Pstat: An R Package to Assess Population Differentiation in Phenotypic Traits

by Stéphane Blondeau Da Silva and Anne Da Silva

Abstract The package **Pstat** calculates P_{ST} values to assess differentiation among populations from a set of quantitative traits and provides bootstrapped distributions and confidence intervals for P_{ST}. Variations of P_{ST} as a function of the parameter c/h^2 are studied as well. The package implements different transformations of the measured phenotypic traits to eliminate variation resulting from allometric growth, including calculation of residuals from linear regression, Reist standardization, and the Aitchison transformation.

Introduction

Understanding the causes governing patterns of morphological variations in the wild represents a fundamental goal of evolutionary biology. In particular, the relative importance of selective and neutral processes behind the observed differentiation remains a crucial question.

Studies comparing differentiation in quantitative traits and neutral markers have significantly increased over the last ten years (Leinonen et al., 2013). Typically, a set of populations is sampled and the degree of genetic differentiation is estimated for a set of molecular markers with the Wright's F_{ST} index (Wright, 1951). For its part, the Q_{ST} index (Spitze, 1993) assesses the degree of phenotypic differentiation over a set of quantitative traits. The logic of F_{ST} and Q_{ST} comparison relies on the assumption that the F_{ST} obtained by the consideration of neutral markers reflects the divergence only induced by genetic drift (Reynolds et al., 1983). Hence, F_{ST} provides a null expectation and allows estimation of the degree of population differentiation that would be reached without selection (Merilä and Crnokrak, 2001).

As a consequence, the comparison between F_{ST} and Q_{ST} leads to three possibilities: (i) $Q_{ST} > F_{ST}$ means that quantitative traits show a higher level of differentiation than what would have been expected under the influence of genetic drift, such that natural selection could induce differentiation between populations by favoring different phenotypes (i.e., heterogeneous selection); (ii) $Q_{ST} < F_{ST}$ could indicate the influence of natural selection, but selecting for same optima among populations (i.e., homogeneous selection); (iii) $Q_{ST} = F_{ST}$ means that no departure from neutral expectations can be detected and that the degree of differentiation in quantitative traits could have been obtained only by genetic drift, even if the contribution of natural selection can neither be excluded nor estimated.

Spitze (1993) introduced and defined the Q_{ST} quantity as follows for diploid species assuming purely additive gene action:

$$Q_{ST} = \frac{\sigma_{A_b}^2}{\sigma_{A_b}^2 + 2\sigma_{A_w}^2} ,$$

where $\sigma_{A_b}^2$ and $\sigma_{A_w}^2$ are the morphological additive genetic variance components between and within populations.

In the wild, the estimation of the additive genetic variance components is challenging as breeding design is impossible. Therefore, Q_{ST} is often approximated by P_{ST} (Leinonen et al., 2006), which is directly calculated from the total phenotypic variance components with no distinction between the relative contribution of genetic and environmental variations:

$$P_{ST} = \frac{c\sigma_b^2}{c\sigma_b^2 + 2h^2\sigma_w^2} \quad \text{or} \quad P_{ST} = \frac{\frac{c}{h^2}\sigma_b^2}{\frac{c}{h^2}\sigma_b^2 + 2\sigma_w^2} , \tag{1}$$

where σ_b^2 and σ_w^2 are the respective phenotypic variances between and within populations, c is an estimate of the proportion of the total variance due to additive genetic effects across populations, and h^2 is heritability, the proportion of phenotypic variance due to additive genetic effects (Brommer, 2011). How well P_{ST} approximates Q_{ST} depends on the parameters c and h^2, such that if the values of c and h^2 are known, then the phenotypic divergence quantified by P_{ST} would equal Q_{ST}. This implies caution in the interpretation obtained from P_{ST} (Brommer, 2011).

