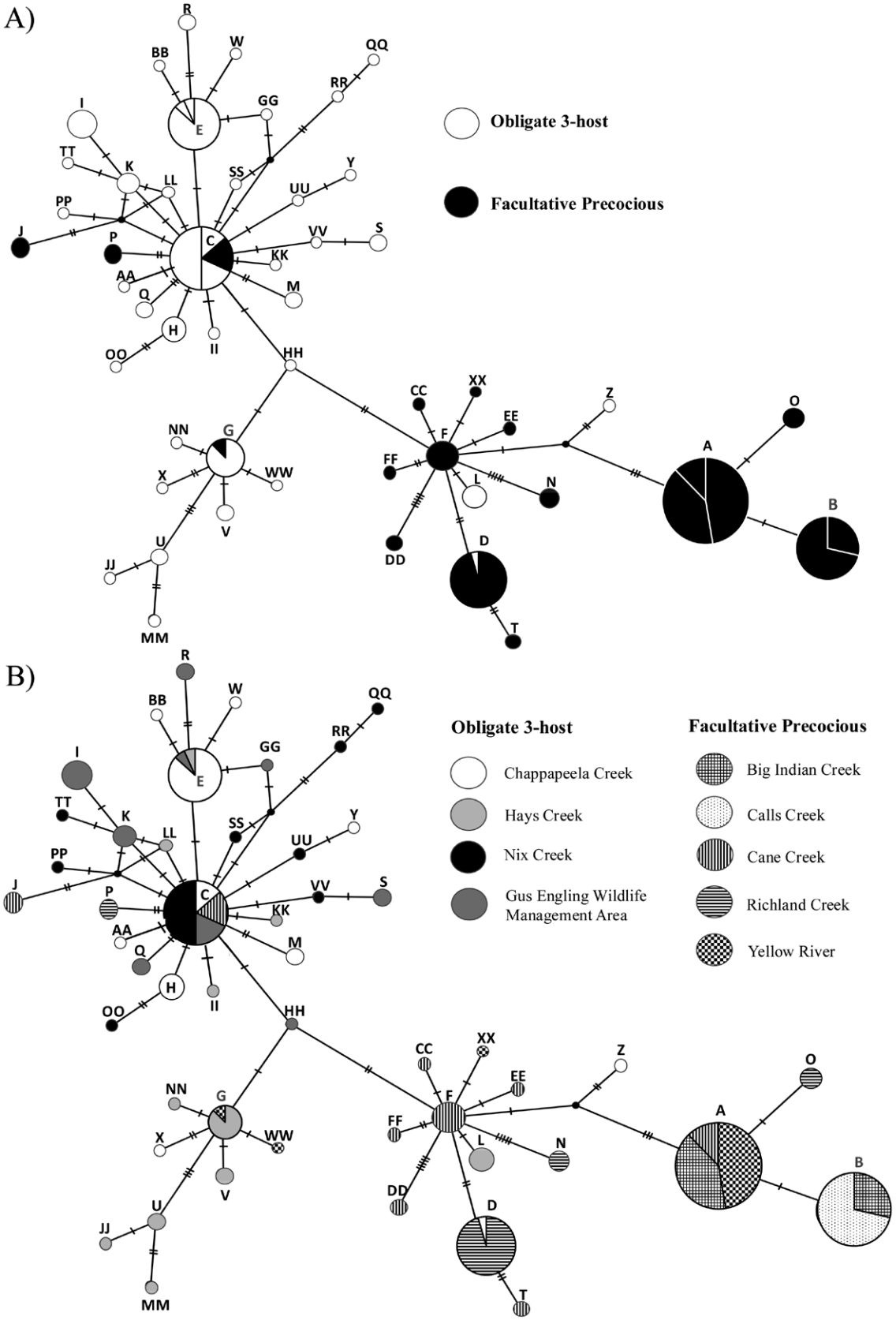

Figure 2. Haplotype network of the ND1 mtDNA for sequences generated in the current study. Symbol shading in the keys denotes sampling origin among the nine *Alloglossidium progeneticum* populations. The same network is shown in A and B. (A) Demarcated by life history: black fills are samples from facultative precocious populations and white fills are from obligate three-host populations. (B) Demarcated by sampling locations: solid fills are samples from populations that have an obligate three-host life cycle and patterned fills are from populations with a facultative precocious life cycle. Each tick mark between haplotypes represents a single nucleotide difference. Haplotype IDs are shown as letters and size of the circle reflects the number of individuals with that haplotype (see Supplementary File S1 for actual numbers and comparison of IDs from Kasl et al., 2015). Notice the samples from facultative precocious populations a predominately isolated on the branch starting with Haplotype F.

