MOLECULAR ECOLOGY

Molecular Ecology (2016) **25**, 2805–2815 doi: 10.1111/mec.13662

Cryptic sex? Estimates of genome exchange in unisexual mole salamanders (*Ambystoma* sp.)

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Abstract

Cryptic sex has been argued to explain the exceptional longevity of certain parthenogenetic vertebrate lineages, yet direct measurements of genetic exchange between sexual and apparently parthenogenetic forms are rare. Female unisexual mole salamanders (*Ambystoma* sp.) are the oldest known unisexual vertebrate lineage (~5 million years), and one hypothesis for their persistence is that allopolyploid female unisexuals periodically exchange haploid genomes 'genome exchange' during gynogenetic reproduction with males from sympatric sexual species. We test this hypothesis by using genome-specific microsatellite DNA markers to estimate the rates of genome exchange between sexual males and unisexual females in two ponds in NE Ohio. We also test the prediction that levels of gene flow should be higher for 'sympatric' (sexual males present) genomes in unisexuals compared to 'allopatric' (sexual males absent) unisexual genomes. We used a model testing framework in the coalescent-based program MIGRATE-N to compare models where unidirectional gene flow is present and absent between sexual species and unisexuals. As predicted, our results show higher levels of gene flow between sexuals and sympatric unisexual genomes compared to lower (likely artefactual) levels of gene flow between sexuals and allopatric unisexual genomes. Our results provide direct evidence that genome exchange between sexual and unisexual *Ambystoma* occurs and demonstrate that the magnitude depends on which sexual species are present. The relatively high levels of gene flow suggest that unisexuals must be at a selective advantage over sexual forms so as to avoid extinction due to genetic swamping through genome exchange.

Keywords: *Ambystoma* salamanders, genome exchange, gynogenesis, unisexual

Received 18 December 2015; revision received 3 April 2016; accepted 14 April 2016

Introduction

The costs and benefits of sexual vs. asexual reproduction have sparked a rich and ongoing discussion about the selective advantages of each mode of reproduction (Agrawal 2001; Butlin 2002; Lehtonen *et al.* 2012). Theoretically, asexual lineages have a twofold fitness advantage over their sexual counterparts due to the cost of meiosis (the genetic contribution of an individual is halved each generation; Williams 1975) and the cost of producing males (males' contribution to population growth is limited by females; Doncaster *et al.* 2000; Maynard Smith 1978). Nonetheless, sexual reproduction is the dominant mode of reproduction in most eukaryotes. This may be due to the potential ecological advantages conferred by the ability to purge deleterious mutations through recombination and generate high levels of genetic variation in their offspring (Hamilton *et al.* 1990). However, evolutionary biologists as far back as Wright (1939) suggested that asexual lineages which occasionally engage in sex could realize the ecological and genetic advantages of both sexual and asexual reproduction (Green & Noakes 1995; Hurst & Peck 1996) and account for the paradox of apparent evolutionarily ancient, asexual lineages of animals in nature (Judson & Normark 1996). To assess this explanation, direct estimates of the extent of gene exchange between

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sexual and putatively asexual forms are needed for natural populations (Shurko *et al.* 2009).

Unisexual (all-female) populations of Ambystomatid salamanders offer a system where it is possible to directly estimate the rate of genetic contribution from sympatric sexual species to putatively asexual forms. These salamanders are distributed across eastern North America (Bogart & Klemens 2008) and are generally triploid (3N), but can have genomes that vary from 2N to 5N in ploidy number (Bogart *et al.* 2007). The nuclear DNA of unisexuals is commonly made up of genomes from up to four diploid, biparental species: the Blue-spotted Salamander (*Ambystoma laterale*), Jefferson Salamander (*A. jeffersonianum*), Small-mouthed Salamander (*A. texanum*) and Tiger Salamander (*A. tigrinum*), designated as L, J, T and Ti, respectively (Lowcock *et al.* 1987). This diversity of genetic sources leads to a large number of possible nuclear genome combinations (biotypes) in unisexuals with the only constant being that all unisexual salamanders have at least one L genome (Bi *et al.* 2008). More than 20 unique genome combinations have been found, with triploid combinations (e.g. LJJ, LLJ, LTT, LTTi) being most common (Bogart *et al.* 2007). Despite the complexity of the nuclear genome combinations, all unisexuals form a monophyletic lineage based on their mitochondrial DNA (Bogart *et al.* 2007). The maternal ancestor of the unisexuals was most closely related to the Streamside Salamander (*A. barbouri*), with the original hybridization event occurring ~5 million years ago (Bi & Bogart 2010).

Female unisexuals require sperm from a co-occurring bisexual species to initiate reproduction; however, they can then either use the sperm solely to activate egg development (i.e. gynogenesis) or incorporate the sperm genome into the resulting zygotes (Bogart *et al.* 2007; Ramsden 2008). Sperm incorporation can take the form of ploidy elevation in the offspring or true replacement, wherein one of the maternal genomes is discarded. The molecular mechanisms that underlie ploidy elevation are better understood than those responsible for the loss of genomes through reduction (Bogart & Bi 2013). Collectively, these processes have been termed 'genome replacement' by Bogart *et al.* (2007) and Bi *et al.* (2008). Because our analyses [and those of Bi *et al.* (2008)] cannot distinguish between ploidy elevation and true replacement as mechanisms for shared variation between sexuals and unisexuals, we refer to this process as genome exchange (GE), which is a mechanism-neutral term.

