

Locomotor endurance predicts differences in realized dispersal between sympatric sexual and unisexual salamanders

Robert D. Denton^{*,1,2}, Katherine R. Greenwald³ and H. Lisle Gibbs^{1,2}

¹Department of Evolution, Ecology and Organismal Biology, Ohio State University, 300 Aronoff Laboratory, 318 West 12th Avenue, Columbus, OH 43210, USA; ²Ohio Biodiversity Conservation Partnership, Ohio State University, 300 Aronoff Laboratory, 318 West 12th Avenue, Columbus, OH 43210, USA; and ³Department of Biology, Eastern Michigan University, 441 Mark Jefferson Science Complex, Ypsilanti, MI 48197, USA

Summary

1. Dispersal is the central mechanism that determines connectivity between populations yet few studies connect the mechanisms of movement with realized dispersal in natural populations. To make such a link, we assessed how physiological variation among individuals predicted dispersal in natural populations of unisexual (all-female) and sexual *Ambystoma* salamanders on the same fragmented landscape in Ohio.

2. Specifically, we assessed variation in a trait that influences long-distance animal movement (locomotor endurance) and determined whether variation in endurance matched patterns of realized dispersal assessed using genetic assignment tests. A possible mechanism for why unisexuals would have lower locomotor endurance than a sympatric sexual species (*Ambystoma texanum*) is the potential energetic cost of evolutionarily mismatched mitochondrial and nuclear genomes within polyploid unisexuals.

3. We found that sexuals walked four times farther than unisexuals during treadmill endurance trials that mimic the locomotor endurance required for dispersal.

4. We then applied landscape genetic methods to identify dispersed adults and quantify realized dispersal. We show that the differences in locomotor endurance between unisexual and sexual salamanders scale to realized dispersal: dispersing sexual individuals travelled approximately twice the distance between presumed natal wetlands and the site of capture compared to dispersing unisexuals.

5. This study links variation in individual performance in terms of endurance with realized dispersal in the field and suggests a potential mechanism (physiological limitation due to mitonuclear mismatch) for the reduced endurance of unisexual individuals relative to sexual individuals although we discuss other possible explanations.

6. The differences in dispersal between these two types of salamanders also informs our understanding of sexual/unisexual coexistence by suggesting that unisexuals are at a competitive disadvantage in terms of colonization ability under a extinction-colonization model of coexistence.

Key-words: *Ambystoma* salamanders, coexistence, dispersal, endurance, genetic assignment, mitonuclear mismatch, unisexuals

Introduction

Dispersal differences within and between species shape patterns of diversity from local to range-wide scales (Berdahl *et al.* 2015). Integrating dispersal rates or distances with the phenotypes that drive these patterns is an important step toward understanding how species influence each

other's dispersal behaviour (Fronhofer *et al.* 2015) and how these differences produce patterns of biodiversity across landscapes (Lowe & McPeck 2014). Intraspecific differences in dispersal behaviour can scale up to shifts in species distributions (Bestion, Clobert & Cote 2015), and dispersal asymmetries between species can have strong effects on the outcomes of competition (Amarasekare 2003). Despite the impact that dispersal has on

*Correspondence author. E-mail: robert.d.denton@gmail.com

evolutionary and ecological processes, there is little data that connects variation in traits that facilitate dispersal of individuals with actual movement of individuals among populations. However, there are a multitude of behavioural, morphological, physiological and genetic traits that can influence such movements (Bowler & Benton 2005; Nathan *et al.* 2008). Understanding how specific traits contribute to successful dispersal events is critical to predicting how changes in environment, phenotypic diversity and species interactions may alter species persistence through time (Salomon, Connolly & Bode 2010).

Studies that link the physiology of dispersal with observed dispersal patterns in the wild can provide important ecological insights. For example, differences in metabolic rate and locomotion propensity can account for differences in dispersal among demes in the Glanville fritillary butterfly (*Melitaea cinxia*; Hanski 2012). Studies of the spread of invasive cane toads (*Rhinella marina*) across Australia have also shown how greater dispersal ability is correlated with specific phenotypes (Shine, Brown & Phillips 2011). For instance, rapidly dispersing range-edge toads have longer limbs (Phillips *et al.* 2006), greater locomotor endurance (Llewelyn *et al.* 2010) and upregulation of genes involved in metabolism and cell repair (Rollins, Richardson & Shine 2015). These toads have also displayed patterns that are contrary to predictions of expected dispersal for specific phenotypes. For example there is a lack of cellular metabolic differences between toads with differing dispersal ability (Tracy *et al.* 2012) and physiological performance may not be related to the magnitude of *in situ* dispersal (Olson & McPherson 1987). Physiology is clearly important for the determination of dispersal ability, but there are few studies that link differences in physiological phenotypes between competing species with their realized capacity for dispersal (Salomon, Connolly & Bode 2010).

Here, we provide an integrated assessment of both a mechanism of dispersal and realized natural dispersal within sympatric populations of sexual and unisexual salamanders (genus *Ambystoma*). Unisexual *Ambystoma* salamanders are the oldest lineage of unisexual vertebrate and reproduce through kleptogenesis, in which polyploid unisexual female salamanders produce clonal offspring after stimulation from a sexual male's sperm but can occasionally 'steal' sperm from the males of congeneric sexual salamanders (Bogart *et al.* 2007; Gibbs & Denton 2016). The result of this mating strategy is a single, distinct mitochondrial lineage combined with 2–5 haploid nuclear genomes from other sexual *Ambystoma* species (*A. laterale*, *A. jeffersonianum*, *A. texanum*, *A. tigrinum*, *A. barbouri*; Robertson *et al.* 2006; Bi & Bogart 2010a). The majority of unisexuals contain nuclear genomes from *A. laterale* and *A. jeffersonianum*, whereas the mitochondrial genome of unisexuals is most closely related to *A. barbouri*, an extremely rare sperm donor to the lineage (Robertson *et al.* 2006; Bogart & Klemens 2008). Unisexuals are currently widespread across northeastern North America, and their range corresponds to large areas of agricultural land (Bogart *et al.* 2007; Bogart & Klemens 2008),

which are some of the most challenging environments for salamander movement and persistence (Compton *et al.* 2007; Greenwald, Gibbs & Waite 2009).

A compelling reason why there may be differences in dispersal ability in unisexual salamanders compared to related sexual species is that unisexuals are a lineage that shows cyto-nuclear discordance similar to that observed in experimentally generated nucleocytoplasmic 'cybrids', which combine the mitochondrion of one species with the nuclear genome of another (Narbonne, Simpson & Gurdon 2011). Specifically, the nuclear genomes within unisexual individuals are more evolutionarily distinct from their mitochondrial genomes than those found in sexual species because the unisexual mitochondrial genome is most closely related to a species (*A. barbouri*) whose nuclear genomes are extremely rare in unisexuals (see above). Genome exchange between sexual males and unisexual females is relatively common (Gibbs & Denton 2016), and therefore presents more opportunity for co-evolution between heterospecific mitochondrial and nuclear genomes compared to strictly asexual taxa. But even small levels of cyto-nuclear mismatch may have a significant impact on physiological processes involved in energy production at the cellular level. A higher likelihood of molecular mismatch within protein complexes that require coding information from both mitochondrial and nuclear genomes in unisexuals and a resultant reduction in the efficiency of ATP production (Harrison & Burton 2006). Although there is evidence that mitochondrial introgression can also be associated with greater mitochondrial respiration (Toews *et al.* 2013), mitonuclear mismatch often causes oxidative stress (Monaghan, Metcalfe & Torres 2009) and can lead to generalized physiological limitations (Wolff *et al.* 2014).

We assessed potential and realized differences in dispersal capacities between unisexual *Ambystoma* and a closely related, sympatric sexual species (small-mouthed salamander, *A. texanum*, Fig. 1) using a two-step procedure. First, we measured walking endurance using treadmill trials on wild-caught individuals from a fragmented landscape in central Ohio. Second, we confirmed differences in endurance by using genetic assignment tests to identify dispersed animals at the same field sites (reviewed in Broquet & Petit 2009). Together, these methods can connect the capacity to disperse with the distribution of dispersed animals on a landscape in relation to their natal population, linking a potential mechanism for differential dispersal to the patterns in realized dispersal inferred from genetic data. These results also have important implications for understanding mechanisms of coexistence between sexual and unisexual forms (Hellriegel & Reyer 2000).

Materials and methods

LOCOMOTOR ENDURANCE

We collected 38 individuals (17 *A. texanum*, 21 unisexuals) from five sites within a largely agricultural landscape in Crawford



Fig. 1. Unisexual *Ambystoma* salamander (top) and small-mouthed salamander (*Ambystoma texanum*, bottom).

