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Genetic data in population viability analysis: case studies with ambystomatid salamanders

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Abstract

Parameterization of population viability models is a complicated task for most types of animals, as knowledge of population demography, abundance and connectivity can be incomplete or unattainable. Here I illustrate several ways in which genetic data can be used to inform population viability analysis, via the parameterization of both initial abundance and dispersal matrices. As case studies, I use three ambysomatid salamander datasets to address the following question: how do population viability predictions change when dispersal estimates are based on genetic assignment test data versus a general dispersal–distance function? Model results showed that no local population was large enough to ensure long-term persistence in the absence of immigration, suggesting a metapopulation structure. Models parameterized with a dispersal–distance function resulted in much more optimistic predictions than those incorporating genetic data in the dispersal estimates. Under the dispersal–distance function scenario all local populations persisted; however, using genetic assignments to infer dispersal revealed local populations at risk of extinction. Viability estimates based on dispersal–distance functions should be interpreted with caution, especially in heterogeneous landscapes. In these situations I promote the idea of model parameterization using genetic assignment tests for a more accurate portrayal of real-world dispersal patterns.

Introduction

Whether isolated populations of organisms are able to persist in fragmented habitats is a question of central importance in conservation biology. Site occupancy and population persistence are affected by both demography (e.g. reproductive rate, immigration/emigration rate), and landscape (e.g. habitat patch size and isolation, and the quality of non-habitat between patches; Fahrig & Merriam, 1994; Fahrig, 2001; Ricketts, 2001; Prugh *et al.*, 2008). In many cases, local persistence requires regional connectivity, and single fragments (patches) may not be sufficient to ensure long-term viability (Marsh, 2008). An improved understanding of the taxon-specific nature of these relationships is critical for the conservation of target organisms. Population viability analysis is an important predictive tool for assessing persistence probability and informing conservation decisions. Perhaps the most common approach to examining population connectivity and viability on a landscape scale is metapopulation modeling (Hanski & Gilpin, 1997; Hanski, 1999), although various other methods have been developed (e.g. Ray, Lehmann & Joly, 2002; Rustigian, Santelmann & Schumaker, 2003; Compton *et al.*, 2007). Metapopulation models use demographic and dispersal parameters to predict metapopulation persistence and size, average local population size and latency to recolonization following local extirpation.

For population viability predictions to be meaningful, they must be based on accurately parameterized models. In many cases, models require input parameters such as initial abundance, dispersal rates and demographic measures (e.g. survival and fecundity), values which may be very difficult to obtain (Halley *et al.*, 1996). Applicability of the metapopulation framework is particularly dependent on the hypothesis of limited dispersal among local populations (Smith & Green, 2005); unfortunately, dispersal is often an especially difficult parameter to estimate (Marsh, 2008). Previous work has taken numerous approaches to this problem, including basing dispersal estimates on mark–recapture data (Schtickzelle & Baguette, 2004) or expert opinion (Gilioli *et al.*, 2008) or modeling dispersal as a constant proportion of individuals per time step (Hels & Nachman, 2002) or a distance function (e.g. Akçakaya & Atwood, 1997; Akçakaya *et al.*, 2004). Use of a dispersal–-distance function assumes that animals are able to disperse equally in any direction; however, real-world landscapes comprise a mosaic of land-cover types that may vary in permeability. Incorporating known movement patterns may lead to vastly altered predictions, a possibility that I explore here.

Genetic assignments of individuals to local populations of origin provide an ideal method for parameterizing metapopulation models, as these data can reveal current dispersal rates when immigrants or their offspring are sampled (Berry, Tocher & Sarre, 2004; Paetkau *et al*., 2004). This method assigns each individual to the most probable population of origin based on background allele frequencies at all sampled sites. Individuals genetically assigned to a site other than their capture location (i.e. 'misassignments') may be immigrants. Despite being widely applied in conservation research, I am not aware of previous studies using assignment tests to parameterize metapopulation models. Here I use three ambystomatid salamander datasets as case studies to address the question: How do viability predictions change when dispersal is parameterized using empirical genetic estimates as opposed to a general dispersal–distance function?

The systems included here are ideal case studies for two important reasons. First, research on population viability is especially important for amphibians, which have undergone global population declines due in large part to habitat loss and degradation (Wake, 1991; Alford & Richards, 1999; Houlahan *et al*., 2000; Stuart *et al*., 2004). Metapopulation models have been used frequently in amphibian studies (e.g. Gill, 1978; Sjögren-Gulve, 1994; Hecnar & M'Closkey, 1996; Driscoll, 1997; Hels & Nachman, 2002) and have been recommended as an important management tool (Semlitsch, 2000; Marsh, 2008). Management actions for amphibians (e.g. the creation of new breeding ponds) are often based on the assumption of a metapopulation dynamic (Marsh, 2008). The popularity of this framework in amphibian research is due to the ongoing creation of discrete patches by habitat fragmentation, as well as the methodological convenience of identifying breeding ponds as habitat patches (Marsh & Trenham, 2001).

Second, several of the conditions for metapopulation structure are already known to be fulfiled in these study systems. These conditions are: (1) habitat patches support local breeding populations; (2) no single population can ensure long-term survival; (3) patches are not too isolated to prevent recolonization; (4) synchronous extinction of all sites is unlikely (Hanski, 1999; Hanski & Gaggiotti, 2004; Smith & Green, 2005). Samples included here were collected from breeding individuals and/or offspring, so is clear that habitat patches support local breeding populations [condition (1)]. Previous research has demonstrated gene flow among patches for all three of the systems, thus patches are sufficiently connected to allow recolonization following local extinction [condition (3); Zamudio & Wieczorek, 2007; Greenwald, Gibbs & Waite, 2009*a*,; Greenwald, Purrenhage & Savage, 2009*b*; Purrenhage, Niewiarowski & Moore, 2009]. Synchronous extinction of all local populations should be unlikely, as the spatial scale of the sampling area is larger than the scale on which we would expect autocorrelation of stochastic extinction events [condition (4)]. Genetic spatial autocorrelation occurs at scales <4.8 km in one of these systems (Zamudio & Wieczorek, 2007), a scale much smaller than the sampling extent (Fig. 1). In this study, I address whether any individual population can ensure long-term persistence [condition (2)]. I also focus on the condition of limited dispersal, and ask how viability predictions change when models are parameterized with distance functions versus genetic assignment test data. Persistence estimates based on empirical dispersal data will allow for more informed management based on an accurate representation of movement in fragmented habitats.

