**Detecting exceptional temporal change in genetic diversity using limited information**

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**ABSTRACT**

Understanding spatio-temporal changes in biodiversity, including genetic diversity, is a cornerstone of landscape ecology and is essential to produce sensible conservation plans. However, detecting atypical local genetic change, relatively to the changes we expect from common processes such as drift, is challenging, and is often dealt with subjectively. Sophisticated methods to detect genetic change in time exist, but they often require large genetic datasets with information beyond simple allele counts, and require a lot of knowledge about the system and its potential evolutionary scenarios. In this paper, we describe Temporal Genetic Indices (TGI), which is a method that utilizes permutations of genotypic matrices to test the significance of indices describing genetic temporal change at sites, given genetic change in other sampling sites in the study landscape. TGI is usable on even small genetic datasets. We tested TGI using demo-genetic simulations to model the genetic legacy of a major external event and evaluated our ability to detect it while varying dispersal, the number of populations affected by the change, and the timing of sampling relative to the event. We found that all three factors influence the performance of TGI and our study serves as a rich template for researchers who would use TGI in their system. We successfully applied TGI on one empirical dataset, with our application providing a straightforward test for genetic change, and supporting previous conclusions about the dataset. An R function to implement the method is now available, as well as convenience functions for those wishing to further simulate and analyze their simulations.

**INTRODUCTION**

Global biodiversity in terms of genes, species, populations, and ecosystems is being lost at an increasing rate with significant consequences for ecosystem functioning and long term viability of the biosphere (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 2012; Dirzo et al., 2014; Leigh, Hendry, Vázquez‐Domínguez, & Friesen, 2019). Novel monitoring techniques are needed to track these losses and to inform conservation efforts. Further, it is recognized that it is no longer sufficient to study spatial patterns in biodiversity loss at a single point in time. Instead, one must examine trends and patterns in biodiversity through both space and time (Bradburd & Ralph, 2019; Fenderson, Kovach, & Llamas, 2019).

Analysis of spatial and temporal variation in genetic diversity can tell us a great deal about demography and population connectivity (Bradburd & Ralph, 2019; Lowe & Allendorf, 2010). Indeed, population genetics has proven essential to translate the observed genetic variation into meaningful inferences regarding connectivity and demography that are essential for conservation efforts (Allendorf, Hohenlohe, & Luikart, 2010; Harrisson, Pavlova, Telonis-Scott, & Sunnucks, 2014; Segelbacher et al., 2010). The field of landscape genetics takes these ideas further and examines interactions between micro-evolutionary processes and landscape features (Manel & Holderegger, 2013; Manel, Schwartz, Luikart, & Taberlet, 2003; Wagner & Fortin, 2013) to improve our understanding of how spatial heterogeneity influences population genetic processes. Landscape genetics has historically mostly had a spatial focus, in studies where sampling and analysis were carried out at a single point in time. Adding a temporal dimension to landscape genetics represents a great opportunity for improving the quality and usefulness of inference (Anderson et al., 2010; Draheim, Moore, Fortin, & Scribner, 2018; Martensen, Saura, & Fortin, 2017; Sun & Hedgecock, 2017). New conceptual approaches and tools that allow for the integration of spatial and temporal variation in studies of genetic variation hold great promises of further elucidating the processes that govern demographically dynamic systems such as insect outbreaks, invasions, and species declines (Allendorf et al., 2010; Bradburd & Ralph, 2019; Fenderson et al., 2019).

Temporal variation in genetic diversity, and its drivers, are indeed at the crux of many conservation and public health issues (Díez-del-Molino, Sánchez-Barreiro, Barnes, Gilbert, & Dalén, 2018; Lauterjung et al., 2019; Moraes et al., 2017). Researchers commonly use spatio-temporal population genetic legacies, which represent the signal that is left after a change (Banks et al., 2013), to study isolation-by-distance (Rousset, 1997; Wright, 1943), population bottlenecks (Gattepaille, Jakobsson, & Blum, 2013; Maruyama & Fuerstt, 1985), migration between isolated populations (Bezemer, Krauss, Roberts, & Hopper, 2019; Buschbom, Yanbaev, & Degen, 2011), and outbreak expansions (Larroque et al., 2019; Wittische, Janes, & James, 2019). Temporal genetic analyses are for example needed to help identify which populations have experienced high mortality as a result of disturbance such as a forest fire, major weather event, or disease outbreaks (Poff et al., 2018; Suárez, Betancor, Fregel, Rodríguez, & Pestano, 2012). Similarly, such analyses could identify which, among a set of previously sampled populations, received migrants from a long-distance dispersal event (Apodaca, Trexler, Jue, Schrader, & Travis, 2013). Spatio-temporal genetic studies have also led to a better understanding of the invasion history of the vector species of major diseases (Maynard et al., 2017) and of the impacts of landscape fragmentation and alteration on food webs (Nair, Fountain, Ikonen, Ojanen, & Van Nouhuys, 2016) and rare species (Baker et al., 2018). Temporal genetic variation reflects the evolutionary potential of a population and the probability of its persistence (Aeschbacher, Selby, Willis, & Coop, 2016; Bolnick & Nosil, 2007; Kremer et al., 2012); relating it to temporal landscape change can give us important insights about the eco-evolutionary dynamics of a species, and be used to inform conservation strategies (e.g. Landguth, Holden, Mahalovich, & Cushman, 2017). An improved capacity to detect meaningful changes in genetic diversity of populations, and from which to infer the effects of historical demographic events, holds great potential to improve management, including guiding the prioritization of areas for conservation or mitigation efforts. However, distinguishing between natural variation in temporal genetic structure due to the processes of recombination, mutation, and demographically-induced genetic drift from the changes wrought by external landscape variation is challenging.

Spatio-temporal population genetics methods to detect such significant demographic events exist, but they are generally purpose-built for information-rich genetic datasets, which span great sections or the genome or are the result of deep sequencing. Such data are usually collected at a single point in time. For example, several sophisticated frameworks have been used to infer demographic history from at least tens of thousands of loci, based on different demographic scenarios and evolutionary assumptions (Excoffier, Dupanloup, Huerta-Sánchez, Sousa, & Foll, 2013; Gutenkunst, Hernandez, Williamson, & Bustamante, 2009; Kamm, Terhorst, Durbin, & Song, 2019). In general, these methods require input beyond simple allele counts, such as information about recombination processes (Gattepaille et al., 2013) and ascertainment bias (Albrechtsen, Nielsen, & Nielsen, 2010; Clark, Hubisz, Bustamante, Williamson, & Nielsen, 2005; Marth, Czabarka, Murvai, & Sherry, 2004) to estimate demographic parameters and history. In practice, many research projects have fewer genetic markers and/or information about those markers because they focus on non-model species (e.g. Legault et al. 2020), or want to use older and potentially more restricted genetic samples or datasets (*e.g.* Moraes et al., 2017) as references to compare newer samples to. Some other studies have directly used genetic differentiation metrics, such as FST, to evaluate temporal changes between genetic datasets (e.g. Larroque et al 2019; Segura-García et al., 2019). However, translating our spatial understanding of FST-based results to the temporal dimension is not straightforward (Bhatia, Patterson, Sankararaman, & Price, 2013). Additionally, disentangling spatial from temporal effects is a challenge because the additivity of genetic drift means than genetic differentiation can be associated with both space and time (Murray et al., 2016; Skoglund, Sjödin, Skoglund, Lascoux, & Jakobsson, 2014). Detecting significant population genetic changes, relative to what would be expected due to drift, based on limited time series of genetic data, remains a challenge.