County, Ohio (~200 km²) during spring 2015. The mean number of individuals per site was 4.25 (range: 3–5; sites C10, C13, C22, C29) for *A. texanum* and seven for unisexuals (range: 6–9; sites C13, C29, C1303). All individuals were acclimated individually in a cold room kept at 13 °C and fed three adult crickets weekly for a month. In addition to sympatric unisexual and *A. texanum* individuals, we included two additional sympatric, sexual species, *A. jeffersonianum* ($N = 5$) and *A. laterale* ($N = 2$), that had been held captive in the same conditions as the wild-caught animals since 2010. Because of the potential confounding factors associated with the length of captivity (diet quality, lack of seasonality, acclimation to human interaction), we do not include these two sexual species in any statistical analysis. However, the data from *A. jeffersonianum* and *A. laterale* individuals are useful for providing a qualitative comparison in levels of endurance between the unisexuals and their parental, sexual species that constitute their nuclear genomes.

We conducted treadmill endurance trials at the same acclimation temperature following the protocol of Johnson, Johnson & Shaffer (2010). Briefly, each randomly selected animal was fed 3 days before their trial, then weight, snout to posterior vent (SVL) length and femur length was recorded. The treadmill used for trials was the same as used by Johnson, Johnson & Shaffer (2010), and rotation speed was maintained at a near constant speed that matched the walking speed of each individual salamander. A metal spatula was used to gently prod animals to maintain initial walking speed. Every 3 min, the animal was removed from the treadmill and tested for fatigue by a righting response test (Johnson, Johnson & Shaffer 2010). If an animal could not right itself after 3-s, the trial was terminated. If an animal refused to continue after 10 min of gently tapping or pinching the tail, the trial was concluded. After a successful righting response, the animal was rehydrated with a spray bottle and returned to the treadmill. Distance travelled was calculated using the speed maintained by each individual multiplied by the duration walked and an analysis of covariance (ANCOVA) was conducted using weight and femur length as covariates and distance travelled as the response variable.

COLLECTION OF GENETIC DATA

We collected salamander tissue samples from the same sites described above and all other breeding wetlands in the ~200 km²

section of southwestern Crawford County, Ohio to infer dispersal distance in wild individuals using genetic assignment tests (Berry, Tocher & Sarre 2004). To improve the confidence associated with identifying dispersed animals using genetic data (Cornuet *et al.* 1999), we chose well-documented sites that have been previously mapped (Weyrauch & Grubb 2004) such that all known breeding sites within the study area were sampled. All sites were characterized as vernal wetlands embedded in small woodlots (<1 km²). We surveyed the 28 sites, including those above, identified as amphibian breeding habitat by Weyrauch & Grubb (2004) over a 4 year period (2012–2015). In 2012, we visited all sites to confirm the presence of wetland habitat and conduct preliminary salamander surveys. From 2013 to 2015, we sampled each site in at least two consecutive years using aquatic minnow traps and constrained searches during the early spring. We sampled each wetland over multiple years to avoid effects from potential relatedness within breeding cohorts (Semlitsch *et al.* 1996). For analysis, we chose only sites in which salamanders were detected over multiple seasons and where at least two consecutive nights of trapping yielded >20 individuals of either group.

GENETIC ANALYSES

We extracted genomic DNA from the tail tips of 294 *A. texanum* and 151 unisexual salamanders using Qiagen DNeasy kits (Qiagen, Venecia, CA, USA). We identified unisexual individuals based on longer snouts, slimmer bodies and longer limbs in comparison to the primary sperm donor species of the area, *A. texanum* (R. Denton, unpubl. data). We confirmed the field identity of the first 200 individuals using a 346 bp section of control region mtDNA (THR; McKnight & Shaffer 1997). We correctly confirmed the identity of all 200 individuals, and so further samples were classified based only on field identification. We amplified 10 species-specific microsatellite loci for *A. texanum* using the PCR conditions recommended by Williams & DeWoody (2003) (Table S1, Supporting Information) and determined the ploidy and genome composition of unisexual samples using a single-nucleotide polymorphism (SNP) assay (Greenwald & Gibbs 2012). After we identified the genomes present in the unisexual individuals, we amplified a combination of species-specific microsatellite loci designed for *A. laterale* and *A. jeffersonianum* (Julian, King & Savage 2003; Denton, Gibbs & Glenn 2015; Table S1). A total of 19 loci were amplified: six specific to *A. laterale*, nine specific to *A. jeffersonianum*, and two that amplify in both species at different size ranges. The genotypes for unisexuals were determined using the ploidy expectation from the SNP assay. If an animal had more than two haploid genomes from either *A. laterale* or *A. jeffersonianum*, alleles were scored based on comparative peak height with any ambiguity coded as missing alleles. The final genotype for a unisexual individual consisted of the combined alleles from all haploid genomes (*A. jeffersonianum* and *A. laterale*). All loci were scored using GENEIOUS (v 7.1.8; Kearsse *et al.* 2012).

We tested for null alleles among the *A. texanum* loci using MICROCHECKER (v 2.2.3; Van Oosterhout *et al.* 2004). We performed tests for linkage and Hardy Weinberg equilibrium (HWE) on the *A. texanum* loci using GENEPOP (v 4.2, Raymond & Rousset 1995; Rousset 2008). Because unisexuals are polyploid, population-level genetic data do not meet the assumptions of most analytical methods that are based on population genetics theory. As such, we used assumption-free, multivariate genetic analyses within the statistical package ADEGENET (v 1.4.1; Jombart 2008) to genetically cluster putative populations and identify individuals dispersed from their natal populations. Populations were described using the *find.clusters* function and evaluated using BIC scores and by evaluating plots of population assignment for all individuals.

We conducted a discriminant analysis of principle components (DAPC; Jombart, Devillard & Balloux 2010) to produce posterior probabilities of individual assignment to the sampled sites (Kraus

et al. 2013; Gotzek *et al.* 2015). The DAPC procedure consists of a principle components analysis (PCA) as a prior step for a discriminant analysis (DA). This allows for the discrimination of individuals to pre-defined groups using the simplified and uncorrelated variables produced by a PCA. Most importantly, this procedure remains assumption-free (HWE or LD) and can be used to infer the group assignment of mixed ploidy (James, Jordan & Griffin 2013). To determine an optimal number of principle components (PCs) for the DAPC, we used both the *optim.a.score* and the *xval.dapc* procedures. The retained PCs were then used for a final DAPC run. To choose a posterior probability threshold for genetic assignment of a dispersed individual, we followed the guidelines developed for assigning birds to natal populations using stable isotope data (Rocque *et al.* 2006; Wunder 2012). Specifically, posterior probability thresholds for assignment to the groups identified in the *find.clusters* analysis ranged from 0.55 to 0.99, which translate to odds ratios from 1.7:1 to 198:1. Jonker *et al.* (2013) refer to a posterior probability of >0.3 as evidence of a recent instance of gene flow in migratory waterfowl using discriminant analysis on genetic data. Using the sample sizes in our study for *A. texanum* ($N = 294$) and unisexuals ($N = 151$), we choose a posterior probability threshold of 0.70. This translates to odds ratios of 28.8:1 and 16.6:1 for *A. texanum* and unisexuals respectively; meaning that an individual that is assigned to a population other than the site where it was sampled at a posterior probability of 0.7 is 28.8 or 16.6 more likely than at random. To validate the use of DAPC for detecting migrant individuals, we also conducted a detection of first generation migrants test in GeneClass2 (Piry *et al.* 2004) using *A. texanum* individuals and compared these results with those obtained using DAPC.

LANDSCAPE ANALYSES

To identify biologically relevant dispersal distances for each individual identified as a disperser, we constructed least-cost paths (LCPs) among assigned and sampled sites using the Landscape Genetics Toolbox developed for ARCGIS 9.3 (Etherington 2011). This requires a base layer indicating the relative cost of each land-cover type. To generate such a layer, we used LANDSAT imagery based on 30 m-resolution landcover data from the National Land Cover Database (NLCD 2011; Homer *et al.* 2015), with encoded costs based on empirical data (Compton *et al.* 2007). The toolbox then calculates every pairwise route among sites in such a way as to minimize the accumulated cost of the journey, and then provides the length of these cost-minimizing paths. Least-cost paths constructed using landscape resistance values derived from natural history knowledge have outperformed other geographic distances in their ability to fit patterns of genetic structure (Michels *et al.* 2001; Coulon *et al.* 2004). However, this has not been true in all cases (e.g. Charney 2012a), and there is ongoing discussion about the optimal way to parameterize such models (Zeller, McGarigal & Whiteley 2012). Therefore, we also calculated straight-line

(Euclidean distance) as an assumption-free means to assess pairwise distances among sites.

