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Original article

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ABSTRACT

Aim

Pleistocene climatic oscillations induced range fluctuations in African rain forest organisms and may have shaped species diversification through allopatric speciation events. We compared the spatial genetic structure of two forest species that live in obligate symbiosis and thus must have experienced the same range fluctuations, as a means to discriminate incipient speciation from transient differentiation simply resulting from past divergence.

Location

Western central Africa.

Methods

We genotyped 765 individuals of the tree *Barteria fistulosa* and 605 colonies of its symbiotic ant *Tetraponera aethiops* at 12 and 13 microsatellite loci respectively. We compared the spatial genetic structure of the two symbionts by using Bayesian clustering algorithms, isolation-by-distance analyses and clines of synthetic alleles. We used species niche modelling (climatic and soil variables) to investigate ecological variables associated with genetic discontinuities in tree populations.

Results

The trees and the ants showed congruent patterns of spatial genetic structure. However, the trees showed a very steep genetic discontinuity between groups north and south of latitude 1°N, which was much weaker in the ants. There was no evidence for effective gene flow between the two tree

lineages in contact at the transition zone, despite the presence of a few hybrids. Niche modelling did not predict the occurrence of northern trees south of this genetic transition, and vice versa.

Main conclusions

The genetic discontinuity near latitude 1°N is inferred to be a tension zone resulting from reproductive incompatibilities between previously allopatric tree lineages. This tension zone may have stabilized at a climatic transition (between boreal and austral seasonal regimes), and matches patterns of genetic structure previously observed in other forest plant species. Our results illustrate independent speciation between two species that live in specific and obligate symbiosis and suggest that a tension zone may separate lineages of several central African forest plants near the thermal equator.

KEYWORDS

Africa, *Barteria*,climatic oscillations, Guineo-Congolian rainforest, incipient speciation, phylogeography, Pleistocene, symbiosis, tension zone, *Tetraponera*.

INTRODUCTION

During the Pleistocene, Central African populations of forest-dependent species experienced cycles of contraction and expansion (Maley, 1996). Because there are few mountainous areas in this zone, populations of forest species had little opportunity to escape climate changes by altitudinal shifts. As a consequence, the spatial extent of forest contraction/expansion cycles must have led to strong population reduction and fragmentation, and species extinctions, possibly explaining the lower biodiversity observed in tropical Africa than in tropical America and Southeast Asia (Morley, 2000). Extinctions may however be balanced by speciation events, as populations occupying fragmented forest cover may evolve in isolation for considerable periods of time. This process may account for several plant species radiations that were shown to have occurred during the Pliocene and the Pleistocene (Harris, 2000; Wieringa & Gervais, 2003; Plana *et al.*, 2004; Couvreur *et al.*, 2011).

The spatial genetic structure of many plant and animal species of the tropical rain forest has recently been described in the domain of Lower Guinea (i.e., western Central Africa) in order to investigate the types and locations of putative forest refugia during the Pleistocene (plants: Hardy *et al.*, 2013; Dauby *et al.*, 2014; Heuertz *et al.*, 2014; Ley *et al.*, 2014; Duminil *et al.*, 2015; Faye, 2015; animals: Smith *et al.*, 2000; Telfer *et al.*, 2003; Bowie *et al.*, 2006; Anthony *et al.*, 2007; Gonder *et al.*, 2011; Nicolas *et al.*, 2011). Notably, 10 of the 11 plant species investigated present a genetic discontinuity between populations north and south of latitude ~1-3°N. A similar genetic discontinuity is reported in the tree *Barteria fistulosa* Mast. (Passifloraceae) (Peccoud *et al.*, 2013). This recurrent genetic discontinuity is even more intriguing because the region does not show any obvious physical barrier. It is therefore tempting to assume the existence of a reproductive barrier between north and south lineages of several Central African forest plants, indicating a broad pattern of incipient allopatric speciation. However, strong genetic discontinuities not matching any obvious physical barrier within continuous species ranges may simply constitute remnants of past

divergences that subsequent gene flow may erode. Addressing this uncertainty requires a detailed investigation of the contact zone between genetic groups.

The tree *B. fistulosa* offers a unique opportunity to test whether the observed north-south discontinuity is a sign of incipient speciation or a transient imprint of past divergence. Indeed, this tree lives in obligate and specific symbiosis with the ant *Tetraponera aethiops* Smith, 1877 (Hymenoptera: Formicidae), which means that both organisms experienced the exact same range fluctuations. Habitat fragmentation should therefore leave comparable genetic discontinuities between the two partners. However, symbionts and hosts usually have very different life history characteristics (e.g., generation time, dispersal range, mutation rate) so that their genetic structures may erode at different rates (Nieberding & Olivieri, 2007). Symbiotic partners may also differ in respect to the strength of reproductive barriers that have evolved during geographical isolation, and which govern the maintenance of genetic discontinuities if secondary contact occurs. As a result, comparing genetic discontinuities in populations of obligate symbionts may inform on the presence of such reproductive barriers and thus, on the evolutionary consequences of forest fragmentation due to climatic fluctuations. This symbiosis is widely distributed in the lowlands of central Africa, though restricted to the tropical rainforest, such that populations of the two species have experienced fragmentation, isolation and recent expansion that are expected to have created spatial genetic structure. Moreover, the two species reproduce by outcrossing, which allows interpreting population genetic results in the same way for both. Finally, the association has to be re-established anew at each generation, which ensures that signatures of population dynamics at neutral markers are independent between the two species.

Here, we distinguish between reproductive isolation and transient differentiation resulting simply from past divergence by confronting the spatial genetic structures of populations of the two obligate symbionts, *B. fistulosa* and *T. aethiops*. We particularly sampled along a transect in a region of supposed contact between two previously identified genetic groups of *B. fistulosa*

(Peccoud *et al*., 2013).

MATERIALS AND METHODS

Study system

Barteria fistulosa trees have swollen and hollow branches presenting cavities called domatia in which nests the large black ant *T. aethiops*. A closely related ant species, *T. latifrons* Emery, 1912, is occasionally associated with *B. fistulosa*, although much less frequently than *T. aethiops*. The two ant species can be distinguished unambiguously and *T. latifrons* is not considered in this paper. Each tree is occupied by a single colony (Yumoto & Maruhashi, 1999; Blatrix and Djiéto-Lordon, unpublished field observations). The ant has a powerful sting and protects its host tree from herbivorous insects (Janzen, 1972; Dejean *et al.*, 2008) and vertebrates (McKey, 1974). It also prunes surrounding vegetation (Janzen, 1972; Yumoto & Maruhashi, 1999). In return, the ant colony gets most of its food resources from the tree through exploitation of extrafloral nectar and of hemipterans reared within domatia (Bequaert, 1922; Janzen, 1972). Fungi grown within domatia constitute an additional food source for the ants (Blatrix *et al.*, 2012).

