Niche partitioning among sexual and unisexual *Ambystoma* salamanders

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Abstract. Organisms that have ecologically similar sexual and asexual forms present an evolutionary puzzle, as theory predicts that eventually one form should eliminate the other. However, both forms may persist if there is niche partitioning between them. Geographical parthenogenesis is a hypothesis that predicts that in terms of niche use the asexual form in such pairs should be more ecologically successful in marginal habitats. This model has rarely been considered for gynogenetic taxa, as they are sperm dependent and thus constrained to the geographical range of sexual "sperm donor" species. Unisexual *Ambystoma* salamanders present a unique opportunity to look for evidence of niche partitioning and geographical parthenogenesis in a gynogenetic lineage, as there are multiple possible sperm donor species and so the geographical distributions of unisexuals are less constrained. We used broad sampling, comparative ecological niche models, and a model selection approach to determine (1) whether niche partitioning occurs among sexual and unisexual salamanders and (2) whether unisexual lineages indeed occupy marginal habitat relative to their most similar sexual species. Nearly all unisexual and sexual types showed significant niche differentiation. Predictions consistent with geographical parthenogenesis were upheld for one sexual–unisexual pair (*A. jeffersonianum* and LJJ unisexuals), but not for a second pair (*A. texanum* and LTT unisexuals). Our study provides evidence that different biotypes within the unisexual lineage have distinct ecological interactions with sexual taxa, supporting a role for these differences as a mechanism promoting coexistence between some sexual and unisexual forms. However, geographical parthenogenesis is only a partial explanation for sexual–unisexual coexistence, suggesting that other ecological and genetic mechanisms also play important roles in mediating coexistence among these salamanders.

Key words: environmental niche models; geographical parthenogenesis; gynogenesis; sexual–unisexual coexistence; unisexual *Ambystoma*.

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INTRODUCTION

The coexistence of sexual and asexual forms of closely related organisms presents an ecological and evolutionary puzzle, as one or the other should ultimately be eliminated due to competition between the forms or costs associated with their respective reproductive modes (Case and Taper 1986, Peck et al. 1999). As such, situations in which long-lived asexual lineages coexist with close sexual relatives are exceptional and provide an opportunity to identify key features that allow the long-term persistence of asexual forms (Vrijenhoek 1994). Niche partitioning between sexual and asexual forms is a long-standing ecological hypothesis for the coexistence of such

lineages (Schlupp 2005). Multiple hypotheses exist as to the mechanisms that underlie niche differentiation (e.g., temporal differentiation, Halkett et al. 2006). One hypothesis that may be most relevant to the unisexual–sexual salamanders that we study here is the Geographical Parthenogenesis Hypothesis (GPH), in which asexual forms coexist with sexual forms by occupying "marginal" habitats not utilized by sexual organisms (Beukeboom and Vrijenhoek 1998, Peck et al. 1998, Vrijenhoek and Parker 2009). This hypothesis stems from observations that parthenogenetic organisms tend to exploit "extreme" habitats that are presumably more environmentally stressful, such as those at higher latitudes, higher elevations, or regions that have undergone disturbance. This pattern was first described in arthropods (Vandel 1928) and since has been documented in a wide variety of taxa such as terrestrial and aquatic invertebrates (Glesener and Tilman 1978, Beaton and Hebert 1988), lizards (Wright and Lowe 1968), and plants (Bierzychudek 1985, Hörandl 2006). Numerous mechanisms have been suggested, including that parthenogens have superior colonization ability, that sexual taxa cope better with increased biotic interactions at the core of the range (Vrijenhoek and Parker 2009, and references therein), or that the pattern is driven by a metapopulation structure at range edges, which gives an advantage to the asexual taxa (Haag and Ebert 2004). The superiority of parthenogens in "fringe" habitat may be due in large part to heterosis rather than to the reproductive mode itself; however, these factors are difficult to disentangle, as most parthenogens are of hybrid origin (Kearney 2005).

Conceptually, whether niche differentiation can explain the coexistence of asexual forms that reproduce via sperm-dependent parthenogenesis (gynogenesis) is uncertain because gynogens cannot expand their geographical distribution beyond the range of their obligate sexual "sperm donor" species (Beukeboom and Vrijenhoek 1998). Despite this, ecological studies of unisexual gynogens and their associated sexual species have repeatedly found evidence for ecological differences between forms (see reviews in Vrijenhoek 1994, Beukeboom and Vrijenhoek 1998, Schlupp 2005, Lampert 2009, Vrijenhoek and Parker 2009) including some degree of spatial segregation suggestive of niche divergence among gynogenetic forms in flatworms (Weinzierl et al. 1999), annelids (Christensen 1980), arthropods (Løyning 2000), and fishes in the families Atherinidae (Echelle and Echelle 1997) and Poeciliidae (Vrijenhoek 1978, Vrijenhoek and Pfeiler 1997, Gray and Weeks 2001). Here, we capitalize on recent innovations in the characterization and analysis of niche space using environmental niche modeling (ENM) techniques (Peterson 2011), joint species distribution models (JSDMs), and model selection approaches in a hypothesis-testing framework based on the Akaike information criterion (Diniz-Filho et al. 2008, Rangel et al. 2010) to examine niche differences in a gynogenetic complex of salamanders. This multifaceted approach, which has only rarely been applied to gynogenetic animals (Costa and Schlupp 2010), allows for a robust representation of ecological niches, allowing us to test for niche differentiation on both regional and local spatial scales and to explore whether the specific environmental factors that underlie differences support the GPH as an explanation for niche differentiation between sexual and unisexual salamanders.

Unisexual (all-female) populations of *Ambystoma* salamanders present an opportunity to assess spatial distribution and habitat use in a lineage that reproduces using a flexible form of sperm-dependent gynogenesis. Unisexual salamanders are widely distributed across northeastern North America, and reproduce using a mode known as kleptogenesis (Bogart et al. 2007). These pond-breeding salamanders co-occur with several sexual (diploid, biparental) species, from which they "steal" gametes by picking up male spermatophores. Much of the time, the spermatophore triggers egg development, but the male genome is not incorporated into the zygote (gynogenesis); however, infrequently the male genome is added, typically resulting in ploidy elevation (Bogart et al. 2007, Gibbs and Denton 2016). As a result, unisexual ploidy levels range from 2N to 5N (Bogart and Klemens 1997, Bogart et al. 2007), and the nuclear DNA is comprised of genomes of any of five sexual species: the Blue-spotted Salamander (*Ambystoma laterale*), Jefferson Salamander (*A. jeffersonianum*), Smallmouth Salamander (*A. texanum*), Streamside Salamander (*A. barbouri*), and Tiger Salamander (*A. tigrinum*), denoted as L, J, T, B, and Ti, respectively (Lowcock et al. 1987, Bogart et al. 2009).