Results

LOCOMOTOR ENDURANCE

Salamanders showed pronounced differences in performance during treadmill trials. Unisexual individuals had significantly longer femurs and body length than *A. texanum* individuals (Femur length: $t = -2.49$, $P = 0.018$; SVL: $t = -4.00$, $P < 0.001$), whereas the groups were not statistically different in weight ($t = -1.74$, $P = 0.09$, Table 1). We converted the duration of each trial into a distance based on the speed of the treadmill, which stayed relatively consistent for both groups at a rate of 19.4 s per rotation. We log-transformed the distance values to correct for non-normality. All covariates measured (weight, SVL and femur length) were significantly, positively correlated with one another (Pearson correlation range = 0.447–0.673, all $P < 0.006$, Fig. S2). Therefore, we only retained the covariate with the largest correlation with distance (SVL) in further analyses. After controlling for SVL, *A. texanum* had significantly greater walking endurance compared to unisexuals ($F = 29.0$, $P < 0.001$, Fig. 2). Unisexuals travelled approximately 25% of the average distance travelled by *A. texanum* individuals (159.25 ± 86.4 m for *A. texanum*, 34.47 ± 28.2 m for unisexuals). Unisexual individual performance did not vary by genome composition, as the single individual composed of one genome from *A. laterale*, *A. texanum* and *A. jeffersonianum* (abbreviated at LTJ) was near the mean for the group (28.9 m travelled). Individuals of two additional species that represent the composite genomes of the unisexuals (*A. jeffersonianum* and *A. laterale*) showed similar treadmill endurance to the *A. texanum*. Finally, all but two unisexual trials (90%) were concluded following a lack of righting reflex. In contrast, only three *A. texanum* individuals (18%) lacked righting response at the end of their trials and were instead concluded due to a >10 min refusal period.

GENETIC ANALYSES OF DISPERSAL

Salamanders were collected from 16 to 9 sites for *A. texanum* and unisexuals respectively. Samples sizes per site

Table 1. Distances travelled during locomotor endurance trials by unisexual and sexual salamanders collected from sites within Crawford County, Ohio with associated morphological measurements

Group	N	Group mean \pm 1 standard deviation			
		Weight (g \pm SD)	Snout–vent length (mm \pm SD)	Femur length (mm \pm SD)	Distance walked (mm \pm SD)
<i>Ambystoma texanum</i>	14	10.05 \pm 1.97	63.6 \pm 5.0	5.73 \pm 0.79	159.25 \pm 86.4
Unisexual <i>Ambystoma</i>	19	11.42 \pm 0.60	70.7 \pm 6.0	6.36 \pm 0.73	34.47 \pm 28.2
<i>A. laterale</i>	2	6.18 \pm 0.88	63.0 \pm 5.0	4.46 \pm 0.23	161.20 \pm 16.09
<i>A. jeffersonianum</i>	4	8.47 \pm 0.70	61.0 \pm 4.2	5.54 \pm 0.87	172.21 \pm 133.49

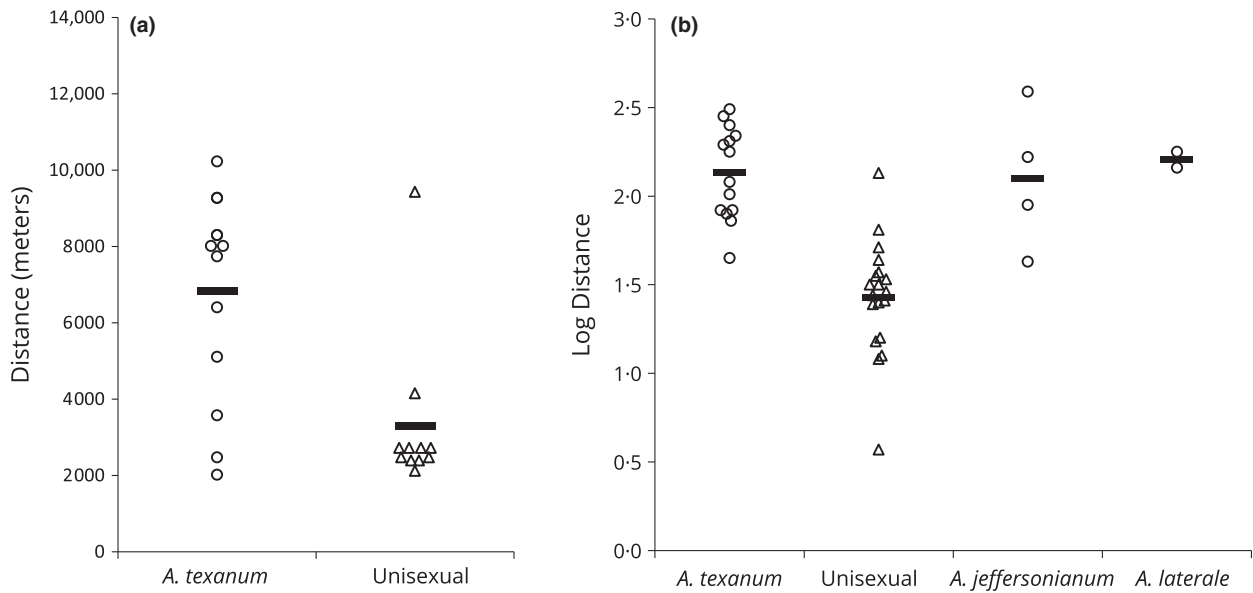


Fig. 2. Univariate plots (Weissgerber *et al.* 2015) for the distance travelled by dispersed sexual salamanders (circles) and unisexual salamanders (triangles) as measured by Euclidean distance (a) and the log Distance travelled during locomotor endurance trials by unisexuals and three sexual species (b). Each point represents one individual and black horizontal bars represent mean values. Standard errors for (a) are 22.25 for *Ambystoma texanum* and 6.31 for Unisexuals. Standard errors for (b) are 0.07, 0.07, 0.18, 0.03 for *A. texanum*, Unisexuals, *Ambystoma jeffersonianum* and *Ambystoma laterale*.

ranged from 10 to 30 individuals for *A. texanum* and from 8 to 24 individuals for unisexuals (Table 2). Unisexuals and *A. texanum* were the two most commonly captured salamander species at the majority of sites, but both *A. maculatum* (spotted salamander) and *A. tigrinum* (tiger salamander) were detected in a minority of sites. While

Table 2. Number of individuals sampled from each site and the proportion of individuals assigned to the site at which they were sampled

Site	Group	<i>N</i>	Proportion assigned to sampled site
C03_C10	<i>Ambystoma texanum</i>	46	0.87
C13	<i>A. texanum</i>	20	0.85
	Unisexual	19	1.00
C22	<i>A. texanum</i>	20	0.60
	Unisexual	8	0.88
C29	<i>A. texanum</i>	17	1.00
	Unisexual	15	0.53
C57_C58	<i>A. texanum</i>	33	0.85
C60	<i>A. texanum</i>	18	0.56
	Unisexual	20	0.95
C61	<i>A. texanum</i>	17	0.59
C63	<i>A. texanum</i>	20	0.40
C63_C64	Unisexual	36	0.94
C64	<i>A. texanum</i>	10	0.20
C77	<i>A. texanum</i>	19	0.37
	Unisexual	9	0.89
C84	<i>A. texanum</i>	19	0.16
C86	<i>A. texanum</i>	16	1.00
C96A	<i>A. texanum</i>	19	0.58
	Unisexual	20	0.85
C99	<i>A. texanum</i>	20	0.35
	Unisexual	24	1.00

A. maculatum were abundant when present, this species does not contribute to the unisexual complex. In contrast, *A. tigrinum* does occasionally contribute genomes to unisexuals, but this species was rare in surveys (less than twenty individuals captured over 4 years).

For *A. texanum*, two loci showed evidence for null alleles in more than half of the populations (t133 and t87). However, we retained these loci in further analyses based on the robustness of assignment testing (Carlsson 2008) and the lack of assumptions made by DAPC assignment methods. No loci showed evidence for linkage disequilibrium based on a Bonferroni-adjusted critical *P* value (0.005). For *A. texanum*, the mean number of alleles (\pm SE) was 8.74 ± 0.32 ($H_o = 0.55$, $H_e = 0.75$). For unisexuals, the mean number of alleles was lower for *A. laterale* loci (1.59 ± 0.114) compared to *A. jeffersonianum* loci (3.58 ± 0.15). Mean pairwise F_{st} for *A. texanum* among all populations was 0.08.