Temporal Beta-diversity Indices (TBI; Legendre 2019) have been used to assess the significance of changes in ecological community composition through time. Given the conceptual similarity between the question of how multi-species communities change through time and how genetic diversity changes through time, we can assume that this analysis could be applied to spatio-temporal multi-locus genotypic data. The TBI approach quantifies temporal changes in sampling sites between two points in time using a dissimilarity index. The significance of these dissimilarities is then tested using permutation. The TBI approach has been extensively tested on simulated community composition data (Legendre, 2019), but its ability to detect meaningful changes in genetic diversity has not yet been examined.

In this study, we expand the TBI framework to apply it to spatio-temporal population genetic data. The objective of our new analysis, Temporal Genetic diversity Indices (TGI), is to quantify and statistically assess temporal variation in spatial genetic diversity. Quantifying relative temporal genetic change among locations will allow us to infer the existence of past demographic events and provide sensible information extracted from genetic data through assumption-light and purpose-designed tests to policymakers and managers, regardless of the availability of very large genetic datasets. Persisting spatial legacies in genetic diversity can also be used to identify sites that were most strongly impacted by previous demographic events. We demonstrate the effectiveness and applicability of the approach using simulated genetic data generated using a spatially-explicit demo-genetic simulator (*CDMetaPOP*; Landguth, Bearlin, Day, & Dunham, 2017). Our general approach was to simulate multiple scenarios in which portions of a landscape are affected by a non-selective demographic change. We then used TGI to measure changes in the genetic diversity of our populations under these different demographic contexts. Specifically, we explored how dispersal ability, the number of populations affected by a demographic event (i.e., spatial extent), and time between two sampling efforts affected our capacity to detect significant temporal variation in genetic diversity. Performance was quantified using standard false positive/negative rates binary classification (Legendre & Legendre, 2012). We predict that our ability to detect historical demographic changes would be lower with increasing dispersal ability because of the homogenizing effect of higher gene flow. We also predict that the longer the time between successive sampling, regardless of when an event occurred between them, the harder it will be to identify where and when a demographic event occurred. Finally, we illustrate our approach by applying it to a real genetic dataset and compare our results with earlier indirect analyses.

**METHODS**

*Adapting Temporal Beta diversity Indices for genetic data*

Calculating TBI involves computing dissimilarities in species composition (see Legendre & Legendre 2012 for an overview of available dissimilarities, and Legendre & De Cáceres, 2013 for criteria to determine the indices that are appropriate for beta diversity studies) between the data sampled at two different times at each site, and testing the significance of these indices through simultaneous permutations of the two site-by-species input matrices. In extending TBI to TGI, we considered population-level genotype frequency matrices as input and used as dissimilarity the genetic distances that measure the genetic separation between populations sampled at two different times, based on some geometrical and evolutionary assumptions (Fig. 1 A). In this case, the null hypothesis is that genetic composition does not differ between the two points in time that were sampled.

One of the challenges, given background genetic processes such as drift, is to evaluate the significance of the temporal changes at the different sites. There are no reference distributions for what constitutes significant temporal genetic change available to researchers and decision-makers. Instead, we will use a permutation-based approach to generate a distribution of values to which the observed value can be compared. Permutation-based methods have been previously developed and applied to the analysis of spatio-temporal changes in community composition (for reviews: Legendre & Gauthier, 2014; Shimadzu, Dornelas, & Magurran, 2015).

Although several permutation approaches have been described, they are not all usable to support meaningful inference for all types of scientific questions (for genetic questions, see Adams & Collyer, 2015). Testing the significance of TGI involved permuting the input genotypic matrices. Here, we permuted the genotypes at each locus in the same way in both time samples (Fig. 1 B) using the *poppr* R package (see *Software*). This permutation was chosen because it maintains allelic structure and heterozygosity (Agapow & Burt, 2001). We used 999 permutations in all analyses.

*Genetic distance*

Genetic distances between points in time for a given location were calculated using the Rogers’ genetic distance (Avise, 1994; Rogers, 1972), which is very similar to the Euclidean genetic distance (see Annex A). It makes no assumptions about base-pair substitutions or time since separation and is suitable to study short-term dynamics. It has recently been used to investigate spatial genetic structure in a pond turtle (Pereira, Teixeira, & Velo-Antón, 2018) and a fungus (Bennett & Stone, 2019). We computed the distance using the *dist.genpop* function from the *adegenet* R package (see *Software*).

*Simulation framework*

To simulate the dynamics of population genetic changes through time, we used the spatially-explicit gene flow simulation software *CDMetaPOP* (Landguth, Bearlin, et al., 2017). *CDMetaPOP* simulates dispersal and mating of individuals across a landscape and allows the user to define the initial genetic structure, spatial distribution of individuals, dispersal characteristics, and life-history traits of the population. The physical landscape we simulated was modelled as a homogeneous and interconnected square grid containing 5 × 5 cells, each cell representing a population. Each population had a maximum carrying capacity of 50 individuals. Structural connectivity between populations was strictly a function of geographic distance. The populated landscape, therefore, contains a maximum of 1250 individuals. Each simulation was run for 100 generations before a demographic event (see below) was imposed on up to three populations in the landscape. 10 more generations were simulated after this event. The mutation rate was set at 10-8 to reflect empirically-derived mutation rates found in many taxa (Allio, Donega, Galtier, & Nabholz, 2017). The genotypic information of each individual was recorded and consisted of 100 neutral, unlinked, bi-allelic SNP loci. Sampling was done before and after the event unless otherwise specified.

We simulated 180 replicates for each scenario (see below). For each replicate, we initialized the simulation with random and unique allocations of alleles among individuals, therefore reaching maximum diversity (Landguth, Bearlin, Day, & Dunham, 2016). Those parameters were chosen as a compromise between realism and computational time limitations, and they seemed appropriate to produce the complex evolutionary dynamics necessary to produce reasonably realistic and useful simulated genetic data.

Many demographic processes may alter the genetic diversity of a local population and we chose our demographic event to be immigration from a previously isolated population because it is a commonly studied process in the empirical literature. When modelling immigration, we simulated immigration from a population that was separate from our 5×5 grid (i.e., population #26). Our goal was to apply the TGI approach to detect historical population changes in genetic data due to immigration. This independent source population otherwise shared the same attributes as other populations in our simulated landscape. Only during simulated demographic events were individuals from the 26th isolated population allowed to disperse into the simulation grid.