Materials and methods

I analyzed landscapes and microsatellite genetic data for two ambystomatid species in three regions (Table 1, Fig. 1; names reflect species and locations). Datasets consisted of 367–665 individuals from 11 to 29 sites, averaging 24 individuals site^{-1} (Table 1). Dataset Aop-OH was composed of marbled salamander *Ambystoma opacum* samples collected from a cluster of 21 ponds in south-eastern Ohio in spring 2005 (Greenwald *et al*., 2009*a*). The other two datasets consisted of spotted salamander *Ambystoma maculatum* samples from north-east Ohio (Amac-OH; Purrenhage *et al*., 2009) and upstate New York (Amac-NY; Zamudio & Wieczorek, 2007). For dataset Amac-OH, I used only a central subset of 11 sites sampled in 2003, as other sites were well beyond the dispersal capabilities observed for these and more vagile ambystomatid salamanders (Trenham *et al*., 2000; Semlitsch & Bodie, 2003; excluded sites were 15–50 km from the central cluster). Details on sampling methodology and genetic analysis can be found in the associated publications (Table 1; Greenwald *et al*., 2009*b*).

Genetic analysis

Previous publications report the basic population genetic structure for these datasets (Zamudio & Wieczorek, 2007; Greenwald *et al*., 2009*a*,*b*; Purrenhage *et al*., 2009). I conducted genetic assignment tests with GeneClass2 (Piry *et al*., 2004), using standard assignment of individuals for datasets consisting of adults (Amac-OH; 12 sites from Amac-NY) and detection of first generation migrants for datasets composed of larval samples (Aop-OH; 19 sites from Amac-NY; Greenwald *et al*., 2009*a*,*b*). I used a Bayesian computation method (Rannala & Mountain, 1997) and Monte-Carlo resampling (Paetkau *et al*., 2004) with 1000 replicates and an α value of 0.05. Use of the resampling procedure helps to avoid inflated Type I error (falsely identifying residents as immigrants; Paetkau *et al*., 2004), a serious problem when further population viability analysis relies on the identification of immigrants. I generated a pairwise matrix of all individuals assigned as migrants for each dataset to parameterize dispersal matrices in metapopulation modeling. I used two approaches for migrant assignment, one stringent (migrants were only counted if the probability of assignment to a source population was $\geq 80\%$ and $P<0.05$ from the simulation) and one lenient (migrants were assigned to the source population

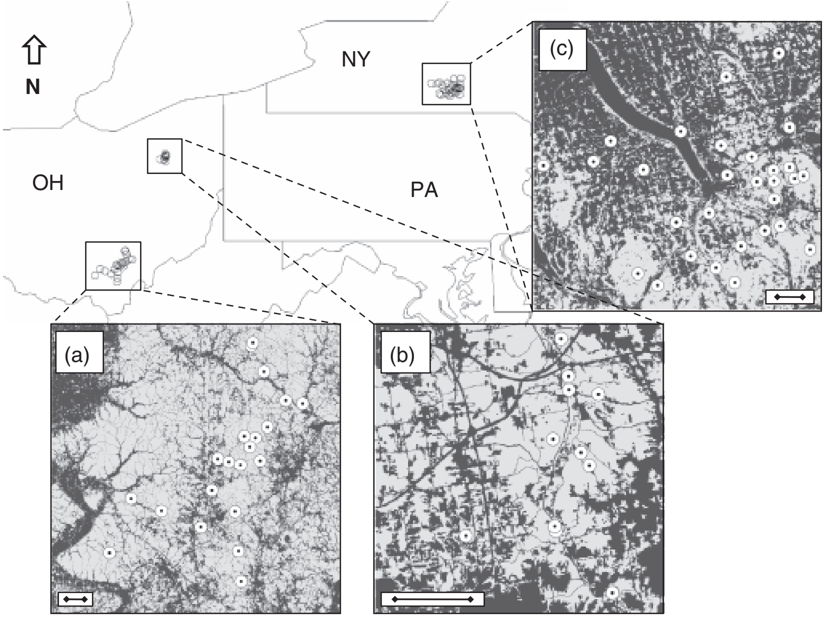

Figure 1 Map showing locations for Ambystomatid salamander datasets Aop-OH [*Ambystoma opacum,* south-east Ohio; inset (a)], Amac-OH [*Ambystoma maculatum*, north-east Ohio; inset (b)] and Amac-NY [*A. maculatum*, upstate New York; inset (c)]. Insets show sampling locations (circles) along with forest (light gray) and non-forest (dark gray) land cover. Inset scale bars each represent 5 km.

Table 1 Three datasets included in this study of the effect of dispersal on population persistence in *Ambystoma* salamanders

Dataset	Aop-OH	Amac-NY	Amac-OH
Species	*Ambystoma opacum*	*Ambystoma maculatum*	*A. maculatum*
Location	Ohio	New York	Ohio
Samples	478 L	404 L/261 A	367 A
Sites	21	29	11
Loci	9	11	8
Spatial extent (km)	1.0–68.2	1.6–46.9	0.1–14.1
Population size	4251 (246–18 493)	704 (150–1355)	20 165 (1698–61 094)
References	Greenwald et al. (2009*a*)	Zamudio & Wieczorek (2007)	Purrenhage *et al.* (2009)

The number of individuals sampled is shown for both larvae (L) and adults (A). Spatial extent indicates the minimum and maximum pairwise distance between sampled sites for each dataset. Population size indicates the means and ranges (in parentheses) estimated by MSVAR. These values are likely overestimated due to gene flow among sites.

with maximum probability of assignment regardless of *P*-value).

I estimated current effective population sizes using MSVAR (Storz & Beaumont, 2002), a program that uses Markov Chain Monte Carlo simulation of coalescence to estimate demographic parameters (e.g. Aspi *et al*., 2006; Goossens *et al*., 2006). I assumed a generation time of 3 years which is typical for these species (Scott, 1994; Pechmann, 1995; Petranka, 1998). I assumed an initial mutation rate for all loci of 1×10^{-4} mutations generation^{-1} and an exponential model of population decline as it is more accurate for recent, sharp declines, which might be expected in populations recently affected by anthropogenic habitat change (Beaumont, 1999). When more than one run was necessary to achieve convergence, each subsequent run was reseeded with a large (four-digit) random number to assure independence between runs. I used a thinning interval of 10 000 steps, resulting in a total of at least 100 000 states (sampled steps in the run) for each population. I used TRACER (Rambaut & Drummond, 2007) to examine output trace files for convergence, and to calculate the effective sample size (ESS) for each variable. I ran the program until every estimated parameter converged (ESS > 200). For dataset Amac-NY I used a random subset of the data (eight loci), as parameter estimates failed to converge with all 11 loci.

