How to improve PCA based methods of genome scan using ecological data: detecting selection using RDA.

Eric Bazin,*,[1] Keurcien Luu,[2] Michael G. B. Blum,[2]
[1]LECA, Université de Grenoble
[2]TIMC, Université de Grenoble
***Corresponding author:** E-mail: eric.bazin@univ-grenoble-alpes.fr
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Abstract

Ordination is a common tool in Ecology that aims at representing complex biological information on a reduced space. For instance, it is frequently used to study geographic distribution pattern of species diversity and to study the link between ecological variable such as temperature, drought, etc, on the species turnover. Recently, these methodologies are becoming quite popular in Landscape Genomic where one wants to study the link between environmental variable and the distribution pattern of genome wide diversity. However, it remains unclear what are the expected outcome of such approaches since genetic diversity has presumably a very different dynamic from species diversity. Simulations studies could help to shed light on this problem but they are still lacking whereas it tends to be broadly accepted as a pertinent approach. Furthermore, recent development have proposed to use ordination methods such as PCA to detect genes under selection. Simulations tend to support this idea has it seems to be quite robust to the underlying population structure and dynamic. Some authors have proposed to use other ordination approaches such as RDA, taking advantage of using environmental data. However no clear statistical framework have been developed to efficiently implement this idea in a robust and efficient test and once again, we don't know what is expected from the outcome of such approaches: which genes will be detected under which selective pressures? This paper aims at proposing a new test based on RDA approaches to search for genes under selection and to compare it to a classical PCA method. Thanks to individual based simulation, we compare both performance and robustness. Additionally, we test the efficiency of constrained ordination method such as RDA to detect relevant selective gradient since this was lacking in the Landscape Genomic literature. Finally, to illustrate the pertinence of such method in concrete example, we apply it to a real dataset.

Key words:

Article

Introduction

Performing genome scan in order to detect genomic region of interest is a common task in population genomic area (Foll and Gaggiotti, 2008; Frichot *et al.*, 2013; Luu *et al.*, 2016; Vatsiou *et al.*, 2015). Some methods aim at detecting genes that has suffered from a loss of genetic diversity and increase of linkage disequilibrium following

the appearance of a beneficial allele and its spread by the mean of selective sweep. Others aim at picking up alleles with strong correlation with some environnemantal variable (e.g. Temperature, drought) with the idea that these alleles may confer a selective advantage to the individuals (Coop *et al.*, 2010; Frichot *et al.*, 2013). Finally, other methods aim at detecting genomic region involved in local adaptation process. These region should have an increased differentiation between population because different alleles tend to be beneficial in each environment. Differentiation between population is excepted under the hypothesis of geographical isolation. Therefore, this region can be detected by quantifying the level of differentiation using some statistics and detecting the regions with unexpectedly high values. A common statistic and very easily comprehensible in population genetic is Fst. Many methods use this parameter as a basis in many different implementation of genome scans (Bazin *et al.*, 2010; de Villemereuil and Gaggiotti, 2015; Foll and Gaggiotti, 2008). These are model based method where parameters such as Fst are usually inferred using likelihood or Bayesian methods. This mean that users must have some a priori on their parameter value and the best model that fits their data in order to expect the best from their analysis. However, it is often difficult to get a satisfactory a priori picture of the demographic and population structure of the species one is interested in. Indeed many species are not clearly structured in different populations but more or less show a pattern of isolation by distance without clear geographical barrier to gene flow. One solution would be to use more complex model that better reflect reality but these are difficult to implement in Bayesian framework. Additionally, these latter methods are very time consuming and the increase of both model complexity and the amount of data to analyze in terms of the number of individuals and loci makes them more and more difficult to use. A new path has opened recently with the use of multivariate methods. The idea is to capture the whole genome geographic structure using an ordination method such as ACP. Following this analysis, outliers loci are detected if they have extremely high correlation with one or more ordination axis (Duforet-Frebourg *et al.*, 2014; Luu *et al.*, 2016). These are very efficient methods and simulations have shown that while they are very fast, they show similar efficiency than classical Bayesian method and sometimes perform better when the simulated demographic model drift from the model implemented in bayesian method, usually the island model. For instance Luu *et al.* (2016) have shown their method to be better when population are structured in hierarchical set or in isolation by distance pattern. Nevertheless, one conundrum of such approaches is the difficulty to interpret ordination axis in term of ecological meanings. These are usually tight to geographical axis (latitudinal or