Study area and sampling

Trees of *B. fistulosa* and associated *T. aethiops* ants were sampled in western Central Africa, across the Lower Guinea domain (Cameroon, Gabon, Republic of Congo) (see Appendices S1 and S2 in Supporting Information). Leaf samples were dried with silica gel immediately upon collection. Additional samples were obtained from the National Herbarium of the Netherlands (WAG) and the Royal Botanic Garden Edinburgh (E), Scotland. Samples of the symbiotic ant *T. aethiops* were stored in 70% ethanol or dried with silica gel. Since each *B. fistulosa* tree is occupied by a single colony of *T. aethiops*, we used only one individual ant from each tree for genetic analyses.

Molecular methods

DNA was extracted from ~0.2 g of dry leaf for each tree using the DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands), and from the head of each ant with the DNeasy Tissue Kit (Qiagen), or with the Extract-N-Amp PCR ReadyMix (Sigma-Aldrich, St. Louis, USA) for both ant and tree specimens, following the manufacturers' instructions. Each individual of *B. fistulosa* was genotyped for 12 microsatellite markers (Bar6, Bar16, Bar31, Bar50, Bar51, Bar53, Bar56, Bar57, Bar58, Bar62, Bar64, Bar69) following a protocol described previously (Molecular Ecology Resources Primer Development Consortium *et al.*, 2012b). Each individual of *T. aethiops* was genotyped for 13 microsatellite markers (Molecular Ecology Resources Primer Development Consortium *et al.*, 2012a). We used a total of 765 and 605 individuals for the tree and the ant respectively, including 358 tree individuals that were genotyped for 11 loci by Peccoud *et al.* (2013).

Spatial genetic structure

To assess spatial genetic structure, we first used the Bayesian clustering algorithm implemented in the software STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), which uses the genotypic data alone. STRUCTURE was run 10 times for each number of groups assumed (*K* from 1 to 10), considering admixture and independent allele frequencies between groups (Falush *et al.*, 2003), with the admixture parameter α inferred from the data and given a uniform prior. Each run consisted of 1 000 000 iterations, including a burn-in of 200 000. Runs for each value of *K* were summarised using CLUMPP 1.1.2 (Jakobsson & Rosenberg, 2007). The most likely number of groups was determined using the method of Evanno *et al.* (2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012).

To take spatial coordinates of sampled individuals into account, we also used the Bayesian clustering algorithm implemented in the software TESS 2.3 (François *et al.*, 2006). This approach is less likely than STRUCTURE to identify genetic discontinuities that may result from uneven sampling. TESS was run 10 times for each *K* from 2 to 10, using the admixture BYM model. Each

run consisted of 200 000 iterations, including a burn-in of 50 000. The interaction parameter was set to 0.6. The most likely number of groups was determined by comparing the deviance information criterion (DIC) among values of *K*. Congruence between the groups defined by STRUCTURE and those defined by TESS was evaluated by computing the proportion of individuals that were not assigned to the same group in the two analyses.

To assess the reality of the genetic discontinuities identified by the Bayesian clustering algorithms, pairwise kinship coefficients (Loiselle *et al.*, 1995) were computed for pairs of individuals within groups, pairs among groups and all pairs. In the presence of a genetic discontinuity, it is expected that kinship between individuals of different groups is lower than that between individuals within the same group, for the same geographical distance. Kinship coefficients were computed using SPAGEDI 1.4 (Hardy & Vekemans, 2002) for each of 10 categories of spatial distances defined so that the number of pairwise comparisons was similar across categories. Jackknife standard errors were computed over loci. For each level of partition of the pairwise kinship coefficients—within groups, among groups and all pairs—we drew either a logarithmic or a linear regression curve, whichever best fitted the data. Best fit was determined by comparing the coefficients of determination computed by SPAGEDI. To test for spatial genetic structure, the slope of the regression was tested by 9 999 random permutations of locations. Pairwise F_{ST} values among groups were calculated with SPAGEDI using the ANOVA approach (Weir & Cockerham, 1984).

We then focused on the north-south genetic discontinuity already noticed in a previous study (Peccoud *et al.*, 2013), which proved to be congruent between the ant and the tree in the present study. We compared tree and ant genetic data in respect to the abruptness of the discontinuity, independently of differences in allelic frequencies, by building synthetic alleles (Bierne *et al.*, 2002) and considering $K = 2$ genetic groups for both species. In each species, allelic frequencies were obtained from SPAGEDI for each locus and for each of the two groups. Each allele was attributed to the group in which its frequency was higher, and was then replaced by this group name in the

individual genotypes, creating a synthetic allele. This allowed computing the frequency of each synthetic allele for each individual (averaged across loci), which we call *y*. A generalized logistic function was fitted using the following formula: $y = a + (b-a)/(1+e^{c\cdot(x-d)})$, where *x* is the individual's coordinates along a transect perpendicular to the genetic discontinuity, *a* is the low asymptote, *b* the high asymptote, *c* the slope of the tangent at the inflexion point, and *d* the abscissa of the inflexion point. Parameters *a*, *b* and *c* describe the shape of the regression and allow comparing the abruptness of the genetic discontinuity between the tree and the ant.

As we highlighted a particularly abrupt genetic discontinuity for the tree, we searched for potential hybrids using the Bayesian method implemented in NEWHYBRIDS 1.1 (Anderson & Thompson, 2002). Individuals were assigned to one of the five following categories: each of the two purebred parents, F1 and each of the two first-generation backcrosses. Uniform priors were used. The Markov chain was run for 1 000 000 iterations, including a burn-in of 500 000.

To assess whether the difference in strength of the genetic discontinuity between ants and trees could be explained by different dispersal abilities, we compared degrees of isolation-by-distance of the two species. To limit the influence of genetic discontinuities that may interfere with the estimate of isolation-by-distance, we restricted this analysis to within genetic groups (for two groups, including the most geographically widespread), that were congruent between the tree and the ant. For pairs of individuals of this group, average pairwise kinship coefficients (Loiselle *et al.*, 1995) established with SPAGEDI were plotted on 10 categories of spatial distances defined so that the number of pairwise comparisons was approximately constant across categories.