Direct DNA-based evidence for GE comes from two sources. First, a microsatellite DNA analysis of egg masses laid by single unisexual females showed that almost half (11 of 26 masses) contained larvae of mixed biotype that could have resulted from genome addition (Bogart *et al.* 2007). Second, a sequence-based analysis of a single marker on the L genome showed that the geographic distribution of the L haplotypes in unisexual populations matched those found in local *A. laterale* populations, suggesting that L genomes in unisexuals are transferred from sympatric populations of *A. laterale* through GE (Bi *et al.* 2008). Both pieces of evidence suggest that while unisexual lineages originated millions of years ago, their nuclear genomes have undergone frequent and repeated shuffling through evolutionary time as a result of GE. However, direct estimates of the rate at which this exchange occurs are not available despite their key importance for evaluating the models assessing the relative fitness of unisexuals compared to their sexual progenitors. In particular, Charney (2012) showed that moderate levels of unidirectional gene exchange between sympatric populations of sexual and unisexual *Ambystoma* over time would lead to the extinction of unisexuals due to the replacement of unisexual genomes by those of the sexual species unless there was compensating positive selection favouring unisexuals. Bogart & Bi (2013) have argued that cytological mechanisms may also play an important role in the dynamics of gene exchange, particularly in terms of genome reduction in unisexuals. Because higher levels of gene exchange would require stronger selection favouring unisexuals, this parameter is essential to understanding the evolutionary processes that facilitate coexistence between sexual and asexual forms.

A conceptual framework for examining the one-way transfer of genes from sexual males to unisexual females at a population level is to assess the rates of asymmetric gene flow between genome-specific gene pools in the sexual and asexual lineages (Fig. 1). In this model, gene exchange occurs in one direction from sexual species' gene pool into the unisexual gene pool via GE from sexual males to unisexual females. Gene loss from the unisexual gene pool via ploidy reduction can occur in unisexual offspring, but empirical studies

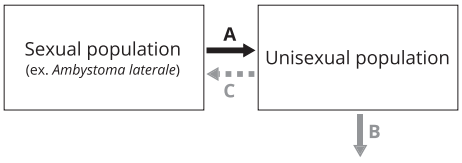

Fig. 1 Conceptual model for asymmetrical exchange of homologous species-specific genomes between sexual males and unisexual females. Exchange of genetic material due to genome exchange (A) is assumed to be much greater than loss of genes from the unisexual gene pool via ploidy reduction (B). Gene flow from a unisexual gene pool (C) is infrequent or nonexistent because 'unisexual males' are extremely rare (Bogart and Klemens 2008).

suggest that it is extremely rare (Bogart & Licht 1986), and so we assume that its impact on levels of standing variation in the unisexual gene pool is minimal. The advantage of modelling GE in this way is that data from highly variable genome-specific genetic markers can be analysed with coalescent-based population genetic programs such as MIGRATE-N (Beerli 2006) to compare the statistical likelihood of models with and without GE between sexual and unisexual gene pools (Carstens *et al.* 2013; Tsai & Carstens 2013) and derive parameter estimates of the rates of genome-specific asymmetric gene flow over evolutionary timescales. To apply this approach, homologous genetic loci are required in sexual and unisexual individuals that may also vary in gene copy number. This is especially challenging in polyploid individuals such as unisexuals, which consist of combinations of related genomes from different species. However, recent advances in DNA-based methods to assess genome identity and number in unisexuals (Greenwald & Gibbs 2012) and the isolation of variable species-specific microsatellite loci for *Ambystoma* species that contribute genomes to the most common unisexual biotypes (Denton *et al.* 2015) have made the generation of data from multiple loci across different species-specific genomes in unisexuals possible.

Here, we use these genetic resources combined with a model-based approach to assess (i) whether GE occurs between like genomes in sexual and unisexual *Ambystoma* and, if so, at what rate and (ii) if genome-specific rates of GE in unisexuals vary depending on which sexual species exist in sympatry with specific unisexual populations. In particular, we predict that if GE occurs, then genome-specific levels of GE in unisexuals will be higher for portions of the unisexual genomes that match coexisting sympatric sexual males than for allopatric sexual males (see Fig. 2). We examine these questions for populations of sexual and unisexual salamanders in Northern Ohio, which consist of two sexual species, Jefferson Salamanders (designed as JJ), Blue-spotted