We examined the influence of dispersal and the spatial extent of demographic events (number of affected populations), which were simulation parameters, on the persistence of genetic spatial legacies, using this simulation model. We examined three levels of dispersal (see below), and three different numbers of populations (1, 2, 3) affected for a total of 9 unique scenarios, each of which was replicated 180 times, for a total of 1620 (9 × 180) unique simulations for this experiment, aside from the control simulations described below (Table 1). In the next sections, we detail how we modelled the two experimental factors.

*Dispersal*

To model dispersal, we weighted the geographic distances between populations using a power law function, , where *B* represents how difficult it is to disperse and high values of *B* correspond to low dispersal capacity (elaborated below). We then rescaled the values, using the maximum and the minimum (0) distances possible in this virtual landscape, as described in the *CDMetaPOP* (Landguth, Bearlin, et al., 2017) user manual (p.63). This produced values in the [0,1] range, which are considered to represent probabilities that an individual disperses to a cell located at that distance (Fig.1). We chose this way of modelling dispersal to allow both within-population movements and long-distance dispersal.

The population to which an individual disperses was selected randomly from the set of populations available at the given distance. Individuals always stay within our simulated landscape, and any individual may disperse to one of our 25 populations at each generation. To investigate the effect of different levels of dispersal, we used three different values of *B*: low (*B* = 2), moderate (*B* = 1.301) and high (*B* = 0.6015) dispersal levels (Fig.1; Table 1).

*Number and position of populations with spatial legacies*

We also wanted to evaluate how the number of populations bearing spatio-temporal population genetic legacies influenced the performance of our testing procedure. To achieve this, we triggered demographic events at 1 to 3 populations randomly selected among the 25. When only 1 population was affected, we partitioned the 180 replicates of that scenario equally among 6 populations in the landscape. The positions of these 6 populations were randomly selected once and identical across runs. Indeed, because our landscape is square and homogeneously resistant to movement, it is therefore symmetric and only 6 positions need to be assessed. When several (2 or 3) populations underwent a demographic event, we randomly sampled 1 position among the 6 previously described and randomly picked 1 or 2 additional populations directly adjacent (when possible) to it. We did this 6 times (30 replicates for each different set of populations). We chose to pick populations this way to respect the spatial autocorrelation often exhibited in demographic events.

*Time since demographic change*

To assess how the time since the simulated demographic event affects our ability to detect genetic changes, we used TGI on simulated data collected up to 9 years before, and after, the event, and compared them with data collected respectively right after, and right before the event year. We chose 9 years as the maximum time between samplings as this time lag would represent most of the “before/after” population genetic studies we encountered, and because most long-term ecological research programs monitor during a shorter time interval. Specifically, for our analyses concerning the timing of sampling, rather than arbitrarily choosing a threshold, we chose the 0.05 *p*-value threshold as it was a good compromise between decent FPR and FNR in our earlier results (next paragraph).

*Statistical performance*

We used the False Positive Rate (FPR) and False Negative Rate (FNR) to assess the statistical performance of the TGI testing procedure. A false positive is a population that we know *a priori* did not undergo the demographic change we imposed but has been found to have done so by the TGI test. A false negative is a population that did experience a demographic event but was not found to have done so. FPR represents the number of false positives over the total number of negative tests, and FNR represents the number of false negatives over the total number of positive tests. A high FPR means that we often select the wrong population(s) as significant, and researchers generally want to keep it as low as possible when there are, for example, heavy costs to focusing on wrong populations, because of the limited money available to invest in conservation actions. The higher the FPR, the lower the selectivity of our testing procedure. A high FNR means that we often miss the population(s) that were actually affected. The higher the FNR, the lower the power of our testing procedure. Researchers may want to minimize the FNR in situations where finding the right population is the most important aspect, for example, if there is limited time to take conservation action. Selecting a proper threshold for permutation tests is often important to identify a compromise between power (1 – FNR) and selectivity (1 – FPR). To characterise this compromise, we evaluated the statistical performance of TGI using a range of thresholds: 0.001, 0.005, 0.01, 0.015, 0.020, 0.025, 0.030, 0.035, 0.040, 0.045, 0.050, 0.055, 0.060, 0.065, 0.070, 0.075, 0.080, 0.085, 0.090, 0.095, and 0.1.

*Controls*

Control simulations were run, in which no populations were affected by demographic events and were therefore only subject to the processes of gene flow, drift, and mutation. Dispersal was the only parameter that varied among the control simulations, resulting in three control scenarios (Table 1). We evaluated the FPR of these control scenarios; there was no need to compute FNR because there were no true positives/false negatives so it was always equal to 0. When describing the performance of other scenarios with similar dispersal parameters, we always used these control values as reference.

*Software  
CDMetaPOP* runs on *Python 2.7* (Landguth, Bearlin, et al., 2017). We used the *R* software (R Core Team, 2019) in the RStudio IDE (RStudio Team, 2018) for all analyses and illustration. We used the *adegenet* (Jombart, 2008; Jombart & Ahmed, 2011), *pegas* (Paradis, 2010), *poppr* (Kamvar, Brooks, & Grünwald, 2015; Kamvar, Tabima, & Gr̈unwald, 2014) and *adespatial* (Dray et al., 2019) *R* packages for calculations. Our *TGI* function is available in the annex.

*Application example: an endangered fish*

To showcase TGI’s general ability to provide sensible information about temporal change in a real system with conservation implications, we chose to apply it to a threatened vertebrate: the Northern tidewater goby. We chose this example because it uses a different genetic data type than we the one we simulated, to make it clear TGI is usable on a diversity of markers, and because the authors suggested that one population had undergone change among other more stable local populations, which allows to test a hypothesis, and go beyond a simple illustration of our method (Kinziger, Hellmair, McCraney, Jacobs, & Goldsmith, 2015). The dataset was available through DRYAD (doi:10.5061/ dryad.871db). We used 9999 permutations for this application.

**RESULTS**

*Dispersal*

As hypothesized, dispersal capacity influenced our ability to detect temporal changes in genetic diversity. FNR generally increased with dispersal intensity (Fig. 3). One scenario (H3; Table 1) was the only one with FNR values above 1% regardless of which threshold was used (Fig. 3). Among the four scenarios (L3, M3, H2, H3) never reaching an average FNR of 0, two are scenarios involving high dispersal. Taking the average from scenarios sharing the same dispersal parameters for the ubiquitous 0.05 threshold (e.g.one value for L1, L2, and L3 grouped together), we had mean FNRs of 0.0037 (0.0007 - 0.0066; 95% CI) for low dispersal, 0.0049 (0.0015 - 0.0083; 95% CI) for moderate dispersal, and 0.0108 (0.0055 - 0.0161; 95% CI) for high dispersal. FNR values overall decreased with threshold, with a sharp decrease (most notable for H3) before 0.025 followed by a slower decrease until 0.1.