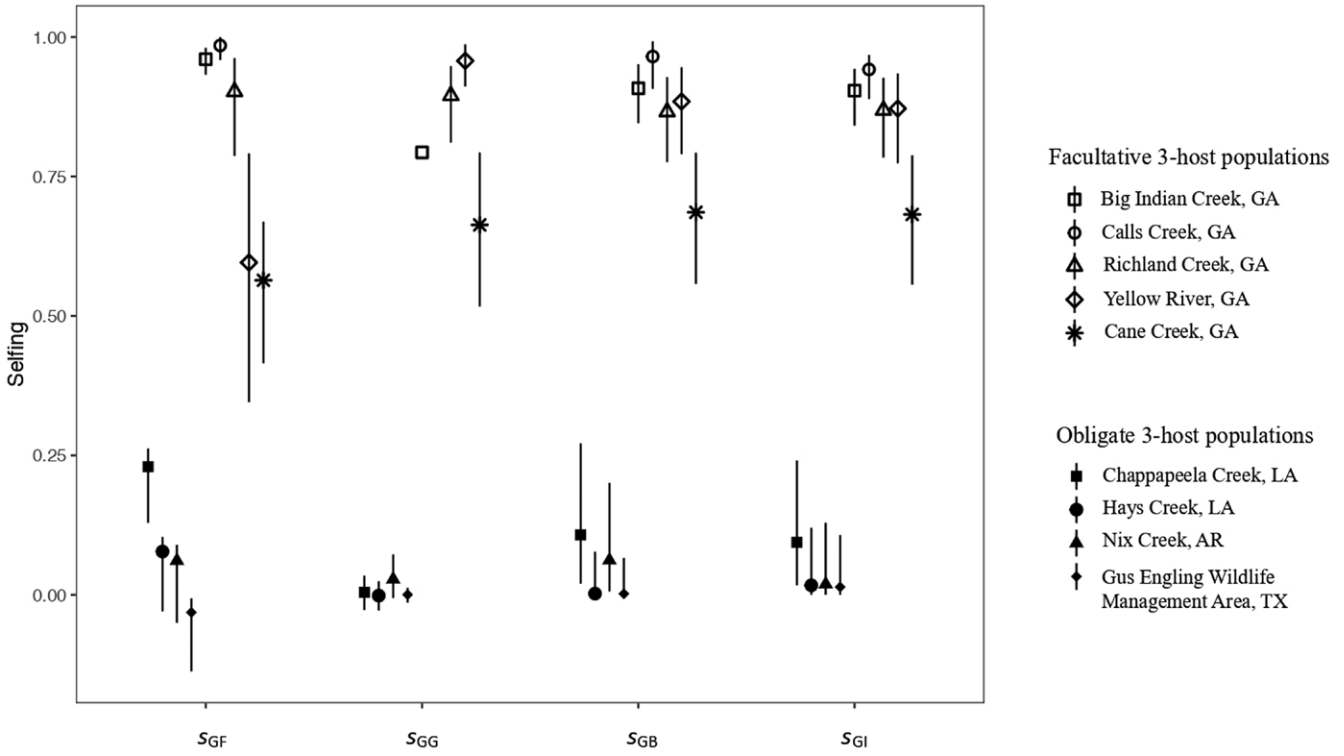

Figure 3. Genetic selfing-rate estimates in each of the nine populations of *Alloglossidium progeneticum*. Genetic selfing-rate estimates are clustered by method: F_{IS} (s_{GF}), g_2 (s_{GG}), BES (s_{GB}), and Instruct (s_{GI}). Point estimates from each location are indicated by the shapes in the legend where filled shapes indicate obligate three-host populations and open shapes are from facultative precocious populations. The 95% CI (or credible intervals for the Bayesian methods) are shown for each estimate. Note a s_{GG} estimate for Calls Creek was not calculable nor was CI for s_{GG} of Big Indian Creek. All of the precocious populations had high levels of selfing (>0.56) and no CI overlapped with the obligate three-host population estimates.

Table 2. Mean abundance of gravid parasites in crayfish hosts (*G*), mean abundance of gravid parasites in catfish hosts (*I*), ratio of crayfish to catfish (*r*), and total number of worms collected from the five facultative precocious populations.

Location	*G*	*I*	*r*	Total number of parasites in crayfish	Total number of gravid parasites in crayfish	% Gravid in crayfish	Total number of gravid parasites in catfish
Big Indian Creek	9.051	0.964	3.265	354	353	0.997	27
Calls Creek	2.263	0.464	1.079	92	86	0.935	13
Cane Creek	4.545	2.1	3.154	166	150	0.904	21
Richland Creek	1.5	4.39	3.25	70	51	0.729	79[a]
Yellow River	4.813	0.893	0.912	200	154	0.77	25

Note.
[a]Eighty total parasites were collected from Richland Creek.

Altman, 2013; Schenker & Gentleman, 2001). However, several studies indicate nonoverlapping 84% CI approximate a significant difference at $p = .05$ (Krzywinski & Altman, 2013; MacGregor-Fors & Payton, 2013; Payton et al., 2003; Zheng, 2015). We used the exact binomial test (α=0.05) to globally determine if there was a difference within a population sample as follows. In Cane Creek, Richland Creek, and Yellow Creek, there were four estimates of s_G, so we deemed significance if the 84% CI of two or more s_G estimates did not overlap the 84% CI of s_D as $p = .014$ ($p = .185$ when one estimate does not overlap). Big Indian Creek and Calls Creek could only be assessed with three s_G estimates because reliable estimates of s_{GG} could not be obtained from these locations (see Results).

Results

Collections summary

Sampling location, GPS coordinates, and sampled stage of *A. progeneticum* development within a given host along with prevalence and mean intensity by host species are reported in Supplementary Table S1.

Within and among population structure and genetic diversity

The number of individuals genotyped for microsatellite markers, multilocus F_{IS}, average H_S, and average allelic richness are reported by location in Table 1. By-locus calculations of F_{IS}, allelic richness, and H_S are presented in Supplementary File S1.