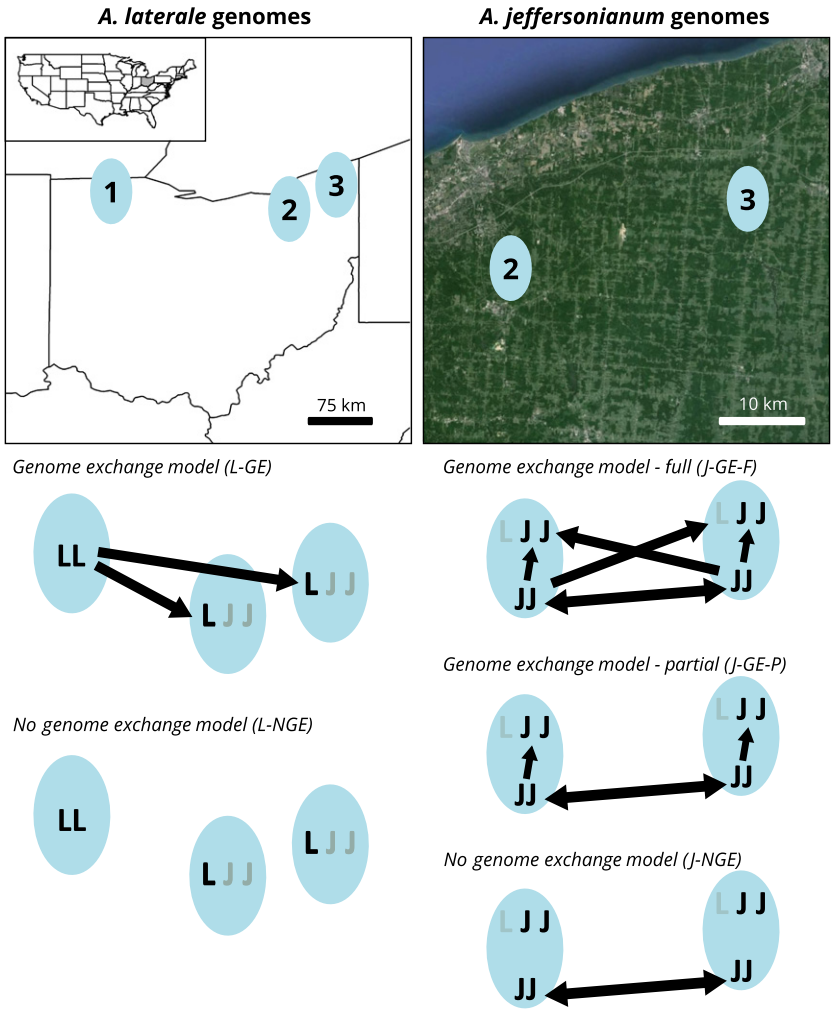

Fig. 2 Sample locations and migration models that were tested for populations of *Ambystoma laterale* (LL), *A. jeffersonianum* (JJ) and unisexual *Ambystoma* (LJJ). Only *Ambystoma laterale* ($N = 24$) were present at Kitty Todd Nature Preserve (1), whereas both *A. jeffersonianum* ($N = 20$) and unisexual salamanders ($N = 19$, 20) were present at Big Creek Park (2) and Grand River Terraces (3). Migration models are shown for analyses based on *A. laterale* genomes only (left) or *A. jeffersonianum* genomes only (right), where ovals represent a wetland area and arrows represent the magnitude and direction of an estimated migration parameter.

Salamanders (LL) and polyploid unisexuals consisting of combinations of L and J genomes (e.g. LLJ, LJJ). Our analyses provide the first quantitative estimate of the rate at which realized GR between sexual and unisexual gene pools occurs over evolutionary timescales and also assesses whether geographic proximity of males from particular species is an important factor influencing this phenomenon.

Methods

Locations and samples

During the spring breeding season (March-April) of 2012, adult salamanders were sampled at vernal pools at three sites in Northern Ohio. Two sites (Grand River Terraces and Big Creek Park) were located in northeastern Ohio (Ashtabula and Geauga County, respectively), while the third (Kitty Todd Nature Preserve) was close to Toledo in Lucas County (Fig. 2). The Grand River Terraces and Big Creek Park sites were chosen because preliminary sampling showed that they contained sexual populations of *A. jeffersonianum* (JJ) and unisexual *Ambystoma* with biotypes consisting of *A. laterale* and *A. jeffersonianum* genomes (LLJ, LJJ and LJJJ). The Kitty Todd site was sampled because it represented the closest site at which *A. laterale* (LL) are present from which we could obtain samples. For all captured salamanders, a small (3- to 4-mm) tissue sample was removed from the tip of the tail for genetic analysis (tails are subsequently regenerated; Ohio State University Institutional Animal Care and Use Committee Protocol #2012A00000039). Tail samples were stored in 95% EtOH until further processing. All salamanders were then released at the site of capture. Samples at Grand River Terraces and Big Creek Park sites were collected by Tim Matson and Roberta Muehlheim, while those at Kitty Todd were collected by Greg Lipps.

Genetic analyses

We followed the methods outlined in Greenwald & Gibbs (2012) to confirm species identification and to assess unisexual biotype. Specifically, we extracted DNA from tissue samples using Qiagen DNeasy extraction kits (Qiagen, Valencia, CA, usa) and then used a two-step process to identify the genetic identity of particular samples. First, we sequenced mitochondrial DNA (primers F-THR and R-651; Shaffer & McKnight 1996; Bogart *et al.* 2007) and used fixed polymorphisms to differentiate between unisexuals and any sexual species that were present. Second, for samples identified as unisexuals, we then used a panel of single nucleotide polymorphisms to determine unisexual biotype (detailed methods are outlined in Greenwald & Gibbs 2012). This yields direct estimates of genome identity (L or J) and numbers of these specific genomes that we used to characterize the unisexual biotypes.