longitudinal) but they are not necessary linked to an environmental variable such as Temperature, drought, diet habit, etc. Therefore, when this information exists, it has to be a posteriori used as a mean of interpretation but are not involved in the inference process. It should be recalled that natural selection is the result of a complex set of environmental pressures and that it most often acts on several characters simultaneously and that these characters are encoded by several genes which generally have weak effects. In order to extract the maximum of all available information, it seems therefore necessary to use approaches that are able to compile all kind of variable (e.g. alleles, phenotypic measurement, biotic and abiotic variables). One natural way to overcome this limitation would be to use more sophisticated ordination method than ACP like methods. Constrained ordination methods (i.e. Redundancy Analysis, RDA, Canonical Correspondence Analyis, CCA) are well-known set of approaches in Ecology for instance to explain the species distribution pattern by the mean of environmental data. They have specifically been designed in order to deal with biological complexity. In the population genomic era, it seems that data amount, complexity and heterogeneity is often a limitation to the use of inference methods based on classical population genetic models. Although they are more difficult to interpret, such approaches would be complementary to the model based method because of their long-term use in ecology and their efficiency on complex and large datasets. These method have sometimes been used in population genomic studies, not as a genome scan but in order to quantify multilocus adaptation to an environmental gradient (De Kort *et al.*, 2014; Hecht *et al.*, 2015; Lasky *et al.*, 2012; Steane *et al.*, 2014). These studies whereby relationships between environmental data and large multilocus data is explored are becoming more and more popular and are often coined as Ecological Genomics or Landscape Genomics studies. However the concept of using constrained ordination methods to analyse genomic data has never been tested on simulated datasets. This paper aims at filling this gap. First, we show how one can make use of a constrained ordination method namely Redundancy Analysis (RDA) as an efficient and robust genome scan method. We discarded the other constraint ordination methods such as CCA since they are very similar in their principles. RDA has already been used for instance by Lasky *et al.* (2012) to perform genome scan in order to detect loci involved in the adaptation to climate in *Arabidopsis thaliana*. Outliers were identified as SNPs with the greatest squared scores along the first RDA axis (i.e. those in the 0.5 % tail). We build on this idea to develop a comprehensive and robust statistical test that allows to search for outliers on an arbitrary number of RDA axis simultaneously and allows to control precisely for the false discovery

rate. Using simulations, we show that it has better results than PCA-based method. Second, thanks to these simulations, we show that RDA can indeed help to identify important environmental gradient that better explain the adaptive variation in the data. It is therefore a proof of concept of the idea of using constrained ordination method as an environmental genomic tool to identify relevent selective gradient in the environmental data. Finally, to give a concrete illustration of RDA approach in population genomics, we apply this method to the detection of outliers on a real data set.

Material and method

Genome scan

Redundancy analysis (RDA) was first introduced by (Rao, 1964) and is broadly described in (Legendre and Legendre, 2012) section 11.1. It is the direct extension of multiple regression to the modeling of multivariate response data. Typically the data to be analysed are separated in two sets, a response matrix Y of variable to be explained (e.g. species abundance in a set of sites; m sites and n species) and an explanatory matrix X (e.g. a set of environmental variable within each site; m sites and p environment). In the following analysis, species are replaced by loci and sites by individuals. In other word, we wish to project on a reduced space the proportion of variance in genetic difference between individuals which is better explained by environmental data. After this ordination, we follow the Luu *et al.* (2016) methodology to compute pvalues. First we compute the test statistic by regressing each of the p SNPs by the K ordination axis $X_1,...,X_K$.