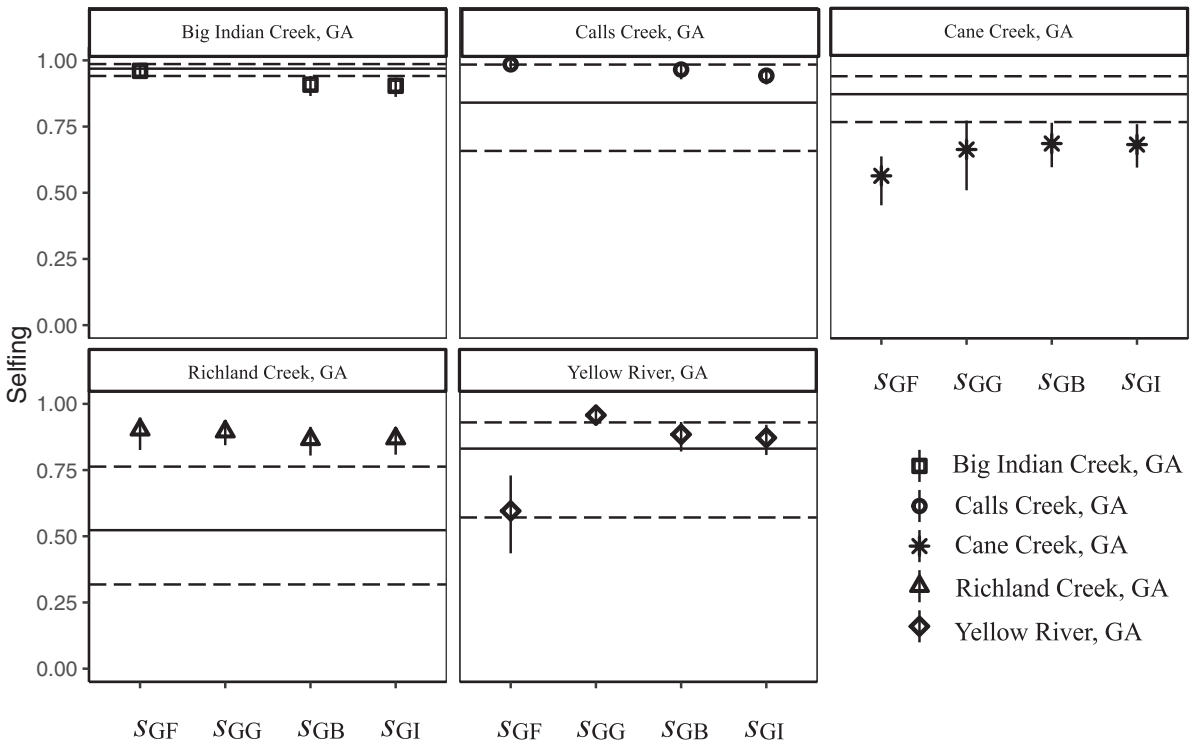

Figure 4. Comparison of genetic (s_G) and demographic (s_D) estimates of selfing for the five facultative precocious populations of *Alloglossidium progeneticum*. Solid black lines denote the point estimates of s_D in each population and dotted lines represent the 84% CI. Genetic selfing-rate estimates are presented by estimation method: F_{IS} (s_{GF}), g_2 (s_{GG}), BES (s_{GB}), and Instruct (s_{GI}). Note a s_{GG} estimate for Calls Creek was not calculable nor was CI for the s_{GG} of Big Indian Creek; hence, these were omitted from comparisons.

Of the nine populations, eight had significantly positive F_{IS} values (Table 1). However, F_{IS} can be influenced by technical factors such as large allelic dropout and null alleles, thus inflating values of F_{IS} (Jarne & David, 2008); we return to this in the discussion. Global F_{ST} among all nine populations was 0.348 and significant ($p < .0001$). Pairwise F_{ST} ranged from 0.0335 to 0.7202 (pairwise values in Supplementary File S1); all pairwise values were significant after Bonferroni correction (all p-values < .001). Allelic richness was significantly less in the facultative precocious populations (mean = 3.97) than in the obligate three-host populations (mean = 11.53) ($t = -5.9$, df = 5.2, $p = .002$). H_S was also less in the facultative precocious populations than the obligate three-host populations with the means being 0.3284 and 0.835, respectively ($t = -4.9$, df = 4.1, $p = .007$).

Genotypic disequilibrium was significant overall for four of the five facultative precocious populations: Big Indian Creek had 12 out of 134 pairwise comparisons that were significant ($p = .0369$), Cane Creek 190 out of 231 ($p < .001$), Richland Creek 126 out of 210 ($p < .001$), and Yellow Creek 202 out of 210 ($p < .001$). Calls Creek had very little genetic variation (Table 1) so there were only 34 pairwise loci comparisons of which only two were significant ($p = .512$). None of the obligate three-host populations had overall significant genotypic disequilibrium with only eight out of 209 pairwise comparisons significant in Chappapeela Creek ($p = .824$), six out of 231 for Hays Creek ($p = .976$), four out of 231 for GEWMA ($p = .997$), and two out of 231 for Nix Creek ($p = .999$).

In total, 237 *A. progeneticum* ($n = 145$ from precocious and $n = 92$ from obligate three-host populations) were sequenced at the ND1 locus resulting in 50 haplotypes from the nine locations (Figure 2, Supplementary Table S1). No premature stop codons were found after translation of the sequences (using amino acid translation code 9 on GenBank). Total $\pi = 0.00769$ and the two most divergent haplotypes differed by 15 nucleotide differences (2.2% difference). These patterns are similar when combined with the previously published ND1 sequences of *A. progeneticum* from Kasl et al. (2015) (Supplementary Figure S2). The average H_d was significantly less in facultative precocious populations (mean = 0.369) than the obligate three-host populations (mean = 0.812) ($t = -3.01$, df = 5.03, $p = .03$). However, π was not significantly lower in the facultative precocious populations (mean = 0.0026) compared to the obligate three-host populations (mean = 0.0037) ($t = -0.8$, df = 5.3, $p = .44$). Among the five facultative precocious populations, there were 17 haplotypes, three of which were also found in an obligate three-host population (Haplotypes C, D, and G, Figure 2). Of the 145 precocious individuals, 134 were isolated to a single branch of the network (starting at Haplotype F, Figure 2). Moreover, this branch was nearly exclusive to precocious individuals having only four individuals (Haplotypes L and Z, Figure 2) from obligate three-host populations. In general, this patterning holds when including the previously published ND1 sequences of *A. progeneticum* from Kasl et al. (2015) (Supplementary Figure S2).

Genetic selfing-rate estimates

With the exception of Chappapeela Creek, all s_G estimates in the obligate three-host populations were very low (range: −0.03 to 0.077) with 95% CI that contained, or effectively contained in the Bayesian CI, 0 (Figure 3, Supplementary File S1). Chappapeela Creek had mixed results with the s_{GG} indicating no significant selfing, an approximate selfing rate

of 10% from both Bayesian inferences (s_{GI} and s_{GB}), and a selfing rate of 22% based on s_{GF}. The five facultative precocious populations had mixed-mating systems with high levels of selfing (Figure 3). Cane Creek had the lowest s_G estimates ranging from 0.56 to 0.69, whereas the remaining precious populations typically had s_G estimates 0.8 or greater (Figure 3, Supplementary File S1). We note that for Calls Creek, s_{GG} was not calculable. This may be due to low marker information. The g_2 statistic relies on the presence of heterozygote genotypes. However, loci where there may be two alleles, but no heterozygotes (i.e., $F_{IS} = 1$) are excluded in the calculation. In Calls Creek, only nine out of the 22 loci had more than one allele, and of those nine, seven had an F_{IS} of 1 indicating that there were no heterozygotes present (Supplementary File S1). A similar issue was likely in the calculation of the s_{GG} CI of Big Indian Creek where bootstraps over individuals might lead to the exclusion of rare heterozygous genotypes in a given bootstrap.

For each of the four genetic selfing rate estimators, s_G was significantly higher (Bonferroni adjusted $p = .0125$) in the facultative precocious populations compared to the obligate three-host populations (s_{GF}: $t = 6.7$, df = 6.3, $p < .001$, mean difference = 0.717; s_{GG}: $t = 12.7$, df = 3.1, $p < .001$, mean difference = 0.819; s_{GB}: $t = 15.3$, df = 6.01, $p < .001$, mean difference = 0.819; s_{GI}: $t = 16.7$, df = 5.4, $p < .001$, mean difference = 0.818).