Species distribution modelling

To explore the possibility that the main spatial genetic discontinuity in *B. fistulosa* could match with an environmental discontinuity (genotype-environment association), we built a species distribution model based on environmental variables for each of the two genetic groups independently and we checked whether each group was predicted to occur on the opposite side of the genetic

212 discontinuity. We used the R package 'biomod2' (Thuiller *et al.*, 2013) with the "ensemble
213 modelling" approach. We selected seven climatic and five soil variables (see below) out of the 19
214 and seven from WorldClim (Hijmans *et al.*, 2005) and Africa Soil Profiles Database
215 (http://www.isric.org/data/soil-property-maps-africa-1-km) respectively. Variables from the Africa
216 Soil Profiles Database contained values for six soil depths that we averaged for this study. The
217 purpose of variable selection was to remove strong correlation among variables used in the
218 modelling procedure. Among correlated variables, we selected the one that appeared the most
219 meaningful biologically according to our knowledge of the organisms. After selection, pairwise
220 correlations between variables were < 0.7. Variables used were (names in parentheses are names of
221 the variables in their respective databases): annual mean temperature (BIO1), maximum
222 temperature of warmest month (BIO5), minimum temperature of coldest month (BIO6),
223 precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of
224 warmest quarter (BIO18), precipitation of coldest quarter (BIO19), pH (PHIHO5), soil organic
225 carbon (ORCDRC), sand content (SNDPPT), silt content (SLTPPT) and bulk density (BLD). All
226 variables were used at a 10 min. resolution. We used presence data for the focal genetic group only.
227 Presence data of the non-focal group were coded as *no data*, like all the cells for which we lacked
228 presence data. As there were no true absence data, five repetitions of 100 pseudo-absences were
229 generated following the *Surface Range Envelope* strategy. Nine different distribution models were
230 built (GLM, GBM, GAM, ANN, SRE, CTA, RF, MARS and FDA; for definition of the models see
231 reference manual of 'biomod2' at https://cran.r-project.org/web/packages/biomod2/biomod2.pdf).
232 Model evaluation was based on a five-fold cross-validation by randomly splitting the data set into
233 two subsets, one for calibrating the model (70 % of the presence data) and one for testing the model
234 (30 % of the presence data). After removing modelling runs with an evaluation TSS score < 0.7,
235 individual models were combined with the committee averaging algorithm (i.e., model probabilities
236 are transformed into binary data which are averaged over models). The resulting meta-model was

projected on the current variables. To determine which environmental variables were most important in explaining the distribution of the focal genetic group we normalized the relative contributions of each variable to each run of each model and plotted the average over the 25 runs of each model for each variable (five independent runs × five repetitions of generation of pseudo-absences).

Statistical analyses and figures, unless specified otherwise, were performed using R 3.1.0 (R Core Team, 2014).

RESULTS

Individual genotypes and collection details are presented in Appendices S1 and S2.

Spatial genetic structure

For the tree, DIC values obtained with TESS began to stabilize at $K = 4$ groups (Appendix S3). However, the ΔK value was highest for $K = 2$. Thus, both values were investigated. For the ant, both DIC values and ΔK indicate that the most likely number of groups is four (Appendix S3). For both the tree and the ant, the genetic groups defined by TESS and STRUCTURE for $K = 4$ were highly congruent (only 9 % and 7 % of the tree and ant individuals respectively were not assigned to the same group), indicating that the two algorithms gave similar results. For $K = 2$, there was no mismatch at all for the tree and only 0.5 % of the ant individuals mismatched. We thus chose to consider only the results obtained with STRUCTURE in the subsequent analyses (Fig. 1a). Kinship coefficients were higher for pairwise comparisons within than among groups, whatever the geographic distance (Fig. 1b), hence the genetic discontinuities are real and not the product of isolation-by-distance coupled with uneven sampling. Regression of kinship coefficient on spatial distance was significant for pairwise comparisons within groups, among groups and among all individuals for both the ant and the tree, indicating spatial genetic structure. Kinship coefficients for pairs of trees belonging to different groups (Fig. 1b, circles), and for all trees (diamonds), were

261 better fitted by a linear than by a logarithmic regression, indicating that a process other than
262 isolation-by-distance is involved in the spatial genetic structure of *B. fistulosa* (Born *et al.*, 2008).
263 This was not the case for *T. aethiops*.

264 In each species, the four genetic groups are spatially segregated and their distributions appear
265 congruent between the tree and the ant (Fig. 1a). However, the southernmost group of trees was
266 highly differentiated from the three other ones (F_{ST} = 0.38), congruent with results from Peccoud *et*
267 *al*. (2013), a pattern that did not occur in the ant (Fig. 1a). In contrast, the main genetic
268 discontinuity in the ant was that between the northernmost group and the others.

269 **Characterisation of the north-south genetic discontinuity**

270 The frequencies of synthetic alleles representative of the northern groups (assuming K = 2 for
271 trees and for ants) along a latitudinal transect (Fig. 2) confirm that the north-south genetic
272 discontinuity is much more abrupt in trees. Not only do asymptotes from either side of the
273 discontinuity represent more extreme (high or low) allelic frequencies in trees, but also the slope of
274 the tangent at the inflexion point is steeper for the tree (parameter c = 6.79) than for the ant
275 (c = 2.81).

276 The NEWHYBRIDS algorithm detected 21 individuals of *B. fistulosa* as potential hybrids
277 between the northern and southern groups, all of which were found within a narrow contact zone of
278 ~100 km (Fig. 3). These hybrid individuals represented 18 % of the individuals sampled in this area.
279 No F1 hybrid was detected as only first-generation backcrosses were found.

280 **Isolation-by-distance within genetic groups**

281 Within two widespread genetic groups separated by the north-south genetic discontinuity,
282 regression of pairwise kinship coefficients on spatial distance was significant both for the tree and
283 the ant, and values were better fit by a logarithmic than a linear regression, indicating a pattern of
284 isolation-by-distance for both organisms (Fig. 1a). In the southern (blue) groups, the decline in

kinship coefficients and the fit of regression are similar in ants (r^2 = 0.2452) and trees (r^2 = 0.2427) (Fig. 4a). In the northern (orange) group, the steeper decline in kinship coefficients and the better fit of regression in ants (r^2 = 0.0766) than in trees (r^2 = 0.0018) showed that isolation-by-distance within this widespread genetic group is more pronounced in ants (Fig. 4b). The same pattern was observed when we considered pairs within groups for the whole sampling of trees and ants (triangles in Fig. 1b).