A large number of studies have assessed the potential for natural selection to affect morphological evolution by comparing phenotypic divergence with neutral genetic divergence via a P_{ST} versus F_{ST} approach (e.g., Mobley et al., 2011; Lima, 2012; He et al., 2013; Shinn et al., 2015). While estimation of F_{ST} values are included in various R packages such as **diveRsity** (Keenan et al., 2013) or **hierfstat**

(Goudet, 2005), no R package exists to deal with the P_{ST} index. In this study, we present the **Pstat** package to handle large datasets of quantitative traits and correct quantitative traits taking into account allometric growth. The package calculates P_{ST} values with their respective bootstrapped confidence intervals, and offers several options to select individuals, traits, or populations. We also provide various plotting tools for the visual evaluation of P_{ST} and F_{ST} values. We will walk through a detailed example to give an overview of the **Pstat** package.

An example to get familiar with the main functions

After loading the package with `library(Pstat)`, load the sample data with `data(test)`. This data frame contains 200 rows, with each row representing an individual in a population of common wetland plants, *Juncus effusus* (see Michalski and Durka 2015 and the Dryad Digital Repository, https://doi.org/10.5061/dryad.bk5hk). The data frame contains the name of the populations (A, B, C, D, and E) to which each individual belongs and eleven quantitative measures. An excerpt from the sample data are presented in Table 1.

	Populations	QM1	QM2	QM3	QM4	...
1	A	0.18487253	0.4001979	0.1694021	42	...
2	B	0.24023500	0.4718000	0.2178500	46	...
3	C	0.23499676	0.4686213	0.2060222	25	...
4	B	0.20495223	0.3746026	0.1846816	51	...
5	C	0.20739220	0.4866461	0.2131618	19	...
6	C	0.22545341	0.3770903	0.1882165	28	...
7	C	0.18371681	0.4992361	0.2167194	25	...
...	...	...	...	...	...	...

Table 1: Sample from the `test` data frame, containing quantitative measures for individual members of *Juncus effusus* (Michalski and Durka, 2015).

The data preparation

The package can be used to transform data to eliminate variation resulting from allometric growth. Users have the choice between three alternatives:

1. Residuals of a linear regression, with one of the quantitative variables used as the regressor (Kuhry and Marcus, 1977);

2. The allometric transformation described in Reist (1985); or

3. Aitchison's log-ratio transform (Aitchison, 1986).

Among a variety of univariate transformations that aim to separate size and shape variations, Reist (1985) showed that adjustments for size using a regression and residuals (the first option) and allometric adjustments to a standard size (the second option) are preferred since they allow the complete removal of size variations and have minimal impact on the correlation and covariance structure of the data. Unlike the first two options, the third transformation offers the benefit of keeping the same number of variables. We provide examples of each of the three alternatives below.

Simple linear adjustments

The first adjustment method provided by **Pstat** is a simple linear regression. Assuming the existence of linear relationships between the dependent variable and one of the quantitative traits, the `Res` function returns a new data frame with the residuals of the regression. The function's arguments are as follows:

- **data**: the studied data frame to be transformed with as many rows as individuals; the first column must contain the population to which the individual belongs and the other columns may contain quantitative variables.

- **reg**: the name or the rank of the variable chosen as the regressor.

- **Rp**: the names of the populations to be deleted. Default value: `Rp=0`, no population removed.

- **Ri**: the line numbers of individuals to be deleted. Default value: `Ri=0`, no individuals removed.

We present sample output from the test data, using one of the quantitative traits as the regressor. A sample of the transformed data output by Res is presented in Table 2.

```
## Using the explanatory variable QM3 as the regressor
Res(data=test, reg="QM3")
```

	Populations	QM1	QM2	QM4	...
1	A	0.0339245264	5.621424e-03	6.23817063	...
2	B	0.1001268662	4.497085e-02	8.44522196	...
3	C	0.0922422473	4.966613e-02	-12.11705813	...
4	B	0.0574228940	-3.014565e-02	14.67271191	...
5	C	0.0662351079	6.293798e-02	-18.38127681	...
6	C	0.0787149904	-3.001126e-02	-8.45810788	...
7	C	0.0433557311	7.315960e-02	-12.51293868	...
...	...	...	...	...	...