Inbreeding depression tests

Mean abundances of gravid parasites in crayfish, mean abundances of gravid parasites in catfish, ratios of crayfish to catfish, and total numbers of *A. progeneticum* from each population are presented in Table 2. For Calls Creek and Yellow River, all s_G estimates had overlapping 84% CI within the s_D estimates ($p = 1$) indicating no inbreeding depression (Figure 4; see Supplementary Files S1 for CI values). For Big Indian Creek, one of the three s_G estimates (i.e., s_{GI}) did not have overlapping 84% CI with s_D ($p = .14$), so there is no support for inbreeding depression. In Richland Creek, all four s_G estimates were greater than the s_D estimates ($p < .0001$) leading to a conclusion of outbreeding depression. In contrast in Cane Creek, only one of the four s_G estimates (i.e., s_{GG}) had overlapping 84% CI with the s_D estimates ($p = .0005$) leading to a conclusion of inbreeding depression.

Discussion

We had three main findings in comparing the two diverged life histories. First, populations with ancestral obligate three-host life cycles had a greater amount of genetic diversity at both microsatellite and ND1 mtDNA loci than the populations with precocious facultative life cycles (Table 1). Second, the populations with derived, facultative precocious life cycles had significantly higher selfing rates compared to the populations with ancestral, obligate three-host life cycles (Figure 3). Indeed, the selfing rates in populations with the obligate three-host life cycles, with the exception of Chappapeela Creek, were at or near zero, indicating predominate outcrossing. While the F_{IS} of Hays and Nix Creeks tested significantly greater than 0, bootstrap 95% CI (and likewise their conversion to s_{GF}) contained 0. Moreover, the 95% CI of the other s_G estimates in these two populations contained 0 (or effectively 0 in the Bayesian methods). Therefore, the elevated F_{IS} values in Hays and Nix Creeks likely represent the susceptibility of F_{IS} to technical artifacts, e.g., null alleles (Jarne & David, 2008). The F_{IS} and hence, s_{GF} of Chappapeela Creek was also elevated compared to the other s_G estimates in this location. However, there were mixed results of the other s_G estimates with the Bayesian methods around 10%, whereas s_{GG} was 0. Thus, there may be some low level of selfing in Chappapeela Creek, but this level is drastically lower than any of the high selfing rates (58%–98%) estimated from the facultative precocious populations. The significant differences in the mating systems of the obligate three-host and the facultative precocious life cycles suggest that the within-species mating system has co-evolved with the life cycle. With the current phylogenetic trait reconstruction analysis indicating an ancestral obligate tree-host life cycle for *A. progeneticum* (Kasl et al., 2018), we assume that the ancestral mating system was outcrossing and that the populations with derived facultative precocious life cycles subsequently evolved selfing. Third, the facultative precocious populations of *A. progeneticum* had no overall evidence for inbreeding depression: three populations do not deviate from a null model where demography explained the mating system, one population had support for an inference of outbreeding depression, and one had support for inbreeding depression (Figure 4).

Genetic diversity within and relationships among populations of *A. progeneticum*

The obligate three-host populations had greater genetic diversity as indicated by greater microsatellite allelic richness and gene diversity, and greater mtDNA haplotype diversity compared to the facultative precocious populations. Based on a completely different set of samples than this study, Kasl et al. (2015) reported that facultative precocious populations of *A. progeneticum* had qualitatively lower ND1-haplotype and -nucleotide diversity relative to obligate three-host populations. They hypothesized that the forced self-mating in the precocious populations could promote bottleneck/founder events. Here, we found that indeed there was high selfing in all the precocious populations. At autosomal loci, the low levels of genetic diversity and higher linkage disequilibrium we observed within the facultative precocious populations are consistent with what has been observed in other highly selfing species (Detwiler & Criscione, 2017; Glémin et al., 2006; Jullien et al., 2019; Mable & Adam, 2007). In finite populations, nonrandom mating increases identity by descent and decreases effective recombination rates, both of which contribute to an increased rate of genetic drift, resulting in linkage disequilibrium and loss of genetic diversity (Charlesworth, 2003; Nordborg, 2000; Pollak, 1987). Moreover, the life history of highly selfing species enables founder events of one or a few individuals, again leading to a large drift effect (Hedrick, 2011; Siol et al., 2008).

The facultative precocious population of Cane Creek was the one exception to the above genetic diversity patterns. Indeed, the selfing rate estimates in Cane Creek were lower (56%–86%; Figure 3) compared to the other facultative precocious populations, but the genetic diversity levels were closer to that of the obligate three-host populations (Table 1). The Cane Creek population had an ND1 haplotype shared with obligate three-host populations (Haplotype C, Figure 2), a haplotype shared with facultative precocious populations (Haplotype A, Figure 2), and its own private haplotypes stemming at the base of the branch supporting most of the haplotypes found among precocious populations (Haplotype F,

Figure 2). Overall, the levels of genetic diversity and network patterning may indicate a larger effective size retaining haplotypes in Cane Creek, but it may also indicate this location has had a history of admixture (possibly via anthropogenic means).

There are a few patterns of the ND1 mtDNA worth noting. First, the overall π of 0.0077 and the max difference of 2.2% between the two most divergent haplotypes are consistent with intraspecific variation among parasitic flatworms (Vilas et al., 2005). Thus, the different set of samples used in our study supports the findings of Kasl et al. (2015) in that the obligate three-host and precocious populations of *A. progeneticum* represent a single species (see also Supplementary Figure S2). Second, haplotypes of the facultative precocious populations are largely restricted to one branch in the network and this branch has very few haplotypes from obligate three-host populations (starting at Haplotype F, Figure 2). Although the facultative precocious life cycles are largely restricted to one branch, these data alone do not enable us to deduce if there are multiple origins of precociousness or a single origin that was followed by gene flow between populations. Nonetheless, there is clear substructure among the facultative precocious populations (pairwise F_{ST} in Supplementary File S1) that enables independent mating system dynamics, which is reflected in the variation in selfing rates among the precocious populations (Figure 3).