Metapopulation parameterization and analysis

I used both geographic configuration and population dynamics in RAMAS/METAPOP to address whether the groups of local populations constitute a metapopulation (Akçakaya, 1998; Akçakaya *et al*., 2004). Specifically, I used the program to address Hanski's second condition, that no single population is large enough to ensure long-term survival. I report the metapopulation occupancy (number of local populations occupied over time) as well as the terminal extinction risk (or quasi-extinction probability), the probability that the metapopulation will fall below a given size by the end of the simulation (Stevens & Baguette, 2008). For each dataset I ran 10 000 replicate simulations over a 100-year period.

I used a discrete, age-structured model (Leslie matrix) for demographic parameters. The four ages were larvae (year 0), 1-year-old juveniles, 2-year-old juveniles and a composite age class for adults (years 3 +). The same input matrix was used for both marbled and spotted salamanders, as demographic parameters from the literature were very similar. Adults were the only reproductive stage, with an average fecundity of 4.0 hatched eggs/female. This number was calculated to incorporate both average clutch size (~100; Mohr, 1930; Noble & Brady, 1933; King, 1935; Savage & Zamudio, 2005) and egg survival (~0.04; Petranka, 1998; Gibbs & Shriver, 2005). I used a wide standard deviation (± 3) to account for potentially high variability in reproductive success. Larval survival was density dependent, and set to 0.14 ± 0.1 (Taylor, Scott & Gibbons, 2006). Survival for both juvenile stages was set to 0.6 ± 0.2, while adult survival was 0.8 ± 0.2 (Taylor & Scott, 1997; Petranka, 1998; Gibbs & Shriver, 2005; Taylor *et al.*, 2006). Both juveniles and adults were allowed to disperse (Savage & Zamudio, 2005; Gamble, McGarigal & Compton, 2007). Stochasticity was incorporated into the dispersal estimate by allowing it to vary by a common coefficient of variation (CV = 0.001). These conditions produced a relatively stable finite rate of increase ($\lambda = 1.001$), allowing me to evaluate the outcome of the various dispersal scenarios described below.

Initial abundances and dispersal matrices were parameterized using genetic data (Fig. 2). For the former, I used effective population sizes as estimated by MSVAR as the initial abundance of adults for each local population; initial abundances for the other stages were estimated by RAMAS/METAPOP from the stable age distribution matrix. A genetic estimate of population size was used due to a lack of ecological data for these populations; additionally, initial population size has been shown to have no effect on the trajectory and quasi-extinction probabilities of simulated populations (Schtickzelle & Baguette, 2004). To determine the sensitivity of the model to variation in dispersal, I compared results parameterizing the model four different ways: no dispersal, a dispersal–distance function (‘distance function’ model) and with genetic assignment tests of both low stringency resulting in many migrants (‘high dispersal’ model) and high stringency resulting in few migrants (‘low dispersal’ model). Under the ‘no dispersal’ model, all pairwise dispersal rates and stochasticity were set to zero. For the distance function model, the dispersal matrix was calculated from a given dispersal-by-distance relationship using pairwise distances between sites. The function was a normal curve with mean 0 and standard deviation 440.1 m as described for dispersing juvenile *A. opacum* by Gamble *et al.* (2007).

The ‘high dispersal’ model assumed symmetrical dispersal, and used low-stringency genetic assignment test results, that is immigrants were assigned to the source population of maximum likelihood regardless of probability level. The ‘low dispersal’ model assumed asymmetrical dispersal and used high-stringency assignment test results; individuals were only included if they were assigned as immigrants with $P<0.05$ in the GENECLASS simulation and with $\geq 80\%$ probability of assignment to a given source population. To illustrate the difference between symmetrical and asymmetrical dispersal values, suppose sites A and B each had 25 sampled individuals. Two individuals were assigned as moving from A to B, while one individual moved from B to A. Under symmetrical dispersal (for the ‘high dispersal’ model), I summed all migrants ($2+1=3$) and divided by the total number of individuals sampled at both sites ($25+25=50$) for a dispersal rate of 0.06. This value was then used as the dispersal rate in both directions (A to B and B to A). However, under asymmetrical dispersal (for the ‘low dispersal’ model), the rate from A to B would be $2/25=0.04$, while dispersal from B to A would be $1/25=0.02$.

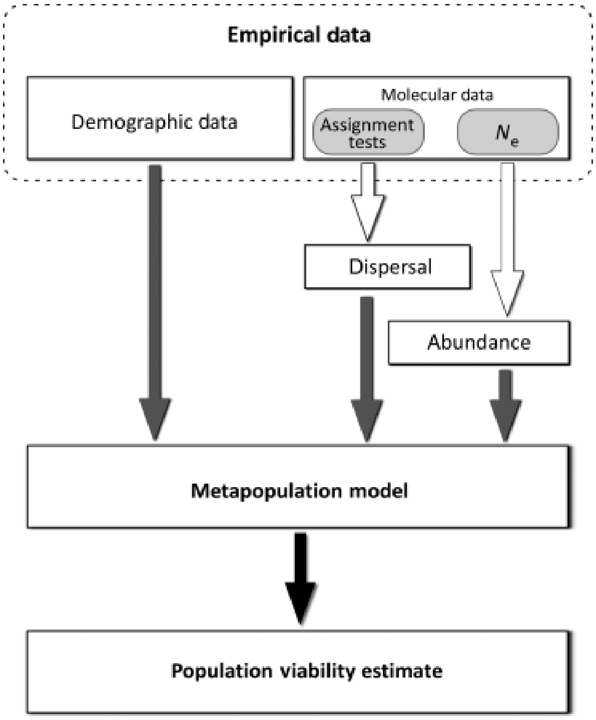

Figure 2 Schematic representation of the methodology used in this study. White arrows represent indirect estimation of population parameters; gray and black arrows represent metapopulation model input and output, respectively. Empirical data (top) can be used either directly (demographic data) or indirectly (molecular data) to parameterize metapopulation models. See the ‘Materials and methods’ for a detailed explanation of how empirical data were converted to model parameters.