Species distribution modelling

Modelling of the spatial distribution of the *B. fistulosa* genetic groups north and south, based on environmental variables, showed similar and symmetrical genotype-environment associations for the two groups, and thus, only those for the northern group are presented. The predicted distribution is restricted to the northern half of Lower Guinea. The model does not predict the northern group to occur where the southern group occurs (Fig. 5). Among the 12 soil and climatic variables used to build the models, one had a particularly strong contribution to the explanation of the distribution of the morthern genetic group: precipitation during the driest quarter (Fig. 6), which is lower in the south than in the north. This was true for each of the nine modelling algorithms used in the ensemble modelling procedure.

DISCUSSION

The tree *Barteria fistulosa* and its obligate ant symbiont *Tetraponera aethiops* both display spatial genetic structure and share the same geographic pattern of genetic discontinuities. These patterns probably result from the multiple successive events of range fragmentation through time (Maley, 1996). The Last Glacial Maximum imposed particularly dry conditions in the lowlands of tropical Africa that resulted in a considerable reduction of the forest, so that most of the surface covered by the current African rainforest has been recolonized only relatively recently (Hessler *et al.*, 2010; Lezine *et al.*, 2013). Genetic discontinuities remain detectable after secondary contact, as long as

gene flow has not completely erased past differentiation. Obligate symbionts are expected to show spatial congruency in their genetic structures because they experienced the same range fluctuations as well as a recent episode of recolonization.

Most values of genetic differentiation between groups are moderate, except that between the southernmost tree group and the others, where differentiation reaches a value (F_{ST} = 0.38) similar to those frequently observed between plant sister species (Friar *et al.*, 2006; Yu *et al.*, 2013). We do not consider that the southern and northern populations deserve formal description as species. We rather consider they are at a stage of incipient speciation that might eventually lead to full speciation. The ant does not show such a strong north-south differentiation, neither in F_{ST} nor in the strength of shift of synthetic allele frequencies (Fig. 2). Notably, the levels of genetic differentiation between the southernmost tree group and northern groups are unaffected by geographical distance (Fig. 1a), indicating absence of significant gene flow across the north-south genetic discontinuity since the divergence of the three northern groups. Contrariwise, the southernmost group of ants is genetically closest to its parapatric group (Fig. 1a). This implies that ant genes have flowed across this boundary, not that the southernmost ant population diverged more recently, since divergence times must be the same as for the host trees. For parapatric ant populations to become genetically closer, the duration of contact must greatly exceed the effective generation time of *B. fistulosa* (~30 years), and should have left enough time for gene flow between tree groups across the north-south boundary. The absence of such gene flow cannot be due to less efficient dispersal, as trees show weaker within-group isolation-by-distance than ants (Fig. 4). Considering that nothing at this contact zone is susceptible to impede seed or pollen dispersal, the only plausible barrier preventing genes flow between *B. fistulosa* groups is reproductive isolation. Incipient allopatric speciation between *B. fistulosa* group South and the other groups can explain the genetic structure of both symbiotic partners, in particular the co-occurring genetic discontinuity between northern and southern populations as the remnant of past allopatric divergence that gene flow has partially eroded

for the ant. Comparing spatial genetic structure between species in obligatory symbiosis (Alvarez *et al.*, 2010) thus proves useful for inferring the existence of reproductive barriers in contact zones.

The north-south transition zone between *Barteria fistulosa* genetic groups is less than 100 km wide, is located near latitude 1°N and within this zone, approximately 20 % of the individuals are of hybrid origin. Absence of effective gene flow between genetic groups despite the presence of hybrids indicates that post-zygotic isolation (hybrid unfitness) is an important component of overall reproductive isolation between these tree groups. Post-zygotic isolation can freely evolve in allopatry, under the Dobzhansky-Muller-Bateson model (Coyne & Orr, 2004), resulting in intrinsic genetic incompatibilities that can kill or sterilize hybrids in many taxa (Presgraves, 2010). These incompatibilities generally do not result from the adaptation of populations to their respective environments, and thus cause “intrinsic” (environmentally-independent) post-zygotic isolation (Turelli *et al.*, 2001). Alternatively or in conjunction, hybrids may suffer from maladaptation to parental niches (ecological speciation, reviewed in Rundle & Nosil, 2005), given that the two tree groups are predicted to populate distinct environments. Known cases of hybrid ecological maladaptation result from divergent selective pressures of considerable strength that are imposed upon parental ecotypes by plainly distinct environments, such as mountain versus plain for plants (Wang *et al.*, 1997), limnetic versus benthic zones for sticklebacks (Rundle, 2002) or different host species for phytophagous insects (Egan & Funk, 2009; Peccoud *et al.*, 2014). By contrast, the predicted niches of the tree groups present subtle differences that only appeared through modelling. These differences seem to us unlikely to impose selection pressures nearly as strong as those imposed upon the aforementioned ecotypes. We therefore believe that ecology would only contribute weakly, if at all, to hybrid deficiency in *B. fistulosa*, especially when it is considered that the contact zone may in fact represent an intermediate environment particularly suited to hybrids (e.g., Wang *et al.* 1997).

Spatial segregation of the northern and southern tree lineages, despite efficient dispersal

359 capacities, would mostly result from reciprocal exclusion by competition. Migrants from the other
360 side of the contact zone are outcompeted by residents, as their progeny would mostly consist of
361 deficient hybrids. The zone of contact between genetic groups can thus be seen as a “tension zone”,
362 a term defining a cline maintained by a balance between dispersal and selection against hybrids
363 (Barton & Hewitt, 1985). The spatial location of the tension zone should move freely according to
364 the relative competitive ability of each lineage at the colonization front. Modelling approaches
365 predict that the spatial location of a tension zone should stabilize on an environmental transition, a
366 phenomenon conceptualised as “the coupling hypothesis” (Bierne *et al.*, 2011, 2013). In *Barteria*
367 *fistulosa*, edaphic and climatic conditions would sufficiently differ between the areas north and
368 south of latitude 1°N to explain the geographic position of the tension zone. One climatic variable
369 contributed particularly heavily to niche differentiation: precipitation during the driest quarter,
370 which was lower in the area occupied by the Southern group. It still remains to be tested whether
371 southern individuals are better adapted than northern ones to a particularly pronounced dry season.
372 Genetic differentiation between groups north and south of latitude 1-3°N has been detected
373 in many plant species in the Lower Guinea domain (see Introduction). Interestingly, these latitudes
374 correspond to the “climatic hinge”, which is defined as the transition between the boreal and austral
375 seasonal regimes. The tension zone we revealed between *Barteria fistulosa* lineages in that region
376 (at 1°N) suggests that other Central African forest taxa may also have undergone incipient allopatric
377 speciation during past episodes of forest fragmentation. This hypothesis, and the existence of other
378 tension zones, can be tested by genetic analyses of candidate species that occur in sufficiently high
379 densities in the equatorial region to permit adequate sampling. In combination, ecological data may
380 inform on the environmental variables—shifts in seasonal phenology, differing soil and climate
381 conditions—that may have a stabilizing effect on secondary contact zones, and on the extent to
382 which these abiotic parameters explain the geographical genetic structure observed in several taxa
383 of the African tropical rain forest.