Impact of parasite life history on their mating systems

As noted in the introduction, the Brown et al. (2001) model assumes that a shorter life cycle leads to more inbreeding. Only a few trematode species with precocious life cycles have had their mating systems and/or inbreeding examined with genetic data: *Coitocaecum parvum* (Lagrue et al., 2007, 2009), *Proctoeces* cf. *lintoni* (Valdivia et al., 2014), *Alloglossidium renale* (Hulke & Criscione, 2024), and *A. progeneticum* (current study). Of these four species, three have facultative precocious life cycles and only *A. renale* has an obligate precocious life cycle. Three of these four species do indeed exhibit high inbreeding, the exception being *P. lintoni* which showed no deviation from HWE (Valdivia et al., 2014). The life cycle of *P. lintoni* consists of a snail first host, a limpet second host, and a clingfish third host. In contrast to *C. parvum* and *A. progeneticum*, *P. lintoni* does not encyst within the second host, and thereby can outcross in both the second and third host. Having free adults at two life cycle stages likely allows for *P. lintoni* to have multiple chances to encounter mates for outcrossing. The life cycle of *C. parvum* (a snail first host, amphipod second host, and fish final host) is similar to that of *A. progeneticum* in that there is forced self-mating while encysted in its second host. In both *C. parvum* and *A. progeneticum* the high levels of inbreeding highlight how a change in developmental pattern (i.e., sexually maturing within the cyst stage) can impact parasite mating systems.

Alloglossidium renale has an obligate two-host life cycle consisting of a snail first host and grass shrimp second host, with no encysted stage. As noted in the introduction, *A. renale* is a sister species to *A. progeneticum* and prior phylogenetic trait reconstruction analysis indicates that the ancestral life cycle for the clade that contains *A. progeneticum* and *A. renale* is an obligate three-host life cycle (Kasl et al., 2018). Thus, based on the findings of Kasl et al. (2018), the precocious life cycle of *A. renale* would also be considered derived. Hulke and Criscione (2024) found high selfing rates within four population samples of *A. renale* that were explained by demography. In particular, *A. renale* infects the grass shrimp's paired antennal glands, which further subdivides the parasite's mating boundaries within a host. Thus, the mean intensities per mating unit (i.e., a gland) are lower (1.31–1.77; Hulke & Criscione, 2024) than mean infection intensities per host (1.85–2.58; Hulke et al., 2021). Even with random mating among the free adults in a gland, these infection intensities result in high selfing rates. The high population selfing rates of *A. progeneticum* can also be largely explained by demography. However, the selfing rates of *A. progeneticum* are dictated by the large number of precocious individuals that are forced to self-mate while encysted within the crayfish rather than low infection intensities.

While three of the four precocious species discussed above show evidence of high inbreeding, our study of *A. progeneticum* is the first to test the mating system of the derived precocious state in a comparison to the ancestral state. The comparison of *A. progeneticum* populations that maintain the ancestral three-host life cycle, which were predominately outcrossing, to populations with the derived facultative precocious life cycles, which had high selfing rates, demonstrates a clear transition from predominately outcrossing to high rates of selfing. Moreover, given the trait reconstruction findings of Kasl et al. (2018), our results herein also suggest that there was a likely transition in the mating system from outcrossing to high selfing in the lineage leading to *A. renale*.

The mating system results of the different *A. progeneticum* populations also support the sex allocation patterns previously detected among the *A. progeneticum* populations (Kasl et al., 2015). Specifically, Kasl et al. (2015) tested the influence of mating system on sex allocation among the populations of *A. progeneticum*. Although Kasl et al. (2015) did not have actual selfing rates at the time, they predicted that precocious populations would have evolved toward more female-biased sex allocation dues to the forced selfing mating in crayfish. Indeed, we find very high selfing in the precocious populations and predominate outcrossing in the populations with ancestral, obligate three-host life cycles. This change in mating system is consistent with subsequent evolution toward female-biased sex allocation in these precocious populations (Kasl et al., 2015).

Inferences on inbreeding depression

Our test of inbreeding depression relies on comparing a demographic estimate of selfing to a genetic estimate. Thus, we first discuss two assumptions made in obtaining the demographic estimate of selfing for *A. progeneticum*. Our first assumption was that all individuals within the catfish hosts are 100% outcrossing. If selfing occurred in catfish hosts, the demographic estimates of selfing in Figure 4 would be elevated. However, the s_G estimates from obligate three-host populations do indeed support that there is near complete outcrossing within catfish hosts as sefling rates were at or near zero (Figure 3). The second assumption is that *A. progeneticum* has random reproductive success across all flukes in the population. The inverse infection intensity model developed by Detwiler et al. (2017) for estimating a demographic selfing rate from hermaphroditic endoparasites can account for random reproductive success or density-dependent fecundity. In the latter, the total number of parasite offspring originating from each host is similar no matter the level of intensity (Dobson, 1986). As

such, the population selfing rate is increased because density-dependence puts more weight (i.e., higher reproductive success) on parasites from lower infection intensities where within-host selfing rates are higher (under random mating in a host; Detwiler et al., 2017). Unfortunately, we were unable to derive a means to incorporate density-dependent fecundity in the demographic selfing rate we developed for *A. progeneticum*. Nonetheless, there may not be a consistent impact of density-dependent fecundity on the overall mating system when there is 100% outcrossing in catfish hosts and 100% selfing in the precocious individuals in crayfish. The reason is that these mating system rates are occurring independent of infection intensities and thus, no greater weight given to selfing as in the estimator derived by Detwiler et al. (2017).