$G_j = \sum_{k=1}^{K} \beta_{jk} X_k + \epsilon_j, j = 1,...,p$

where β_{jk} is the regression coefficient corresponding to the j-th SNP regressed by the k-th ordination axis, and ϵ_j is the residuals vector. To summarize the result of the regression analysis for the j-th SNP, we return a vector of z-scores $z_j = (z_{j1},...,z_{jK})$ where z_{jk} corresponds to the z-score obtained when regressing the j-th SNP by the k-th ordination axis. The test statistic is a robust Mahalanobis distance D computed using covRob function of the robustR package. We retain $K = 5$ axis to compute Mahalanobis distances as it seems to explain most of the variance. D should be Khi2 distributed after a correction with inflation factor (Luu et al., 2016). Pvalues are computed using K degree of freedom. We use the FDR approach to control for false positives. Qvalue are computed with qvalueR package and a loci is considered as an outlier if its qvalue is less than 10%. For the analysis of simulated dataset (see below), we retain the first four ordination axis to compute Mahalanobis distances as they seem to explain most of the variance in the data. To peform the ordination, we use the 10th environmental variables as input in the explanatory matrix. In the following example, we don't use phenotypic informations since these informations are often laking in environmental genomics. Neither we use

geographical coordinates (i,j) which is sometimes added to control for the geographical covariation in the differentiation pattern (Frichot *et al.*, 2013).

To emphasize the utility of RDA, we compared to pcadapt from which the idea of using multivariate method for genome scan is based. On the simulated dataset, we retain for both methods $K=5$ axis to compute Mahalanobis distances as it seems to explain the main amount of variance in the data using scatter plots. To control for false positive, we used the same qvalue threshold (i.e. ¡= 10%).

Environmental genomic

Once outliers have been identified, we isolate them in a separate matrix A defining an "adaptively enriched genetic space" as coined by Steane *et al.* (2014). Following their methodology, we perform a second constrained ordination (RDA) on matrix A against environmental data. The rational of this analysis is to remove neutral variation before performing ordination in order to have a better picture of which environmental gradients have the strongest association with the adaptive genetic space. On the simulated dataset, we report the R^2 statistics between env1, env2 and env3 and the first three ordination axis to have an idea of which they are better associated with and if the ordination space succeed in seperating the environmental effect on different axis.

Simulations

To test for the efficiency of RDA in population genomic, we performed simulations using simuPop python library (Peng and Kimmel, 2005). We compared our approach to PCAdapt method to perform genome scans. Both approach are equivalent except their ordination method. Finally we use these simulations to evaluate RDA approach as a mean to detect selective environmental gradient. A lattice of 8x8 populations is simulated (i.e. 64 populations in total). Each population is initialized with 200 diploid individual with random genotypes. Migration is set to 0.1 so that population structure must be very smooth and genetic differentiation must show an isolation by distance pattern over the 64 populations. This is where pcadapt is best designed for. Loci are biallelic (0 or 1) like SNPs. Allele frequency of the whole population is initialized at 0.5. 1000 loci are defined. They are separated in 200 chuncks of 5 SNPs in physical linkage with recombination rate between adjacent loci fixed at 0.1. 3 different Traits are coded by a group of 10 different loci. The first trait is coded by loci 1, 11, 21, ..., 91. The trait value is simply the sum of genotype value and therefore can take value between 0 an 20. For the sake of realism, we add to each trait a random noise (non heritable variation) drawn from a normal distribution $N(0,2)$. The second trait is coded by loci 101, 111, ..., 191 and the third is coded by loci 201, 211, ..., 291. Each trait