The BIC scores produced by the *find.clusters* procedure in *adeget* suggested fewer genetic groups than the number of sampled sites (six groups from 16 populations for *A. texanum*; five groups from nine populations for unisexuals). We evaluated each sampled population's assignment probabilities and chose to combine populations that were closest in geographic distance, had extensive overlap in the assignment of individuals, and had pairwise $F_{st} < 0.04$ (suggested F_{st} for number of loci from Paetkau *et al.* [2004]). This resulted in four of the total populations being collapsed into two new sites for *A. texanum* ('C03_C10' and 'C57_C58') and one newly combined site for unisexuals ('C63_C64').

Both sexual and unisexual salamanders showed similar percentages of individuals that were identified as

dispersers. For *A. texanum*, the DAPC procedure accounted for 60% of the variance in the dataset and assigned 64% (188/294) of the total individuals to a population (28 PCs and 6 DFs retained). The percentage of individuals from a given population that were assigned to their sampled site (non-dispersers) ranged from 16 to 100%. Thirteen individuals (4% of total; Table 3) were assigned to a population other than where they were sampled (disperser) with a posterior probability of ≥ 0.70 (4% of total, posterior probabilities = 0.70–0.94). Seven of these thirteen were also identified as migrants by GeneClass2 ($P < 0.01$) and 50% of individuals were confidently assigned to a population. For unisexuales, the DAPC procedure accounted for 79% of the variance in the data set and assigned 90% of the total individuals (136/151) to a population (14 PCs and six DFs retained). The percentage of individuals assigned to their sampled site (non-dispersers) ranged from 50 to 100%. Eleven individuals were identified as dispersers (7% of total, posterior probabilities = 0.83–1.00, Table 3). Nine of the eleven unisexual dispersers were successfully genotyped, and the majority of all sampled individuals were tetraploids consisting of a single *A. laterale* genome and three *A. jeffersonianum* genomes (LJJ = 32, LJJJ = 54, LTJ = 16, LTJJ = 11). Three of the nine dispersers also included a single genome derived from *A. texanum*, but the proportion of

A. texanum genomes within the identified dispersers was similar to that of the total population (0.33 vs. 0.24 respectively).

LANDSCAPE ANALYSES

Sexual salamanders travelled farther between their sampled site and their presumed natal pond when compared to unisexual salamanders. The average Euclidean distance between sites was significantly farther among *A. texanum* dispersers ($N = 13$, mean = 6826 m) compared to unisexual dispersers ($N = 11$, mean = 3300 m; $t = 3.5$, $P < 0.01$, Table 3). The LCPs generated based on landscape resistance values for amphibians (Compton *et al.* 2007; Greenwald, Purrenhage & Savage 2009) displayed a similar difference between groups (*A. texanum* mean = 9926 m, unisexual mean = 5291 m, $t = 2.5$, $P = 0.02$, Figs 2, 3). However, due to the homogeneous landscape of the study area, many calculated routes favoured the use of roads as corridors avoid travelling across row crop agriculture. To address this biologically unrealistic result, we built additional LCPs using a resistance layer composed of 30-m resolution percent tree canopy cover (TCC; Homer *et al.* 2015). This resistance layer produced LCPs that were more likely to reflect travel among drainage ditches and tree lines but still maintained the same relationship

Table 3. Individuals identified as dispersers (mismatch between sampled site and genetically assigned site). Discriminant Analysis of Principal Components (DAPC) was used to identify animals with a ≥ 0.70 posterior probability of assignment and GeneClass2 was used to identify first generation migrants

Individual	Group	Biotype	Population		Distance between sampled and assigned population (m)			
			Sampled	Assigned (DAPC)	Assigned (GeneClass2)	Euclidean	Least-cost path (canopy cover)	Least-cost path (NLCD)
A1977	<i>Ambystoma texanum</i>		C03_C10(C10)	C57_C58	C57_C58	8013	9520	10 111
A1980	<i>A. texanum</i>		C03_C10(C10)	C57_C58		8013	9520	10 111
A1549	<i>A. texanum</i>		C22	C61		7743	9097	14 914
A1964	<i>A. texanum</i>		C57_C58(C58)	C03_C10		6407	7061	7504
A2324	<i>A. texanum</i>		C60	C96A	C96A	2474	2830	2989
A2365	<i>A. texanum</i>		C61	C03_C10	C03_C10	9275	11 511	11 686
A2366	<i>A. texanum</i>		C61	C57_C58	C57_C58	5108	5276	5850
A2367	<i>A. texanum</i>		C61	C03_C10	C03_C10	9275	11 511	11 686
A2516	<i>A. texanum</i>		C61	C86		3578	4080	3997
A2255	<i>A. texanum</i>		C84	C57_C58		8299	14 058	14 358
A2259	<i>A. texanum</i>		C84	C57_C58	C57_C58	8299	14 058	14 358
A2475	<i>A. texanum</i>		C84	C86	C99	2020	2529	2473
A2424	<i>A. texanum</i>		C96A	C29		10 228	12 010	18 998
A1510	Unisexual	LJJJ	C29	C22		2390	2651	2693
A1511	Unisexual	LTJJ	C29	C22		2390	2651	2693
A2013	Unisexual	LJJJ	C29	C13		2718	6383	5865
A2015	Unisexual	LJJJ	C29	C13		2718	6383	5865
A2016	Unisexual	LJJJ	C29	C13		2718	6383	5865
A2017	Unisexual	LJJJ	C29	C13		2718	6383	5865
A2539	Unisexual		C63_C64(C63)	C99		4149	5944	5740
A2418	Unisexual	LJJ	C63_C64(C64)	C22		9429	15 165	15 399
A2511	Unisexual		C77	C63_C64		2118	2286	2260
A2445	Unisexual	LTJJ	C96A	C60		2474	2830	2979
A2446	Unisexual	LTJ	C96A	C60		2474	2830	2979