Table 2: Sample from the adjusted data frame output by Res, using QM3 as the explanatory variable.

Reist transformation

In the second adjustment method provided by **Pstat**, all morphometric measurements are standardized using the transformation proposed by Reist (1985).

Let n be the number of individuals and p the number of quantitative traits such that $\exists\ k \in \{1, \ldots, p\}$ and the kth trait is the explanatory variable. Let us denote this variable $(x_i)_{1 \leq i \leq n}$ and the other traits as $j \in \{1, \ldots, p\} \setminus \{k\}$, $(y_{ij})_{1 \leq i \leq n}$. The Reist transformation is

$$\forall i \in \{1, \ldots, n\} \quad \text{and} \quad \forall j \in \{1, \ldots, p\} \setminus \{k\},$$
$$Y_{ij} = log(y_{ij}) - b_j(log(x_i) - log(\overline{x})),$$

where Y_{ij} is the size adjusted measurement of the jth trait for the ith individual, y_{ij} the original morphometric measurement, $\overline{x}$ the population mean of the explanatory variable, and x_i the value of the explanatory variable for the ith individual. For all $j \in \{1, \ldots, p\} \setminus \{k\}$, the parameter b_j is estimated for the quantitative trait y_j (i.e. $(y_{ij})_{1 \leq i \leq n}$) and represents the slope of the linear regression of $log(y_j)$ on $log(x)$.

The ReistTrans function returns a corrected data frame. Using QM3 as the explanatory variable, we present a sample of the transformed data frame in Table 3.

```
## Using QM3 as the explanatory variable (identified by column number)
ReistTrans(test, reg=3)
```

	Populations	QM1	QM2	QM4	...
1	A	-0.7445410	-0.3859875	1.631722	...
2	B	-0.6004703	-0.3462059	1.648348	...
3	C	-0.6167708	-0.3421063	1.388608	...
4	B	-0.6893556	-0.4255755	1.708186	...
5	C	-0.6669355	-0.3300087	1.266323	...
6	C	-0.6456670	-0.4250906	1.446049	...
7	C	-0.7175846	-0.3210021	1.384003	...
...	...	...	...	...	...

Table 3: Sample from the Reist adjusted data frame using QM3 as the explanatory variable.

Aitchison transformation

The third adjustment method provided by **Pstat** performs the Aitchison log-ratio transformation to account for individual size-effects (Aitchison, 1986).

Let n be the number of individuals and p the number of morphological traits. For $j \in \{1, \ldots, p\}$, let $(y_{ij})_{1 \leq i \leq n}$ represent the quantitative variables. The formula formula for the Aitchison transformation

is as follows:

$$\forall i \in \{1, \ldots, n\} \quad \text{and} \quad \forall j \in \{1, \ldots, p\},$$

$$Y_{ij} = log(y_{ij}) - \frac{1}{p} \sum_{k=1}^{p} log(y_{ik}),$$

where Y_{ij} is the transformed measure of the jth trait for the ith individual, and y_{ij} is the original value for the ith individual and the jth trait.

The `AitTrans` function returns a corrected data frame. Sample output are included in Table 4.

```
AitTrans(test)
```

	Populations	QM1	QM2	QM3	QM4	...
1	A	-1.947544	-1.6121417	-1.985498	0.408832854	...
2	B	-1.910214	-1.6170919	-1.952692	0.371908012	...
3	C	-1.834151	-1.5343910	-1.891299	0.192727037	...
4	B	-1.901481	-1.6395625	-1.946710	0.494436831	...
5	C	-1.832709	-1.4622885	-1.820792	0.129251912	...
6	C	-1.801889	-1.5785008	-1.880288	0.292211929	...
7	C	-1.938699	-1.5045418	-1.866950	0.195092172	...
...	...	...	...	...	...	...