FPR did not substantially change as a function of dispersal capacity (Fig. 4). Low dispersal did not consistently result in higher FPR than moderate dispersal, or high dispersal (Fig. 4). Indeed, given the same number of affected populations we can see that L1 has slightly higher values than M1 and H1, L2 has slightly lower values than M2 and H2, and L3 has intermediate values, between that of M3 and H3. Conservatively defining FPR as acceptable by whether it is below the threshold used in the test, we notice that low dispersal scenarios had, on average, acceptable FPR values at more thresholds than higher dispersal scenarios, which overall presented the same performance by this metric (Table 2). FPR averages from scenario sharing the same dispersal parameters, for the 0.05 threshold, were 0.0599 (0.0558 - 0.0641; 95% CI) for low dispersal, 0.0621 (0.0580 - 0.0662; 95% CI) for moderate dispersal, and 0.0600 (0.0562 - 0.0638; 95% CI) for high dispersal. FPR values overall increased with threshold, with a sharp increase at low thresholds followed by a continued but saturating increase until threshold 0.1).

While the order of the performances of scenarios differing in dispersal capacity stayed the same, independently of the number of populations affected, for FNR (H>M>L for a given number of populations affected; Fig. 3), it was not the case for FPR where this order changes depending on the number of populations affected (Fig. 4).

*Number of populations affected*

The number of populations affected by a migration event also influenced our ability to detect meaningful temporal change. Scenarios with a lower number of populations consistently performed better according to FNR, while the opposite is true for FPR (Figs. 3, 4).

All scenarios with only one affected population (L1, M1, H1) showed perfect FNR (0; Fig. 3), while L2 and M2 reached this perfect FNR only for liberal thresholds, from 0.03 and on. Taking the average from scenarios sharing the same number of populations affected, for the ubiquitous 0.05 threshold (e.g.one value for L1, M1, and H1 grouped together), we had mean FNRs of 0 for scenarios with one affected population as previously stated, 0.0028 (0 - 0.0059; 95% CI) for scenarios with two affected populations, and 0.0167 (0.0105 – 0.0228; 95% CI) for scenarios with three affected populations. FNR values overall decreased with threshold, with a sharp decrease (most notable for H3) before 0.025 followed by a slower decrease until 0.1.

As shown by the overlap of FPR values across scenarios with similar numbers of populations affected rather than across scenarios with similar dispersal, the number of populations affected generally influenced the FPR performance more than dispersal, for the levels we used in the simulations (Table 2; Figs. 3, 4). Using the same conservative definition of an acceptable FPR as previously described (< alpha), we can see that scenarios with only one affected population never offered acceptable FPR values while those with two and three populations offered acceptable FPR values at thresholds higher than 0.0750 and 0.0450, respectively (Table 2). FPR averages from scenario sharing the same number of populations affected, for the 0.05 threshold, were 0.0820 (0.0778 - 0.0863; 95% CI) for scenarios with one affected population, 0.0553 (0.0516 - 0.0591; 95% CI) for scenarios with two affected populations, and 0.0447 (0.0413 - 0.0481; 95% CI) for scenarios with three affected populations.

*Time interval between samplings*

As hypothesized from the nature of genetic processes in connected populations, the genetic signal of the demographic event inflicted upon populations decays over time. Generally, the longer the interval between a pre-event and a post-event sampling, the lower the power available to detect the demographic event, as evidenced by the increase in false positives and false negatives for several scenarios (Fig. 5, 6). However, this is strongly affected by the dispersal level present in the landscape and by the number of populations affected. The timing of sampling prior to a simulated event was, as expected, generally less important than that of the posterior sampling.

For example, for the worst-performing scenario for sampling undertaken 9 years before the punctual migration event (M3), the FNR was more than 30 times that of the FNR for sampling undertaken the year immediately before, almost halving the power of the test (Fig. 5). For second samplings done 9 years after a first sampling done before the event, we observe high FNR values especially with high and moderate dispersal scenarios (Fig. 5). One very interesting point is that the number of populations affected is the main factor increasing FNR values in older first samplings (3>2>1; left side of Fig. 5), while dispersal capacity is the main factor increasing FNR values with the time lag of second samplings (H>M>L; right side of Fig. 5). For a same number of populations affected, moderate dispersal scenarios showed the worst performance for first sampling time lags, while high dispersal scenarios generally showed the worst performance for second sampling time lags (Fig .5). FNR changed the least for the L1 scenario, and the most for the H3 scenario(Fig. 5).

While the relative differences in FPR performance given different time lags were not as high as for FNR, FPR nonetheless increased with sampling time lag, for example, up to more than twice the value of the threshold for first samplings for L1, and even higher for the largest time lags possible for second samplings (Fig. 6). There are not clear patterns for whether dispersal or the number of populations affected most influenced FPR change for pre-event samplings (Fig .6). However, for time gaps attributed to the second sampling, dispersal becomes the main factor in driving FPR (Fig. 6). The previously described strong relationship between FPR and the number of populations affected by the demographic event, therefore, changed as dispersal became more influential. FPR did not change much for the L1 scenario, and changed the most dramatically for the H3 scenario (Fig. 6), again denoting large differences in the influence of time between our two most extreme scenarios.

Although FNR became quickly became pathological for second samplings done after event for high dispersal scenarios, FNR also increased with time lag for the posterior sampling for low and moderate dispersal scenarios, but more linearly, and never reaching 30% in the scope of our analyses, even after 9 years, for low dispersal scenarios (Fig. 5). The increase in FPR with posterior time lag was also the strongest for high dispersal scenarios.

When considering the scenario most likely to preserve the signal according to earlier results on FNR and FPR (L1), the TGI approach was still able to keep false negatives below 15% and false positives below 10%, even for a second sampling done 9 years after the event (Fig. 5, 6), regardless of whether the first or second sampling is responsible for the time lag. Given the large variation in performance for each parameter that we considered, the parameter values we chose to define different scenarios produced sufficiently complex, and useful simulation results.

*Control simulations*

Experimental FPR values consistently stayed below control FPR values corresponding to their dispersal scenario for low and moderate dispersal (Fig. 4). This means that in the presence of an actual migration event, we were always less likely to incorrectly identify a population as having been affected, than without such an event. The higher the dispersal, the lower the control FPR (Fig. 4). Control FPR values were generally at least twice as high as the maximum experimental FPR values (L1, M1), regardless of the threshold used. This means that even for the worst scenarios, TGI was much more effective at avoiding false positives, in the presence of an event, than in its absence (as shown in the control simulations).

*Application example*

TGI results show that the Elk River population of Northern tidewater goby (Kinziger et al., 2015) has significantly changed relative to the other populations sampled in the study area (p.value = 0.0005), even after using strict adjustments (Holm-Bonferroni; p.value = 0.004). The results describe a loss of genetic diversity in that population between 2006 and 2011. The Elk River population was the population where unexpected temporal genetic change was suggested to have taken place (Kinziger et al., 2015), so our results support the original study and provided a more direct hypothesis test.