Results

Dispersal matrices using low-stringency versus high-stringency assignment test results varied considerably within dataset. With low-stringency assignment tests, the number of individuals assigned as immigrants (and included in

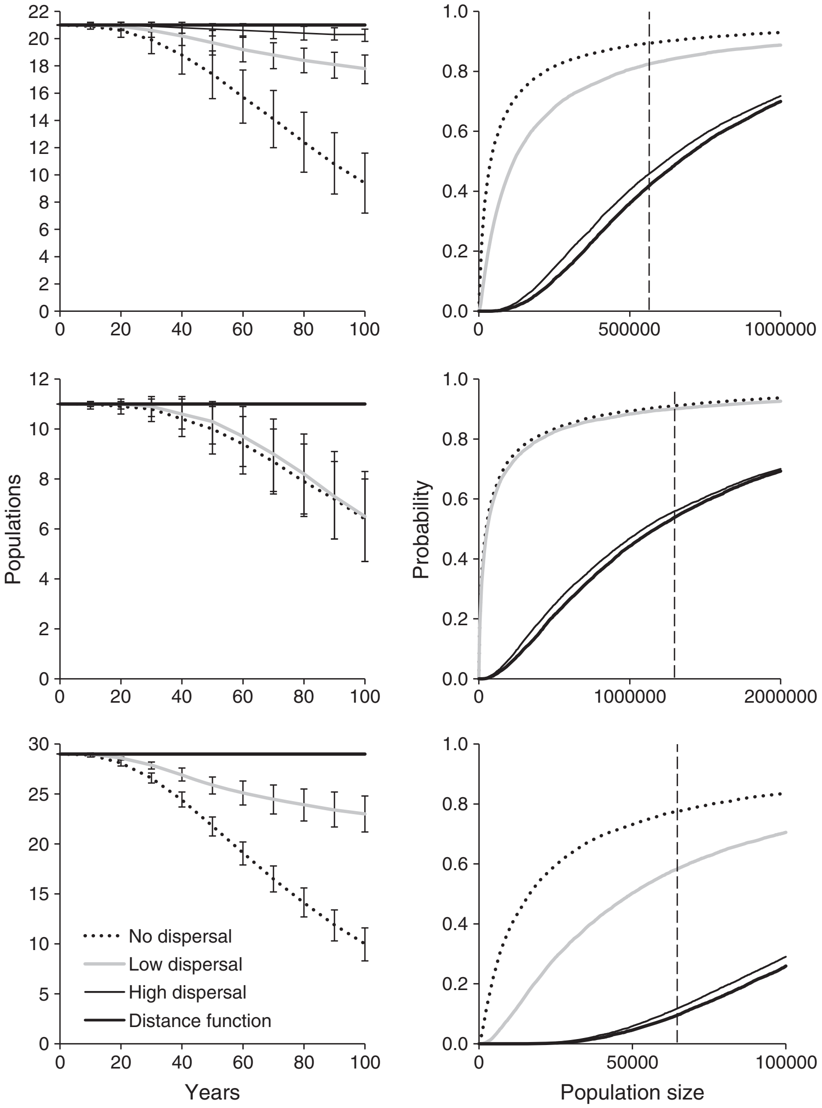

Figure 3 Local population occupancy (left) and terminal extinction risk (right) for datasets Aop-OH (top), Amac-OH (middle) and Amac-NY (bottom). Local population occupancy shows the number of local populations occupied across time over the 100-year simulation. Terminal extinction risk shows the probability that the metapopulation will be below a given population size at the end of the simulation. The vertical dashed lines indicate the approximate starting population sizes for each metapopulation.

dispersal matrices) was 198, 117 and 377 for datasets Aop-OH, Amac-OH and Amac-NY, respectively. However, with high-stringency assignment tests these numbers were vastly reduced, to 32, 9 and 58, respectively. This variation considerably affected predictions for population persistence and terminal extinction risk. Current population size as estimated by MSVAR were also variable, ranging from 246 to 18 493 (mean = 4251) for dataset Aop-OH, 1698 to 61 094 (mean = 20 165) for dataset Amac-OH and 150 to 1355 (mean = 704) for dataset Amac-NY (Table 1); however, this affected predictions much less than variation in dispersal regimes.

The three datasets had similar population trajectories under the extreme dispersal scenarios (no dispersal and the dispersal–distance function based on purely demographic inferences of dispersal; Fig. 3). Under no dispersal, all local populations of all three datasets eventually went extinct. I conducted longer *a posteriori* simulations (300 years) to estimate time to metapopulation extinction for each dataset under this scenario. With no dispersal, Aop-OH fell below one remaining population after 234 years, Amac-OH after 218 years and Amac-NY after 226 years. Within the 100-year simulation, Aop-OH and Amac-OH had over 90% probability of decline, while Amac-NY had a 78% probability of decline (Table 2). On the other hand, the dispersal–distance function scenario resulted in persistence of all local populations and low probabilities of decline (Fig. 3; Table 2). The local populations do not constitute a metapopulation under these scenarios, as the probability of recolonization of local populations is either 0% (no dispersal) or 100% (dispersal–distance function).

Patterns of population persistence were more variable when genetic data were used to parameterize dispersal matrices (Fig. 3; Table 2). Under high dispersal (using assignments with no probability cutoff), Amac-OH and Amac-NY results were identical to the dispersal–distance

Table 2 Probability of decline (population size at end of simulation $<$ population size at beginning of simulation) for each of three datasets under four dispersal scenarios

Scenario	Aop-OH	Amac-OH	Amac-NY
No dispersal	0.90 ± 0.01	0.91 ± 0.01	0.78 ± 0.01
Low dispersal	0.83 ± 0.01	0.90 ± 0.01	0.60 ± 0.01
High dispersal	0.47 ± 0.01	0.57 ± 0.01	0.13 ± 0.01
Distance function	0.43 ± 0.01	0.55 ± 0.01	0.11 ± 0.01

Under 'no dispersal,' all pairwise dispersal values were set to 0. Low and high dispersal values were based on genetic assignment tests of different stringencies (see 'Materials and methods'). Under the distance function, dispersal was a function of distance among sites based on empirical data (Gamble *et al.*, 2007).

function. That is, no local populations went extinct, and the probability of decline was low. However, Aop-OH had one local population that received no immigrants even under the high dispersal scenario. This population was lost and accounts for the difference between the distance function and high dispersal scenarios. The probability of decline under high dispersal ranged from 13 (Amac-NY) to 57% (Amac-OH; Table 2). Under low dispersal (using assignments with $P < 0.05$ and 80% assignment likelihood), Amac-OH followed a nearly identical extinction trajectory as under no dispersal, while Aop-OH and Amac-NY lost some local populations (4 and 6, respectively) but then stabilized (Fig. 3). The probability of decline under low dispersal ranged from 60 (Amac-NY) to 90% (Amac-OH; Table 2). Longer simulation under low dispersal showed that Amac-OH fell below one remaining population after 224 years, while Aop-OH and Amac-NY never fell below one local population even in 300-year simulations.