This flexibility in both ploidy level and genome complement results in a large number of possible nuclear biotypes (genome combinations) in the unisexuals, with the most common being triploid genome combinations (e.g., LJJ, LLJ, LTT; Bogart et al. 2007). Despite the complexity of the nuclear genome, all unisexuals form a distinct monophyletic lineage (~5 Ma) based on their mitochondrial DNA (Bi and Bogart 2010).

This system is unique in that there are multiple potential "sperm donor" species, making it possible for unisexual biotype lineages to potentially expand their ranges beyond the bounds of their current or historical host species (e.g., LJJ unisexuals may leave the range of *A. jeffersonianum* and move into the range of *A. texanum*, while LTT unisexuals might do the opposite). These salamanders therefore provide a unique opportunity to test predictions based on geographical parthenogenesis in gynogens when the gynogenetic lineage is not "locked" into the range of a particular sexual species due to sperm dependence. Here, we use distribution data from unisexual and sexual ambystomatid salamander populations in Ohio, United States, to test two predictions stemming from the GPH related to the distribution of unisexuals relative to their sexual congeners: (1) The ecological niche in which we find a unisexual biotype should differ from that of the "parental" (most similar) sexual species. Specifically, in our analyses, the niche of LJJ unisexuals should differ from that of *A. jeffersonianum* and the niche of LTT unisexuals should differ from that of *A. texanum* (Hypothesis 1). (2) More specifically, when comparing a unisexual–sexual pair as above, the unisexuals should be found in areas that are "marginal" in some way: higher latitude, higher elevation, and/or more impacted by anthropogenic disturbance (Hypothesis 2).

MATERIALS AND METHODS

Collection of samples and genetic identification

With the assistance of collaborators (see *Acknowledgments*), we used a combination of minnow traps and dip netting to capture over 1800 adult salamanders at nearly 100 sites across Ohio during the spring breeding seasons (February and March) of 2010 and 2011 (Data S1). We removed sites with <5 salamanders captured to avoid samples that were largely non-representative of the population composition. Thus, the current analysis includes 61 remaining sites (mean salamanders sampled in retained sites: 19.7; Fig. 1), which resulted in a total of 132 occurrence points among all groups (*A. texanum, A. jeffersonianum*, and unisexuals). For all captured salamanders, we removed a small (3- to 4-mm) tissue sample from the tail tip for genetic analysis (tails are subsequently regenerated). Tissue samples were stored in 95% EtOH until further processing. All salamanders were then released at the site of capture. All field equipment (traps, nets, waders) was disinfected with a dilute bleach solution between sites so as to avoid transferring parasites or pathogens between sites.

We extracted DNA from tissue samples using Qiagen DNeasy extraction kits (Qiagen, Valencia, California, USA) and then used a two-step process to identify population composition as described in Greenwald and Gibbs (2012). First, we sequenced a region of mitochondrial DNA (amplified using primers F-THR and R-651; Shaffer and McKnight 1996, Bogart et al. 2007) to differentiate between unisexuals and any sexual species that were present. Second, for samples identified as unisexuals, we used a panel of single nucleotide polymorphisms to determine unisexual biotype (for detailed methods, see Greenwald and Gibbs 2012).

Selection of environmental variables

We address two hypotheses (outlined above) related to (1) broad-scale environmental niche and (2) local-scale patch- and habitat-level metrics. We therefore analyzed two sets of environmental variables (Table 1). For the broad-scale analysis (Hypothesis 1), we followed other studies that use climatic variables to make evolutionary inferences (Carstens and Richards 2007, Rissler and Apodaca 2007) and analyzed 19 climate variables from the WorldClim BIOCLIM database (Hijmans et al. 2005). We used a correlation analysis to identify and reduce correlations among these variables using ArcGIS version 9.3 (Environmental Systems Research Institute, Redlands, California, USA) and SDMtoolbox (Brown 2014), a collection of Python script-based tools in ArcGIS. We extracted all BIOCLIM variables from an area encompassing the border of Ohio with an additional ~100-km buffer around
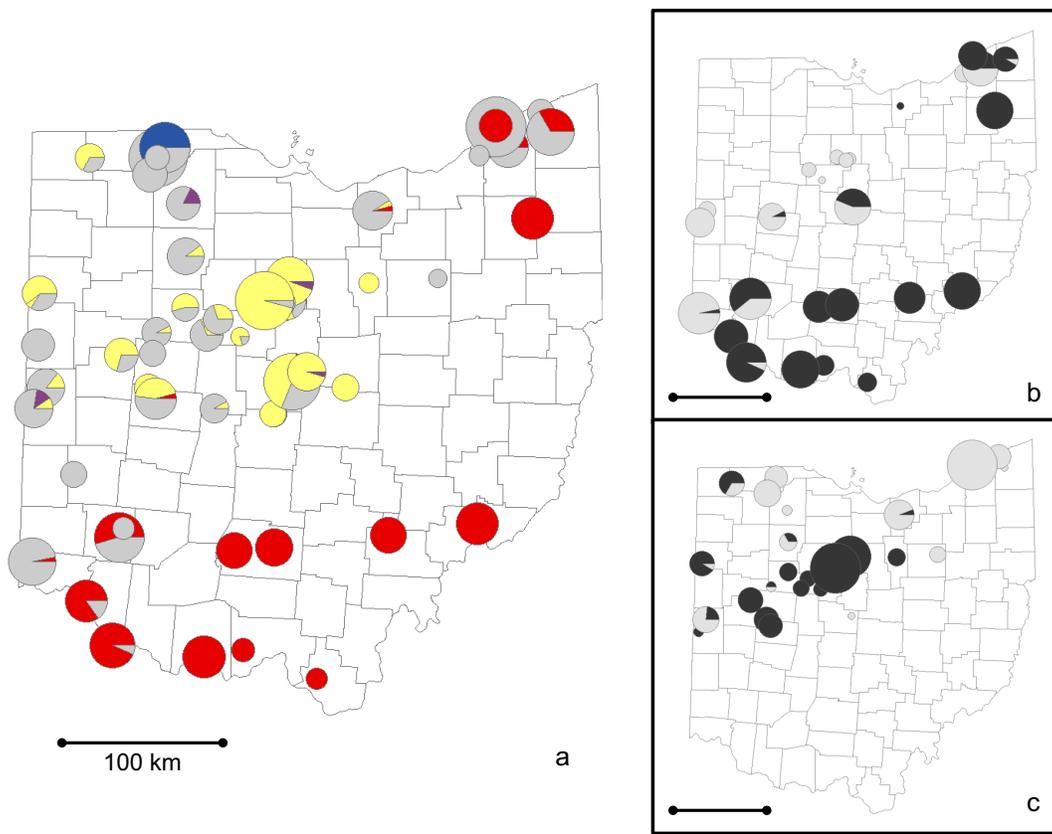
Fig. 1.   Sample collection sites in Ohio, United States. Pie chart symbols are proportional to sample size and indicate the proportion of each population consisting of sexual vs. unisexual salamanders. (a) All sites with unisexual salamanders (gray) or sexual species *Ambystoma jeffersonianum* (red), *A. texanum* (yellow), *A. laterale* (blue), *A. tigrinum* (purple). (b) Subset of sites with *A. jeffersonianum* (black) and LJJ unisexuals (gray). (c) Subset of sites with *A. texanum* (black) and LTT unisexuals (gray). The scale bar in all panels indicates 100 km.