To conclude, our results show that reproductive isolation can evolve independently between obligatory symbionts, so that diversification in ant-plant symbioses does not necessarily involve co-speciation. Although this has already been inferred from the taxonomic distribution of ant-plant symbioses and phylogenetic approaches (Davidson & McKey, 1993; Chomicki & Renner, 2015), our study constitutes the first snapshot of ongoing disjoint speciation in these symbioses. Surprisingly, we show that isolated populations of trees can evolve stronger reproductive isolation than their ant symbionts, even though insects have shorter generation times and generally present higher speciation rates than trees. This unexpected outcome of allopatric divergence illustrates the inherent stochasticity in the evolution of reproductive incompatibilities, which depends on the occurrence and location of mutations, so that reproductive barriers need not emerge in taxa that look more likely to speciate *a priori*.

In addition, our results provide insight into the process of incipient speciation after secondary contact. In central Africa, re-colonization is little constrained by landforms, allowing for more accurate perception of the effects of environmental factors other than topography on the spatial distribution of genetic discontinuities. The existence of a tension zone between lineages of *Barteria fistulosa* that co-localizes with an environmental transition at the climatic hinge informs us on possible ongoing speciation in other Central African forest species, and on the ecological factors that govern the location of secondary contact zones in general.

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REFERENCES

Alvarez, N., McKey, D., Kjellberg, F. & Hossaert-McKey, M. (2010) Phylogeography and historical biogeography of obligate specific mutualisms. *The biogeography of host-parasite interactions* (ed. by S. Morand and B. Krasnov), pp. 31–39. Oxford University Press, Oxford.

Anderson, E.C. & Thompson, E.A. (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, **160**, 1217–1229.

Anthony, N.M., Johnson-Bawe, M., Jeffery, K., Clifford, S.L., Abernethy, K.A., Tutin, C.E., Lahm, S.A., White, L.J., Utley, J.F., Wickings, E.J. & Bruford, M.W. (2007) The role of Pleistocene refugia and rivers in shaping gorilla genetic diversity in central Africa. *Proceedings of the National Academy of Sciences USA*, **104**, 20432–20436.

Barton, N. H. & Hewitt, G. M. (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, **16**, 113-148.

Bequaert, J. (1922) Ants in their diverse relations to the plant world. *Bulletin of the American*

Museum of Natural History, **45**, 333–583.

Bierne, N., David, P., Langlade, A. & Bonhomme, F. (2002) Can habitat specialisation maintain a mosaic hybrid zone in marine bivalves? *Marine Ecology Progress Series*, **245**, 157–170.

Bierne, N., Gagnaire, P.A. & David, P. (2013) The geography of introgression in a patchy environment and the thorn in the side of ecological speciation. *Current Zoology*, **59**, 72–86.

Bierne, N., Welch, J., Loire, E., Bonhomme, F. & David, P. (2011) The coupling hypothesis: why genome scans may fail to map local adaptation genes. *Molecular Ecology*, **20**, 2044–2072.

Blatrix, R., Djiéto-Lordon, C., Mondolot, L., La Fisca, P., Voglmayr, H. & McKey, D. (2012) Plant-ants use symbiotic fungi as a food source: new insight into the nutritional ecology of ant-plant interactions. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 3940–3947.

Born, C., Kjellberg, F., Chevallier, M.-H., Vignes, H., Dikangadissi, J.-T., Sanguié, J., Wickings, E.J. & Hossaert-McKey, M. (2008) Colonization processes and the maintenance of genetic diversity: insights from a pioneer rainforest tree, *Aucoumea klaineana*. *Proceedings of the Royal Society of London B: Biological Sciences*, **275**, 2171–2179.

Bowie, R.C., Fjeldsa, J., Hackett, S.J., Bates, J.M. & Crowe, T.M. (2006) Coalescent models reveal the relative roles of ancestral polymorphism, vicariance, and dispersal in shaping phylogeographical structure of an African montane forest robin. *Molecular Phylogenetics and Evolution*, **38**, 171–188.

Chomicki, G. & Renner, S.S. (2015) Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytologist*, **207**, 411–424.

454 Couvreur, T.L.P., Porter-Morgan, H., Wieringa, J.J. & Chatrou, L.W. (2011) Little ecological
455 divergence associated with speciation in two African rain forest tree genera. *BMC*
456 *Evolutionary Biology*, **11**, 296.

457 Coyne, J.A. & Orr, H.A. (2004) *Speciation.* Sinauer Associates, Sunderland, MA.

458 Dauby, G., Duminil, J., Heuertz, M., Koffi, G.K., Stévart, T. & Hardy, O.J. (2014) Congruent
459 phylogeographical patterns of eight tree species in Atlantic Central Africa provide insights
460 into the past dynamics of forest cover. *Molecular Ecology*, **23**, 2299–2312.

461 Davidson, D.W. & McKey, D. (1993) The evolutionary ecology of symbiotic ant-plant
462 relationships. *Journal of Hymenoptera Research*, **2**, 13–83.

463 Dejean, A., Djiéto-Lordon, C. & Orivel, J. (2008) The plant ant *Tetraponera aethiops*
464 (Pseudomyrmecinae) protects its host myrmecophyte *Barteria fistulosa* (Passifloraceae)
465 through aggressiveness and predation. *Biological Journal of the Linnean Society*, **93**, 63–69.

466 Duminil, J., Mona, S., Mardulyn, P., Doumenge, C., Walmacq, F., Doucet, J.L. & Hardy, O.J.
467 (2015) Late Pleistocene molecular dating of past population fragmentation and demographic
468 changes in African rain forest tree species supports the forest refuge hypothesis. *Journal of*
469 *Biogeography*, **42**, 1443–1454.

470 Earl, D.A. & vonHoldt, B.M. (2012) STRUCTURE HARVESTER: a website and program for
471 visualizing STRUCTURE output and implementing the Evanno method. *Conservation*
472 *Genetics Resources*, **4**, 359–361.

473 Egan, S.P. & Funk, D.J. (2009) Ecologically dependent postmating isolation between sympatric
474 host forms of *Neochlamisus bebbianae* leaf beetles. *Proceedings of the National Academy of*
475 *Sciences of the United States of America*, **106**, 19426–19431.