Discussion

This study demonstrates the utility of genetic data in population viability analysis, a promising method that has been suggested but not extensively used (Storfer *et al.*, 2007). I show that incorporating empirical dispersal data from genetic assignment tests can greatly alter population viability predictions from metapopulation models. The sensitivity of the persistence estimates to the connectivity matrix illustrates the point that PVA results may be misleading if dispersal is not parameterized accurately. I suggest that this point might be especially relevant for studies conducted in fragmented or otherwise heterogeneous habitats, as the assumption that an organism can move with equal probability in any direction (i.e. parameterization with a dispersal–distance function) is clearly violated in those conditions.

The case studies included here illustrate that population viability predictions change dramatically when dispersal estimates are based on genetic data as opposed to a general dispersal–distance function. Models using distance functions predicted persistence in all cases, while those with genetic data were less optimistic. For one dataset (Amac-OH) so few individuals were assigned as immigrants with high likelihood that the low dispersal model was functionally equivalent to the no dispersal model (although this may be misleading). The remaining two datasets lost four and six local populations under the low dispersal model. No individuals were assigned as immigrants at these sites, and thus recolonization was impossible following stochastic local extinction. In the absence of immigration, all local populations went extinct even under relatively optimistic population size and vital rate estimates, suggesting that these local populations likely do function as a metapopulation. Altogether, these results suggest that conservation of single local populations may be insufficient to protect ambystomatid salamanders and that population connectivity is also critical for persistence, a result complementing previous research in amphibian conservation (Hels & Nachman, 2002; Cushman, 2006; Marsh, 2008).

Connectivity matrices parameterized with genetic data could be subject to two sources of error. First, the reliance on assignment tests to estimate dispersal presents the possible issue of 'false positives,' that is individuals that are assigned as immigrants but are in fact residents. Use of the stringent ($P = 0.05$, likelihood $> 80\%$) cutoff for assignment was intended to avoid this issue; however, inclusion of such individuals may still occur and may cause dispersal values to be overly optimistic. Second, dispersal could in fact be underestimated if the sampled region contained high numbers of unsampled (ghost) populations. This would result in immigrants from unsampled source populations never being assigned with high likelihood; thus it would appear that there were few immigrants when in fact there could be many. This seems likely to be the case for dataset Amac-OH, as a previous analysis showed relatively high levels of gene flow in this system (Purrenhage *et al.*, 2009). For this dataset and other similar cases, use of the 'high dispersal' parameterization scenario is likely the best approach, as metapopulation model results may be overly pessimistic using more stringent assignment tests. Additionally, it should be noted that the high and low dispersal scenarios presented here are in fact points on a continuum. While useful for illustrating the methodology, alternative assignment methods or likelihood cutoffs might be more appropriate for other systems.

The use of genetic data to estimate population size also has potential to introduce error into persistence estimates, although I suggest that this is a less important issue. Sensitivity analyses have shown that initial population size has very little effect on persistence estimates (Schtickzelle & Baguette, 2004), so any error introduced with these estimates should not greatly skew PVA results. Indeed, *a posteriori* analysis of the datasets included here showed that initial population size had no qualitative effect on the simulation trajectories. Additionally, any bias introduced this way would be positive, that is populations would be represented as larger than they actually are, and persistence would then be more likely. This is because effective population size estimates are affected by migration, reflecting an estimate of the 'genetic neighborhood' size rather than a single sampled site. However, local extinctions still occurred in simulations despite this potentially optimistic bias.

In conclusion, these results suggest that genetic data can be an extremely valuable tool for population viability analysis. Genetic estimates of dispersal may be especially useful in heterogeneous landscapes, as dispersal–distance functions do not account for variation in landscape permeability and may therefore overestimate population persistence and viability. Genetic data can be used to identify source populations as well as those at risk of local extinction, which may aid in allocating limited conservation resources. For the datasets examined here, management on a local scale may temporarily protect functional subpopulations of ambystomatid salamanders; however, this approach does not appear sufficient to ensure long-term persistence. Steps must be taken to maintain or enhance regional connectivity, as no local population was large enough to persist in isolation. Improvement of connectivity in these and similar landscapes could help shift metapopulation dynamics further toward the 'high dispersal' end of the continuum, thus improving persistence and viability estimates.

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COMMENTARY

Cautious optimism for applied conservation genetics and metapopulation viability analysis

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Among conservation biologists there is little argument that habitat loss and fragmentation are the greatest threats to future biodiversity. We know from elementary population ecology that, if isolated, even large populations will ultimately be driven to extinction by demographic stochasticity, and that this will occur more rapidly for small populations in small fragments. Theory and observations also show that post-fragmentation species losses can occur slowly, sometimes requiring more than a century for the total 'extinction debt' to be realized (Tilman *et al.*, 1994; Vellend *et al.*, 2006). For long-lived animals, such as ambystomatid salamanders, isolated populations doomed to demographic irrelevance and ultimate extinction may persist for decades or longer (Greenwald, 2010). I think of the animals occupying these isolated habitats with little chance of long-term survival as 'zombie' populations. As imperfect as our population models are, they represent our best chance for understanding the threats to populations at risk and possibly recovering them from zombie status.

Population and metapopulation viability analyses are valuable tools for illustrating, in an understandable way, the risk of extinctions, and for exploring probabilities of local and regional persistence under different scenarios (Morris & Doak, 2002; Marsh, 2008). I see the greatest value of these modeling exercises in their ability to help land managers to evaluate alternative management actions and appreciate their relative costs and likely benefits. As Greenwald (2010) shows, actions to promote connectivity can enhance population viability. Restoring connectivity to fragmented landscapes is often extremely costly (think wildlife overpasses), and avoiding fragmentation completely may be even more controversial (think not building the freeway at all). So, models evaluating the importance of habitat connectivity must be convincing to those people on the hook for making big decisions. The greatest barrier to constructing convincing models is obtaining parameter estimates in which we have confidence, which traditionally means labor- and time-intensive mark–recapture studies. Fortunately, the recent work of Greenwald (2010) in addition to others indicates that molecular tools may be surprisingly robust to the challenge of more rapidly and painlessly estimating at least some demographic parameters needed for landscape-scale population modeling.