the state line. We identified all pairwise correlations greater than 0.80 between all variables, then evaluated groups of variables that measured similar features (precipitation, temperature, and indirect measurements such as isothermality), and then removed variables that were highly correlated within these groups. This resulted in retaining 12 of 19 original variables: annual mean temperature (BIO1), mean diurnal range (BIO2), isothermality (BIO3), temperature seasonality (BIO4), temperature annual range (BIO7), mean temperature of wettest quarter (BIO8), mean temperature of the driest quarter (BIO9), mean temperature of the warmest quarter (BIO10), mean temperature of the coldest quarter (BIO11), annual precipitation (BIO12), precipitation seasonality (BIO15), and precipitation of coldest quarter (BIO19). We then extracted site estimates

for these variables for each unique salamander locality record.

For our local-scale analysis (Hypothesis 2), we required additional site-specific information on the environmental "marginality" of specific locations (Table 1). To generate this information, we used seven a priori predictor variables as described below: five that summarized local physical and climatic factors (ELEV, SIZE, SOIL, BIO1, and BIO12) and two specifically related to anthropogenic modification (ORAM and RES). The physical variables used to assess Hypothesis 2 include elevation (ELEV), pond size (SIZE; coded categorically during ORAM assessment as (1) <0.04 ha; (2) 0.04 to <0.12 ha; (3) 0.12 to <1.2 ha; or (4) 1.2 to <4 ha), and soil type (SOIL; coded categorically based on SSURGO-certified data layers from

Table 1. Variables used in this study, along with associated information (name, definition, units, and source), and which hypothesis they were used to test (HYP column).

Name	Definition	Units	HYP	Source
BIO1	Annual mean temperature	°C × 10	1, 2(+)	1
BIO2	Mean diurnal range	Mean of monthly (max temp − min temp)	1	1
BIO3	Isothermality	(BIO2/BIO7) (×100)	1	1
BIO4	Temperature seasonality	SD × 100	1	1
BIO7	Temperature annual range	°C × 10	1	1
BIO8	Mean temperature of wettest quarter	°C × 10	1	1
BIO9	Mean temperature of driest quarter	°C × 10	1	1
BIO10	Mean temperature of warmest quarter	°C × 10	1	1
BIO11	Mean temperature of coldest quarter	°C × 10	1	1
BIO12	Annual precipitation	mm	1, 2(+)	1
BIO15	Precipitation seasonality	Coefficient of variation	1	1
BIO19	Precipitation of coldest quarter	mm	1	1
ELEV	Elevation	m above sea level	2(−)	1
SIZE	Pond size	Categorical: (1) <0.04 ha; (2) 0.04 to <0.12 ha; (3) 0.12 to <1.2 ha; or (4) 1.2 to <4 ha	2(+)	2
SOIL	Soil type	Categorical: sand, sandy loam, loam, silt loam, silty clay loam, silty clay, clay, or impervious	2(−)	3
ORAM	Site quality	Total score (see text for details)	2(+)	2
RES	Landscape resistance	Scaled proportions within 1-km buffer around site (see text for details)	2(−)	4

Notes: For those variables used to test Hypothesis 2, we indicate the predicted relationship between each variable and the proportion of the population consisting of sexual individuals (i.e., "+" indicates higher proportion of sexual individuals, and "−" indicates higher proportion of unisexuals). For justification for these predictions, see Appendix S1. Sources are 1: WorldClim BIOCLIM database; 2: Ohio Rapid Assessment Method; 3: USDA SSURGO Database; 4: National Land Cover Database.

the United States Department of Agriculture (USDA) Natural Resources Conservation Services as sand, sandy loam, loam, silt loam, silty clay loam, silty clay, clay, or impervious). To summarize local climate, we included two summary BIOCLIM variables that were also used in the ENM analysis (BIO1: annual mean temperature; and BIO12: annual precipitation).

To describe anthropogenic modification at the level of the individual pond (ORAM), we collected habitat quality data at 58 of the 61 sites to assess local level of disturbance, following the methodology of the Ohio Rapid Assessment Method for Wetlands developed by the Ohio Environmental Protection Agency (Mack 2001). This method integrates the following wetland characteristics to rank each site based on its degree of disturbance: wetland area; upland buffers and surrounding land use; hydrology (sources and intactness); habitat alteration and development; special wetlands (pre-identified high-quality and rare wetland systems receive extra points and extremely low-quality sites lose

points); and plant communities, interspersion, and microtopography (Micacchion 2004). There are 100 possible points based on these metrics; wetlands scoring over 65 points are considered priority high-quality sites for conservation by Ohio EPA. This metric has been shown to correlate well with an index of biotic integrity based on amphibian species presence and sensitivity (Micacchion et al. 2015, Stapanian et al. 2015), and so we interpret it as a proxy for breeding site integrity.

To describe upland habitat quality surrounding each breeding site (RES), we used the National Land Cover Database to assess landscape resistance within a 1000-m buffer around each breeding site. This scale was chosen in order to encompass and document presence of critical upland habitat (Herrmann et al. 2005). For each site, we determined the proportional amount of each landcover category (e.g., deciduous forest, row crops) within the buffer area. These categories were multiplied by resistance metrics empirically derived for a related species, *Ambystoma*

opacum (Greenwald et al. 2009), and then summed (Mack 2006). For example, for a site buffer composed of 60% forest and 40% agriculture, with corresponding resistance values of 1 and 10.2, respectively, then RES = (0.6 × 1) + (0.4 × 10.2) = 4.68. Thus, a larger RES value implies a higher proportion of high-resistance landcover types in the surrounding area, while a lower value indicates more intact natural habitat (i.e., forest cover).

Based on previous work on both geographical parthenogenesis and the specific organisms in question, we interpret the following as constituting marginal habitat: *lower* temperature, *less* precipitation, *higher* elevation, *smaller* pond size, *sandier* soil, and *more* anthropogenic modification. Our justification for these classifications is based on the responses of salamanders and more generally amphibians to specific environmental conditions and is described in detail in Appendix S1.