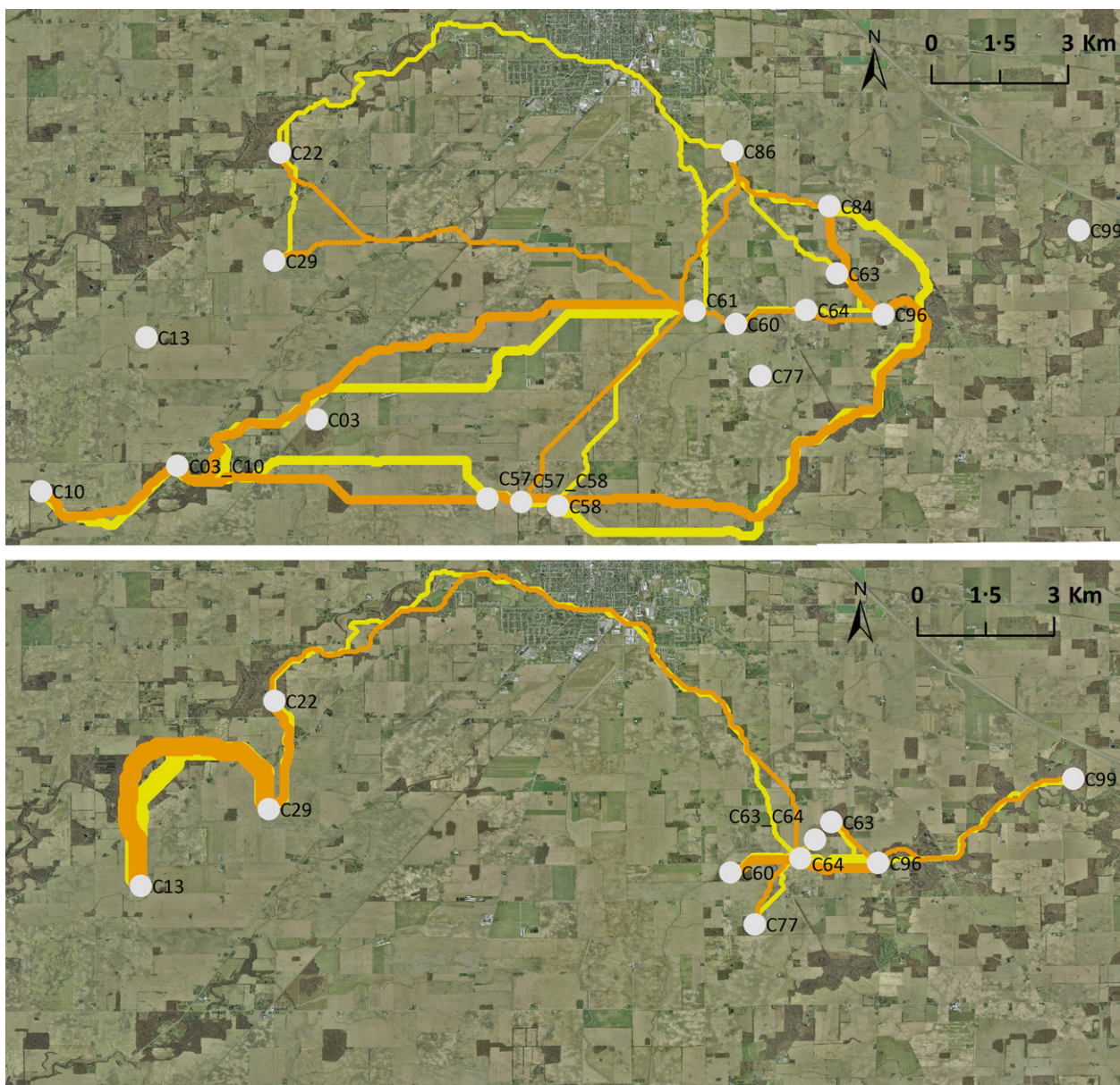


Fig. 3. Predicted least-cost paths travelled by genetically identified dispersers (top: *Ambystoma texanum*, bottom: unisexual *Ambystoma*). Paths were constructed by using resistance surfaces derived from canopy cover (orange lines) or using national land cover data base values from other *Ambystoma* species (yellow lines). All lines are scaled by the number of individuals that moved between the same wetland pairs.

between groups (*A. texanum* mean = 8697 m, unisexual mean = 5444 m, $t = 2.0$, $P = 0.05$). However, this change in resistance layer still produced LCPs that followed roads to avoid agricultural land cover, even suggesting the unlikely scenario of unisexual animals traversing urban areas.

Discussion

Unisexual *Ambystoma* salamanders are inferior dispersers compared to a sympatric sexual species, *A. texanum*. This conclusion was supported by both the superior endurance by sexual salamanders in treadmill trials and the greater distance from home populations displayed by sexual dispersers. If only the locomotor trials were considered, it

would be unclear if the differences between the groups translated to differential dispersal and subsequent breeding in natural populations, but here we demonstrate such a link. The addition of genetic data to the locomotor endurance measurements provides strong support for a physiological basis to dispersal asymmetry between these taxa. The unisexuals' limitation documented here is consistent with negative impacts on cellular metabolism due to mismatches between mitochondrial and nuclear-encoded proteins in unisexuals compared to sexuals, although other mechanisms are possible (see below). This study adds to the growing literature (see Bowler & Benton 2005; Lowe & McPeck 2014) that emphasizes the complexity of animal dispersal and the requirement for integrative approaches in

understanding the mechanisms and ecological and evolutionary consequences of animal dispersal.

CAUSES OF DISPERSAL DIFFERENCES

Despite morphological features that favour locomotor ability (larger body size, longer limbs; Bennett, Garland & Else 1989), unisexuals performed poorly in treadmill endurance trials compared to *A. texanum*. While this difference in endurance could be due to the influence of either *A. jeffersonianum* or *A. laterale* genomes within unisexuals, trials with captive adults of each parental species closely resemble those of the *A. texanum* individuals. In addition, *A. texanum* individuals travelled similar distances as another sexual species, *A. tigrinum*, using the same treadmill methodology (Johnson, Johnson & Shaffer 2010). Furthermore, these trials likely underrepresent the true endurance capacity of *A. texanum* due to the large proportion (82%) of individuals that maintained a righting response but refused to continue a trial, suggesting that the majority of *A. texanum* individuals had the capacity to walk for even greater distances.

Given the similar performance by the three sexual species in this study and one *Ambystoma* species from other work, the reduction in locomotor endurance that is specific to the unisexuals could potentially be related to limitations of cellular metabolism imposed by their unique mode of reproduction that reduce the efficiency of the oxidative phosphorylation (OXYPHOS) system due to a mismatch in proteins separately encoded by mitochondrial and nuclear genes (Lane 2011). Mitochondrial- and nuclear-encoded subunits that produce basic energetic functions such as the OXYPHOS pathway show evidence for positive selection that indicates co-evolution (Gershoni *et al.* 2010; Zhang & Broughton 2013). Mitochondrial replacement experiments in both rodents (McKenzie *et al.* 2003) and primates (Kenyon & Moraes 1997) show a negative correlation between cellular respiration and the phylogenetic distance between donor mitochondria and recipient cells. The evolutionary mismatch between mitochondrial and nuclear genomes of unisexual salamanders is expected to be high: all unisexual individuals harbour a phylogenetically distinct mitochondrial haplotype (Robertson *et al.* 2006; Bogart *et al.* 2007) compared to the species from which they derive their nuclear genomes (Bi & Bogart 2010b; Gibbs & Denton 2016). This mismatch may produce a physiological disadvantage that manifests here through limited locomotor endurance compared to sexual species. The quantification of the mitonuclear mismatch at a genotype level in unisexual salamanders and the relationship between mitonuclear mismatch in unisexuals and impacts on their physiology are important goal for future research.

While mitonuclear mismatch is a plausible explanation for dispersal differences, other mechanisms not explored in this study could also account for this difference. Two possibilities are (1) differences in behavioural motivation to

disperse or (2) differences in other life-history characteristics that promote dispersal. In terms of behaviour, the environmental cues or genetic basis for dispersing is not well understood in salamanders (Semlitsch 2008), and we have no evidence for differences in salamander density between our study sites that could lead to greater density-dependent dispersal effects on sexuals (Bitume *et al.* 2014). However, it is possible that there are unmeasured behavioural variables that may help explain the discrepancy in realized dispersal between *A. texanum* and the unisexuals. Differences in realized dispersal could also be affected by other life-history differences between unisexuals and sexuals. For example unisexuals could have a greater site fidelity compared to *A. texanum*. However, this is unlikely because most *Ambystoma* species show similar levels of site fidelity (Petranka 1998; Gamble, McGarigal & Compton 2007). Alternatively, this result could be an artefact of sex biased dispersal. All but one dispersed *A. texanum* were male, and unisexuals may share the dispersal behaviour of female *A. texanum*. However, both unisexuals and *A. texanum* showed a similar proportion of dispersed individuals (7% and 4% for unisexuals and *A. texanum* respectively). This indicates that, if present, the effect of sex is more likely associated with dispersal distance and not frequency. However, differences in dispersal ability between sexes seem to be driven by morphological dimorphism that affects locomotion or the additional weight of reproductive females that are carrying eggs (Bowler & Benton 2005). In the case of our study animals, male and female *Ambystoma* are not sexually dimorphic beyond a slightly swollen cloacal area in males during the breeding season and both types of females used in the locomotor trials were collected after depositing eggs when such differences are minimal. Finally, the connection between locomotor endurance and realized dispersal distance may be obscured by potential performance differences between unisexuals and *A. texanum* on realistic landscapes that may not be represented by the wet treadmill (e.g. grass, forest, soil). The direction of this bias is not understood because there are currently no comparative studies between species (Stevens *et al.* 2004; Lee-Yaw, Sechley & Irwin 2015). Because the landscape of Crawford County is largely homogeneous (field, road, small forest patches), we expect that there is little opportunity for differential dispersal among substrate types that could account for differences in movement between sexuals and unisexuals.