Overall, there was no evidence for global inbreeding depression among the facultative precocious populations of *A. progeneticum*. We recognize the observation of little to no inbreeding depression could be a consequence of genetic purging rather than directly reflecting the ancestral condition at the time of transition to high selfing. Unfortunately, we do not have a means of assessing inbreeding depression in the obligate three-host populations. Nevertheless, the lack of inbreeding depression within the precocious populations is consistent with the Brown et al. (2001) model. The Brown et al. (2001) model predicts that a shorter life cycle is more likely to evolve in the absence of inbreeding depression. There are only three studies (including this study) from precocious trematode species that test for inbreeding depression. Lagrue and Poulin (2009) tested for inbreeding depression in *C. parvum* by comparing selfed offspring produced while encysted in the second amphipod to offspring produced from the third fish host, assumed to be a product of outcrossing. Overall, they found no significant differences between egg size, hatching rates, infection success, and asexual multiplication within the snail host from the two different mating systems (Lagrue & Poulin, 2009). Tests of inbreeding depression for *A. renale* were done by comparing genetic estimates of selfing with demographic estimates; the latter estimator used demographic data on infection intensities as developed by Detwiler et al. (2017) (Hulke & Criscione, 2024). In three *A. renale* populations and an additional temporal sample from one of these populations, there was no evidence for inbreeding depression (Hulke & Criscione, 2024). Herein, we tested for inbreeding depression in *A. progeneticum* also using a comparison of genetic selfing-rate estimates to demographic estimates, though deriving a different demographic estimator to account for the different life history of *A. progeneticum*. Three of the five of the facultative precocious populations did not deviate from their demographic estimate and thus, do not show evidence for inbreeding depression. There were two populations that significantly deviated from the demographic estimates with one population, Cane Creek, exhibiting less selfing than predicted by its demographic estimates, and the other population, Richland Creek, exhibiting more selfing. Thus, the inferences would be inbreeding depression and outbreeding depression, respectively. For several demographic or selection-specific causes, selfing rates are known to vary among populations of a species for a variety of different organisms including snails (Lounnas et al., 2017), plants (Whitehead et al., 2018), and cnidarians (Olsen & Levitan, 2023). Likewise, different locations may have site-specific selection pressures that result in differences in inbreeding depression (Cheptou & Donohue, 2011). Regardless, results from four of the five precocious populations did not show evidence for inbreeding depression. We also indicate there is no strong support for the selection of selfing. For example, Busch and Delph (2012) highlight that reproductive assurance (being able to self in the absence of mates) can select for maintenance of selfing, but only if seed (egg) discounting and pollen (sperm) discounting are allowed. Sperm discounting in *A. progeneticum* is high as encysted individuals cannot donate sperm to other individuals. Hence, reproductive assurance driving the selfing seems unlikely. Moreover, only one of five populations showed evidence of outbreeding depression, so in general, there is little support that selfing is driven by anything more than the demographics (i.e., trematode abundances among the crayfish and catfishes) of the parasite. Additional studies on precocious trematodes are needed, but to date, the three studied precocious species discussed above show support for the prediction of the Brown et al. (2001) model.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All raw data have been provided in the Supplementary Material.

Author contributions

C.D. Criscione designed the research project. J.M. Hulke generated the genetic data. J.M. Hulke and C.D. Criscione performed the analyses and wrote the manuscript.

Funding

C.D. Criscione's studies on the population genetics and evolution of parasite life cycles are supported by National Science Foundation Grant DEB-1655147.

Conflict of interest: The authors declare no conflicts of interest.

Acknowledgments

We thank W. Ellenburg, E. Kasl, H. Kusy, B. Trejo, C. McAllister, and A. Sakla, for helping with field collections and to G. Hernandez, N. Hein, and S. Crosby for assistance in the lab. Portions of this research were conducted with the advanced computing resources provided by Texas A&M High Performance Research Computing.

References

Ball, M. A., Parker, G. A., & Chubb, J. C. (2008). The evolution of complex life cycles when parasite mortality is size- or time-dependent. *Journal of Theoretical Biology*, *253*(1), 202–214. https://doi.org/10.1016/j.jtbi.2008.02.025

Belkhir, K., Borsa, P., Chikhi, L., Rausfast, N., & Bonhomme, F. (2004). *GENETIX 4.05, Windows software for population genetics*. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université Montpellier II.

Benesh, D. P. (2011). Intensity-dependent host mortality: What can it tell us about larval growth strategies in complex life cycle helminths? *Parasitology*, *138*(7), 913–925. https://doi.org/10.1017/S0031182011000370

Benesh, D. P., Chubb, J. C., Lafferty, K. D., & Parker, G. A. (2022). Complex life-cycles in trophically transmitted helminths: Do the benefits of increased growth and transmission outweigh generalism and complexity costs? *Current Research in Parasitology & Vector-Borne Diseases*, *2*, 100085. https://doi.org/10.1016/j.crpvbd.2022.100085

Benesh, D. P., Chubb, J. C., & Parker, G. A. (2013). Complex life cycles: Why refrain from growth before reproduction in the adult niche? *The American Naturalist*, *181*(1), 39–51. https://doi.org/10.1086/668592

Benesh, D. P., Chubb, J. C., & Parker, G. A. (2014). The trophic vacuum and the evolution of complex life cycles in trophically transmitted helminths. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1793), 20141462. https://doi.org/10.1098/rspb.2014.1462

Benesh, D. P., Parker, G. A., Chubb, J. C., & Lafferty, K. D. (2021). Trade-offs with growth limit host range in complex life-cycle helminths. *The American Naturalist*, *197*(2), E40–E54. https://doi.org/10.1086/712249

Brown, S. P., Renaud, F., Guégan, J. F., & Thomas, F. (2001). Evolution of trophic transmission in parasites: The need to reach a mating place? *Journal of Evolutionary Biology*, *14*(5), 815–820. https://doi.org/10.1046/j.1420-9101.2001.00318.x

Busch, J. W., & Delph, L. F. (2012). The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. *Annals of Botany*, *109*(3), 553–562. https://doi.org/10.1093/aob/mcr219

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology*, *83*(4), 575–583.

Cézilly, F., Thomas, F., Médoc, V., & Perrot-Minnot, M. J. (2010). Host-manipulation by parasites with complex life cycles: Adaptive or not? *Trends in Parasitology*, *26*(6), 311–317. https://doi.org/10.1016/j.pt.2010.03.009

Charlesworth, D. (2003). Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, *358*(1434), 1051–1070. https://doi.org/10.1098/rstb.2003.1296

Cheptou, P. O., & Donohue, K. (2011). Environment-dependent inbreeding depression: Its ecological and evolutionary significance. *New Phytologist*, *189*, 395–407.

Clement, M., Posada, D., & Crandall, K. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1660.

Cribb, T. H., Bray, R. A., Olson, P. D., & Littlewood, D. T. J. (2003). Life cycle evolution in the Digenea: A new perspective from phylogeny. *Advances in Parasitology*, *54*, 197–254.

Criscione, C. D., & Blouin, M. S. (2004). Life cycles shape parasite evolution: Comparative population genetics of salmon trematodes. *Evolution*, *58*(1), 198–202. https://doi.org/10.1111/j.0014-3820.2004.tb01587.x

Criscione, C. D., & Blouin, M. S. (2006). Minimal selfing, few clones, and no among-host genetic structure in a hermaphroditic parasite with asexual larval propagation. *Evolution*, *60*(3), 553–562.