**DISCUSSION**

We investigated how dispersal, the spatial extent of a demographic event, and the timing of sampling affects our ability to identify populations having undergone significant changes in genetic diversity. Using a new permutation-based testing procedure, TGI, we showed that useful information about temporal changes in the genetic structure of populations can be obtained. Performance of the new procedure was evaluated using data generated using a spatially-explicit gene flow simulation software (Landguth, Bearlin, et al., 2017). Using this model, we explored our ability to detect punctual and significant demographic events in one to three local populations, within a larger landscape of connected populations bearing more than a thousand individuals in total. We aimed at evaluating how often TGI would fail to identify populations that truly experienced significant genetic changes under different dispersal intensities, event spatial extents, and sampling timing scenarios. We found that those three factors all influence our ability to detect exceptional temporal changes in genetic diversity, using limited genetic information. Beyond the interest of our new approach for population genetics and the fact that we tested its performance in an extensive simulation study, our results could serve as a guide on how to use the new method, alongside simulations, to evaluate the information loss of different sampling schemes. Therefore, TGI has clear implications on the design of empirical field studies

Detecting significant change depends on the level of landscape functional connectivity. Indeed, our simulations showed that false negatives increased with dispersal ability, whereas false positives did not show a trend. This has important implications as there are clear benefits in avoiding the detection of unaffected populations. The influence of dispersal on our ability to avoid such populations becomes even greater with an increasing time lags between an event and the subsequent sampling. While this influence exists even with only one generation separating two temporal samples (right before and right after the event), it becomes even stronger when time between samplings increases. Considering that connectedness among the local populations increases dispersal ability, this suggests that studying highly connected systems might require more frequent sampling if the researchers’ objective is to ensure they have detected the affected populations no matter the investment in monitoring false positives. High dispersal, and higher gene flowing through it (Cayuela et al., 2018), is implicated in many short-term or long-term mechanisms and lowers our ability to understand the eco-evolutionary dynamics of species. For example, high dispersal during range expansion lowers our ability to correctly detect loci under natural selection (Mayrand, Filotas, Wittische, & James, 2019). However, high gene flow may not always be associated with a strong decrease in measured structure (Landguth, Cushman, Murphy, & Luikart, 2010) or early detection of barriers to gene flow (Landguth, Cushman, Schwartz, et al., 2010).

The spatial extent of an event, represented by the number of local populations affected by the punctual demographic event in our study, displayed a performance trade-off event without time lag, increasing FNR, and decreasing FPR. Indeed, the spatial extent increases our ability to correctly reject populations that have not truly changed, but it decreases our ability to correctly detect populations that have truly changed. This trade-off is apparent for all scenarios but whether it is substantial depends on the dispersal level within the landscape. Although the spatial extent of a legacy may help researchers detect the legacy as the chance of the legacy being sampled increases, it may also increase the risk of not identifying the legacy at all, especially in high dispersal landscapes. When several populations were targeted by the punctual demographic event, we chose adjacent populations; whether lowering the degree of spatial autocorrelation in the spatial genetic legacy, that is targeting populations not necessarily adjacent to each other, influences detection, has not been investigated in our paper. Spatial autocorrelation may greatly affect many genetic analyses, and solutions are being developed to integrate it within them (Rousset & Ferdy, 2014). We believe that explicitly taking spatial autocorrelation into account in temporal analyses of genetic diversity (Bradburd & Ralph, 2019) represents a promising and challenging avenue of research.

As expected, spatial genetic legacies tend to decay over time. However, two main points emerged from our analysis of the timing of sampling required to detect significant genetic change. First, spatial extent is important to take into account when we have access to a sampling done promplty after a suspected event. This is true when we compare it to old or recent pre-event sampling. The main implication of this result is that while it could reassure researchers that they may compare an older sample to a recent one obtained shortly after the event, the power to detect change decreases sharply if the event affected a large part of the landscape. However, the timing of the pre-event sampling is generally less important than that of the post-event sampling. Indeed, dispersal becomes the most important factor in driving the performance of TGI, especially its power, when the time lag between the event and the post-event sampling increases. This means that researchers may for example have to accept as many as 50% of false negatives and 10% of false positives after sampling only a few years after an event if their system presents high dispersal. This has serious implications: if the demographic parameters of the models of a study system would be similar to our inputs (moderate dispersal for example), researchers might systematically spend a substantial part of their resources on monitoring or treating unaffected populations, while missing half the affected populations. For a cartoon example, if 10 guards would be hired to protect populations of a threatened salamander identified as having recently lost more genetic diversity than expected, 1 of them would protect populations that have actually been stable, and may, therefore, be less important to protect, and the guards would not protect 10 populations that should be protected. Although the spatial legacy of a past demographic event could be perceptible in richer genomic data (*e.g.* probability of mutational configurations in sequence blocks), limited biallelic gene frequency data may not retain most of the signal beyond a few years, even in the best situations. In contrast, the previous investigations using TBI, which used community composition data, have not focused on the timing of sampling. Although community composition data (species x sites) generally varies at a larger time scale than genetic data, we encourage future investigations of the influence of timing on TBI performance.

Our analyses have shown that our TGI testing procedure is adequate to study genetic change, but there are certain considerations to keep in mind when using it. For example, stricter values (lower values) for the TGI *p*-value threshold expectedly bring a better FPR but may also bring a worse FNR (lower power). Regarding FNR, lower performance is not very dependent on user choice for thresholds past a certain, regardless of dispersal level and spatial extent. Finding this threshold value would be valuable to better understand the trade-offs of different sampling schemes in specific systems. TGI can also readily be used on other types of genetic data, such as microsatellites. Although TGI already represents a more transparent alternative to arbitrarily comparing pairwise genetic differentiation, or node-based genetic diversity values, future work is needed to explore how the performance of TGI, as well as other methods, varies with other factors not considered in this study. Among the most interesting factors would be the choice of the genetic distance used in the algorithm, the influence of the degree of spatial autocorrelation in genetic legacies, varying effective population sizes, and spatial heterogeneity in landscape resistance to movement. Successful implementation of TGI will require some a priori understanding of the range of useful threshold values to use. Simulation is a powerful tool for investigating how demography and spatial context influence population genetic dynamics (Epperson et al., 2010), and can be used to help identify those appropriate threshold values, as we have demonstrated here. Several programs such as *CDMetaPOP* (Landguth, Bearlin, et al., 2017), *Nemo* (Guillaume & Rougemont, 2006), *SPLATCHE* (Currat, Ray, & Excoffier, 2004), or *SLIM* (Haller & Messer, 2019) provide very flexible and sophisticated ways to implement such simulations. We expect greater sensitivity to threshold selection in systems that exhibit dramatic demographic fluctuations, as is the case in outbreaking or invasive species.

Identifying changes in genetic diversity, beyond what one would expect due to background micro-evolutionary processes, can help researchers and conservation managers identify locations that have experienced important past demographic events. These events could be detrimental (*e.g.* loss of diversity, maladaptation) or beneficial (*e.g.* higher effective population size, genetic rescue). Such sites could then be prioritized for increased monitoring and further investigation into the origin of these changes. As shown in our application of TGI on an endangered animal system, our method provides a direct test of the hypothesis of exceptional temporal genetic change. Our approach to detecting temporal genetic differentiation does not require extensive genomic information and can be used to explore the temporal dynamics of demographically induced genetic diversity using relatively small genetic datasets (*e.g.* hundreds of SNPs). As such, our approach holds great promises to facilitate spatio-temporal analysis of wild, non-model organisms for which extensive genomic resources are yet to be developed.