As someone who has invested years in the field marking and recapturing animals, I am optimistic at the promise these methods potentially hold (Trenham *et al.*, 2000; Trenham, Koenig & Shaffer, 2001). The ability of genetic assignment tests to accurately reveal residents and immigrants in samples could change the study of dispersal. My caution in getting too excited about the current abilities of these methods stems from my understanding of several issues that can substantially bias the results. The author addressed the issue of unsampled 'ghost' populations. This is a real issue for animals like ambystomatid salamanders where large fractions of the adult population commonly skip breeding and are completely undetectable, thus samples from a single year are unlikely to accurately represent the standing genetic variation present in a population. Despite these issues, one recent study is very encouraging. A detailed study with marked skinks, occupying and dispersing among patches of rocky habitat, showed that assignment tests usually recognized immigrants with high confidence. And further, using skink tissues collected over 3 months, the assignment tests yielded estimates of interpatch dispersal rates essentially identical to those estimated over 7 years of mark–recapture efforts (Berry, Tocher & Sarre *et al.*, 2004). At this point, I think there is still need for more cross validation of these methods with mark–recapture studies in other taxa and landscapes, especially if we are to use these data predicting metapopulation viability.

If these methods produce robust predictions they have the potential to greatly assist conservation planners. For example, the California tiger salamander *Ambystoma californiense* is the species about which I know the most, and one whose management would benefit greatly from the types of genetic data and the coupled modeling approach explored by Greenwald (2010). The most extreme situation exists in a 15 km by 5 km region of Sonoma County, California, USA, which is home to the entirety of a federally endangered Distinct Population Segment of *A. californiense*. The entire area is severely fragmented by a dense road network, development and agriculture. There are roughly 50–100 ephemeral breeding pools in which breeding has been observed during the last 10 years, and many of these are situated on small regional preserves – the largest encompasses just 70 ha. Because my empirically based estimate is that >125 ha is required to encompass the normal migratory movements of$\sim$95% of salamanders around a single pond, I have serious concerns about long-term population viability on any of these preserves (Trenham & Shaffer, 2005). Although annual larval sampling indicates that the species remains widely distributed, there is essentially no recent information on breeding population sizes nor has there been any study of movement. Because California tiger salamanders can live 10 years or more, without additional information it is unclear whether populations are viable or entering the realm of the zombies. If genetic data could provide reliable estimates of population size or recent dispersal among ponds, or ideally contribute to a realistic metapopulation viability analysis, I can imagine many ways that available resources might be more effectively focused to improve the prospects for this highly endangered population segment.

Although approaches that make use of molecular genetic data are potentially very useful, for some purposes there is still no substitute for capture–recapture studies; I know of no other means for estimating survival. The modeling approach used by Greenwald (2010) relied heavily on generalized survival probabilities estimated at sites far from the study sites. This was a necessary simplification, but it limits my confidence in the specific predictions of the model. There is a great need for additional work to estimate survival in differing landscapes. Most capture–recapture studies are conducted on protected lands where the species of interest is abundant and conditions are generally benign. In contrast, the focal areas in this study, and those where managers often target conservation efforts, are often far less ideal. There was no real discussion in the paper regarding the habitat quality, level of fragmentation, and the degree to which these sets of ponds were thought to be isolated from other possible source populations. If mortality in these landscapes is substantially higher than the assumed values, model populations and metapopulations would most likely decline to extinction rapidly, even with the high dispersal values.

Finally some words about amphibians and metapopulations. My personal observations are that just because many amphibians use naturally patchy breeding ponds, the metapopulation label cannot be accurately applied to pond-breeding amphibians as a group. My own studies, and an extensive literature review indicated that in unfragmented habitat there is often too much dispersal and not enough extinction for classical metapopulation dynamics to be observed (Marsh & Trenham, 2001; Trenham *et al.,* 2001). However, in marginal and fragmented habitats many amphibian populations do exhibit dynamics consistent with classical or non-equilibrium (i.e. declining) metapopulations (Marsh & Trenham, 2001). I see real value in the metapopulation modeling approach advocated by Greenwald's (2010) work, especially as a tool for exploring general guidelines for land management. If we hope to make confident predictions about the probability of extinction/persistence for a regional population of salamanders or other animals, survival, dispersal and other potentially important parameters should be estimated within the system of interest. With improving genetic methods to model dispersal, and powerful computational tools to estimate demographic parameters from marked populations, our ability to apply Greenwald's (2010) vision of landscape-scale population modeling is nearly within reach. Ultimately we need a tool like this to help convince decision makers that maintaining and promoting habitat connectivity remains our most important approach in the pursuit to minimize the number of zombie populations.

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COMMENTARY

Predicting the fate of metapopulations is aided by DNA fingerprinting of individuals

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Population viability analysis (PVA) and genetic assignment tests are two important items in the toolbox of contemporary conservation biologists, but appear to have little in common as they are applied to scientific problems of different scale and scope. PVAs use life history and environmental data to predict whether populations are able to survive a certain period of time, for example comparing different management strategies. Assignment tests, on the other hand, are statistical procedures applied to individual multi-locus DNA fingerprints (most often derived from microsatellites), and measure the likelihood with which a genetic profile originates from a specific gene pool. Applied to wild populations, assignment tests can, for example, identify individual migrants, which bear genetic signatures that match better to a distant population rather than to the population where they were sampled.

In a remarkable paper that uses three datasets from temperate urodele amphibians (North American salamanders of the genus *Ambystoma*), Greenwald (2010) explores assignment tests to quantify the degree of connectivity between demes comprising a metapopulation, an important parameter to predict whether a species is locally able to survive. The general idea is that empirically collected genetic data should provide a more flexible and realistic measure of migration than the commonly applied approach of spatial dispersal modelling (assuming a general dispersal function calibrated with field data, in which short-distance movements are common and long-distance movements are rare). Greenwald (2010) shows that assignment tests, although they only approximate the true figures of dispersal depending on statistical power and theoretical assumptions, lead to a potentially more realistic parameterisation of migration rates in PVA models, and, as an important consequence, to markedly lower estimates of metapopulation survival. Moreover, in the absence of population census data such as derived from capture–mark–recapture, Greenwald (2010) also used genetic data to measure individual abundances in each dataset approximated through effective population sizes (N_e).