We then generated genome-specific (L or J) microsatellite genotypes for all individuals using primer sets for L- or J-specific loci and methods reported in Denton *et al.* (2015). Briefly, we first used the methods described above to characterize the type and the number of genomes present in each sample and the used appropriate primer sets to genotype specific samples. Sexual individuals were genotyped using either 11 (*A. laterale*) or 14 (*A. jeffersonianum*) polymorphic microsatellite loci as described by Denton *et al.* (2015) and Julian *et al.* (2003; see Table S1, Supporting information for the list of loci used) and all unisexuals were genotyped using both sets of loci. To generate microsatellite genotypes for all samples, we followed the protocols described in Denton *et al.* (2015). Briefly, PCRs for all loci were carried out using a mixture of tagged forward (CAG or M13 tag) and untagged reverse primers combined with M13R/CAG primers labelled with FAM, HEX or NED fluorescent dyes. Amplification reactions were run using a touchdown temperature profile. Amplified products were then analysed on either a Applied Biosystems 3100 or 3730 DNA sequencer using a ROX-labelled internal size standard (GeneScan 500 ROX; Applied Biosystems) and scored using GeneMapper software (version 4.1; Applied Biosystems). Scoring of microsatellite profiles in the unisexuals was guided by the results of the initial biotype screening. Specifically, the number of L- or J-specific alleles that were scored in a unisexual matched the number estimated to be present from the results of the biotype assay and was determined by comparisons of peak heights in the profiles. For example, if a SNP-identified LJJJ individual displayed two distinct microsatellite peaks for a J-specific locus and one was twice the height of the other, that individual would be scored as having three alleles: two representing the size of the larger peak and one representing the size of the smaller peak. In a limited number of cases, there was ambiguity in terms of matches between peak heights and the expected number of alleles based on the SNP assay (e.g. two similar size peaks present in a triploid individual). In this situation, we scored the individual as having two known alleles corresponding to the observed peaks and one unknown allele.

Population genetic methods

We followed the conceptual framework of Halkett *et al.* (2005) and Ramsden (2008) by calculating population genetic parameters that are predicted to show the

differences between sexual and asexual organisms. Specifically, we estimated the G:N ratio, which represents the number of unique multilocus genotypes (G) relative to the number of individuals sampled (N) by hand, and then used GENEPOP (Rousset 2008) and FSTAT (Goudet 1995) where appropriate to determine the proportion of loci in Hardy–Weinberg disequilibrium, linkage disequilibrium between pairs of loci, locus-specific and overall expected (H_e) and observed (H_o) heterozygosity and F_{is} values. These parameters were estimated using all available data for the two sexual species. To make unisexual genotypes based on J genomes comparable, we followed Ramsden (2008) and 'diploidized' the data set by excluding all haploid J genotypes and randomly excluding one genotype in unisexuals that had three copies of a J genome. Because all unisexuals had a single L genome, it was only possible to calculate a G/N ratio and the mean number of alleles per locus because the other parameters required diploid genotypes.

To gain a description of the level of differentiation between different gene pools, we also estimated overall F_{ST} values based on allele frequencies for specific loci between different combinations of sexual and unisexual genomes using GENEPOP. To explore the potential role of retained ancestral polymorphism as an influence on our results for the comparisons involving the geographically separate L genomes, we used published data from Greenwald *et al.* (2009) on genetic (microsatellite loci) and geographic distances between populations of *A. laterale* from a site in Wisconsin to estimate the isolation-by-distance (IBD) relationship for these populations (see Greenwald *et al.* 2009 for details).

Estimates of genome exchange via model testing

We used the program MIGRATE-N (version 3.3.1; Beerli 2006) under the framework outlined in Fig. 1 to directly estimate the levels of genome-specific gene exchange between sexual and unisexual individuals. MIGRATE-N is appropriate for estimating gene flow with data where ploidy varies between 'populations' because it uses analyses of the coalescent histories of individual alleles and not genotypes to estimate migration (Beerli 2006). We used the explicit model testing framework outlined by Beerli & Palczewski (2010) to compare the statistical fit of the data to two types of models: genome exchange (GE – see Fig. 2) models that included parameter for positive, unidirectional gene exchange from sexuals to unisexuals ($M = m/\mu$, where m is the proportion of migrants exchanged per generation between gene pools and μ is the mutation rate) and no genome exchange (NGE) models that were identical except that they excluded this parameter (Fig. 2). A model testing approach offers two important advantages over simple parameter estimation. First, it provides an explicit statistical test of whether a model with this additional parameter for gene flow provides a better fit to the data. Second, it allows a simultaneous estimate of levels of gene flow when other important demographic parameters are included in the model, which results in a more accurate estimate (Tsai & Carstens 2013). If GE models provide a better fit to the data, then the point estimate of the migration parameter M between sexuals and unisexuals can be used as measure of the observed level of gene exchange between specific genomes. This conceptual approach has been used by Jørgensen *et al.* (2011) to estimate levels of gene flow between natural populations of sexual and polyploid plants albeit at a different evolutionary scale using a different coalescent-based program.

We conducted separate analyses for L and J genome-specific data to ask whether the sympatric (JJ) or allopatric (LL) distribution of potential donor sexual males impacts the magnitude of gene exchange with unisexuals. In both these analysis, GE models included unidirectional gene flow from sexuals to unisexuals, while the NGE models included no such parameter (J comparisons) or an extremely low value (M = 0.001; L comparisons), which was necessary to allow us to compare the fit of GE vs. NGE models with no other migration parameter in the model (P. Beerli, pers. comm.). Finally, for the J-based GE models, we included two submodels that enabled us to test the importance of within-vs. between-pond gene exchange. We did this by including one model with parameters for GE between sexual JJ males and unisexuals sampled from different ponds (GE – full) vs. a model that only included a within-pond GE parameter (GE – partial; Fig. 2).