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**DATA ACCESSIBILITY**

All simulation data used for this paper will be deposited online upon acceptance. Functions used to analyze the simulations will be available on a public repository on *GitHub*.TGI, the function that would be most useful to potential users of our approach, will continue to be maintained and developed and may be contributed to a CRAN package in the near future.

**AUTHOR CONTRIBUTIONS**

J.W. designed the study, created the simulation inputs, ran the simulations, transformed the TBI function to TGI, and performed the analyses. P.L. and P.M.A.J. provided advice on the study design, analysis, and the vizualisation. J.W., P.L. and P.M.A.J. wrote the paper.

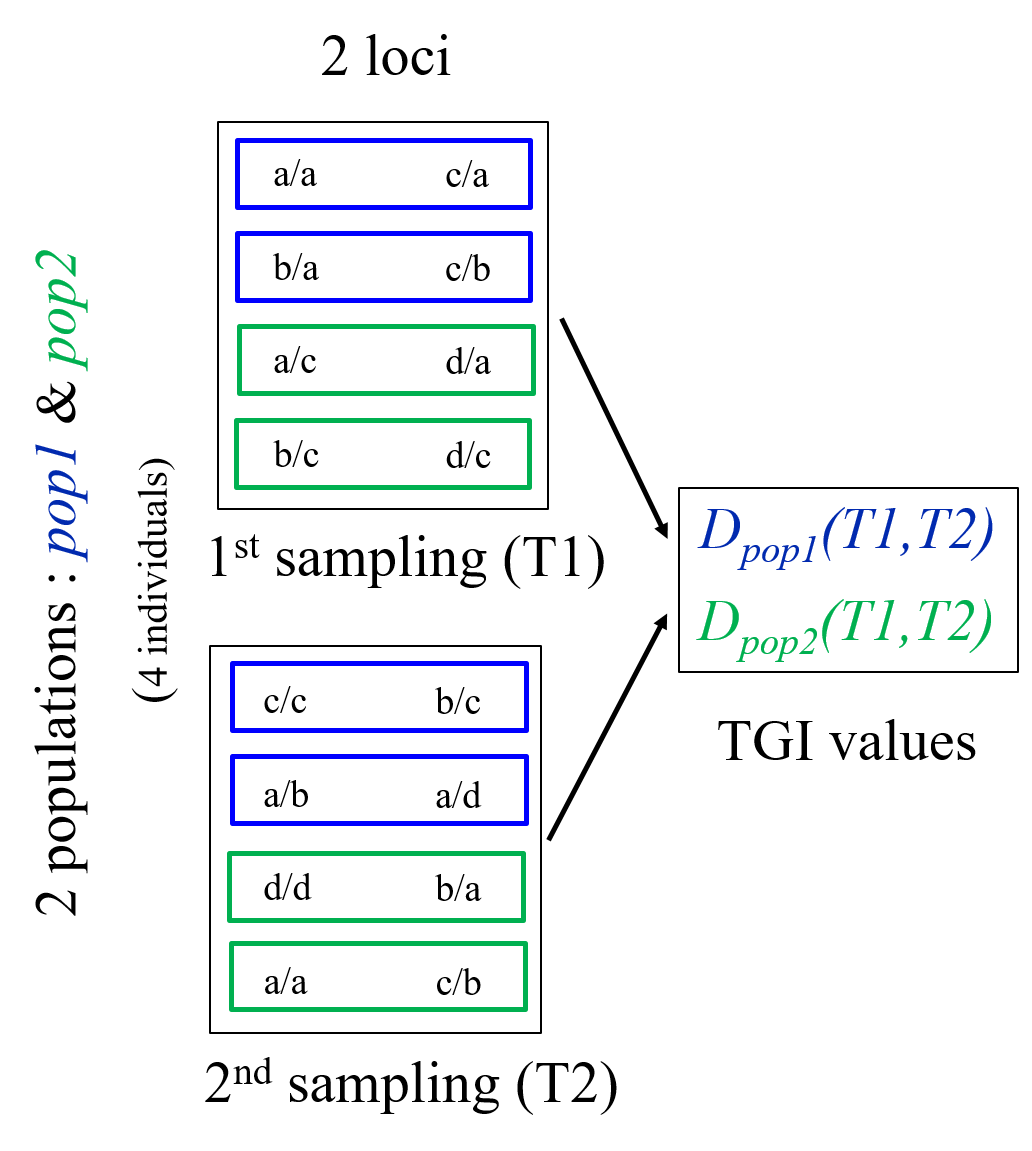
**TABLES AND FIGURES**

**Table 1:** Two-factor simulation experiment with scenario abbreviations used throughout the manuscript. Rows: number of populations with spatio-temporal population genetic legacies. Columns: dispersal values. Numbers in parentheses indicate the number of unique simulations ran for each factor level or combination of factor levels. We ran 2160 simulations in total.

|  |  |  |  |
| --- | --- | --- | --- |
| *Dispersal (B)*  *No. populations* | **Low** (720) | **Moderate** (720) | **High** (720) |
| **1** (540) | L1 (180) | M1 (180) | H1 (180) |
| **2** (540) | L2 (180) | M2 (180) | H2 (180) |
| **3** (540) | L3 (180) | M3 (180) | H3 (180) |
| **0: control** (540) | CL (180) | CM (180) | CH (180) |

**Table 2.** Are average FPR values staying below the thresholds used in the TGI tests? True (T) or False (F).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Scenarios*  *--- Thresholds* | **L1** | **L2** | **L3** | **M1** | **M2** | **M3** | **H1** | **H2** | **H3** |
| **0.0010** | **T** | **T** | **T** | **T** | **T** | **T** | **T** | **T** | **T** |
| **0.0050** | F | F | F | F | F | F | F | F | F |
| **0.0100** | F | F | F | F | F | F | F | F | F |
| **0.0150** | F | F | F | F | F | F | F | F | F |
| **0.0200** | F | F | F | F | F | F | F | F | F |
| **0.0250** | F | F | F | F | F | F | F | F | F |
| **0.0300** | F | F | F | F | F | F | F | F | F |
| **0.0350** | F | F | F | F | F | F | F | F | F |
| **0.0400** | F | F | **T** | F | F | **T** | F | F | F |
| **0.0450** | F | F | **T** | F | F | **T** | F | F | **T** |
| **0.0500** | F | **T** | **T** | F | F | **T** | F | F | **T** |
| **0.0550** | F | **T** | **T** | F | F | **T** | F | F | **T** |
| **0.0600** | F | **T** | **T** | F | F | **T** | F | F | **T** |
| **0.0650** | F | **T** | **T** | F | F | **T** | F | F | **T** |
| **0.0700** | F | **T** | **T** | F | F | **T** | F | **T** | **T** |
| **0.0750** | F | **T** | **T** | F | **T** | **T** | F | **T** | **T** |
| **0.0800** | F | **T** | **T** | F | **T** | **T** | F | **T** | **T** |
| **0.0850** | F | **T** | **T** | F | **T** | **T** | F | **T** | **T** |
| **0.0900** | F | **T** | **T** | F | **T** | **T** | F | **T** | **T** |
| **0.0950** | F | **T** | **T** | F | **T** | **T** | F | **T** | **T** |
| **0.1000** | F | **T** | **T** | F | **T** | **T** | F | **T** | **T** |