DISPERSAL IN *AMBYSTOMA* SALAMANDERS

The magnitude of dispersal identified by our genetic methods is similar to those described for other species of *Ambystoma* salamanders. At spatial scales similar to those in this study, *Ambystoma* salamanders display high levels of gene flow between populations (Newman & Squire 2001; Zamudio & Wiczorek 2007; Purrenhage, Niewiarowski & Moore 2009; Coster *et al.* 2015). However, this connectivity is spatially dependent, with greater population

differentiation at larger scales, as would be predicted by an isolation-by-distance model. Large *Ambystoma*, such as *A. tigrinum*, can regularly move 1–3 km from breeding habitat (Searcy & Shaffer 2008), and other *Ambystoma* have been documented dispersing >1 km (Smith & Green 2005; Gamble, McGarigal & Compton 2007). Local colonization and extinction of *Ambystoma* populations can be affected by landscape resistance (Cosentino, Schooley & Phillips 2011a), and even different crop species affect movement decisions by salamanders (Cosentino, Schooley & Phillips 2011b). However, *A. texanum* is mainly associated with open habitats (fields, bottomland forests, farmland) and displays some of the lowest dispersal distances among *Ambystoma* (Parmelee 1993; Petranksa 1998; Smith & Green 2005). Because small differences in estimated dispersal distance (~350 m; Peterman *et al.* 2015) can produce large differences in genetic structure of sympatric salamanders, both the magnitude (>3000 m for both species) and differences (at least 3000 m between species) of dispersal distance are significant in the context of previous research. Since the spatial genetic structure of *Ambystoma* varies both between and within species and is related to various landscape factors or life-history characters, this makes comparisons of dispersal ability difficult across studies. Importantly, no landscape genetics studies of *Ambystoma* salamanders include the combination of (1) species that are sexually parasitized by unisexuals and (2) geographic areas where unisexuals are present. Further analyses of *A. laterale*, *A. jeffersonianum* and *A. texanum* in areas of allopatry/sympatry would better reveal if differences in the sexual species dispersal patterns are a consequence of unisexual presence. Finally, it is important to caution that all of the locomotor endurance trials were conducted on adult animals, whereas the majority of dispersal takes place in the juvenile stage (Gamble, McGarigal & Compton 2007). Because long-term repeatability in locomotor endurance is low for salamanders (Austin & Shaffer 1992), our locomotor endurance trials might not represent the life-history stage most relevant to dispersal. In contrast, the dispersal differences calculated from genetic data represent realized dispersal unbiased by life stage effects.

COEXISTENCE OF SEXUALS AND ASEXUALS

If unisexual *Ambystoma* can obtain the benefits of both asexual and sexual reproduction through kleptogenesis, they would be predicted to quickly out-compete other *Ambystoma* species (Lampert & Schartl 2010). However, unisexuals are rarely identified as the only *Ambystoma* species in amphibian communities across their range (Noël, Labonté & Lapointe 2011). Primary life-history traits that are predicted to dictate the coexistence between a sexual parasite and their host favour sexual *Ambystoma*, including mate choice (Dawley & Dawley 1986), fecundity (Uzzell 1969), competitive ability of larvae (Brodman & Krouse 2007) and now dispersal ability. Using these four traits within the model of coexistence developed in a

similar frog system (Hellriegel & Reyer 2000) predicts a local extinction scenario for unisexual salamanders. Not only is the inferior dispersal ability of unisexuals surprising within the context of these coexistence models, two other lines of evidence would also predict greater dispersal ability in the unisexuals. First, vertebrates that lack sexual reproduction are often all-female, and differential dispersal in relation to competing sexual species can be a stabilizing force for unisexual population persistence (Kokko, Heubel & Rankin 2008). In cases where the unisexual lineage is a sexual parasite, colonization ability can drive persistence because the unisexual lineage is likely to out-compete local hosts and rely on locating new sexual populations (Kerr *et al.* 2006). Second, unisexual salamanders are polyploids, a commonly hypothesized driver of increased dispersal ability (Linder & Barker 2014) due to an association with marginal habitats (Greenwald, Denton & Gibbs 2016). However, in our study, ploidy did not appear to be a driver of dispersal since the ratio of triploids to tetraploids among unisexuals was similar between dispersing and non-dispersing individuals (57% tetraploids in total sampled individuals vs. 64% tetraploids in dispersers).

In combination with previous comparisons of life-history traits, inferior dispersal ability suggests that unisexuals should not be able to coexist with sexuals based on current models of coexistence. Yet, unisexual populations are abundant in this and many locations across a large area of North America (Bogart & Klemens 2008), suggesting coexistence may be better explained by other models or factors. For example *A. texanum* possesses both higher fecundity and longer dispersal range compared with unisexuals, two life-history traits associated with an 'inferior competitor' that is traditionally predicted to exploit resources in novel habitats before superior competitors arrive (the fugitive strategy; Bolker & Pacala 1999; Amarasakare 2003). Alternatively, if unisexual *Ambystoma* are considered sexual parasites, these results support a model of coexistence similar to that of host–parasite systems, in which over-exploitation of the host (*A. texanum*) can be prevented by mating selection against the parasite (Dawley & Dawley 1986) in combination with low levels of dispersal by the parasite (shown in this study, discussed by Kokko, Heubel & Rankin 2008; and demonstrated by Kerr *et al.* 2006). Unisexual *Ambystoma* may be intermediate between these different models of coexistence due to the frequency of genome exchange between sexual males and female unisexuals (Ramsden 2008; Charney 2012b; Gibbs & Denton 2016). The predictions for a taxon that reproduces clonally while occasionally incorporating novel genetic material from another species, such as in unisexual *Ambystoma*, may differ than other unisexual taxa with limited or no introgression.

Author's contribution

This project was conceived by R.D.D., K.R.G. and H.L.G.; R.D.D. collected field and laboratory data; R.D.D. and K.R.G. analysed the data;

R.D.D. led the writing of the manuscript with contributions by K.R.G. and H.L.G. All authors contributed critically to the drafts and gave final approval for publication.

Acknowledgements

We thank the Crawford County landowners who granted us access to their properties. We thank B. Johnson and M. Gray for providing the treadmill used in this study. We thank J. Dyer, S. Hedge, M. Holding, M. Saccucci, M. Parsley, P. Hudson, K. Costello, C. Ries, J. Lorenz, B. Arnold and J. Diaz for assistance with both field and laboratory work. We thank G. Gerald for helpful discussions and preliminary work. Finally, we thank B. Carstens, S. Matthews, J. Williams and the members of the Gibbs lab for comments on this manuscript. 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. Funds were also provided by the American Society of Ichthyologists and Herpetologists, the Ohio State University Graduate School, and those who contributed to a SciFund crowdfunding campaign by R.D.D. This work was conducted under Ohio State Institutional Animal Care and Use Protocol #2012A00000039.

Data accessibility

Genetic data and input files for DAPC analyses are available on the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.9d9s3> (Denton, Greenwald & Gibbs 2016).