Data analysis (Hypothesis 1)

We used Maxent version 3.2.19 (Phillips et al. 2004, Phillips and Dudík 2008), a maximum entropy algorithm to estimate the biotic and/or abiotic requirements of a particular organism, to construct environmental niche models (ENMs) for each sexual species and unisexual biotype. We used SDMtoolbox to determine background sampling, spatially rarify occurrence data, jackknife SDMs, and calibrate model parameters as follows. First, we spatially rarefied the occurrence points to avoid the overfitting of models when spatial clusters of localities exist (Boria et al. 2014). The environmental layers were used to calculate climate heterogeneity over the study extent, and this layer was combined with buffers around each occurrence record. Sites that were within 10 km of one another in the same heterogeneity class were removed (Brown 2014). Second, to select background points that avoided overprediction (Merow et al. 2013), we sampled by buffered local adaptive convex-hull around occurrence points, which is a conservative intermediate between sampling from a set distance from observations (Thuiller et al. 2009) and sampling by a buffered minimum convex polygon (Brown 2014). Lastly, we used the *Spatially Jackknife* tool in SDMtoolbox to conduct spatial jackknifing (Shcheglovitova and Anderson 2013),

test for most appropriate combination of feature class type and regularization multiplier(s) (Radosavljevic and Anderson 2014), and select the best model using area under the receiver-operating characteristic curve (AUC). The final step of this script then executes the best-choice model using all the occurrence points, which becomes the input for measuring the niche overlap between the groups. We also assessed the permutation importance of each climate variable, which quantifies the amount that model fit (training AUC) decreases without the contribution of that variable.

We used the "niche overlap" function in ENMTools (Warren et al. 2008, Warren and Seifert 2011) to calculate Schoener's D (Schoener 1968) and the *I* statistic (Warren et al. 2008) to describe similarity among all pairs of species and biotypes. Because the groups range from non-overlapping to partially overlapping, we then used the "background tests" function to test for significant niche divergence. We ran 100 replicates for each pairwise comparison and conducted one-sample *t* tests to compare measured niche overlap with the distribution of overlaps from pseudoreplicates drawn from the background files described above.

The ENM analyses described above estimate niche overlap between species based on environmental predictors alone (Elith and Leathwick 2009). Because unisexual *Ambystoma* directly depend on sexual males for reproduction, the environmental variation associated with presence/absence data may not capture important species interactions that could influence distributions. To account for this, we used the same occurrence points to construct joint species distribution models (JSDMs), a recent advance in ecological niche modeling that models the distributions of multiple species simultaneously and produces the shared environmental correlations ($P_{jj'}$) and residual correlations ($P_{jj'}$) between species (Clark et al. 2014, Pollock et al. 2014). The shared environmental correlations between species are similar to those of other ENM methods (Elith and Leathwick 2009), but other ENM methodologies assume that species occurrence probabilities are uncorrelated (Calabrese et al. 2014). The estimation of residual correlation between species can be interpreted as either unmeasured environmental variation or interactions between

species that can result in positive correlations in presence (e.g., commensalism) or negative correlations in presence (e.g., competition, Pollock et al. 2014).

Because well-documented climate variables have previously been shown to correctly predict the habitat suitability of closely related *Ambystoma* species (Micheletti and Storfer 2015), the residual correlations produced by JSDMs can be reasonably interpreted as explaining biotic interactions between groups (Clark et al. 2014). While antagonistic species interactions, such as competition, could be the major mechanism for the intermediate range of unisexual *Ambystoma*, unisexuals may also be limited by a stronger reliance on one or more of the sperm donor species (Landmann et al. 1999, Kim et al. 2014). We identified and compared the strength of the residual correlations between unisexuals of each biotype and each sexual species using the methodology described in the supplementary information from Pollock et al. (2014) implemented in R (Plummer 2013, R Core Team 2013) using the same occurrence and environmental data described above. Each run consisted of three chains of 1,000,000 iterations (15,000 iterations of burn-in) thinned by a factor of 1000. Convergence was determined through inspecting diagnostic graphs of the Gelman-Rubin statistic (Gelman and Rubin 1992) as described in Pollock et al. (2014); both Rho and EnvRho produced mean values close to 1 (mean Gelman-Rubin statistic for Rho = 1.20 and EnvRho = 1.03).

Data analysis (Hypothesis 2)

To assess whether unisexuals are found in areas that are marginal, we used a spatially explicit model selection approach based on the Akaike Information Criterion (AIC) implemented in the Spatial Analysis in Macroecology (SAM) software package (Diniz-Filho et al. 2008, Rangel et al. 2010). We compared the two most common sexual species with their associated unisexuals: *A. jeffersonianum* vs. the LJ/LJJ/LJJJ unisexual group (hereafter "LJJ"), and *A. texanum* vs. the LT/LTT/LTTT unisexual group (hereafter "LTT"). For these species/biotype comparisons, we included only populations with one or both of the focal taxa; for example, when comparing *A. jeffersonianum* and LJJ unisexuals, we removed from the data set populations that had neither of

these groups (i.e., ponds with only *A. texanum*, LTT, or LTJ). This resulted in a subset of 32 sites for each of the species/biotype comparisons; there were a small number of sites with multiple species/biotypes, so there is some overlap among these sites. For this analysis, the response variable was proportion of each sampled population consisting of sexual individuals (0 = entirely unisexual, e.g., LTT, but no *A. texanum*; 1 = entirely sexual, e.g., *A. texanum*, but no LTT).

The SAM model selection routine uses the Akaike Information Criterion metric to establish minimum adequate models explaining variation in the response variable. We included an autoregressive term as a fixed predictor variable present in all models to account for the effects of spatial autocorrelation. This term is calculated by SAM as ϱWy, where ϱ is the autoregressive coefficient estimate, W is the connectivity matrix, and y is species richness (Diniz-Filho et al. 2008). Using this approach, habitat and environmental data can be analyzed in a spatial context to determine which predictors best describe the relative abundance of each sexual species and unisexual biotype.

Results

Geographical distribution of Ambystoma

The proportion of unisexuals across all 61 populations ranged from 0 to 1 (mean ± SD = 0.42 ± 0.40), with a strong geographical gradient from low/no unisexual presence in the southeast of the state to high proportions of unisexuals in the northwestern populations (Fig. 1a). Qualitative comparison of frequencies of sexual species and their most closely allied unisexual biotype also revealed some geographical separation. *A. jeffersonianum* was most common in the southeast and northeast, while LJJ dominated populations in the central and western part of the state (Fig. 1b). *A. texanum* constituted the majority of numerous populations in central Ohio, while the LTT biotype extended across the northern edge of the state (Fig. 1c). The LTJ biotype was relatively rare and widely scattered throughout the northern half of the state.

Hypothesis 1: The niche of LJJ unisexuals differs from that of A. jeffersonianum *and the niche of LTT unisexuals differs from that of* A. texanum.

Table 2. Best model parameters and importance of climate variables to ecological niche models for both sexual species (JEFF: *Ambystoma jeffersonianum* and TEX: *A. texanum*), all unisexual salamanders as a group (UNIS), and each representative biotype (LJJ, LTT, LTJ) in Ohio, United States.