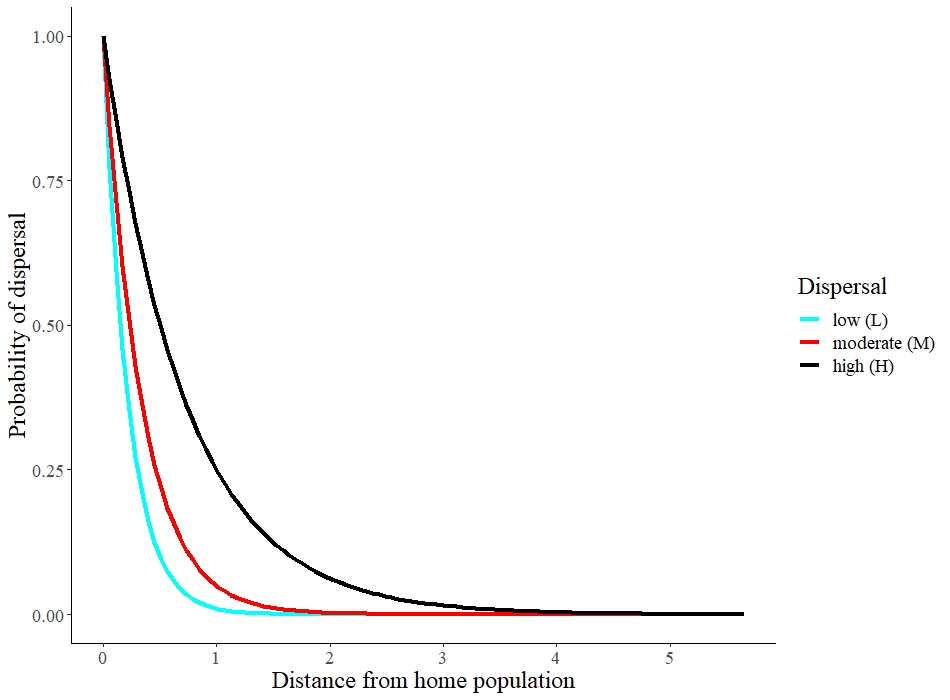
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A)

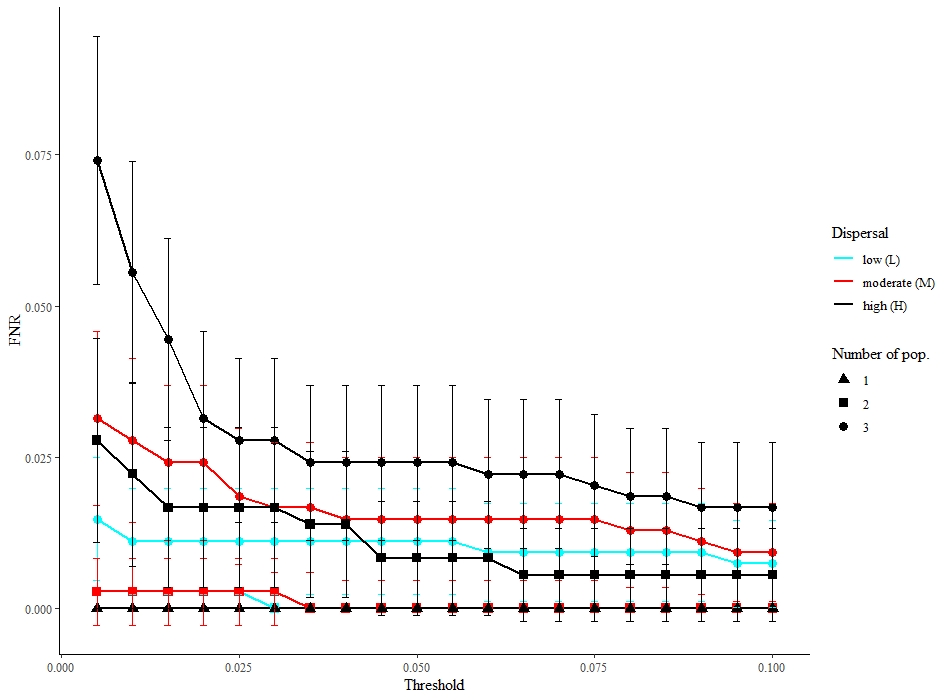
****

B)

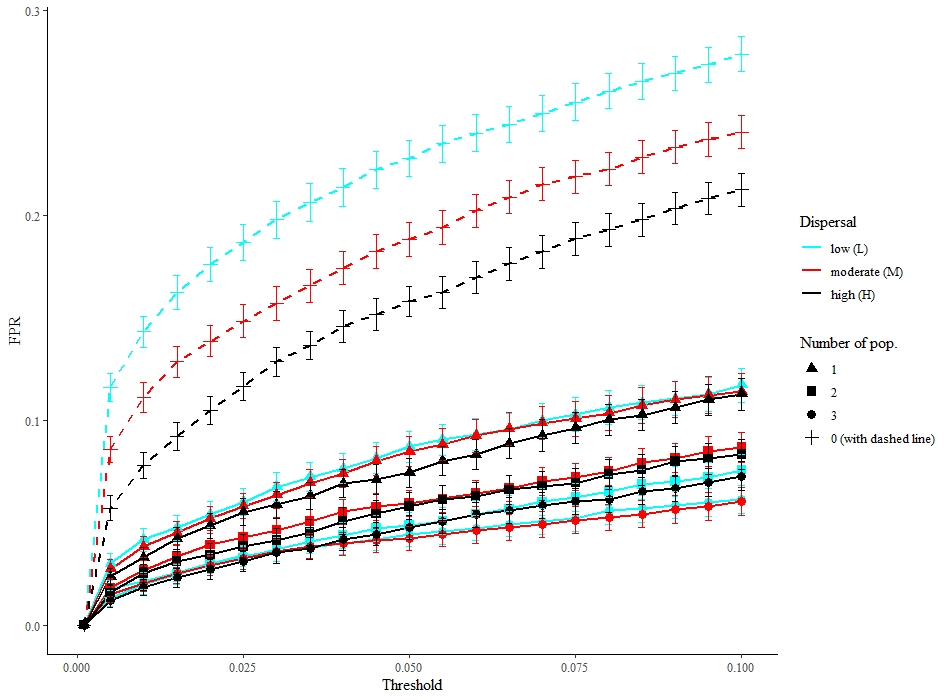
**Figure 1.** Schematic representations of A) the computation of the original TGI values and B) the way we permutated input genotypic matrices to create a distribution to test TGI significance.