For each MIGRATE-N analysis, we generated initial theta and migration values using the default F_{ST} calculation with the initial genealogies sampled started from a random tree. We used broad uniform priors (due to a lack of a priori information on possible parameter values) and slice sampling for parameter distributions. During MCMC sampling, static heating was used with temperatures of 1, 1.5, 3 and 1×10^6. We ran three long chains with 1×10^4 burn-in repetitions followed by 1×10^6 recorded steps for every 25 steps, resulting in a total of 2.5×10^7 sampled genealogies. Convergence was assessed by the consistency of results across two separate runs. The probability of different models in fitting the data was then compared using Bayes factors as described in Beerli & Palczewski (2010). For estimates of M and theta, we used the mean values from the posterior distributions for each parameter estimated across all loci under the best-fit model. These are given for L and J genomes separately.

Results

Identification of individuals

Based on the methods in Greenwald & Gibbs (2012) for genetically identifying sexual species, we identified 19 and 20 adult JJ individuals in the BC and GR populations and 24 adult LL individuals in the KT populations (see Fig. 2 for population designations and locations). A major difference between the genetic composition of unisexuals in these two populations is the presence of *texanum* genomes (T) in 17 of 21 individuals in the GR population, whereas only L and J genomes were found in the BC unisexuals. We did not further analyse the variation in T genomes. Only a single L genome was found in all unisexuals. More specifically, in the BC population, we identified individuals with LJ (n = 2), LJJ (7) and LJJJ (10) biotypes, while in the GR population we detected LJJ (3), LJJJ (1), LTJ (15) and LTJJ (2) individuals.

Population genetic signatures of asexual reproduction

We compared sexual and unisexual genomes to look for genetic signatures of asexual reproduction in the unisexual genomes. 'Diploidizing' unisexual individuals from both BC and GR populations resulted in a total of 22 individuals retained in the analysis. We then compared the genetic characteristics of the JJ genomes in unisexuals with a pooled sample of JJ individuals from both BC and GR populations (n = 39) or unisexual L genomes with LL individuals from KT (n = 24).

We found genetic signatures of reduced variation and increased genetic disequilibrium consistent with a pattern of asexual reproduction and isolation in unisexual genomes, but these patterns were not as pronounced (at least for J genomes) as those found in Ontario populations by Ramsden (2008). For J genomes, G:N ratios were the same, indicating no increase in the relative numbers of clonal genotypes in unisexuals. However, the average number of alleles per locus in unisexuals was almost half that observed in sexuals (10.3 vs. 5.8), and both observed and expected heterozygosity values were lower in unisexuals, as expected (Table 1). Also, the proportion of loci in HW disequilibrium, loci in linkage disequilibrium and mean F_{is} values were all higher in unisexuals, which is consistent with a predicted increase in genetic disequilibrium in unisexuals. However, comparisons of the magnitude of these differences for most (but not all) parameters with those reported by Ramsden (2008) show smaller differences between sexuals and unisexuals in Ohio as compared to Ontario. For example, all loci in Ontario unisexuals were in HW disequilibrium and showed evidence for linkage disequilibrium, whereas the observed heterozygosity is similar between Ontario sexuals and unisexuals (Table 1). Comparisons of diversity for L genomes in sexual and unisexual salamanders in Ohio are limited because most unisexuals only contain a single L genome. However, as predicted, both the G:N ratio and the number of alleles per locus are substantially lower in unisexuals (Table 1).

Levels of differentiation based on overall F_{ST} values are high for genome-specific sexual/unisexual comparisons but are lower between populations of sexual JJ

Table 1 Measures of population genetic diversity in sexual and unisexual *Ambystoma* salamander populations analysed in this study. Results from a previously published study by Ramsden (2008) are included for comparison. To estimate the measures of genetic diversity for unisexuals, the *A. jeffersonianum* genomes were reduced to diploids through the random removal of an allele if more than two alleles were present

	Ambystoma jeffersonianum genomes				*Ambystoma laterale* genomes	
Parameter/sampling	Gibbs & Denton (Ohio)		Ramsden (2008; Ontario)		Gibbs & Denton (Ohio)	
Sampled group	*A. jeffersonianum*	Unisexuals*	*A. jeffersonianum*	Unisexuals	*A. laterale*	Unisexuals†
Number of samples	39	22	168	337	24	39
G:N ratio	1.00	1.00	1.00	0.83	1.00	0.50
Mean alleles/locus	10.27	5.82	9.80	10.70	4.27	2.73
Proportion of loci in HW disequilibrium	0.00	0.09	0.00	1.00	0.00	NA
Proportion of pairs of loci in LD	0.13	0.22	0.19	1.00	0.14	NA
Mean H_o	0.70	0.57	0.84	0.81	0.58	NA
Mean H_e	0.76	0.61	0.82	0.60	0.48	NA
Mean F_{is} (SE)	0.06 (0.06)	0.10 (0.08)	−0.02 (0.008)	−0.39 (0.08)	NA	NA

*No haploids, random alleles removed from triploids.
†*Ambystoma laterale* genomes only.

individuals, suggesting that gene exchange between sexual and unisexual gene pools is limited compared to those of a sympatric sexual species (Table 2). For example, F_{ST} values for sexual/unisexual J comparisons sampled from the same site were ~0.18, whereas the value comparing the two sexual JJ populations 25 km apart was 0.044. Differentiation between J gene pools in different sites was also high (F_{ST} = 0.21), suggesting substantial isolation between unisexual populations in different ponds. Finally, these patterns were similar for comparisons of L genomes: F_{ST} values for comparisons between LL individuals and the two L unisexual gene pools were high (F_{ST} ~ 0.56) likely reflecting the large distance between the populations being compared (Table 2). Differentiation between the two L unisexual gene pools was much higher (F_{ST} = 0.70) than between J gene pools in the same individuals, which reflects their increased isolation.