Parameters	JEFF	TEX	UNIS	LJJ	LTJ	LTT
Regularization multiplier	0.5	0.5	0.5	0.5	1	0.5
Feature class number†	5	3	4	5	3	5
AUC	0.667	0.529	0.818	0.769	0.529	0.738
BIO1	0.0	0.0	0.00	0.0	0.0	0.0
BIO2	16.8	0.3	0.36	13.4	0.0	1.4
BIO3	20.3	13.6	**29.98**	0.0	0.0	0.0
BIO4	6.3	13.8	0.00	12.0	0.0	2.3
BIO7	0.0	**24.0**	23.95	18.4	0.0	3.3
BIO8	15.5	6.4	22.69	2.8	0.0	8.0
BIO9	0.0	0.0	7.50	13.2	0.0	29.3
BIO10	**32.6**	0.0	7.46	0.0	0.0	0.0
BIO11	0.3	10.1	4.41	0.0	**100.0**	12.4
BIO12	8.3	0.0	0.29	**27.3**	0.0	0.0
BIO15	0.0	15.1	3.35	5.0	0.0	**37.0**
BIO19	0.0	16.7	0.00	7.9	0.0	6.3

Notes: Permutation importance values represent the decrease to the fit of the model if a given variable is removed; larger values therefore indicate that a variable contributes heavily to the niche model. The largest contributor to each niche model is highlighted in boldface. See Table 1 for definitions of variables.

† Number represents the following feature groups: 3 = hinge; 4 = linear, quadratic; and hinge, 5 = linear, quadratic, hinge, product, and threshold.

After spatial rarefaction of the occurrence points, the total number of observations for all groups decreased from 132 to 109 and the total number of represented sites decreased from 61 to 56. Environmental niche models described species and biotype distributions conservatively (AUC range: 0.529–0.769 for all species/biotypes). The most important variables that contributed to the niche models were related to precipitation and temperature, but the specific variables differed between groups. The most important parameter for most groups involved temperature. The best model for unisexuals as a whole included isothermality as the most important variable, which was a composite variable composed of joint variation in temperature annual range and mean diurnal range. Temperature parameters were also most important for models describing *A. jeffersonianum* (temperature of warmest quarter), *A. texanum* (annual temperature range), and LTJ unisexuals (mean temperature of coldest quarter). Different variations in precipitation were most important for LJJ biotypes (annual precipitation) and LTT biotypes (precipitation seasonality; Table 2; Appendix S2).

The niche models of the sexual species *A. jeffersonianum* and *A. texanum* were significantly different ($I = 0.045$, $t = 118$, $P < 0.001$; Table 3). Additionally, *A. jeffersonianum* differed significantly from all unisexual biotypes (LJJ: $I = 0.736$, $t = 67$, $P < 0.001$; LTT: $I = 0.145$, $t = 161$, $P < 0.001$; LTJ: $I = 0.189$, $t = 164$, $P < 0.001$). *Ambystoma texanum* also had significant niche differentiation from all unisexual biotypes (LJJ: $I = 0.193$, $t = 156$, $P < 0.001$, LTT: $I = 0.790$, $t = 25$, $P < 0.001$, LTJ: $I = 0.755$, $t = 41$, $P < 0.001$). For the Schoener's D statistic only, *A. texanum* showed significantly greater niche overlap with LTT unisexuals than would be expected at random. When combined with the relatively high, but statistically insignificant, overlap between *A. texanum* and LTT unisexuals using the *I* statistic, these two groups showed the most similar niches as measured by the background tests. Most importantly, individual unisexual biotypes all had significant niche differentiation from unisexuals as a whole, indicating that nuclear genome composition is associated with niche in specific biotypes (Table 3).

Environmental correlations between groups produced by the JSDMs differed from the patterns of niche overlap from ENMTools. While *A. jeffersonianum* displayed negative environmental correlations with all other groups except LJJ biotypes (Fig. 2), all other species pairs except

Table 3. Niche differentiation among sexual species (AJEFF: *Ambystoma jeffersonianum*; ATEX: *A. texanum*), unisexuals combined (UNI), and unisexual biotypes (LJJ, LTT, LTJ) in Ohio, United States.

	AJEFF	ATEX	UNI	LJJ	LTJ	LTT
AJEFF	–	**0.045**	**0.609**	**0.736**	**0.189**	**0.146**
ATEX	**0.025**	–	**0.525**	**0.193**	**0.755**	**0.790**
UNI	**0.547**	**0.277**	–	**0.816**	**0.689**	**0.626**
LJJ	**0.649**	**0.122**	**0.670**	–	**0.347**	**0.292**
LTJ	**0.141**	**0.568**	**0.476**	**0.291**	–	**0.904**
LTT	**0.101**	**0.657**	**0.394**	**0.225**	**0.808**	–

Notes: We used ENMTools to calculate two measures of niche overlap: I (above diagonal) and Schoener's D (below diagonal). Bolded values indicate significant niche differentiation according to background tests.

LJJ and LTJ had positive environmental correlations with SE that did not overlap zero, suggesting similar environmental niches. The residual correlations showed only strong positive associations between unisexual biotypes and sexual species, representing co-occurrence beyond that predicted by environment alone. While LTJ biotypes had positive residual correlations with both sexual species and all other biotypes, LTT and LJJ biotypes showed positive correlations with *A. texanum* only. In contrast, *A. jeffersonianum* was not positively correlated with LTT biotypes or the unisexual biotype with the greatest number of *A. jeffersonianum* genomes (LJJ).

Hypothesis 2: When comparing a unisexual–sexual pair, the unisexuals are found in areas that are marginal, namely those at higher latitude (i.e., lower temperature and lower precipitation), higher elevation, or anthropogenically disturbed.

Ambystoma jeffersonianum vs. LJJ unisexuals.— The proportion of *A. jeffersonianum* across populations ranged from 0 to 1 (mean ± SD = 0.510 ± 0.477). There were only two supported
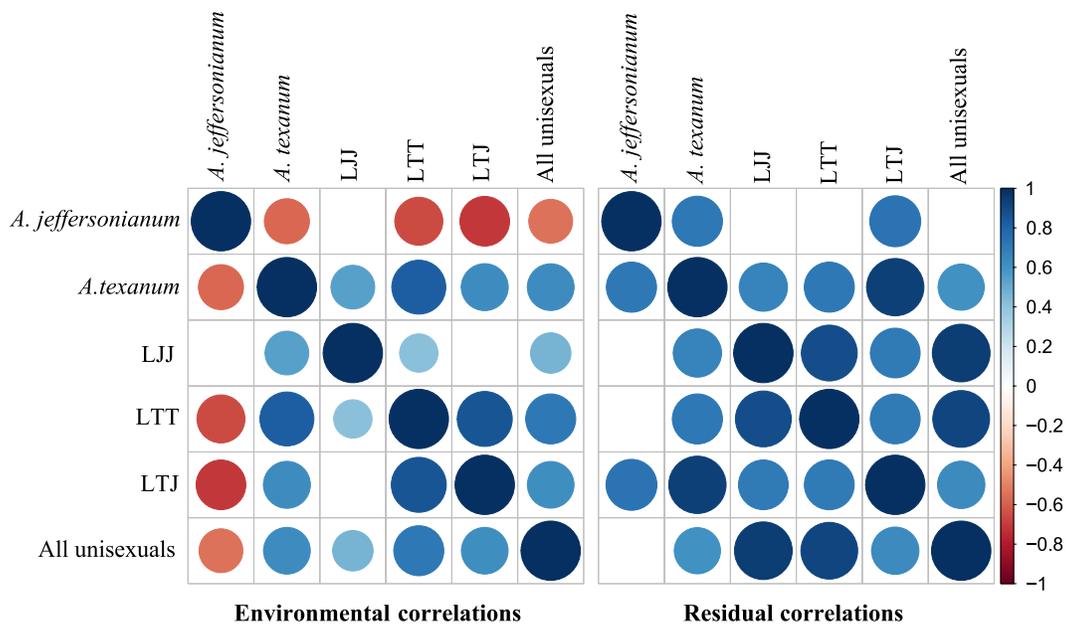