476 Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using
477 the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

478 Falush, D., Stephens, M. & Pritchard, J.K. (2003) Inference of population structure using multilocus
479 genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

480 Faye, A. (2015) *Dynamique évolutive des forêts tropicales humides d'Afrique Centrale: cas d'étude*
481 *de la famille des palmiers (Arecaceae).* PhD Thesis. Université de Montpellier, France.

482 François, O., Ancelet, S. & Guillot, G. (2006) Bayesian clustering using hidden Markov random
483 fields in spatial population genetics. *Genetics*, **174**, 805–816.

484 Friar, E.A., Prince, L.M., Roalson, E.H., McGlaughlin, M.E., Cruse-Sanders, J.M., Groot, S.J.D. &
485 Porter, J.M. (2006) Ecological speciation in the east Maui-endemic *Dubautia* (Asteraceae)
486 species. *Evolution*, **60**, 1777–1792.

487 Gonder, M.K., Locatelli, S., Ghobrial, L., Mitchell, M.W., Kujawski, J.T., Lankester, F.J., Stewart,
488 C.-B. & Tishkoff, S.A. (2011) Evidence from Cameroon reveals differences in the genetic
489 structure and histories of chimpanzee populations. *Proceedings of the National Academy of*
490 *Sciences of the United States of America*, **108**, 4766–4771.

491 Hardy, O.J., Born, C., Budde, K., Dainou, K., Dauby, G., Duminil, J., Ewédjé, E.E.B.K., Gomez,
492 C., Heuertz, M., Koffi, G.K., Lowe, A.J., Micheneau, C., Ndiade-Bourobou, D., Pineiro, R.
493 & Poncet, V. (2013) Comparative phylogeography of African rain forest trees: a review of
494 genetic signatures of vegetation history in the Guineo-Congolian region. *Comptes Rendus*
495 *Geoscience*, **345**, 284–296.

496 Hardy, O.J. & Vekemans, X. (2002) SPAGEDI: a versatile computer program to analyse spatial
497 genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–

620.

Harris, D. (2000) Rapid radiation in *Aframomum* (Zingiberaceae): evidence from nuclear ribosomal DNA internal transcribed spacer (ITS) sequences. *Edinburgh Journal of Botany*, **57**, 377–395.

Hessler, I., Dupont, L., Bonnefille, R., Behling, H., Gonzalez, C., Helmens, K.F., Hooghiemstra, H., Lebamba, J., Ledru, M.P., Lezine, A.M., Maley, J., Marret, F. & Vincens, A. (2010) Millennial-scale changes in vegetation records from tropical Africa and South America during the last glacial. *Quaternary Science Reviews*, **29**, 2882–2899.

Heuertz, M., Duminil, J., Dauby, G., Savolainen, V. & Hardy, O.J. (2014) Comparative phylogeography in rainforest trees from Lower Guinea, Africa. *Plos One*, **9**, e84307.

Hijmans, R.H., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Jakobsson, M. & Rosenberg, N.A. (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.

Janzen, D.H. (1972) Protection of *Barteria* (Passifloraceae) by *Pachysima* ants (Pseudomyrmecinae) in a Nigerian rain-forest. *Ecology*, **53**, 885–892.

Ley, A.C., Dauby, G., Köhler, J., Wypior, C., Röser, M. & Hardy, O.J. (2014) Comparative phylogeography of eight herbs and lianas (Marantaceae) in central African rainforests. *Frontiers in Genetics*, **5**, 403.

Lezine, A.M., Assi-Kaudjhis, C., Roche, E., Vincens, A. & Achoundong, G. (2013) Towards an

520 understanding of West African montane forest response to climate change. *Journal of*
521 *Biogeography*, **40**, 183–196.

522 Loiselle, B.A., Sork, V.L., Nason, J. & Graham, C. (1995) Spatial genetic structure of a tropical
523 understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, **82**,
524 1420–1425.

525 Maley, J. (1996) The African rain forest - main characteristics of changes in vegetation and climate
526 from the Upper Cretaceous to the Quaternary. *Proceedings of the Royal Society of*
527 *Edinburgh. Section B: Biology*, **104**, 31–73.

528 McKey, D. (1974) Ant-plants: selective eating of an unoccupied *Barteria* by a *Colobus* monkey.
529 *Biotropica*, **6**, 269–270.

530 Molecular Ecology Resources Primer Development Consortium, A'Hara, S.W., Amouroux, P., *et*
531 *al.* (2012a) Permanent genetic resources added to Molecular Ecology Resources Database 1
532 August 2011-30 September 2011. *Molecular Ecology Resources*, **12**, 185–9.

533 Molecular Ecology Resources Primer Development Consortium, Arias, M.C., Arnoux, E., *et al.*
534 (2012b) Permanent genetic resources added to Molecular Ecology Resources Database 1
535 December 2011-31 January 2012. *Molecular Ecology Resources*, **12**, 570–2.

536 Morley, E.J. (2000) *Origin and evolution of tropical rain forest.* Wiley, Chichester, United
537 Kingdom.

538 Nicolas, V., Missoup, A.D., Denys, C., Kerbis Peterhans, J., Katuala, P., Couloux, A. & Colyn, M.
539 (2011) The roles of rivers and Pleistocene refugia in shaping genetic diversity in *Praomys*
540 *misonnei* in tropical Africa. *Journal of Biogeography*, **38**, 191–207.

541 Nieberding, C. M. & Olivieri, I. (2007) Parasites: proxies for host genealogy and ecology? *Trends*

in Ecology & Evolution, **22**, 156-165.

Peccoud, J., de la Huerta, M., Bonhomme, J., Laurence, C., Outreman, Y., Smadja, C.M. & Simon, J.-C. (2014) Widespread host-dependent hybrid unfitness in the pea aphid species complex. *Evolution*, **68**, 2983–2995.

Peccoud, J., Piatscheck, F., Yockteng, R., Garcia, M., Sauve, M., Djiéto-Lordon, C., Harris, D.J., Wieringa, J.J., Breteler, F.J., Born, C., McKey, D. & Blatrix, R. (2013) Multi-locus phylogenies of the genus *Barteria* (Passifloraceae) portray complex patterns in the evolution of myrmecophytism. *Molecular Phylogenetics and Evolution*, **66**, 824–832.

Plana, V., Gascoigne, A., Forrest, L.L., Harris, D. & Pennington, R.T. (2004) Pleistocene and pre-Pleistocene *Begonia* speciation in Africa. *Molecular Phylogenetics and Evolution*, **31**, 449–461.