is therefore coded by free recombining SNP loci. In other words, there are 30 coding SNPs among 1000. Selection can have an effect on linked loci, for instance, loci 2, 3, 4 and 5 can be impacted by selection on locus 1. However, recombination is high enough (0.1) to expect a limited linkage effect. We have defined 10 different environmental variables. The first one determines the selective pressure on trait 1, the second one on trait 2 and the third one on trait 3. The first environment variable is a quadratic gradient coded by function $env1 = -(\cos(\theta) * (i - 3.5))^2 - (sin(\theta) * (j - 3.5))^2 + 18$,$\theta = \pi/2$, i and j being the population indicator on the 8x8 lattice. The second one is a linear plan gradient coded by function $env2 = h * \cos(\theta) * (i - 1) + h * \sin(\theta) * (j - 1) + k$ with $h = 2$, $\theta = \pi/4$ and $k = 3$. The third environment variable simulates a coarse environment with value $env3 = 2$ for all populations except population (i,j) = (2,2), (2,3), (3,2), (3,3), (6,2), (6,3), (7,2), (7,3), (2,6), (2,7), (3,6), (3,7), (6,6), (6,7), (7,6), (7,7) for which $env3 = 18$. Env4, env5 and env6 have exactly the same equation than env1, env2 and env3 respectively. The remaining 4 environment variable are similar to env2 but with different value of h and θ. Env7 has $h = 2$, $\theta = 0$ and $k = 3$. Env8 has $h = 2$, $\theta = \pi/4$ and $k = 0$. Env9 has $h = 1$, $\theta = \pi/4$ and $k = 4$. Env10 has $h = 0.5$, $\theta = \pi/4$ and $k = 8$. Graphical representation of mean environmental value for environment 1, 2 and 3 is given in Fig. **??**. Environment 4, 5 and 6 have respectively the same mean value spatial distribution. For a graphical representation of environment 7 to 10, see supplementary material. Environmental equation gives a mean value of the environment variable. To avoid colinearity between environments variable, we added noise by drawing an environment value within a normal distribution $N(\mu = env, \sigma = 1)$. Fitness for each trait is set to be $-e^{((x-env)^2/(2*\omega^2))}$, x being the quantitative trait value, env the environmental value and ω is defining selection strength and has been set to 20 which in our experience seems sufficient for loci to be often detected. To get the overall fitness for a given individual, fitness associated to each trait are multiplied. Fitness are relative and selection arises on parents and determine their number of offsprings. Simulations are made across 500 generations. At the end of simulation, we sample 10 individuals per population. Therefore, we have a sample of 640 individuals with 1000 SNP-like loci.

Real dataset

The *Populus trichocarpa* dataset is a sample of 424 individuals genotyped on 33,070 SNPs from 25 drainages (i.e., topographic units separated by watershed barriers) (Geraldes *et al.*, 2014). Genotyping of each accession was performed as described in (Geraldes *et al.*, 2013) using a 34K Populus SNP array targeting 34,131 SNPs mostly within (plus 2kb upstream and downstream) 3543 genes. Details of SNP and gene selection can be found in (Geraldes *et al.*, 2013). 21 climatic