Estimates of genome exchange

For JJ sexual and J unisexual genomes, both models with gene flow from sexual males to unisexual females (J-GE-F and J-GE-P) had substantially higher likelihoods (Bezier lmL > −23 821) than the null model (J-NGE; Bezier lmL = −394,129; Table 3). This provides strong evidence for gene exchange between sexual JJ males and unisexual females with J genomes in these sites. Of the two gene flow models, the model that restricted gene flow to occurring only between sexual males and unisexual females that were captured at the same pond had a higher probability (J-GE-P; model probability = 1.00) than the model that allowed gene exchange both with and between ponds (J-GE-F; model probability = 0.00; Table 3). This is consistent with the interpretation that the primary mode of gene exchange is between resident sexual males and resident unisexual females. Parameter estimates of unidirectional migration rates (M) from the best-fit model provide measures of the level of gene exchange (Fig. 3). Within-population estimates of M from JJ males to unisexual females were similar for both ponds (GR: M = 3.23; BC: M = 3.37). For comparison, the value for symmetric gene flow between JJ males from each pond was M = 4.89. Finally, theta values estimated for the sexual males (GR: θ = 3.13; BC: θ = 5.76) were larger than for J genomes in unisexual females (both 0.033), which reflects the reduced levels of variation present in the unisexual females.

For L sexual and unisexual genomes, the best-fit model was also the gene flow model (Bezier lmL = −16,546; L-GE model probability, P = 1.00) compared to the 'no gene flow' model (Bezier lmL = −27,268; L-NGE model probability, P = 0.00) (Table 3). This result is surprising given the lack of sexual LL individuals in the ponds containing the female unisexuals with L genomes and the distance between the sampled population of sexual LL males and the sampled populations of unisexuals, perhaps representing an artefact of retained ancestral variation on the estimates of gene flow. The low estimated M (0.55, 0.62)

Table 2 Overall F_{st} values for population comparisons between J and L genomes in sexual and unisexual salamanders. Population key: JJ sexuals at Grand River (JJ-GR); JJ sexuals at Big Creek (JJ-BC); unisexuals at Grand River (U-GR); unisexuals at Big Creek (U-BC); LL sexuals at Kitty Todd (LL-KT); L unisexuals at GR (U-GR)

	JJ-GR	JJ-BC	U-GR	U-BC
Ambystoma jeffersonianum genomes				
JJ-GR	—	0.044	0.176	0.248
JJ-BC	0.044	—	0.119	0.182
U-GR	0.176	0.119	—	—
U-BC	0.248	0.182	—	—
	LL-KT	U-GR	U-BC	
Ambystoma laterale genomes				
LL-KT	—	0.595	0.557	
U-GR	0.595	—	0.697	
U-BC	0.557	0.697	—	

Table 3 Ranks for models comparing the levels of genome exchange between sexual and unisexual *Ambystoma* in Ohio and Ontario (using data from Ramsden 2008). Bezier log-likelihood values were produced with MIGRATE-N and probabilities were calculated as in Beerli & Palczewski (2010). Parameter estimates for the best-fit models are given in Fig. 3

Model name	Bezier lmL	Bezier difference (rank)	Model probability
Ambystoma jeffersonianum genomes			
Genome exchange – partial (J-GE-P)	−20,496.73	0 (1)	1.0
Genome exchange – full (J-GE-F)	−23,821.03	−3324.30 (2)	0.0
No genome exchange (J-NGE)	−394,129.01	−373632.28 (3)	0.0
Ambystoma laterale genomes			
LL gene exchange (L-GE)	−16,545.52	0 (1)	1.0
LL No gene exchange (L-NGE)	−27,268.82	−10723.30 (2)	0.0

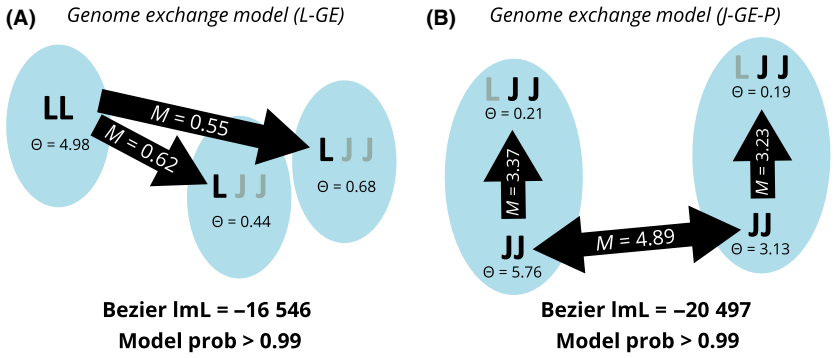

Fig. 3 Best-supported MIGRATE-N models and associated estimates of M and θ parameters for *A. laterale* genomes (A) and *A. jeffersonianum* genomes (B). Values of M and θ were estimated as the mean values of each parameter from the posterior distribution estimated across all loci under the best-fit model. Distance between sites (ovals) is not representative of true distances which are shown in Fig. 2. Model selection was performed as described in Beerli & Palczewski (2010).

and theta (0.68 and 0.44 for GR and BC populations, respectively) values reflect the isolation of the L genomes in the unisexual females in these populations (Fig. 3). Finally, *A. laterale* data from Wisconsin from Greenwald *et al.* (2009) show a curvilinear relationship between F_{ST} values and geographic distance between populations that asymptotes at a F_{st} value of 0.02 between populations that are >10 km apart (Fig. S1, Supporting information). This suggests that the level of shared polymorphism between distant populations of this species is limited.