References

- Amarasekare, P. (2003) Competitive coexistence in spatially structured environments: a synthesis. *Ecology Letters*, **6**, 1109–1122.
- Austin, C. & Shaffer, H. (1992) Short-, medium-, and long-term repeatability of locomotor performance in the tiger salamander *Ambystoma californiense*. *Functional Ecology*, **6**, 145–153.
- Bennett, A., Garland, T. & Else, P. (1989) Individual correlation of morphology, muscle mechanics, and locomotion in a salamander. *The American Journal of Physiology*, **256**, R1200–R1208.
- Berdahl, A., Torney, C.J., Schertzer, E. & Levin, S.A. (2015) On the evolutionary interplay between dispersal and local adaptation in heterogeneous environments. *Evolution*, **69**, 1390–1405.
- Berry, O., Tocher, M. & Sarre, S. (2004) Can assignment tests measure dispersal? *Molecular Ecology*, **13**, 551–561.
- Bestion, E., Clobert, J. & Cote, J. (2015) Dispersal response to climate change: scaling down to intraspecific variation. *Ecology Letters*, **18**, 1226–1233.
- Bi, K. & Bogart, J.P. (2010a) Time and time again: unisexual salamanders (genus *Ambystoma*) are the oldest unisexual vertebrates. *BMC Evolutionary Biology*, **10**, 1–14.
- Bi, K. & Bogart, J.P. (2010b) Probing the meiotic mechanism of intergenomic exchanges by genomic in situ hybridization on lampbrush chromosomes of unisexual *Ambystoma* (Amphibia: Caudata). *Chromosome Research*, **18**, 371–382.
- Bitume, E.V., Bonte, D., Ronce, O., Olivieri, I. & Nieberding, C.M. (2014) Dispersal distance is influenced by parental and grand-parental density. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141061.
- Bogart, J.P. & Klemens, M.W. (2008) Additional distributional records of *Ambystoma laterale*, *A. jeffersonianum* (Amphibia: Caudata) and their unisexual kleptogens in northeastern North America. *American Museum Novitates*, **3627**, 1–58.
- Bogart, J.P., Bi, K., Fu, J., Noble, D.W.A. & Niedzwiecki, J.H. (2007) Unisexual salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. *Genome*, **50**, 119–136.
- Bolker, B.M. & Pacala, S.W. (1999) Spatial moment equations for plant competition: understanding spatial strategies and the advantages of short dispersal. *The American Naturalist*, **153**, 575–602.
- Bowler, D.E. & Benton, T.G. (2005) Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biological Reviews*, **80**, 205–225.
- Brodman, R. & Krouse, H.D. (2007) How blue-spotted and small-mouthed salamander larvae coexist with their unisexual counterparts. *Herpetologica*, **63**, 135–143.
- Broquet, T. & Petit, E.J. (2009) Molecular estimation of dispersal for ecology and population genetics. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 193–216.
- Carlsson, J. (2008) Effects of microsatellite null alleles on assignment testing. *Journal of Heredity*, **99**, 616–623.
- Charney, N.D. (2012a) Evaluating expert opinion and spatial scale in an amphibian model. *Ecological Modelling*, **242**, 37–45.
- Charney, N.D. (2012b) Relating hybrid advantage and genome replacement in unisexual salamanders. *Evolution*, **66**, 1387–1397.
- Compton, B.W., McGarigal, K., Cushman, S.A. & Gamble, L.R. (2007) A resistant-kernel model of connectivity for amphibians that breed in vernal pools. *Conservation Biology*, **21**, 788–799.
- Cornuet, J.M., Piry, S., Luikart, G., Estoup, A. & Solignac, M. (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.
- Cosentino, B.J., Schooley, R.L. & Phillips, C.A. (2011a) Spatial connectivity moderates the effect of predatory fish on salamander metapopulation dynamics. *Ecosphere*, **2**, 1–14.
- Cosentino, B.J., Schooley, R.L. & Phillips, C.A. (2011b) Connectivity of agroecosystems: dispersal costs can vary among crops. *Landscape Ecology*, **26**, 371–379.
- Coster, S.S., Babbitt, K.J., Cooper, A. & Kovach, A.I. (2015) Limited influence of local and landscape factors on fine-scale gene flow in two pond-breeding amphibians. *Molecular Ecology*, **24**, 742–758.
- Coulon, A., Cosson, J.F., Angibault, J.M., Cargnelutti, B., Galan, M., Morellet, N., Petit, E., Aulagnier, S. & Hewison, A.J.M. (2004) Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. *Molecular Ecology*, **13**, 2841–2850.
- Dawley, E. & Dawley, R. (1986) Species discrimination by chemical cues in a unisexual-bisexual complex of salamanders. *Journal of Herpetology*, **20**, 114–116.
- Denton, R.D., Gibbs, H.L. & Glenn, T.C. (2015) Development of 31 new microsatellite loci for two mole salamanders (*Ambystoma laterale* and *A. jeffersonianum*). *Conservation Genetics Resources*, **7**, 167–170.
- Denton, R.D., Greenwald, K.R. & Gibbs, H.L. (2016) Data from: Locomotor endurance predicts differences in realized dispersal between sympatric sexual and unisexual salamanders. *Dryad Digital Repository*, <http://dx.doi.org/10.5061/dryad.9d9s3>
- Etherington, T.R. (2011) Python based GIS tools for landscape genetics: visualising genetic relatedness and measuring landscape connectivity. *Methods in Ecology and Evolution*, **2**, 52–55.
- Fronhofer, E.A., Klecka, J., Melián, C.J. & Altermatt, F. (2015) Condition-dependent movement and dispersal in experimental metacommunities. *Ecology Letters*, **18**, 954–963.
- Gamble, L.R., McGarigal, K. & Compton, B.W. (2007) Fidelity and dispersal in the pond-breeding amphibian, *Ambystoma opacum*: implications for spatio-temporal population dynamics and conservation. *Biological Conservation*, **139**, 247–257.
- Gershoni, M., Fuchs, A., Shani, N., Fridman, Y., Corral-Debrinski, M., Aharoni, A., Frishman, D. & Mishmar, D. (2010) Coevolution predicts direct interactions between mtDNA-encoded and nDNA-encoded subunits of oxidative phosphorylation complex I. *Journal of Molecular Biology*, **404**, 158–171.
- Gibbs, H.L. & Denton, R.D. (2016) Cryptic sex? Estimates of genome replacement in asexual mole salamanders (*Ambystoma* sp.). *Molecular Ecology*, **25**, 2805–2815.
- Gotzek, D., Axen, H.J., Suarez, A.V., Helms Cahan, S. & Shoemaker, D. (2015) Global invasion history of the tropical fire ant: a stowaway on the first global trade routes. *Molecular Ecology*, **24**, 374–388.
- Greenwald, K.R., Denton, R.D. & Gibbs, H.L. (2016) Niche partitioning among sexual and unisexual *Ambystoma* salamanders. *Ecosphere*, **7**, e01579.
- Greenwald, K.R. & Gibbs, H.L. (2012) A single nucleotide polymorphism assay for the identification of unisexual *Ambystoma* salamanders. *Molecular Ecology*, **12**, 354–362.
- Greenwald, K.R., Gibbs, H.L. & Waite, T.A. (2009) Efficacy of land-cover models in predicting isolation of marbled salamander populations in a fragmented landscape. *Conservation Biology*, **23**, 1232–1241.
- Greenwald, K.R., Purrenhage, J. & Savage, W. (2009) Landcover predicts isolation in *Ambystoma* salamanders across region and species. *Biological Conservation*, **142**, 2493–2500.
- Hanski, I. (2012) Dispersal and eco-evolutionary dynamics in the Glanville fritillary butterfly. *Dispersal Ecology and Evolution* (eds J. Clobert,