Table 4: Sample from the Aitchison adjusted data frame.

Phenotypic differentiation evaluation and confidence intervals

P_{ST} **values**

We are interested in determining the phenotypic differentiation across the five populations for each of the eleven quantitative traits of the example dataset. The function `Pst` can determine the P_{ST} values of each trait with the associated bootstrapped confidence intervals (Efron and Tibshirani, 1993). The arguments to `Pst` are as follows:

- ○ **data**: the input data frame with as many rows as individuals; the first column must contain the population label and the others quantitative variables.
- ○ **ci**: if `ci=1`, the confidence intervals are added to P_{ST} values. Default value: `ci=0`.
- ○ **csh**: the $\frac{c}{h^2}$ value. Default value: `csh=1`.
- ○ **va**: a vector containing the names or column numbers of the quantitative measures under consideration. If `va=0`, all the variables are selected. Default value: `va=0`.
- ○ **boot**: the number of data frames generated to determine the confidence interval with the bootstrap method. Default value: `boot=1000`.
- ○ **Pw**: the names of the two populations considered to obtain pairwise P_{ST}. Default value: `Pw=0`, no pairwise analysis.
- ○ **Rp**: the names of the populations to be deleted. Default value: `Rp=0`, no populations removed.
- ○ **Ri**: the line numbers of individuals to be deleted. Default value: `Ri=0`, no individuals removed.
- ○ **pe**: the confidence level of the calculated interval. Default value: `pe=0.95`.

Let us apply the `Pst` function to the `test` dataset. The output from `Pst` will be a data frame:

```
## Example 1:  Pairwise Pst values using populations C and D
Pst(test, csh=0.2, Pw=c("C","D"))
[1] "Populations sizes are:"
 C  D
76 32
   Quant_Varia Pst_Values
 1        QM1  0.1749659
 2        QM2  0.7460913
...        ...        ...
```

```
4        QM10  0.9800028

## Example 2: Pst for the 2nd variable and QM7 with 99% confidence intervals
Pst(test, va=c(2,"QM7"), ci=1, boot=10000, Ri=c(5,117:121), pe=0.99)
[1] "Populations sizes are:"
 A  B  C  D  E
12 76 72 30  4
  Quant_Varia Pst_Values 99 %_LowBoundCI 99 %_UpBoundCI
1         QM2  0.8561307        0.7826177      0.9198395
2         QM7  0.8851413        0.7722856      0.9376501
```

Distribution of P_{ST}

The bootstrapped P_{ST} values output from `BootPst` form a distribution for the selected quantitative trait. In addition to arguments that are shared with `Pst`, the `BootPst` function has the following additional arguments specific to the bootstrap procedure:

- ○ **opt**: if opt=0, all the boot values of P_{ST} are returned; if opt="ci", the ordered values and the confidence interval are returned; and if opt="hist", the ordered values and the distribution histogram of P_{ST} are returned. Default value: opt=0.
- ○ **va**: the name or column number of the quantitative measure considered.
- ○ **bars**: the maximum number of bars the histogram may have. On the x-axis, the interval $[0, 1]$ is divided into bars parts (there may exist unfilled bars). Default value: bars=20.

The output from the `BootPst` function is a vector with the bootstrapped values.