Criscione, C. D., Hulke, J. M., & Goater, C. P. (2022). Trematode clone abundance distributions: An eco-evolutionary link between parasite transmission and parasite mating systems. *The Journal of Parasitology*, *108*(6), 565–576. https://doi.org/10.1645/22-68

David, P., Pujol, B., Viard, F., Castella, V., & Goudet, J. (2007). Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology*, *16*(12), 2474–2487. https://doi.org/10.1111/j.1365-294X.2007.03330.x

Detwiler, J. T., Caballero, I. C., & Criscione, C. D. (2017). Role of parasite transmission in promoting inbreeding: I. Infection intensities drive individual parasite selfing rates. *Molecular Ecology*, *26*(17), 4391–4404. https://doi.org/10.1111/mec.14211

Detwiler, J. T., & Criscione, C. D. (2017). Role of parasite transmission in promoting inbreeding: II. Pedigree reconstruction reveals sib-transmission and consequent kin-mating. *Molecular Ecology*, *26*(17), 4405–4417. https://doi.org/10.1111/mec.14210

Dobson, A. P. (1986). Inequalities in the individual reproductive success of parasites. *Parasitology*, *92*(Pt 3), 675–682. https://doi.org/10.1017/s0031182000065537

Font, W. F., & Corkum, K. C. (1975). *Alloglossidium renale* n. sp. (Digenea: Macroderoididae) from a fresh-water shrimp and *A. progeneticum* n. comb. *Transactions of the American Microscopical Society*, *94*(3), 421–424.

Gao, H., Williamson, S., & Bustamante, C. D. (2007). A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics*, *176*(3), 1635–1651. https://doi.org/10.1534/genetics.107.072371

Glémin, S., Bazin, E., & Charlesworth, D. (2006). Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1604), 3011–3019. https://doi.org/10.1098/rspb.2006.3657

Goudet, J. (1995). FSTAT (version 1.2): A computer program to calculate F-statistics. *Journal of Heredity*, *86*(6), 485–486. https://doi.org/10.1093/oxfordjournals.jhered.a111627

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

Hardy, O., & Vekemans, X. (2002). SPAGeDI: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, *2*, 618–620.

Hedrick, P. W. (2011). *Genetics of populations*. Jones & Bartlett Publishers.

Hulke, J. M., & Criscione, C. D. (2023). Characterization of 21 microsatellite loci for the precocious, grass-shrimp trematode *Alloglossidium renale*. *Molecular and Biochemical Parasitology*, *254*, 111563. https://doi.org/10.1016/j.molbiopara.2023.111563

Hulke, J. M., & Criscione, C. D. (2024). Testing the mating system model of parasite complex life cycle evolution reveals demographically driven mixed mating. *The American Naturalist*, *204*(6), 600–615. https://doi.org/10.1086/732807

Hulke, J. M., Ellenburg, W. H., Zelmer, D. A., & Criscione, C. D. (2021). Quantifying bilateral infection patterns in the trematode *Alloglossidium renale*. *The Journal of Parasitology*, *107*(5), 731–738. https://doi.org/10.1645/21-2

Hurd, H., Warr, E., & Polwart, A. (2001). A parasite that increases host lifespan. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1477), 1749–1753. https://doi.org/10.1098/rspb.2001.1729

Iwasa, Y., & Wada, G. (2006). Complex life cycle and body sizes at life-history transitions for macroparasites. *Evolutionary Ecology Research*, *8*, 1427–1443.

Jarne, P., & David, P. (2008). Quantifying inbreeding in natural populations of hermaphroditic organisms. *Heredity*, *100*(4), 431–439. https://doi.org/10.1038/hdy.2008.2

Jullien, M., Navascués, M., Ronfort, J., Loridon, K., & Gay, L. (2019). Structure of multilocus genetic diversity in predominantly selfing populations. *Heredity*, *123*(2), 176–191. https://doi.org/10.1038/s41437-019-0182-6

Kasl, E. L., Font, W. F., & Criscione, C. D. (2018). Resolving evolutionary changes in parasite life cycle complexity: Molecular phylogeny of the trematode genus *Alloglossidium* indicates more than one origin of precociousness. *Molecular Phylogenetics and Evolution*, *126*, 371–381. https://doi.org/10.1016/j.ympev.2018.04.027

Kasl, E. L., McAllister, C. T., Robison, H. W., Connior, M. B., Font, W. F., & Criscione, C. D. (2015). Evolutionary consequence of a change in life cycle complexity: A link between precocious development and evolution toward female-biased sex allocation in a hermaphroditic parasite. *Evolution*, *69*(12), 3156–3170. https://doi.org/10.1111/evo.12805

Krzywinski, M., & Altman, N. (2013). Error bars. *Nature Methods*, *10*(10), 921–922. https://doi.org/10.1038/nmeth.2659

Lagrue, C., & Poulin, R. (2009). Heritability and short-term effects of inbreeding in the progenetic trematode *Coitocaecum parvum*: Is there a need for the definitive host? *Parasitology*, *136*(2), 231–240. https://doi.org/10.1017/S0031182008005325

Lagrue, C., Poulin, R., & Keeney, D. B. (2009). Effects of clonality in multiple infections on the life-history strategy of the trematode

Coitocaecum parvum in its amphipod intermediate host. *Evolution*, *63*(6), 1417–1426. https://doi.org/10.1111/j.1558-5646.2009.00619.x

Lagrue, C., Waters, J. M., Poulin, R., & Keeney, D. B. (2007). Microsatellite loci for the progenetic trematode, *Coitocaecum parvum* (Opecoelidae). *Molecular Ecology Notes*, *7*(4), 694–696. https://doi.org/10.1111/j.1471-8286.2007.01680.x

Lefebvre, F., & Poulin, R. (2005). Progenesis in digenean trematodes: A taxonomic and synthetic overview of species reproducing in their second intermediate hosts. *Parasitology*, *130*(Pt 6), 587–605. https://doi.org/10.1017/s0031182004007103

Leigh, J. W., & Bryant, D. (2015). POPART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116. https://doi.org/10.1111/2041-210x.12410

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*(11), 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Lounnas, M., Correa, A. C., Vázquez, A. A., Dia, A., Escobar, J. S., Nicot, A., & Hurtrez-Boussès, S. (2017). Self-fertilization, long-distance flash invasion and biogeography shape the population structure of *Pseudosuccinea columella* at the worldwide scale. *Molecular Ecology*, *26*, 887–903.