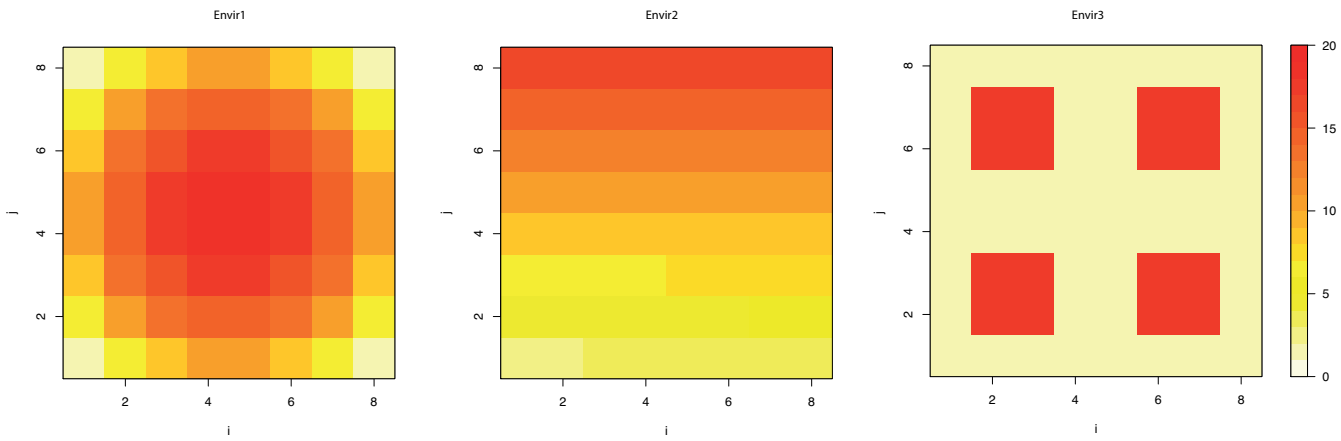

FIG. 1. Graphical representation of mean environmental value for environment 1, 2 and 3

variables are available on each sampling site (details in Table S1). For the RDA and pcadapt analysis, we retained respectively $K = 6$ and $K = 10$ axis as they seem to explain the majority of variance in the data. From the 33,079 SNPs, we removed the SNPs with missing values, leaving us 19,336 SNPs.

Results

Genome scan

When looking at the analysis on one simulation, the pcadapt method seems successful at detecting QTL2 SNPs (Fig. 2), is less performant at detecting QTL1 SNPs and fails entirely to pick-up QTL3 SNPs. On the other hand, RDA performs better at detecting QTL2 SNPs and retain a larger amount of the QTL1 and QTL2 SNPs (Fig. 3). Therefore, the ordination seems to correctly detect environmental variable 1, 2 and 3 as drivers of genetical variance in the data. Over the 100 simulations, we have measured the average FDR and power for both pcadapt and RDA (Fig 4).

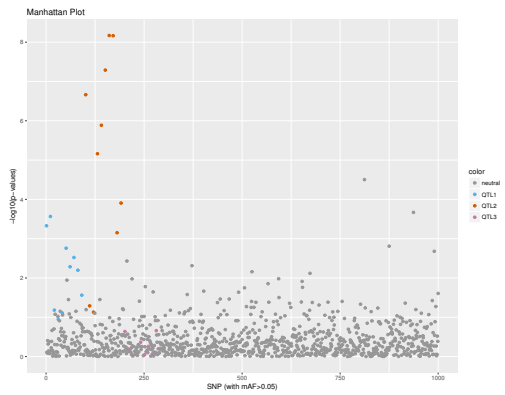

FIG. 2. Manhattan plot of the result of pcadapt on a simulated data set.

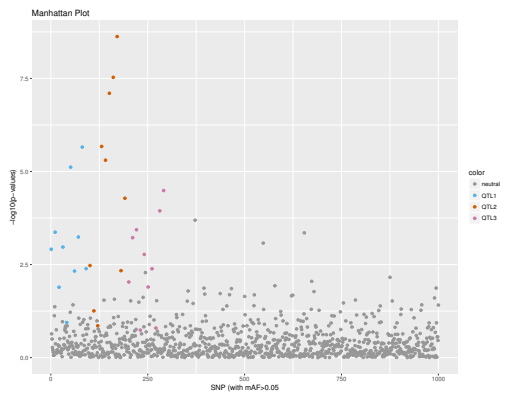

FIG. 3. Manhattan plot of the result of genome scan using RDA on a simulated data set.