Fig. 2. Matrices of environmental and residual correlations produced by a joint species distribution modeling procedure (JSDM; Pollock et al. 2014) for both sexual species (*Ambystoma jeffersonianum* and *A. texanum*), combined unisexuals, and individual unisexual biotypes (LJJ, LTJ, LTT). The size and color of each circle indicates the magnitude and direction of each mean correlation value, and only those values with SD that do not overlap zero are displayed.

Table 4. Model selection (Akaike information criterion) analysis for comparisons of sexual–unisexual pairs (a) *Ambystoma jeffersonianum* vs. LJJ and (b) *A. texanum* vs. LTT.

Model variables	ΔAIC_c	w_i	$r2$
(a) *A. jeffersonianum* vs. LJJ			
SIZE, RES, ELEV, BIO1, SOIL	0	0.390	0.868
RES, ELEV, BIO1, SOIL	1.72	0.165	0.844
SIZE, ORAM, RES, ELEV, BIO1, SOIL	2.67	0.102	0.873
SIZE, RES, ELEV, BIO1, BIO12, SOIL	3.85	0.057	0.868
RES, BIO1, SOIL	4.01	0.053	0.814
ORAM, RES, ELEV, BIO1, SOIL	4.88	0.034	0.846
SIZE, RES, ELEV, BIO12, SOIL	5.17	0.029	0.844
RES, ELEV, BIO1, BIO12, SOIL	5.29	0.028	0.844
SIZE, RES, BIO1, SOIL	5.54	0.024	0.824
RES, ELEV, SOIL	5.98	0.020	0.802
SIZE, RES, ELEV, SOIL	6.14	0.018	0.821
RES, ELEV, BIO12, SOIL	6.62	0.014	0.818
SIZE, ORAM, RES, ELEV, BIO1, BIO12, SOIL	6.96	0.012	0.873
RES, BIO1, BIO12, SOIL	7.09	0.011	0.815
ORAM, RES, BIO1, SOIL	7.21	0.011	0.814
(b) *A. texanum* vs. LTT			
RES, ELEV, SOIL	0	0.137	0.767
RES, SOIL	0.46	0.109	0.740
RES, ELEV	1.04	0.082	0.735
ORAM, RES, ELEV, SOIL	1.71	0.058	0.778
ORAM, RES, ELEV	1.86	0.054	0.753
RES, ELEV, BIO12, SOIL	2.51	0.039	0.772
RES, BIO12	3.08	0.029	0.717
RES, ELEV, BIO1, SOIL	3.08	0.029	0.768
ORAM, RES, SOIL	3.23	0.027	0.742
RES, BIO1, SOIL	3.27	0.027	0.742
RES, BIO12, SOIL	3.29	0.027	0.741
SIZE, RES, ELEV, SOIL	3.30	0.026	0.767
RES	3.44	0.025	0.688
SIZE, RES, ELEV	3.45	0.025	0.740
SIZE, RES, SOIL	3.50	0.024	0.740
RES, ELEV, BIO1	3.59	0.023	0.739
RES, ELEV, BIO12	3.84	0.020	0.737
ORAM, RES, ELEV, BIO12, SOIL	4.09	0.018	0.786
ORAM, RES, ELEV, BIO1	4.78	0.013	0.756
ORAM, RES, ELEV, BIO12	5.12	0.011	0.753
SIZE, ORAM, RES, ELEV	5.12	0.011	0.753
SIZE, ORAM, RES, ELEV, SOIL	5.13	0.011	0.779
ORAM, RES, ELEV, BIO1, SOIL	5.13	0.011	0.779

Notes: See Table 1 for definitions of variables. All models also contained a fixed variable (ARM) accounting for spatial autocorrelation (Diniz-Filho et al. 2008). Models with $\Delta AIC_c < 2$ contain the subset of variables that best explain differences in habitat between each sexual–unisexual pair. We show only models with AIC weight > 0.01. Overall importance and direction of each variable are shown in Table 5.

models ($\Delta AIC_c < 2$; Burnham and Anderson, 2002) that described the relative frequency of *A. jeffersonianum* vs. LJJ unisexuals, and one was a subset of the other (Table 4a). Both contain the variables RES, ELEV, BIO1, and SOIL; the top-ranked model has the additional variable SIZE (all models also included the fixed autoregressive term accounting for spatial autocorrelation; this is not shown in the tables for simplicity). Together, the two best models have a weight of 0.555, and each describes over 84% of the variation in the dependent variable. The most important variable overall was RES (importance, or the sum of model weights for models including this variable, = 1; Table 5, Fig. 3). Sites with low surrounding landscape resistance tended to have higher frequencies of *A. jeffersonianum*, while high-resistance sites had more unisexuals. SOIL, BIO1, and ELEV also had importance values at or near 0.9 (Table 5). High frequency of *A. jeffersonianum* relative to LJJ unisexuals was observed in ponds with sandier soil, higher average annual temperature, and lower elevation. Pond size was moderately important ($I = 0.651$), with larger ponds having higher frequencies of *A. jeffersonianum*. We interpret these results as supporting the predictions of the GPH, as unisexuals were more prevalent at sites with lower habitat quality, cooler temperatures, and higher elevation.