Mable, B. K., & Adam, A. (2007). Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*. *Molecular Ecology*, *16*(17), 3565–3580. https://doi.org/10.1111/j.1365-294X.2007.03416.x

MacGregor-Fors, I., & Payton, M. E. (2013). Contrasting diversity values: Statistical inferences based on overlapping confidence intervals. *PLoS One*, *8*(2), e56794. https://doi.org/10.1371/journal.pone.0056794

McAllister, C. T., Kasl, E. L., Robison, H. W., Connior, M. B., Font, W. F., Trauth, S. E., & Criscione, C. D. (2016). New host records for *Alloglossidium progeneticum* (Digenea: Alloglossiidae) in crayfishes (Decapoda: Cambaridae) from Arkansas and Oklahoma, USA. *Comparative Parasitology*, *83*(2), 255–259. https://doi.org/10.1654/4820i.1

Nei, M. (1987). *Molecular evolutionary genetics*. Columbia University Press.

Nordborg, M. (2000). Linkage disequilibrium, gene trees and selfing: An ancestral recombination graph with partial self-fertilization. *Genetics*, *154*(2), 923–929. https://doi.org/10.1093/genetics/154.2.923

Olsen, K. C., & Levitan, D. R. (2023). Interpopulation variation in inbreeding is primarily driven by tolerance of mating with relatives in a spermcasting invertebrate. *Journal of Evolutionary Biology*, *36*(1), 95–108. https://doi.org/10.1111/jeb.14125

Olson, P. D., Cribb, T. H., Tkach, V. V., Bray, R. A., & Littlewood, D. T. J. (2003). Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology*, *33*(7), 733–755. https://doi.org/10.1016/s0020-7519(03)00049-3

Parker, G. A., Ball, M. A., & Chubb, J. C. (2015a). Evolution of complex life cycles in trophically transmitted helminths. I. Host incorporation and trophic ascent. *Journal of Evolutionary Biology*, *28*(2), 267–291. https://doi.org/10.1111/jeb.12575

Parker, G. A., Ball, M. A., & Chubb, J. C. (2015b). Evolution of complex life cycles in trophically transmitted helminths. II. How do life-history stages adapt to their hosts? *Journal of Evolutionary Biology*, *28*(2), 292–304. https://doi.org/10.1111/jeb.12576

Parker, G. A., Chubb, J. C., Ball, M. A., & Roberts, G. N. (2003). Evolution of complex life cycles in helminth parasites. *Nature*, *425*(6957), 480–484. https://doi.org/10.1038/nature02012

Payton, M. E., Greenstone, M. H., & Schenker, N. (2003). Overlapping confidence intervals or standard error intervals: What do they mean in terms of statistical significance? *Journal of Insect Science*, *3*, 34.

Pollak, E. (1987). On the theory of partially inbreeding finite populations. I. Partial selfing. *Genetics*, *117*(2), 353–360. https://doi.org/10.1093/genetics/117.2.353

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Redelings, B. D., Kumagai, S., Tatrenkov, A., Wang, L., Sakai, A. K., Weller, S. G., Culley, T. M., Avise, J. C., & Uyenoyama, M. K. (2015). A Bayesian approach to inferring rates of selfing and locus-specific mutation. *Genetics*, *201*, 1171–1188.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution*, *43*(1), 223–225. https://doi.org/10.1111/j.1558-5646.1989.tb04220.x

Ritland, K. (1990). Inferences about inbreeding depression based on changes of the inbreeding coefficient. *Evolution*, *44*(5), 1230–1241. https://doi.org/10.1111/j.1558-5646.1990.tb05227.x

Rousset, F. (2008). Genepop'007: A complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Schenker, N., & Gentleman, J. F. (2001). On judging the significance of differences by examining the overlap between confidence intervals. *American Statistician*, *55*(3), 182–186. https://doi.org/10.1198/000313001317097960

Schmidt, G. D., & Roberts, L.S. (2009). *Foundations of parasitology* (8th Ed.). McGraw Hill, New York.

Siol, M., Prosperi, J. M., Bonnin, I., & Ronfort, J. (2008). How multilocus genotypic pattern helps to understand the history of selfing populations: A case study in *Medicago truncatula*. *Heredity*, *100*(5), 517–525. https://doi.org/10.1038/hdy.2008.5

Stoffel, M. A., Esser, M., Kardos, M., Humble, E., Nichols, H., David, P., & Hoffman, J. I. (2016). inbreedR: An R package for the analysis of inbreeding based on genetic markers. *Methods in Ecology and Evolution*, *7*(11), 1331–1339. https://doi.org/10.1111/2041-210x.12588

Sullivan, J. J., & Heard, R. W. (1969). *Macroderoides progeneticus* n. sp. a progenetic trematode (Digenea: Macroderoididae) from antennary gland of crayfish *Procambarus spiculifer* (Leconte). *Transactions of the American Microscopical Society*, *88*(2), 304–308. https://doi.org/10.2307/3224507

Valdivia, I. M., Criscione, C. D., Cárdenas, L., Durán, C. P., & Oliva, M. E. (2014). Does a facultative precocious life cycle predispose the marine trematode *Proctoeces* cf. *lintoni* to inbreeding and genetic differentiation among host species? *International Journal for Parasitology*, *44*(3-4), 183–188. https://doi.org/10.1016/j.ijpara.2013.10.008

Vilas, R., Criscione, C. D., & Blouin, M. S. (2005). A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Parasitology*, *131*(Pt 6), 839–846. https://doi.org/10.1017/S0031182005008437

Villa, M., & Lagrue, C. (2019). Progenesis and facultative life cycle abbreviation in trematode parasites: Are there more constraints than costs? *International Journal for Parasitology*, *49*(5), 347–354. https://doi.org/10.1016/j.ijpara.2018.11.009

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Whitehead, M. R., Lanfear, R., Mitchell, R. J., & Karron, J. D. (2018). Plant mating systems often vary widely among populations. *Frontiers in Ecology and Evolution*, *6*, 38.

Zheng, Q. (2015). Methods for comparing mutation rates using fluctuation assay data. *Mutation Research*, *777*, 20–22. https://doi.org/10.1016/j.mrfmmm.2015.04.002