Environmental genomics

We then performed a second RDA on the "adaptively enriched genetic space" as performed by Steane *et al.* (2014) on the same simulated

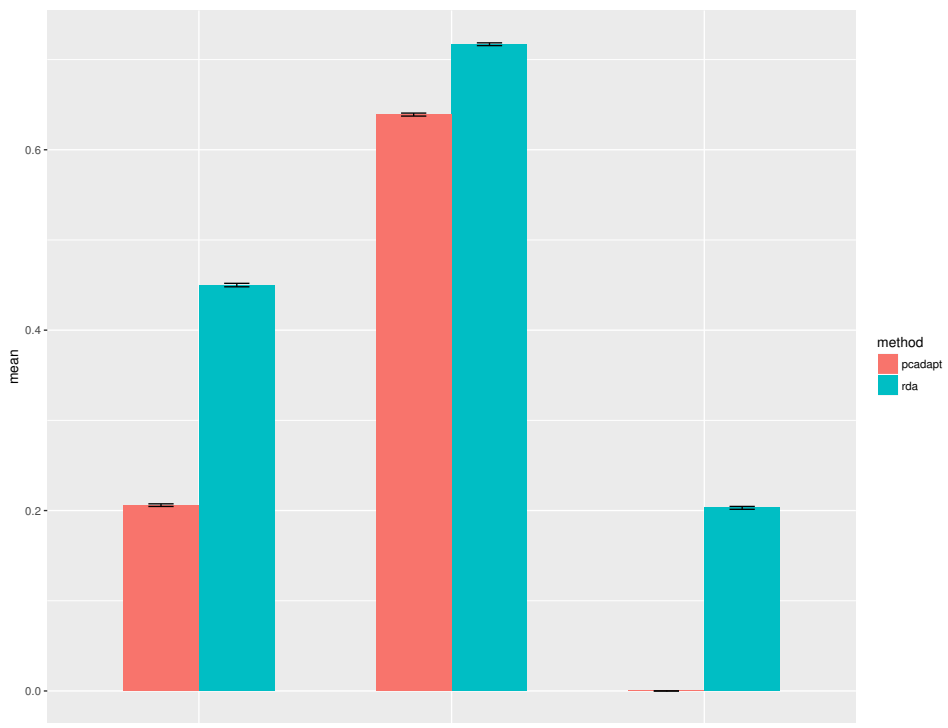

FIG. 4. Performance results of rda and pcadapt methods. Each performance value is averaged over 100 simulated dataset (error bars are displayed but hardly visible since they are very scarce). Power is given seperately for loci coding for quantitative trait 1, 2 and 3.

dataset as in Fig. 2 and 3 and display its results on Fig. 5. We did the same analyis and measured the mean R^2 between env1, env2 and env3 and each of the first three ordination axis. This is summarized in Fig. 6.

Populus trichocarpa

Analysis of *P. trichocarpa* with fdr of 0.1 leads to a list of 105 outliers for RDA and 122 for pcadapt. Interstingly, both methods have 52 outliers in common so that 53 and 70 SNPs are outliers specific to respectively RDA and pcadapt. When we compared RDA genome scan results with the outliers found by Geraldes *et al.* (2014) based on Fdist, bayescan and bayenv methods, we found that substential overlap between them. However, RDA based genome scan found 35 SNPs that no other methods (including pcadapt) had detected as outlier (see Table S2).

Discussion

Fig 2 shows that pcadapt approach works well when the environmental gradient and the selective pressures are correlated to the geographical pattern of isolation by distance. Whereas when the environment is a coarse environment (QTL3) it fails dramatically. Indeed, we can hypothesize that the PCA ordination in this case is not able to orientiate the genetic space differentiation into the direction of environment 3 therefore leaving no chance to detect any outliers on the QTL influenced by this environmental variable. Fig 3 shows that RDA has a much better behavior than pcadapt by taking advantage of using informations of environmental local conditions. It

can be attributed to a better alignment between the genetic space and the environmental variable improving the power to detect true positives.