Ambystoma texanum vs. LTT unisexuals.—The proportion of *A. texanum* across populations ranged from 0 to 1 (mean ± SD = 0.611 ± 0.457). There were five good models describing the relative frequency of *A. texanum* vs. LTT unisexuals (Table 4b), all of which contained the variable RES. Four of five contained ELEV, three of five contained SOIL, and two of five contained ORAM. The five good models had an Akaike weight of 0.440, and each described ~75% of the variation in the dependent variable. RES was again the most important variable (importance = 0.981), but in this case the direction of the relationship was reversed (Table 5, Fig. 3). Sites with low surrounding landscape resistance tended to have more unisexuals, while high-resistance sites had higher frequencies of the sexual species *A. texanum*. ELEV and SOIL were also moderately important variables (importance = 0.637 and

Table 5. Importance (IMP: sum of AIC model weights for all models including each variable) and coefficient (COEF: direction of relationship) for habitat comparisons between each sexual–unisexual pair.

| Variables | *A. jeffersonianum* vs. LJJ | | *A. texanum* vs. LTT | |
	IMP	COEF	IMP	COEF
RES	1.000	NEG	0.981	POS
SOIL	0.998	NEG	0.629	POS
BIO1	0.904	POS	0.184	NEG
ELEV	0.890	NEG	0.637	POS
SIZE	0.651	POS	0.175	NEG
ORAM	0.186	POS	0.279	NEG
BIO12	0.171	POS	0.245	POS

Notes: See Table 1 for definitions of variables. The dependent variable is the proportion of the population consisting of the sexual species (*Ambystoma jeffersonianum* or *A. texanum*). Therefore, we find a higher proportion of *A. jeffersonianum* (lower proportion of LJJ unisexuals) in sites that have lower landscape resistance, more sandy soil, warmer average annual temperature, lower elevation, larger pond size, higher ORAM (habitat quality) score, and higher average annual precipitation. For the *A. texanum*–LTT comparison, the direction of all relationships is reversed with the exception of precipitation.

0.629, respectively). High frequency of *A. texanum* relative to LTT unisexuals was observed in ponds at higher elevations and with more clay-like soil. All variables except for BIO12 were opposite in their directional effect for the *A. texanum*–LTT comparison vs. the *A. jeffersonianum*–LJJ comparison (Table 5). As above, all models included the fixed autoregressive term (ARM) to account for spatial autocorrelation (Diniz-Filho et al. 2008). These results are opposite the predictions of the GPH, as unisexuals were more prevalent at sites with higher habitat quality and lower elevation.

Discussion

There is evidence for ecological differentiation between sexual and unisexual *Ambystoma* salamanders, and furthermore, niches of specific unisexual biotypes differ from each other. The ENM analysis shows that all unisexual biotype niches differ significantly from both sexual species; however, the magnitude of that differentiation varies. Unisexuals are more similar in terms of niche to the sexual species with which they share genomes (LJJ to *A. jeffersonianum*, LTT to *A. texanum*) than they are to the other sexual species or to each other. However, these sexual–unisexual

pairs still show significant niche differentiation, supporting our initial prediction (Hypothesis 1).

The JSDM analysis yielded two important conclusions: First, within the geographical range that we sampled, unisexuals as a group are more strongly associated with *A. texanum* than they are with *A. jeffersonianum*. In fact, *A. jeffersonianum* has *negative* environmental correlations with all species and biotypes except LJJ, and lacks positive residual correlations with any biotype except for LTJ. In contrast, *A. texanum* shows positive environmental and residual correlations with all unisexual biotypes. A gap in sampling in eastern Ohio (within the putative range of *A. jeffersonianum*) could potentially explain the weaker relationships between *A. jeffersonianum* and unisexuals, especially if this gap included sites where both groups were present. However, few records include unisexual *Ambystoma* in unglaciated areas of the Midwest United States
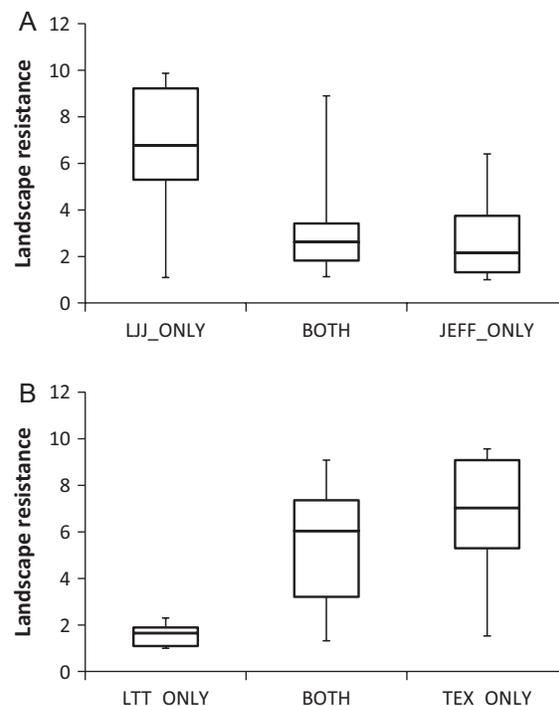
Fig. 3. Landscape resistance (RES metric) at (A) sites with only LJJ unisexuals, only *Ambystoma jeffersonianum*, or both; and (B) sites with only LTT unisexuals, only *A. texanum*, or both. Higher-resistance sites have more agriculture or developed land, while lower-resistance sites have more intact forest.

(Bogart et al. 2007). Paradoxically, the environmental correlations produced through the JSDM procedure are overwhelmingly positive for all groups except *A. jeffersonianum*, which contradict the result of niche dissimilarity detected using the ENM methods. This seemingly contradictory result can be explained by the fact that the niche overlap procedure measures similarity in the predictions of habitat suitability as specified by the ENM models, which predict suitability across areas that were not sampled. In contrast, the JSDM procedure measures the correlation between raw environmental variables at each site. It follows that when environmental conditions at sites where two groups coexist are similar, the environmental correlations should be positive even if the areas of predicted suitability produced by ENMs are not. Second, with the exception of *A. jeffersonianum* noted above, the JSDM analysis revealed nearly universal positive residual correlations among the unisexual lineages and sexual species (Fig. 2). This result indicates that the distributions of these groups are likely greatly influenced by their biological interactions, not just the climate. That is, despite the differentiation uncovered by the niche overlap analysis, these salamander types are found together more frequently than one would predict based on environment alone. While this result could also be indicative of unmeasured environmental variation (Clark et al. 2014), we consider this unlikely due to the scope of environmental variation used across this study (from site-specific to climate variables) and the known relationship between unisexual and sexual salamanders.

The LTJ biotype is an interesting case, as we had no a priori prediction about which sexual species it would be more strongly associated with in terms of niche. However, both ENM and JSDM analyses showed it to be much more similar to *A. texanum* than *A. jeffersonianum*. Furthermore, LTJ and LTT were the only pair that did not differ in either measurement of the niche overlap analysis (Table 3). Our sample size for LTJ sites was relatively small ($n = 19$), but our data suggest that this biotype may be functionally equivalent to LTT. This pattern might suggest a difference in the influence of particular species' genomes (i.e., a T genome has a powerful effect in driving distribution and use of ecological space), a different likelihood of genome exchange between sexual species, or perhaps temporal effects (e.g., LTJ populations are very recently derived from the LTT lineage).

Because all unisexual biotypes contain at least one *A. laterale* genome, the *A. laterale* genome may be the most likely candidate for generating a genome-specific impact on habitat use (Bi et al. 2008). This species has the most northern range of any ambystomatid, so the presence of this genome may also contribute to unisexuals being found at higher-latitude sites, which in our data set tended to have lower temperature and lower precipitation. *A. laterale* also have a stronger association with dry, sandy soils than other ambystomatids (Minton 1954, Vogt 1981). Because of the unisexuals' ubiquitous L genome, this leads us to predict that they will be more strongly associated with sandy soils in comparison with their sexual counterparts. However, the fact that there is mixed support for the unisexuals studied here occupying these types of habitats suggests a limited impact of L genomes alone on habitat use.