- M. Baguette, T.G. Benton & J.M. Bullock), pp. 290–303. Oxford University Press, Oxford, UK.
- Harrison, J.S. & Burton, R.S. (2006) Tracing hybrid incompatibilities to single amino acid substitutions. *Molecular Biology and Evolution*, **23**, 559–564.
- Hellriegel, B. & Reyer, H. (2000) Factors influencing the composition of mixed populations of a hemiclinal hybrid and its sexual host. *Journal of Evolutionary Biology*, **13**, 906–918.
- Homer, C.G., Dewitz, J.A., Yang, L. *et al.* (2015) Completion of the 2011 national land cover database for the conterminous United States - representing a decade of land cover change information. *Photogrammetric Engineering and Remote Sensing*, **81**, 345–354.
- James, E.A., Jordan, R. & Griffin, P.C. (2013) Spatial genetic analysis of two polyploid macrophytes reveals high connectivity in a modified wetland. *Freshwater Biology*, **58**, 2102–2113.
- Johnson, J.R., Johnson, B.B. & Shaffer, H.B. (2010) Genotype and temperature affect locomotor performance in a tiger salamander hybrid swarm. *Functional Ecology*, **24**, 1073–1080.
- Jombart, T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403–1405.
- Jombart, T., Devillard, S. & Balloux, F. (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics*, **11**, 94.
- Jonker, R.M., Kraus, R.H.S., Zhang, Q. *et al.* (2013) Genetic consequences of breaking migratory traditions in barnacle geese *Branta leucopsis*. *Molecular Ecology*, **22**, 5835–5847.
- Julian, S.E., King, T.L. & Savage, W.K. (2003) Novel Jefferson salamander, *Ambystoma jeffersonianum*, microsatellite DNA markers detect population structure and hybrid complexes. *Molecular Ecology*, **3**, 95–97.
- Kearse, M., Moir, R., Wilson, A. *et al.* (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647–1649.
- Kenyon, L. & Moraes, C.T. (1997) Expanding the functional human mitochondrial DNA database by the establishment of primate xenomitochondrial cybrids. *Proceedings of the National Academy of Sciences of the USA*, **94**, 9131–9135.
- Kerr, B., Neuhauser, C., Bohannan, B.J.M. & Dean, A.M. (2006) Local migration promotes competitive restraint in a host-pathogen 'tragedy of the commons'. *Nature*, **442**, 75–78.
- Kokko, H., Heubel, K.U. & Rankin, D.J. (2008) How populations persist when asexuality requires sex: the spatial dynamics of coping with sperm parasites. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 817–825.
- Kraus, R.H.S., Van Hooff, P., Megens, H.J., Tsvey, A., Fokin, S.Y., Ydenberg, R.C. & Prins, H.H.T. (2013) Global lack of flyway structure in a cosmopolitan bird revealed by a genome wide survey of single nucleotide polymorphisms. *Molecular Ecology*, **22**, 41–55.
- Lampert, K.P. & Scharl, M. (2010) A little bit is better than nothing: the incomplete parthenogenesis of salamanders, frogs and fish. *BMC Biology*, **8**, 78.
- Lane, N. (2011) Mitonuclear match: optimizing fitness and fertility over generations drives ageing within generations. *BioEssays*, **33**, 860–869.
- Lee-Yaw, J.A., Sechley, T.H. & Irwin, D.E. (2015) Conflicting effects of microhabitats on long-toed salamander (*Ambystoma macrodactylum*) movement: implications for landscape connectivity. *Canadian Journal of Zoology*, **93**, 1–7.
- Linder, H.P. & Barker, N.P. (2014) Does polyploidy facilitate long-distance dispersal? *Annals of Botany*, **113**, 1175–1183.
- Llewelyn, J., Phillips, B.L., Alford, R.A., Schwarzkopf, L. & Shine, R. (2010) Locomotor performance in an invasive species: cane toads from the invasion front have greater endurance, but not speed, compared to conspecifics from a long-colonised area. *Oecologia*, **162**, 343–348.
- Lowe, W.H. & McPeck, M.A. (2014) Is dispersal neutral? *Trends in Ecology and Evolution*, **29**, 444–450.
- McKenzie, M., Chiotis, M., Pinkert, C.A. & Trounce, I.A. (2003) Functional respiratory chain analyses in murid xenomitochondrial cybrids expose coevolutionary constraints of cytochrome b and nuclear subunits of complex III. *Molecular Biology and Evolution*, **20**, 1117–1124.
- McKnight, M.L. & Shaffer, H.B. (1997) Large, rapidly evolving intergenic spacers in the mitochondrial DNA of the salamander family Ambystomatidae (Amphibia: Caudata). *Molecular Biology and Evolution*, **14**, 1167–1176.
- Michels, E., Cottenie, K., Neys, L., De Gelas, K., Coppin, P. & De Meester, L. (2001) Geographical and genetic distances among zooplankton populations in a set of interconnected ponds: a plea for using GIS modelling of the effective geographical distance. *Molecular Ecology*, **10**, 1929–1938.
- Monaghan, P., Metcalfe, N.B. & Torres, R. (2009) Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecology Letters*, **12**, 75–92.
- Narbonne, P., Simpson, D.E. & Gurdon, J.B. (2011) Deficient induction response in a *Xenopus* nucleocytoplasmic hybrid. *PLoS Biology*, **9**, e1001197.
- Nathan, R., Getz, W.M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D. & Smouse, P.E. (2008) A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences of the USA*, **105**, 19052–19059.
- Newman, R.A. & Squire, T. (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology*, **10**, 1087–1100.
- Noël, S., Labonté, P. & Lapointe, F. (2011) Genomotype frequencies and genetic diversity in urban and protected populations of blue-spotted salamanders (*Ambystoma laterale*) and related unisexuals. *Journal of Herpetology*, **45**, 294–299.
- Olson, R.R. & McPherson, R. (1987) Potential vs. realized larval dispersal: fish predation on larvae of the ascidian *Lissoclinum patella* (Gottschaldt). *Journal of Experimental Marine Biology and Ecology*, **110**, 245–256.
- Paetkau, D., Slade, R., Burden, M. & Estoup, A. (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology*, **13**, 55–65.
- Parmelee, J.R. (1993) Microhabitat segregation and spatial relationships among four species of mole salamander (genus *Ambystoma*). *Occasional Papers of the Museum of Natural History, University of Kansas*, **160**, 1–33.
- Peterman, W.E., Anderson, T.L., Ousterhout, B.H., Drake, D.L., Semlitsch, R.D. & Eggert, L.S. (2015) Differential dispersal shapes population structure and patterns of genetic differentiation in two sympatric pond breeding salamanders. *Conservation Genetics*, **16**, 59–69.
- Petranka, J.W. (1998) *Salamanders of the United States and Canada*. Smithsonian Institution Press, Washington, DC, USA.
- Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. (2006) Invasion and the evolution of speed in toads. *Nature*, **439**, 803.
- Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L. & Estoup, A. (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. *The Journal of Heredity*, **95**, 536–539.
- Purtenhage, J.L., Niewiarowski, P.H. & Moore, F.B.-G. (2009) Population structure of spotted salamanders (*Ambystoma maculatum*) in a fragmented landscape. *Molecular Ecology*, **18**, 235–247.
- Ramsden, C. (2008) Population genetics of *Ambystoma jeffersonianum* and sympatric unisexuals reveal signatures of both gynogenetic and sexual reproduction. *Copeia*, **2008**, 586–594.
- Raymond, M. & Rousset, F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Robertson, A.V., Ramsden, C., Niedzwiecki, J.H., Fu, J. & Bogart, J.P. (2006) An unexpected recent ancestor of unisexual *Ambystoma*. *Molecular Ecology*, **15**, 3339–3351.
- Rocque, D.A., Ben-David, M., Barry, R.P. & Winker, K. (2006) Assigning birds to wintering and breeding grounds using stable isotopes: Lessons from two feather generations among three intercontinental migrants. *Journal of Ornithology*, **147**, 395–404.
- Rollins, L.A., Richardson, M.F. & Shine, R. (2015) A genetic perspective on rapid evolution in cane toads (*Rhinella marina*). *Molecular Ecology*, **24**, 2264–2276.
- Rousset, F. (2008) GENEPOP'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology*, **8**, 103–106.
- Salomon, Y., Connolly, S.R. & Bode, L. (2010) Effects of asymmetric dispersal on the coexistence of competing species. *Ecology Letters*, **13**, 432–441.
- Searcy, C.A. & Shaffer, H. (2008) Calculating biologically accurate mitigation credits: insights from the California tiger salamander. *Conservation Biology*, **22**, 997–1005.
- Semlitsch, R.D. (2008) Differentiating migration and dispersal processes for pond-breeding amphibians. *Journal of Wildlife Management*, **72**, 260–267.
- Semlitsch, R.D., Scott, D.E., Pechmann, J.H.K. & Gibbons, J.W. (1996) Structure and dynamics of an amphibian community: evidence from a 16-year study of a natural pond. *Long-term Studies of Vertebrate*

- Communities* (eds M.L. Cody & J.A. Smallwood), pp. 217–247. Academic Press, San Diego, CA, USA.
- Shine, R., Brown, G.P. & Phillips, B.L. (2011) An evolutionary process that assembles phenotypes through space rather than through time. *Proceedings of the National Academy of Sciences of the USA*, **108**, 5708–5711.
- Smith, M.A. & Green, D.M. (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, **28**, 110–128.
- Stevens, V.M., Polus, E., Wesselingh, R.A., Schtickzelle, N. & Baguette, M. (2004) Quantifying functional connectivity: experimental evidence for patch-specific resistance in the Natterjack toad (*Bufo calamita*). *Landscape Ecology*, **19**, 829–842.
- Toews, D.P.L., Mandic, M., Richards, J.G. & Irwin, D.E. (2013) Migration, mitochondria, and the yellow-rumped warbler. *Evolution*, **68**, 241–255.
- Tracy, C.R., Christian, K.A., Baldwin, J. & Phillips, B.L. (2012) Cane toads lack physiological enhancements for dispersal at the invasive front in Northern Australia. *Biology Open*, **1**, 37–42.
- Uzzell, T. (1969) Notes on spermatophore production by salamanders of the *Ambystoma jeffersonianum* complex. *Copeia*, **1969**, 602–612.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology*, **4**, 535–538.
- Weissgerber, T.L., Milic, N.M., Winham, S.J. & Garovic, V.D. (2015) Beyond bar and line graphs: time for a new data presentation paradigm. *PLOS Biology*, **13**, e1002128.
- Weyrauch, S.L. & Grubb, T.C. Jr (2004) Patch and landscape characteristics associated with the distribution of woodland amphibians in an agricultural fragmented landscape: an information-theoretic approach. *Biological Conservation*, **115**, 443–450.
- Williams, R.N. & DeWoody, J.A. (2003) Fluorescent dUTP helps characterize 10 novel tetranucleotide microsatellites from an enriched salamander (*Ambystoma texanum*) genomic library. *Molecular Ecology*, **4**, 17–19.
- Wolff, J.N., Ladoukakis, E.D., Enriquez, J.A. & Dowling, D.K. (2014) Mitonuclear interactions: evolutionary consequences over multiple biological scales. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **369**, 20130443.
- Wunder, M.B. (2012) Determining geographic patterns of migration and dispersal using stable isotopes in keratins. *Journal of Mammalogy*, **93**, 360–367.
- Zamudio, K.R. & Wiczorek, A.M. (2007) Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. *Molecular Ecology*, **16**, 257–274.
- Zeller, K.A., McGarigal, K. & Whiteley, A.R. (2012) Estimating landscape resistance to movement: a review. *Landscape Ecology*, **27**, 777–797.
- Zhang, F. & Broughton, R.E. (2013) Mitochondrial-nuclear interactions: compensatory evolution or variable functional constraint among vertebrate oxidative phosphorylation genes? *Genome Biology and Evolution*, **5**, 1781–1791.

Received 5 May 2016; accepted 18 November 2016

Handling Editor: Timothy Higham

Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. Loci used for small-mouthed salamanders (*Ambystoma texanum*) and unisexual *Ambystoma* individuals.

Fig. S1. Correlations between log distance travelled during locomotor endurance trials and three potential morphological covariates.