Presgraves, D.C. (2010) The molecular evolutionary basis of species formation. *Nature Reviews Genetics*, **11**, 175–180.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

R Core Team (2014) *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rundle, H.D. (2002) A test of ecologically dependent postmating isolation between sympatric sticklebacks. *Evolution*, **56**, 322–329.

Rundle, H.D. & Nosil, P. (2005) Ecological speciation. *Ecology Letters*, **8**, 336–352.

Smith, T.B., Holder, K., Girman, D., O'keefe, K., Larison, B. & Chan, Y. (2000) Comparative avian phylogeography of Cameroon and Equatorial Guinea mountains: implications for

564 conservation. *Molecular Ecology*, **9**, 1505–1516.

565 Telfer, P.T., Souquiere, S., Clifford, S.L., Abernethy, K.A., Bruford, M.W., Disotell, T.R., Sterner,
566 K.N., Roques, P., Marx, P.A. & Wickings, E.J. (2003) Molecular evidence for deep
567 phylogenetic divergence in *Mandrillus sphinx*. *Molecular Ecology*, **12**, 2019–2024.

568 Thuiller, W., Georges, D. & Engler, R. (2013) *Biomod2: Ensemble platform for species distribution*
569 *modeling.* R package version 3.1-18. http://CRAN.Rproject.org/package=biomod2

570 Turelli, M., Barton, N.H. & Coyne, J.A. (2001) Theory and speciation. *Trends in Ecology &*
571 *Evolution*, **16**, 330–343.

572 Wang, H., McArthur, E.D., Sanderson, S.C., Graham, J.H. & Freeman, D.C. (1997) Narrow hybrid
573 zone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae). IV.
574 Reciprocal transplant experiments. *Evolution*, **51**, 95–102.

575 Weir, B.S. & Cockerham, C.C. (1984) Estimating F statistics for the analysis of population
576 structure. *Evolution*, **38**, 1358–1370.

577 Wieringa, J.J. & Gervais, G.F. (2003) Phylogenetic analyses of combined morphological and
578 molecular data sets on the *Aphanocalyx-Bikinia-Tetraberlinia* group (Leguminosae,
579 Caesalpinioideae, Detarieae s.l.). *Advances in legume systematics, part 10, Higher level*
580 *systematics* (ed. by B.B. Klitgaard and A. Bruneau), pp. 181–196. Royal Botanic Garden,
581 Kew, UK.

582 Yu, Q., Wang, Q., Wu, G., Ma, Y., He, X., Wang, X., Xie, P., Hu, L. & Liu, J. (2013) Genetic
583 differentiation and delimitation of *Pugionium dolabratum* and *Pugionium cornutum*
584 (Brassicaceae). *Plant Systematics and Evolution*, **299**, 1355–1365.

585 Yumoto, T. & Maruhashi, T. (1999) Pruning behavior and intercolony competition of *Tetraponera*

(Pachysima) aethiops (Pseudomyrmecinae, Hymenoptera) in *Barteria fistulosa* in a tropical forest, Democratic Republic of Congo. *Ecological Research*, **14**, 393–404.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 List of *Barteria fistulosa* samples and data.

Appendix S2 List of *Tetraponera aethiops* samples and data.

Appendix S3 Selection of the number of genetic groups.

BIOSKETCHES

Rumsaïs Blatrix and the team at CEFE are interested in the functional ecology and evolutionary biology of species interactions, with a particular emphasis on ant-plant symbioses.

Author contributions: D.M. and R.B. conceived the ideas; R.B., D.M., C.B., C.D.-L., C.A., D.J.H. and J.J.W. contributed to field work; J.P., C.B., F.P., L.B., M.S., C.D.-L. and R.B. performed lab work and analysed molecular data; R.B., D.M., J.P., C.B., F.P., J.J.W. and D.J.H. interpreted the results; and R.B., J.P. and D.M. wrote the paper. All co-authors revised and approved the manuscript.

Editor: Richard Ladle

606 **FIGURE LEGENDS:**

607 **Figure 1**: (a) Spatial distribution of the genetic groups defined by STRUCTURE for *K* = 4, for the tree
608 *Barteria fistulosa* and its symbiotic ant *Tetraponera aethiops*. Each individual is represented by a
609 pie chart indicating the proportions of ancestry to each of the four groups. Groups are coded by
610 colour. Pie charts are slightly shifted from true location to reduce overlap. The map was generated
611 using QGIS 2.8.1 with a Mercator projection. (b) Average kinship coefficient as a function of spatial
612 distance for all pairs of individuals (black diamond), for pairs of individuals within genetic groups
613 (triangles) and for pairs of individuals among groups (circles). Genetic groups (*K* = 4) were defined
614 using the software STRUCTURE. Dotted lines represent regression curves, either linear or logarithmic
615 according to the best fit.

616 **Figure 2**: Variation of the frequency of the synthetic allele *n* (representative of the Northern group)
617 with latitude when considering the two genetic groups defined by STRUCTURE. A generalized
618 logistic function is fitted on the scatter-plot, the equation of which is given for each species.