Both methods have a good control of false discovery rate although slightly better for RDA (12.0×10^{-2} for pcadapt and 10.6×10^{-2} for RDA). Results summarized on Fig 4 is confirming that overall RDA shows better performance at detecting true outliers since it succeeds to detect quite often QTL1 and QTL3 SNPs. It seems however less efficient at detecting QTL3 outliers but this might be due to the fact that local adaptation on a coarse environment is more difficult that adaptation on a smooth environmental gradient as environment 1 and 2. These simulations plead in favor of using constrained ordination method instead of PCA when non genetic data such as environmental variable are available in order to orientate the axis in the direction of informative gradients.

When performing an RDA on the "adaptively enriched genetic space", Fig. 5 and 6 show that the method succeed at detecting the relevant selective gradient and separating them on different axis at least on our simulations. This therefore serves as a proof of concept of Steane *et al.* (2014)'s approach to represent multilocus selective gradient and the possibility to use the ordination axis it to devise a metric that provides a holistic measure of genomic adaptation. Indeed, in RDA1 is strongly associated with envir2, RDA2 with envir1 and RDA3 with envir3 whereas poorly associated with the other axis. As expected, the correlated environment are also strongly associated with this respective axis. This is reflecting the fact that in reality it is difficult on an environmental gradient to distinguish among the covariable which one has a causal effect on the individual fitness. However, it is often sufficient for biologists when performing an exploratory analysis to identify combination of environment variable having a strong association with adaptive variation without knowing precisely the underlying mechanical process.

From the analysis of *Popular trichocarpa*, we picked up some genes that can be interpreted regarding to the environmental variable. For instance, among the top SNPs, some of them are located within or nearby, POPTR_0015s00640 implicated in cellular response to cold, POPTR_0015s00440 involved in the regulation of circadian clock in plants. One outlier, POPTR_0017s06840 that is not detected by pcadapt is involved in amylase catabolism, an activity that is suspected to depend of climatic conditions. Interestingly, Table S2 shows that a substantial amount of RDA genome scan outliers (35) are not detected by any other genome scan method including pcadapt. For instance, four SNPs are assigned to POPTR_0007s04340, a dehydration-responsive protein-related which is similar to similar to early-responsive to dehydration stress ERD3 protein. However, its function remains mainly unknown. These results seem to support the