Our second prediction was that unisexuals would inhabit "marginal" areas (higher elevation, drier, cooler, and more anthropogenically disturbed) relative to sexual species. Comparisons of one of the sexual–unisexual pairs (*A. jeffersonianum* vs. LJJ) support this hypothesis. AIC analysis shows that landscape resistance is the most important variable, followed by soil type, temperature, and elevation; pond size is also moderately important. In line with the prediction, LJJ unisexuals are in areas that are more anthropogenically disturbed, cooler, and at higher elevation. In contrast, ecological differences between *A. texanum* and LTT unisexuals are not consistent with predictions of the GPH. Although LTT unisexuals are more abundant in sites that are drier (potentially consistent with the GPH), they are also more common at locations that are lower elevation, warmer, and less anthropogenically disturbed. These results do not support the idea that LTT unisexuals are more abundant in marginal environments.

Ecological interactions among unisexual biotypes and their closest sexual species are clearly complex. For some of these variables, we suggest that the salamander types examined here exist on a continuum. For example, along the axis of landscape resistance, we propose that the species and

biotypes are arrayed from low to high as: *A. jeffersonianum* < LJJ < LTT < *A. texanum*. Therefore when we compare sets of sexual–unisexual pairs, we see a reversal in the direction of the relationship. This hypothesis could be tested using experimental approaches comparing species and biotypes, for example, in terms of locomotor performance in habitats of varying resistance (e.g., Rothermel and Semlitsch 2002). It would also be fruitful to assess demography of species and biotypes across a range of environmental variables examined here (e.g., assessing egg or larval survivorship across a range of temperatures). Experimental approaches would allow for the isolation of single variables that might have a disproportionate effect on the natural distribution of ambystomatid salamanders.

Methodological considerations

One important assumption we make is that we have accurately assessed the population composition of ponds, despite the fact that our sampling occurred during a single year at each of the sites and so our data provide only a snapshot of the population composition at a single point in time. Little is known about variation in population composition of sexual and unisexual *Ambystoma* across years; however, Bogart and Klemens resampled eight sites a decade apart (Bogart and Klemens 1997, 2008) and reported that "species and genomotypes of the unisexuals were similar for most of the resampled populations" (Bogart and Klemens 2008:19). Capture–mark–recapture data across four years reveal no significant changes in population composition at four ponds in Michigan (K. R. Greenwald, *unpublished data*). These results suggest that populations may be relatively stable on a year-to-year timescale, and that if cyclical changes occur (as discussed below), they may take place on a longer (multi-generational) timescale.

Second, it is possible that our classifications of marginal habitat are inaccurate, which would undermine our assessment of the impact of habitat on niche use by unisexuals. However, we used a comprehensive literature that describes biological, genetic, and physiological characteristics of these salamanders and other amphibians to generate our classifications (additional detail in Appendix S2). Additional work that used species- or lineage-specific assessments of

the impact of specific environmental conditions on salamander viability and performance (e.g., Horne and Dunson 1994, Johnson et al. 2010) would be useful in refining environmental niche modeling analyses like those described here.

Mechanisms for sexual–asexual coexistence

Several hypotheses have been generated to describe the mechanisms underlying the pattern of niche differentiation between sexual and asexual forms. Two of the most prominent are the general purpose genotype (GPG) hypothesis and the frozen niche variation (FNV) hypothesis (Vrijenhoek and Parker 2009). There are difficulties in applying either mechanistic hypothesis to the *Ambystoma* system. The GPG hypothesis states that if a sexual population is variable in terms of the breadth of its environmental tolerance, and clones are generated over the entire range of this distribution, then natural selection will favor those clones with broader environmental tolerance (Vrijenhoek and Parker 2009). However, unisexual biotypes are not strict "clones," but rather genetically alterable components of a single matriline. Due to genome addition and replacement, a single biotype (e.g., LJJ) might include all of the variations found in its "parental" sexual species *A. jeffersonianum*. It is therefore feasible that a single unisexual biotype could have an equal range of environmental tolerance to that of its most similar sexual species.

The frozen niche variation (FNV) hypothesis argues that if a sexual population is broadly variable in terms of its ability to make use of a particular natural resource, and produces clones that contain only a subset of this variation, competition will ensue (Vrijenhoek and Parker 2009). Two possible outcomes are (1) stable coexistence, in which clones with extensive overlap with the sexual species are eliminated by natural selection, but more dissimilar clones are able to persist; and (2) sexual exclusion, in which the sexual progenitor is outcompeted and eliminated by clones that are produced at a rapid rate. Again, as stated above, *Ambystoma* biotypes are not strict clones and therefore their genetic composition and related phenotypic expression are not "frozen"; genome replacement appears to be common on an evolutionary timescale (Bi et al. 2008). Furthermore, kleptogenetic reproduction will cause biotypes to continually converge

genetically with the sexual species with which they coexist, making the "stable coexistence" outcome implausible.

Despite the difficulties in applying the FNV hypothesis to this system, it does appear feasible that some degree of sexual exclusion is occurring. Because unisexual biotypes might cover the entire range of variation in resource use of a sexual species, and because they do have advantages in growth rates and competitive interactions (Licht and Bogart 1989, Brodman and Krouse 2007), it is possible that unisexuals might drive sexual species to very low numbers or local extinction, an idea first proposed by Clanton (1934). We documented 11 ponds in which our sampling did not recover a single sexual salamander. Because only inseminated eggs develop (Bogart et al. 1989), unisexual salamanders may be unlikely to persist at these sites (but see Charney et al. 2014). We hypothesize that long-term dynamics might reveal a cyclical pattern, wherein competition results in local extinction of sexual salamanders, followed by reduction or extinction of the unisexual population (Kokko et al. 2008). Recolonization of the site by sexual animals could then lead to a rebound of the unisexual population. This cyclical dynamic may be more prevalent at the margins of the unisexual distributions, where unisexuals would be expected to have a stronger competitive advantage. Long-term sampling at individual sites and evaluation of dispersal capabilities of unisexuals are necessary to determine whether this hypothesis has merit.

Our results demonstrate that there are ecological differences between sexual and unisexual *Ambystoma.* Ecology, in the form of niche differentiation, needs to be considered as a mechanism facilitating coexistence of the forms. Importantly, unisexuals should not be considered a single ecological entity; their niches differ from one another and from their parental sexual species. The mechanisms underlying this differentiation warrant further work. For example, it is unclear whether *A. jeffersonianum* are competitively excluding LJJ unisexuals from higher-quality habitat, or whether LJJ unisexuals are simply better able to persist at lower-quality sites. Additional research assessing fitness measures of the various species and biotypes in the *Ambystoma* complex would be a valuable contribution to further our understanding of how these sexual and semi-sexual forms continue to persist in the wild.

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