With regard to expanding our knowledge about the dynamics of populations in fragmented habitats, Greenwald (2010) usefully bridges different analytical levels that are usually unconnected with each other. This can be illustrated by looking at two further studies conducted on a pond-breeding urodele amphibian, the European crested newt *Triturus cristatus*. Using a predominately genetic approach, Jehle *et al.* (2005) revealed that between-deme dispersal rates are usually asymmetric (mostly, large demes serve as sources for small demes, which constitute sinks), and that dispersal events appear to some degree stochastic (not all ponds that could exchange individuals based on their geographic distance actually do so). However, despite detailed gene flow information combined with genetic N_e estimates, the study failed to draw any conclusion about population size fluctuations and metapopulation viability. Griffiths, Sewell & McCrea (2010), on the other hand, investigated connected *T. cristatus* populations based on known life histories of adults, and predicted that population persistence was largely dependent on adult survival (negatively affected by mild winters) and modelled pond connectivity via juveniles, which were only produced in some pond–year combinations. As recruits were impossible to mark in the field, measuring actual rates of gene flow and migration across all life stages remained however elusive. What Greenwald (2010) did with *Ambystoma* is precisely what would be desirable for subdivided *T. cristatus* populations: completing our picture of present and future population dynamics by incorporating powerful tools from demography and genetics across different scales.

Building upon the work of Greenwald (2010), aspects that deserve further investigation relate to the distinctions between census and effective population size, as well as migration *per se* and gene flow through reproduction of immigrants. In tailed amphibians, the former two differ from each other by a factor of about 2–10 (e.g. Jehle *et al.* 2005). No empirical data are available on the reproductive success of immigrants in recipient populations, but it is likely that not all are successful in finding mates. In Greenwald's three study systems, the differential sampling regimes (either adults, which can be migrants, or larvae, which can only be descendants of migrants) allow a quantification of either of the two connectivity measures, and it would be possible to discern between them through adjusted PVA models. Another, perhaps more important aspect of consideration is that genetic assignment tests are only able to measure dispersal over a timescale of usually 1–2 generations. Over the longer periods that are typically projected with PVAs (in the case of Greenwald, 100 years), the extent and direction of migration is, however, likely subject to temporal fluctuations: source demes can turn into sinks and vice versa, and the actual migration rate can for example depend on the yearly amount of recruitment (in a good year, an excess of offspring in one pond might result in a temporary wave of immigration in a neighbouring pond, see for example Griffiths *et al.* 2010). Although a practically tedious endeavour, it would therefore be worthwhile to repeatedly obtain dispersal measures from assignment tests across several seasons or generations. A further question that should be addressed in the future is whether assignment tests as a general rule produce more pessimistic viability estimates for metapopulations than estimates based on spatial dispersal modelling, or whether this result is specific to Greenwald (2010). This needs the incorporation of alternative distance functions based on further field-derived migration data, and/or an extension of the approach to other study species and systems.

Although it has been demonstrated in many occasions, Greenwald (2010) once again brings to our attention that the precision of PVAs crucially depends on the extent to which the model parameters reflect the real world. The probably most often cited quote in ecological modelling, 'essentially all models are wrong, but some are useful' (by George Box, a son-in-law of Sir Ronald Fisher) can be easily translated into a more positive 'the best models are the most useful ones'. Greenwald (2010) builds a remarkable bridge between analysing individual DNA fingerprints and predicting the fate of whole metapopulations, and the approach has a lot of potential to improve existing PVA models whenever sufficient population genetic data are available.

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COMMENTARY

Genetic estimation of dispersal in metapopulation viability analysis

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For geographically closed populations, it has long been known that the dynamics of rare populations are affected by demographic stochasticity, and that a short string of 'bad luck' (of the random variable demographic outcomes in a finite population) can easily drive rare populations to extinction. The commonly taught approximation for calculating this extinction risk, $P(\text{extinction}) = (\frac{d}{b})^{N_0}$ (where d, b and N_0 are the respective death rate, birth rate, and initial abundance), clearly shows how sensitive population fate can be to initial abundance (Gotelli, 2008). However, when populations are geographically connected to others, immigration can 'rescue' rare populations from becoming extinct, and the connectivity of multiple populations created by dispersal greatly bolsters the persistence probability of the overall population. As dispersal amongst fragmented habitats increases a population becomes panmictic; overall abundance is enhanced through connectivity and the impact of demographic stochasticity on extinction risk decreases. Conversely, if dispersal is completely impeded by the loss of connectivity among habitat patches, the dynamics in each local population will be more greatly affected by local abundance, birth and death (Clobert *et al*., 2001). Between the realms of zero and panmictic dispersal resides the concept of a metapopulation (Levins, 1969, 1970).

Theoretical studies have demonstrated the positive nonlinear relationship between metapopulation viability and the rate of dispersal among the component sub-populations (Hanski, 1999). Thus, it is not surprising that Greenwald (2010) found metapopulation viability in ambystomatid salamanders to be affected by methodological approaches to estimating dispersal that produce disparate results. The genetically based approach to estimating contemporary (as opposed to historical) rates of dispersal in a metapopulation viability framework will nevertheless be of use to many. The unique viability issues that a rare or declining population faces often requires development of original population models that match the available data and knowledge. For future users of Greenwald's approach, I suggest using a programming language to develop a population model that matches the situation (Caswell, 2001; Bolker, 2008). Use of canned software packages can produce biased predictions when the user is forced to make complex modeling assumptions about levels of density dependence, environmental stochasticity, probability density functions describing stochastic processes, mutation rates and more (Morris & Doak, 2002). Moreover, it is not universally true that population viability is insensitive to initial abundance (Greenwald, 2010). Because of demographic stochasticity, Allee effects, inbreeding depression and other factors, a small population's risk of extinction can be highly sensitive to abundance (e.g. see the simple equation above). Thus, careful attention should be paid to both the actual and genetically effective levels of abundance that viability projections are initiated at.

Nevertheless, the assumptions made in Greenwald's (2010) models should not take away from the novel use of genetically based estimates of dispersal in a metapopulation viability analysis. In the past, many have used dispersal–distance functions to parameterize dispersal in population models. Underlying these functions is the biological assumption that a landscape is homogeneous; a single probability density function describes an individual's random chance of moving a given distance, regardless of where it is coming from, where it is going to, and the habitat it must cross to get there (i.e. akin to the dispersion of gas molecules in a room). Dispersal–distance functions might adequately describe wind dispersal of seeds, movement of invertebrate larvae in oceans and other passive forms of dispersal. However, a single homogeneous function cannot capture the complex processes involved in the movement of a vagile animal from one location to another across a heterogeneous landscape that is reap with both costs and benefits to dispersal and philopatry.