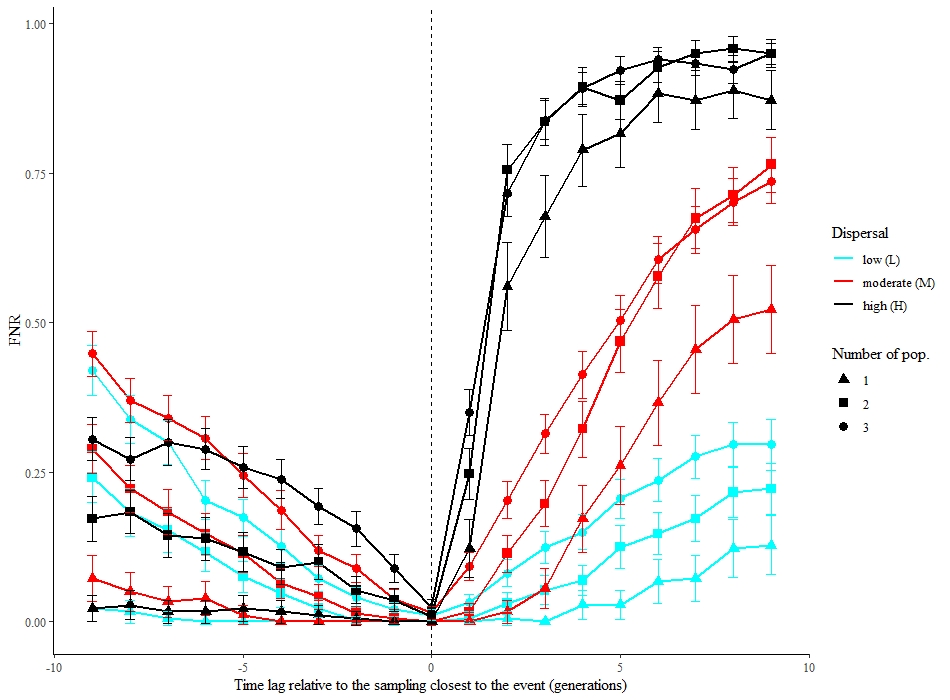
**Figure 2.** Probability of dispersal of an individual as a function of geographic distance, in three different dispersal scenarios.



**Figure 3.** FNR across all threshold and scenarios. There are no control experiment results displayed for FNR because there are no possible true positives in control experiments, hence no false negatives either. Those values are for samplings done at generations 100 and 101, i.e. right before and after the migration event. 95% confidence intervals of the FNR estimates are displayed by bars. For better visualization, we included only thresholds with FNR values not equal to 1.

****

**Figure 4.** FPR across all threshold and scenarios. Control experiments are shown with dashed lines. Those values are for samplings done at the 100 and 101 generations 100 and 101, i.e. right before and after the migration event. 95% confidence intervals of the FPR estimates are displayed by bars.

****

Influence of the timing of the posterior sampling

(0 represents sampling right after the event)

Influence of the timing of the prior sampling

(0 represents sampling right before the event)

**Figure 5**. FNR from TGI tests performed between samplings carried out up to 9 generations before or after the migration event (arrow) when compared with sampling done the generation after the event for prior samplings, or the generation before the event for posterior samplings. 95% confidence intervals are displayed by bars.

****

Influence of the timing of the posterior sampling

(0 represents sampling right after the event)

Influence of the timing of the prior sampling

(0 represents sampling right before the event)

**Figure 6**. FPR from TGI tests performed between sampling executed up to 9 generations before or after the event (arrow) when compared with sampling done the generation after the event for prior samplings, or the generation before the event for posterior samplings. 95% confidence intervals are displayed by bars.

**ANNEX A:** Roger’s genetic distance

Given loci and alleles:

**ANNEX B:** TGI function

# mat1: the genotypic matrix associated with the first sampling; must be a genind object

# mat2: the genotypic matrix associated with the second sampling; must be a genind object

# nperm: the the number of permutations used in the evaluation of significance

# seed.: you may specify a seed by using this argument

# method: see ?adegenet::dist.genpop

# correc: correction for multiple inference; see ?p.adjust

# thresh\_for\_GL: indicate here the threshold you want to use

TGI2 <- function (mat1, mat2, nperm = 999, replace = FALSE, seed. = NULL,

method = 4, correc = "holm", thresh\_for\_GL = 0.05) {

#### Dependency on packages

library(adegenet)

library(poppr)

#### Conversion from genind to genpop objects

mat1p <- genind2genpop(mat1)

mat1p <- mat1p[,order(colnames(mat1p@tab))]

mat2p <- genind2genpop(mat2)

mat2p <- mat2p[,order(colnames(mat2p@tab))]

##### Function to compute genetic distances

dissim <- function(mat1p, mat2p, method) {

dis <- vector(mode = "numeric", length = nrow(mat1p@tab))

for (i in 1:nrow(mat1p@tab)){

if (i == 1){

trick <- 2

} else {

trick <- 1

}

temp\_genpop <- mat1p

temp\_genpop@tab[trick,] <- mat2p@tab[i,]

dis[i] <- dist.genpop(temp\_genpop[c(trick, i),], method = method)

}

list(dis = dis)

}

##### Initialization of seed, tolerance

if (!is.null(seed.)){

set.seed(seed.)

}

epsilon <- sqrt(.Machine$double.eps)

##### Dimensions check

n <- nrow(mat1p@tab)

p <- ncol(mat1p@tab)

if ((nrow(mat2p@tab) != n) | (ncol(mat2p@tab) != p)){

stop("The matrices are not of the same size!")

}

##### Empirical genetic distances

tmp <- dissim(mat1p, mat2p, method)

dis.ref <- tmp$dis

##### Permutations

if (nperm > 0) {

my.vec <- sample(1:(10 \* nperm), size = nperm)

outlier.count = rep(1, n)

for (iperm in 1:nperm) {

set.seed(my.vec[iperm])

mat1.perm <- mat1p

mat1.perm <- shufflepop(mat1.perm, method=4)

set.seed(my.vec[iperm])

mat2.perm <- mat2p

mat2.perm <- shufflepop(mat2.perm, method=4)

tmp <- dissim(mat1.perm, mat2.perm, method)

dis.perm <- tmp$dis

ge <- which(dis.perm + epsilon >= dis.ref)

if (length(ge) > 0) {

outlier.count[ge] <- outlier.count[ge] + 1

}

}

p.dist <- outlier.count/(nperm + 1)

}

p.adj <- p.adjust(p.dist, method = correc)

##### Simple gain or loss?

n.pop1 <- seppop(mat1)

n.pop2 <- seppop(mat2)

mean.hexp1 <- do.call("c", lapply(n.pop1, function(x) mean(summary(x)$Hexp)))

mean.hexp2 <- do.call("c", lapply(n.pop2, function(x) mean(summary(x)$Hexp)))

mean.hexp1[is.nan(mean.hexp1)] <- NA

mean.hexp2[is.nan(mean.hexp2)] <- NA

simple\_diff <- mean.hexp2 - mean.hexp1

# Please only take note of the sign of this difference, not the absolute value

output <- list(TBI = dis.ref, p.TBI = p.dist, p.adj = p.adj, gainloss = simple\_diff[p.adj < thresh\_for\_GL])

class(output) <- "TGI"

return(output)

}