Discussion

Estimates of genome exchange

Assessing the level of gene exchange between sexual and 'asexual' lineages is a key step in explaining the paradox of evolutionarily ancient, parthenogenetic lineages of animals in nature (Judson & Normark 1996; Schurko *et al.* 2009). If high levels of cryptic sex occur, the paradox disappears because such lineages can potentially accrue the advantages of sexual reproduction that promote their evolutionary persistence. If not, the paradox remains and other explanations must be sought, such as mechanisms that control the effects of mutational load (Gabriel *et al.* 1993). Previous work provided qualitative evidence that gene exchange was occurring between unisexual and sexual *Ambystoma*, but direct measures of the magnitude of cryptic sex were lacking despite their importance in understanding why unisexuals persist (Charney 2012). Our study represents a significant advance in that it uses a population genetic modelling framework to provide the first direct evidence that gene flow occurs and an estimate of its magnitude.

The approach of using coalescent-based evolutionary models to estimate gene flow across ploidy barriers has been used for sexual-descendant polyploid pairs of plant lineages (Jørgensen *et al.* 2011). However, those analyses focused on introgression over a longer timescale at a phylogenetically distinct lineage level in which the time of divergence between lineages is important to incorporate into estimates. Our question of interest was more local: within a given pond, what is the best estimate of the equilibrium level of gene exchange over evolutionarily recent timescales? This scale is more relevant to understanding the processes that drive the dynamics of sexual–unisexual populations at local scales, which represents an open question of interest (Lehtonen *et al.* 2013). This necessitates the use of a modelling approach incorporated in a program like MIGRATE-N, which has the advantages of allowing the specification of realistic gene flow models between local gene pools while incorporating key biological features of the system (i.e. unidirectional gene flow) and directly testing alternative models of population processes. However, MIGRATE-N assumes that populations are at drift–migration equilibrium and hence that shared polymorphism is due to gene flow alone and any influence of shared ancestral polymorphism, which could inflate our estimates, is minimal (Beerli 2009). Based on indirect evidence, we feel such effects are small.

Our per-generation estimates demonstrate that realized gene exchange between JJ males and J genomes in unisexual females is common over ecological timescales. Our two estimates of M averaged ~3.3. Estimates of M between sexual populations of JJ in each of the ponds (30 km apart) were only slightly higher (M = 4.89). Direct comparisons of these values are complicated by the fact that there were an unknown number of additional populations of JJ animals between the ponds that were compared and represent 'ghost' populations in our analyses. However, these appear to have a limited effect on estimates of M in MIGRATE-N (Beerli 2004). With

this caveat in mind, we conclude that within-pond levels of gene exchange are the same magnitude as that between sexual populations of JJ at limited (10's kms) spatial scales. If we assume a general mutation rate value of 5×10^{-4} per generation (Goldstein *et al.* 1995), then m (the proportion of migrants per generation) is approximately 0.2% per generation. This percentage can be interpreted to be the proportion of J genomes in unisexuals that result from gene exchange. If we assume continuing replacement of 'resident' genomes, the complete turnover of J genomes in unisexuals could occur as soon as 500 generations. Although the reproductive mechanism by which this process occurs is unknown and needs to be demonstrated (Ellison *et al.* 1992), this work provides the strongest evidence to date that 'unisexual' *Ambystoma* salamanders engage in frequent cryptic sex.

Two observations suggest that there may be a significant geographic influence on levels of gene exchange in specific populations of unisexuals/sexuals that has not previously been appreciated. First, levels of gene exchange should impact other genetic signatures that reflect the frequency of asexual reproduction in a unisexual population (Halkett *et al.* 2005). As noted above, the Ohio populations of unisexuals show lower levels of linkage disequilibrium and fewer loci in HW disequilibrium than the Ontario populations studied by Ramsden (2008). This may reflect lower levels of gene exchange in Ontario relative to Ohio for as yet unknown reasons. Second, our analysis shows that genome-specific levels of gene exchange in Ohio are dependent on the proximity of specific sexual males. When we test models for gene flow between the LL genomes in the nearest population of *A. laterale* and L genomes in the unisexual populations, we find support for the gene flow model. This is surprising because gene exchange between unisexuals in the sampled population and LL individuals is biologically impossible due to a lack of physical proximity of sexual males and unisexual females during reproduction. We suspect this is due to the influence of a low level of shared ancestral polymorphism between unisexuals and LL males (as shown in Fig. S1, Supporting information) that inflates the estimate of migration by MIGRATE-N. Regardless, the point estimate of the M value is fivefold lower than that for JJ individuals and J genomes in unisexuals (0.6 vs. 3.3), demonstrating that even within individual unisexuals, different genomes experience different degrees of genetic isolation and are on distinct evolutionary trajectories.