Let us apply the `BootPst` function to `test` dataset:

```
## Example 1: Bootstrapped 95% confidence intervals for three populations (B, C, and D).
## Note that populations A and E are dropped
BootPst(test, opt="ci", va="Body_length", Rp=c("A","E"))
[1] "The studied quantitative variable is:"
[1] "Body_length"
[1] "Populations sizes are:"
 B  C  D
76 76 32
[1] "95 % confidence interval determined by 1000 bootstrap values:"
[1] 0.8757057 0.9585423
   [1] 0.7938426 0.8338286 0.8510682 0.8512374 0.8545911 0.8551115 0.8552097
   [8] 0.8637057 0.8641575 0.8644145 0.8659723 0.8671139 0.8671265 0.8676122
  [15] 0.8686147 0.8702277 0.8708352 0.8711419 0.8718030 0.8721783 0.8734932
   ...
 [995] 0.9621794 0.9625852 0.9634700 0.9644283 0.9650500 0.9689611

## Example 2: Histogram for the trait in column 3 (output in Figure 1)
BootPst(test, opt="hist", va=3, bars=50)
[1] "The studied quantitative variable is:"
[1] "QM3"
[1] "Populations sizes are:"
 A  B  C  D  E
12 76 76 32  4
[1] "1000 bootstrap values and Pst distribution:"
   [1] 0.1062747 0.1076470 0.1269888 0.1593121 0.1775196 0.2050347 0.2111617
   [8] 0.2327508 0.2401064 0.2487401 0.2588179 0.2589942 0.2623706 0.2722956
  [15] 0.2827915 0.2860497 0.2935858 0.2947525 0.2954878 0.2995198 0.3003267
   ...
 [995] 0.8211326 0.8253874 0.8293417 0.8318546 0.8420100 0.8635299
```

Variations of P_{ST} values and visual comparison with Wright's F_{ST} index

Brommer (2011) and Lima (2012) offer plots that demonstrate how F_{ST} and P_{ST} depend on the $\frac{c}{h^2}$ ratio. The **Pstat** package provides plotting tools to perform these analyses with the function `TracePst`. Arguments specific to `TracePst` include:
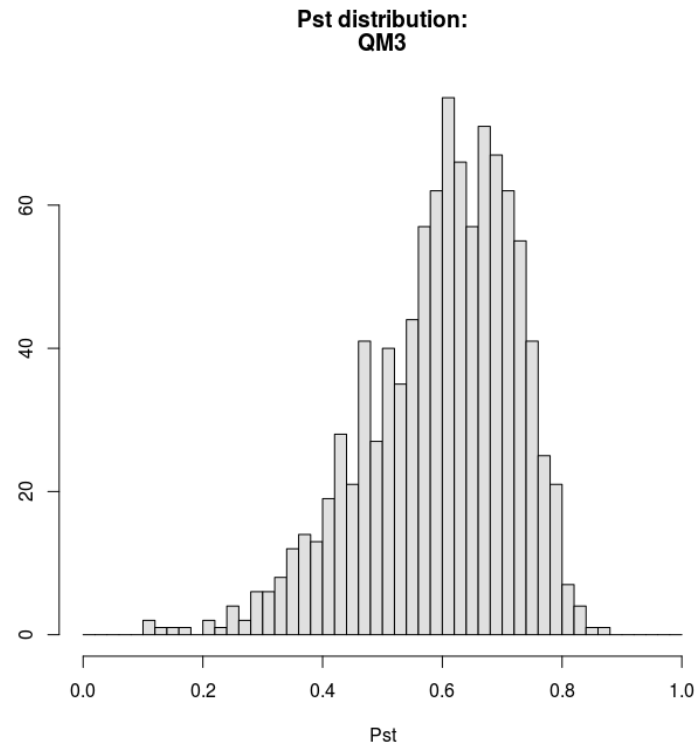
Pst distribution:
QM3

Figure 1: P_{ST} distribution histogram of QM3.

- **va**: a vector containing the selected variables names or numbers (*i.e.* those of the quantitative measures considered). If va=0, all the variables are selected. Default value: va=0.
- **ci**: if ci=1, the confidence interval of P_{ST} is plotted. Default value: ci=1.
- **Fst**: the value of Wright's F_{ST}, if available. Default value: Fst=-1, value of F_{ST} is unavailable.
- **xm**: x-axis maximum. Default value: xm=2.
- **pts**: the number of points used to plot the curves. Default value: pts=30.