Greenwald's (2010) use of genetic-assignment tests for estimating contemporary dispersal in and out of specific locales (Berry, Tocher, & Sarre, 2004; Paetkau *et al.*, 2004) offers a realistic alternative for incorporating dispersal into spatial population models, especially for animals that make active dispersal decisions. For each patch of suitable habitat (e.g. breeding ponds for ambystomatid salamanders) within a region, biologists can assay a range of loci for a sample of individuals and determine the most common genetic make-up for each sub-population (or geographic locale). The genetic make-up for each individual can then be compared to the sub-population mean to determine the likelihood that the individual was born in the local sub-population relative to the likelihood that the individual is an immigrant that was born elsewhere. The more this process is repeated across suitable habitats within a region, the greater the chances that an immigrant's origin can be assigned; thus providing information about the emigration, transfer and immigration phases of dispersal rates between specific locales in a heterogeneous landscape.

A further appeal of the genetic-assignment test for estimating contemporary dispersal is that all organisms have DNA. Given that appropriate tests can be developed for an organism's mode of reproduction (sexual, asexual, etc.) and level of chromosome ploidy, the genetic-assignment approach could serve as a universal way of estimating heterogeneous dispersal amongst locales within a metapopulation. We must nevertheless remember that assignment based on analysis of a small set of gene loci could lead to high rates of misclassification and biased estimates of dispersal. In the future, it would be useful to compare estimates of dispersal attained from genetic-assignments tests to those attained from a rigorous multi-location (i.e. multi-state) capture–mark–recapture analysis (Lebreton & Pradel, 2002). Such comparisons could help identify sources of bias in dispersal estimated from genetic-assignment tests. If sources of bias were consistent, then they could be accounted for with advancement of the statistical models.

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RESPONSE

Genetic data in population viability analysis: guidelines for future research

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The use of genetic data in population viability analysis has promise as a broadly applicable approach to addressing conservation questions (Greenwald, 2010). However, the commentaries on my article in this issue (Jehle, 2010; Koons, 2010; Trenham, 2010) bring up several important points that warrant reiteration for researchers interested in conducting similar analyses. Toward that end, I outline here three issues that should be considered before applying this methodology, and include for each the 'ideal world' scenario. Researchers must decide on a case-by-case basis how well a particular dataset or study system conforms (or fails to conform) to the ideal, as this will determine the accuracy and broader applicability of model results.

(1) *Appropriate conceptual framework*. Since the development of the metapopulation framework, it has been used extensively, especially in amphibian studies. However, as Trenham (2010) points out, the underlying assumptions are not always addressed or tested (Marsh & Trenham, 2001; Smith & Green, 2005). For example, of 53 studies reviewed by Smith & Green (2005), only 10 tested whether any single population was large enough to ensure long-term survival, and only three tested whether local dynamics were asynchronous. Regardless of the organism of interest, this is a critical first step to consider if the goal is to use RAMAS/Metapop (Akçakaya, 1998) or another modeling method that assumes metapopulation structure. This framework is not appropriate for organisms in large, continuous populations (i.e. no probability of local extinction) or for those in completely isolated populations (i.e. no probability of recolonization); in these cases alternative population models must be developed that do not assume metapopulation structure. Indeed, Koons (2010) suggests development of situation-specific population models, which allows for greater flexibility in defining the underlying assumptions. Clearly, the goal should be as close a match as possible between population models and the 'real world' scenario, and careful consideration should be given to population structure and demographic parameters before model development. All models will provide an answer; the accuracy and relevance of that answer depend entirely on the quality of model development and parameterization.

(2) *Availability of relevant demographic data*. Ideally, data on population parameters such as mortality and fecundity would be available from long-term capture–-mark–recapture (CMR) studies of the populations of interest. These data would serve three important purposes. First, they would act as a comparison or replacement for genetic estimates of effective population size, which (as noted by the commentaries) may influence model results in some systems (Koons, 2010) and may vary greatly from census population sizes (Jehle, 2010). Second, dispersal events documented by CMR could validate assignment test results (Berry, Tocher & Sarre, 2004; Koons, 2010; Trenham, 2010). Finally, I reiterate an excellent point made by Trenham (2010): demographic parameters likely vary depending on landscape context. For example, mortality might be underestimated by using values derived from populations in pristine landscapes, and this could clearly influence model results. As he noted, this was a necessary simplification in my study, but ideally demographic data would come from the populations of interest or from populations in similar landscapes.

The assessment of demographic parameters along fragmentation gradients, while logistically challenging, would be an extremely valuable direction for future research. This would allow for accurate model parameterization across a range of landscapes, as well as further comparisons of models based on dispersal–distance curves versus genetic assignment tests (e.g. is there a certain critical level of habitat heterogeneity above which assignment tests outperform dispersal–distance functions?).

(3) *Thorough and representative genetic sampling*. It is important to consider the spatial and temporal extent of sampling when designing a population genetic study. An ideal study system would consist of all potential source populations within a biologically meaningful dispersal

distance. If this is not feasible, awareness of the quantity and location of these unsampled 'ghost' populations would aid interpretation of assignment test results. If there are numerous ghost populations, a less stringent assignment test approach might be most appropriate (e.g. the 'high dispersal' scenario; Greenwald, 2010). The ideal temporal extent of sampling will depend on the organism of interest; multiyear sampling would be especially valuable in cases with potential temporal variation in population genetic structure (e.g. different breeding cohorts) or in dispersal across years (Jehle, 2010; Trenham, 2010).

Another important consideration is balancing the number of sampled individuals and the number of loci. Several simulation studies have explored the power of assignment tests under various combinations of number of alleles, loci, individuals, populations and levels of population differentiation. For moderately differentiated populations ($F_{ST} = 0.1$) one study showed that 100% correct assignment could be obtained using a Bayesian assignment method with 10 microsatellite loci from 30 to 50 individuals from each of 10 populations (Cornuet *et al*., 1999). Adding additional loci appears to increase power much more than adding polymorphism (alleles/locus; Bernatchez & Duchesne, 2000) or increasing the number of sampled individuals (Paetkau *et al*., 2004).

In summary, the approach of combining population genetic data and viability analysis can be a useful tool for conservation research, with the potential to identify source populations, populations at risk of local extinction, and persistence estimates for population networks. It may be especially valuable for organisms in fragmented or degraded habitat, where the assumption underlying dispersal–distance curves (equal probability of dispersal in any direction) is likely to be violated. I will be interested in the success of this technique in other systems, especially for long-term study systems where the 'ideal' conditions outlined above can be met or approached (e.g. the European crested newt *Triturus cristatus*, Jehle, 2010; or the California tiger salamander *Ambystoma californiense*, Trenham, 2010). However, in the 'crisis discipline' (Soulé, 1985) of conservation biology, the need for expedient decisions may preclude this level of thorough information gathering. If time does not permit a long-term CMR study, genetic assignment tests can provide a rapid empirical estimate of population connectivity for use in viability modeling.

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