Biological implications

Our work has implications for understanding the mechanisms that account for the persistence of unisexual *Ambystoma* over long-term evolutionary timescales. First, they provide evidence for a mechanism (genome exchange) hypothesized to allow unisexuals to escape the evolutionary costs of mutation accumulation in asexual lineages (Bogart *et al.* 2007). However, because our analyses used neutral markers as proxies for genome-wide patterns involving genes coding for adaptive variation, we have yet to demonstrate whether exchange actually leads to a reduction in mutational load. Our results point the way to a test of this hypothesis by exploiting the observation that in unisexuals with mixed genomes in specific locations, 'sympatric' genomes in unisexuals (those present in coexisting sexual males, hence undergoing frequent exchange, hence mutational purging) are hypothesized to undergo higher rates of gene exchange than 'allopatric' unisexual genomes (those for which there no sexual males present and hence no opportunity for mutational purging). If mutational purging is effective via frequent gene exchange, then the number of nonsynonymous mutations in the exons of coding genes should be less in homologous loci in sympatric vs. allopatric loci in unisexuals. No difference in the relative number of mutations would argue that gene exchange has not been effective for reducing mutational load and hence cannot account for the persistence of unisexual lineages. This could be tested by using gene capture array methods (Bi *et al.* 2012) to assay the relative mutation rates of homologous genes on sympatric vs. allopatric genomes in unisexuals that are scaled to the relative effective population sizes of these different genome components.

A second important implication of our work is that it provides an estimate for a key parameter in a recent model exploring the role of selection in the long-term persistence of unisexuals in the face of ongoing genome exchange. Specifically, Charney (2012) made the important observation that unidirectional gene flow between sexual males and unisexual females would eventually result in the complete replacement of all allopatric genomes in unisexuals over evolutionary time. This process would lead to extinction unless there was compensating selection favouring unisexuals over sexual individuals. Charney (2012) used a simulation model to explore the dynamics of this process over specific timescales and identified the per-generation rate of gene exchange between sexuals and unisexuals as a key parameter. If genome exchange per reproductive bout occurred at a rate >1/10 000, he found that there must be compensating positive selection in order to maintain genetically distinct unisexual individuals. His model was not parameterized with population genetic parameters, but if we assume a rough correspondence between this parameter (0.0001%) and our estimate of m (0.2%) per generation, then it is clear that our estimate of the rate

of gene exchange per generation is orders of magnitude higher than the threshold value estimated by Charney (2012). If our estimate is accurate, we argue that unisexuals must be at a significant selective advantage relative to coexisting sexuals. However, the overall advantage may be less due to the impact of cytological mechanisms alone as potential determinants of genome reduction (Bogart & Bi 2013). The high level of gene exchange reported here is consistent with the observation that unisexuals can be ecologically successful, often constituting 70% of the individuals in some *Ambystoma* communities (Bogart & Klemens 2008). Despite their success, the identification of specific ecological advantages possessed by unisexuals has been largely unsuccessful, as unisexuals have lower fecundity (Bogart & Licht 1986), are discriminated against by males of sexual species (Dawley & Dawley 1986), have no competitive advantage during the larval stage (Brodman & Krouse 2007) and have reduced dispersal ability (R.D. Denton, K. Greenwald, H.L. Gibbs, in prepartion). In contrast, Saccucci *et al*. (2016) have shown that unisexuals have a physiological advantage in terms of increased rates of tissue regeneration. The relationship between the rate of local gene exchange and the phenotypic expression of these genes is an important area of future research.

This work demonstrates the feasibility of a genetic mechanism operating through gene exchange that would allow unisexual *Ambystoma* to gain the advantages of periodic sexual reproduction. Future work should focus on assessing whether gene exchange is common across the range of all unisexuals in which populations differ in the presence of different combinations of sexual hosts and unisexual biotypes, if the putative advantage of a reduction in the accumulation of harmful mutations is in fact realized, and the relative importance and molecular bases of the two mechanisms – ploidy elevation and reduction – that underlie genome exchange.

Acknowledgements

We thank Tim Matson, Roberta Muehlheim, Greg Lipps, Katherine Greenwald and Matt Holding for providing samples and assistance, Bryan Carstens for advice about analyses and Jose Diaz and Mónica Saccucci for help with laboratory work. We acknowledge the contributions of Katy Greenwald at the early stages of this project and for making the data used for Fig. S1 (Supporting information) available. We dedicate this paper to Jim Bogart in recognition of his pioneering work on unisexual *Ambystoma* that has both inspired and made possible work on the evolutionary biology of these salamanders such as that described in this article. Analyses were performed on the Ohio Biodiversity Conservation Partnership Computing Cluster. This work was supported by the State Wildlife Grants Program, administered jointly by the U.S. Fish and Wildlife Service and the Ohio Division of Wildlife, with funds provided by the Ohio Biodiversity Conservation Partnership between Ohio State University and the Ohio Division of Wildlife and by funds from Ohio State University.

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H.L.G. conceived this study and wrote the manuscript. R.D.D. coordinated sample collection, carried out data collection and analysis and assisted with preparation of the manuscript.

Data accessibility

All genetic data and input, parameter and model comparison files for MIGRATE-N analyses are deposited on Dryad at doi:10.5061/dryad.kt314.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Isolation by distance relationship between *A. laterale* populations based on data from Greenwald *et al.* (2009).

Table S1 *Ambystoma jeffersonianum* and *A. laterale*-specific loci used in this study.