Let us apply the TracePst function to the test dataset. The plots output are in Figure 2.

```
# Aitchison adjustment method:
trans_test=AitTrans(test)

# Plots illustrating how comparisons between Fst and Pst depends on c/h^2:
TracePst(trans_test, Fst=0.3, xm=3)
[1] "Populations sizes are:"
 A  B  C  D  E
12 76 76 32  4
```

Conclusion

The use of P_{ST} versus F_{ST} comparison has increased rapidly in the last few years in the field of evolutionary and ecological genetics. The **Pstat** package is the counterpart of existing R packages dealing with *F*-statistics. It calculates P_{ST} values, and also provides bootstrapped confidence intervals, several graphical tools, as well as three ways of transforming data to remove variation resulting from allometric growth.

Bibliography

J. Aitchison. *The Statistical Analysis of Compositional Data*. Chapman and Hall, London, New York, 1986. 416 pp. [p448, 449]
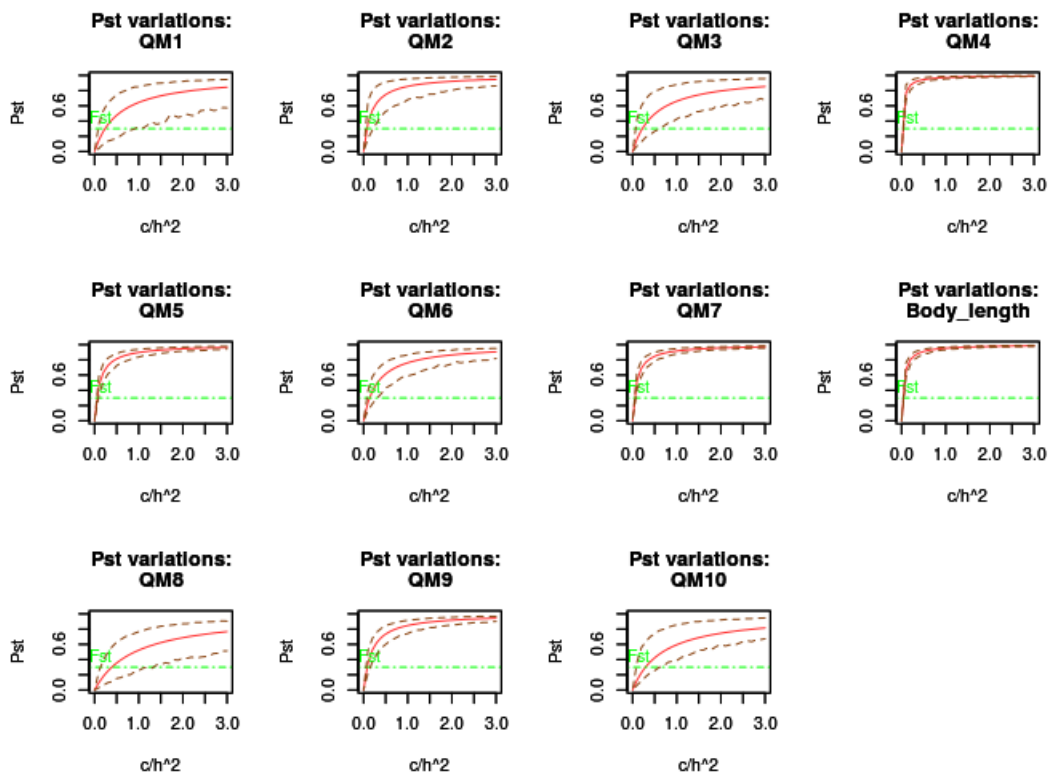
Figure 2: Plots illustrating comparisons between F_{ST} and P_{ST}. The horizontal dotted green line marks the value of F_{ST}. P_{ST} values and associated 95% confidence intervals are plotted in red.