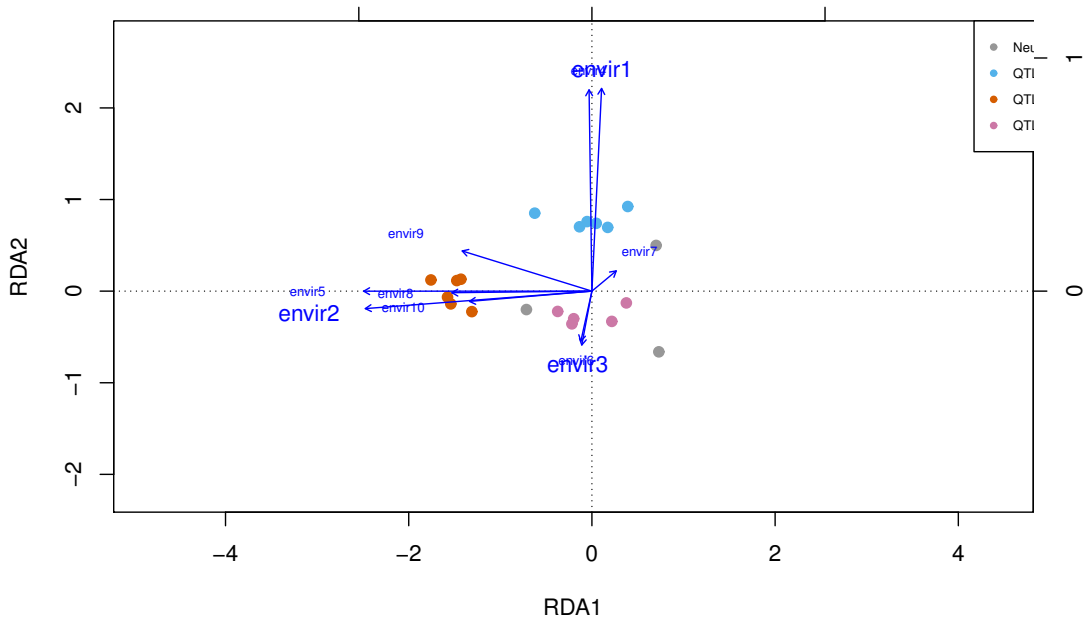

FIG. 5. RDA on the adaptively enriched genetic space. We discarded the individual points for readability. Dots represents outliers SNPs. R^2 of envir1 with the first, second and third axis is (0.172%, 77.6%, 17.6%), envir2 is (94.5%, 0.560%, 0.236%) and envir3 is (0.189%, 5.34%, 90.6%)

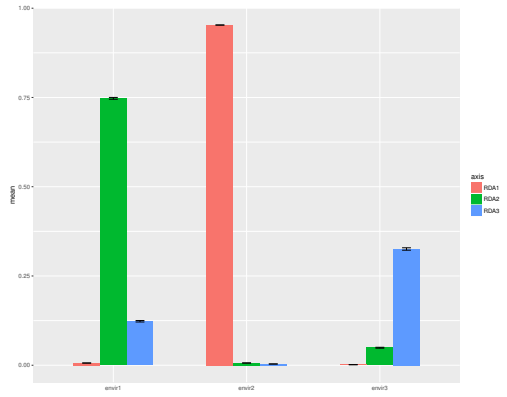

FIG. 6. R^2 between envir1, envir2 and envir3 and each of the first three ordination axis. Values are averaged across the 100 simulated datasets.

simulation analysis conclusions that RDA based genome scan is able to pick up more genes in relation to some environmental gradient than pcadapt. It is however noticeable that a large proportion of outliers are shared between PCA and RDA based methods which highlight the fact that both approaches are very similar. The lack of interpretability of PCA method is compensated by the fact that it is a blind method in regard to environmental data so it can pick up genes that we will miss using RDA because some crucial environmental data are lacking.

When performing an RDA analysis on the adaptively enriched genetic space (RDA and pcadapt outliers merged together), we noticed that first and second axis of RDA explains a large amount of constrained variance and can be used to identify important environmental gradient (Fig. S2). RDA1 is mainly explained by MAT, DD_0 and DD_18 (Fig. S3) which are temperature related climatic variables and RDA2 is mainly explained by SHM, Eref and CMD which are moisture related climatic variables. Although there is a large covariance between

climatic variable, this analysis proves that RDA can be used to identify and distinguish different environmental gradients that are responsible for the adaptive differentiation across the geographic distribution of *Populus trichocarpa*. This was of course expected since the ordination is constrained by environmental axis and is supported by simulations studies as explained previously.

In conclusion, this paper shows that both methods are complementary and should be used simultaneously on the same dataset. RDA will be more easily interpretable in terms of mechanism and should pick up more genes when some important selective gradient are identified but PCA might be able to pick up outliers where no environmental data are available. However this latter method will miss outliers when selective gradient are poorly correlated to the population structure (i.e. geographical distance between populations and individuals). It is therefore crucial when performing landscape genomics to clearly characterize the environmental condition by measuring important variable that are suspected to put selective pressures on the species of interest. This can be done through RDA as it has been validated in our simulations and in the real dataset on *Populus trichocarpa*.

A myriad of constrained ordination method exist that could be used and allows to control for confounding variable (i.e. partial RDA) for instance altitude, latitude and longitude or allow to perform none linear regression between genetic and environmental data (i.e. LVM - Latent Variable Model). This is still to be tested and explored using both simulation and real data set validation.

Supplementary Material

Acknowledgments

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