J. E. Brommer. Whither Pst? The approximation of Qst by Pst in evolutionary and conservation biology. *Journal of Evolutionary Biology*, 24:1160–1168, 2011. URL https://doi.org/10.1111/j.1420-9101.2011.02268.x. [p447, 451]

B. Efron and R. Tibshirani. *An Introduction to the Bootstrap*. Chapman and Hall, New York, 1993. [p450]

J. Goudet. HIERSTAT, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Resources*, 5(1):184–186, 2005. URL https://doi.org/10.1111/j.1471-8286.2004.00828.x. [p448]

Y. He, R. Li, J. Wang, S. Blanchet, and S. Lek. Morphological variation among wild populations of chinese rare minnow (Gobiocypris rarus): Deciphering the role of evolutionary processes. *Zoological Science*, 30:475–483, 2013. URL https://doi.org/10.2108/zsj.30.475. [p447]

K. Keenan, P. McGinnity, T. F. Cross, W. W. Crozier, and P. A. Prodöhl. diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, 4(8):782–788, 2013. URL https://doi.org/10.1111/2041-210X.12067. [p447]

B. Kuhry and L. F. Marcus. Bivariate linear models in biometry. *Systematic Zoology*, 26(2):201–209, 1977. URL https://doi.org/10.2307/2412842. [p448]

T. Leinonen, J. M. Cano, H. Makinen, and J. Merilä. Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology*, 19:1803–1812, 2006. URL https://doi.org/10.1111/j.1420-9101.2006.01182.x. [p447]

T. Leinonen, R. J. McCairns, R. B. O'Hara, and J. Merilä. Qst–Fst comparisons: Evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews Genetics*, 14:179–190, 2013. URL https://doi.org/10.1038/nrg3395. [p447]

M. R. Lima. Genetic and morphometric divergence of an invasive bird: The introduced house sparrow (Passer domesticus) in Brazil. *PloS One*, 7(12), 2012. URL https://doi.org/10.1371/journal.pone.0053332. [p447, 451]

J. Merilä and P. Crnokrak. Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology*, 14:892–903, 2001. URL https://doi.org/10.1046/j.1420-9101.2001.00348.x. [p447]

S. G. Michalski and W. Durka. Separation in flowering time contributes to the maintenance of sympatric cryptic plant lineages. *Ecology and Evolution*, 5(11):2172–2184, 2015. URL https://doi.org/10.1002/ece3.1481. [p448]

K. B. Mobley, D. Lussetti, F. Johansson, G. Englund, and F. Bokma. Morphological and genetic divergence in swedish postglacial stickleback (Pungitius pungitius) populations. *BMC Evolutionary Biology*, 2011. URL https://doi.org/10.1186/1471-2148-11-287. [p447]

J. D. Reist. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. *Canadian Journal Zoology*, 63:1429–1439, 1985. URL https://doi.org/10.1139/z85-213. [p448, 449]

J. Reynolds, B. S. Weir, and C. C. Cockerham. Estimation of the coancestry coefficient: Basis for a short-term genetic distance. *Genetics*, 105:767–779, 1983. [p447]

C. Shinn, S. Blanchet, G. Loot, S. Lek, and G. Grenouillet. Phenotypic variation as an indicator of pesticide stress in gudgeon: Accounting for confounding factors in the wild. *Science of the Total Environment*, 538:733–752, 2015. URL https://doi.org/10.1016/j.scitotenv.2015.08.081. [p447]

K. Spitze. Population structure in Daphnia obtusa: Quantitative genetic and allozymic variation. *Genetics*, 135:367–374, 1993. [p447]

S. Wright. The genetical structure of populations. *Annals of Human Genetics*, 15:323–354, 1951. URL https://doi.org/10.1111/j.1469-1809.1949.tb02451.x. [p447]

Stéphane Blondeau Da Silva, corresponding author
XLIM-Mathis, UMR n°7252 CNRS-Université de Limoges
123, avenue Albert Thomas 87060 Limoges
France
blondeaudasilva@xlim.fr

Anne Da Silva
INRA, UMR n°1061 Génétique Moléculaire Animale
Université de Limoges, UMR n°1061 Génétique Moléculaire Animale
123, avenue Albert Thomas 87060 Limoges
France
anne.blondeau@unilim.fr