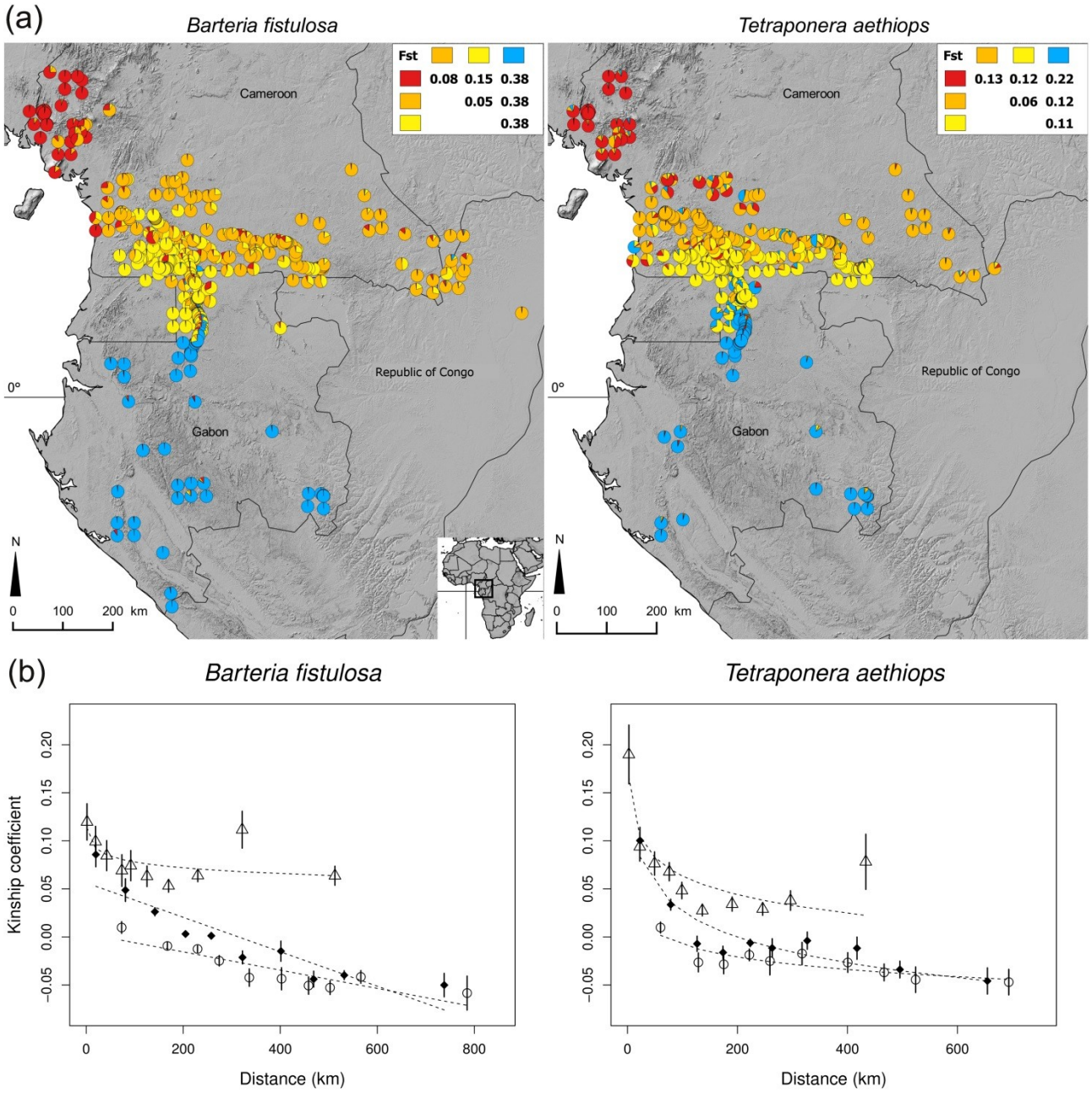
619 **Figure 3**: Spatial distribution of the genetic groups of the tree *Barteria fistulosa* defined by
620 STRUCTURE for *K* = 2. Potential hybrids between the two groups were identified using
621 NEWHYBRIDS. The right panel shows a close-up of the rectangle, where all potential hybrids occur.
622 The map was generated using QGIS 2.8.1 with a Mercator projection.

623 **Figure 4**: Average pairwise kinship coefficient as a function of geographical distance for pairs of
624 individuals of the ant *Tetraponera aethiops* (empty circles) and of the tree *Barteria fistulosa* (filled
625 circles) within two genetic groups defined by STRUCTURE: (a) the southernmost group, coloured in
626 blue in Fig. 1a, and (b) the most widespread of the northern groups, coloured in orange in Fig. 1a.

627 **Figure 5**: Projection of the consensus model (committee averaging over nine modelling algorithms)
628 of spatial distribution of the *Barteria fistulosa* genetic group north. Points represent all the *Barteria*
629 *fistulosa* individuals sampled. Individuals classified in group north by STRUCTURE for *K* = 2 were
630 used as presence data for building the distribution model. Distribution of group south was also

631 modelled and results for both groups were similar and symmetrical. Thus, only those for group
632 north are presented. The map was generated using QGIS 2.8.1 with a Mercator projection.
633 **Figure 6**: Normalized relative contributions of each of 12 environmental variables to nine different
634 models of species distribution run for the northern genetic group of the tree *Barteria fistulosa*
635 defined by STRUCTURE for $K = 2$. Variables are: sand content (SNDPPT), silt content (SLTPPT), pH
636 (PHIHO5), soil organic carbon (ORCDRC), and bulk density (BLD), annual mean temperature
637 (BIO1), maximum temperature of warmest month (BIO5), minimum temperature of coldest month
638 (BIO6), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17),
639 precipitation of warmest quarter (BIO18) and precipitation of coldest quarter (BIO19).
640

641 Figure 1



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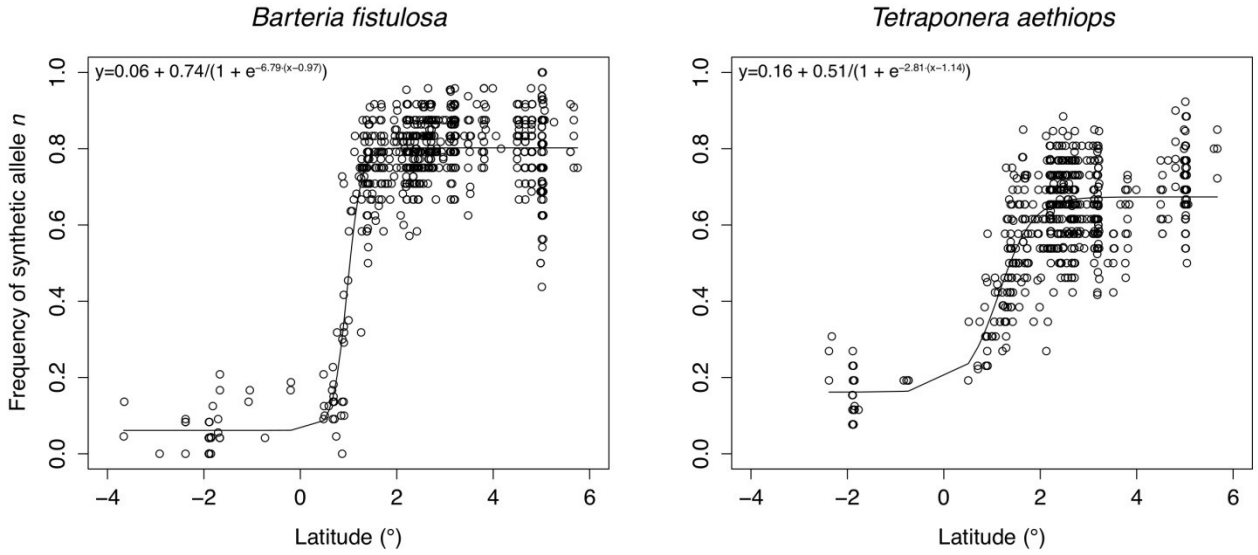
644 Figure 2



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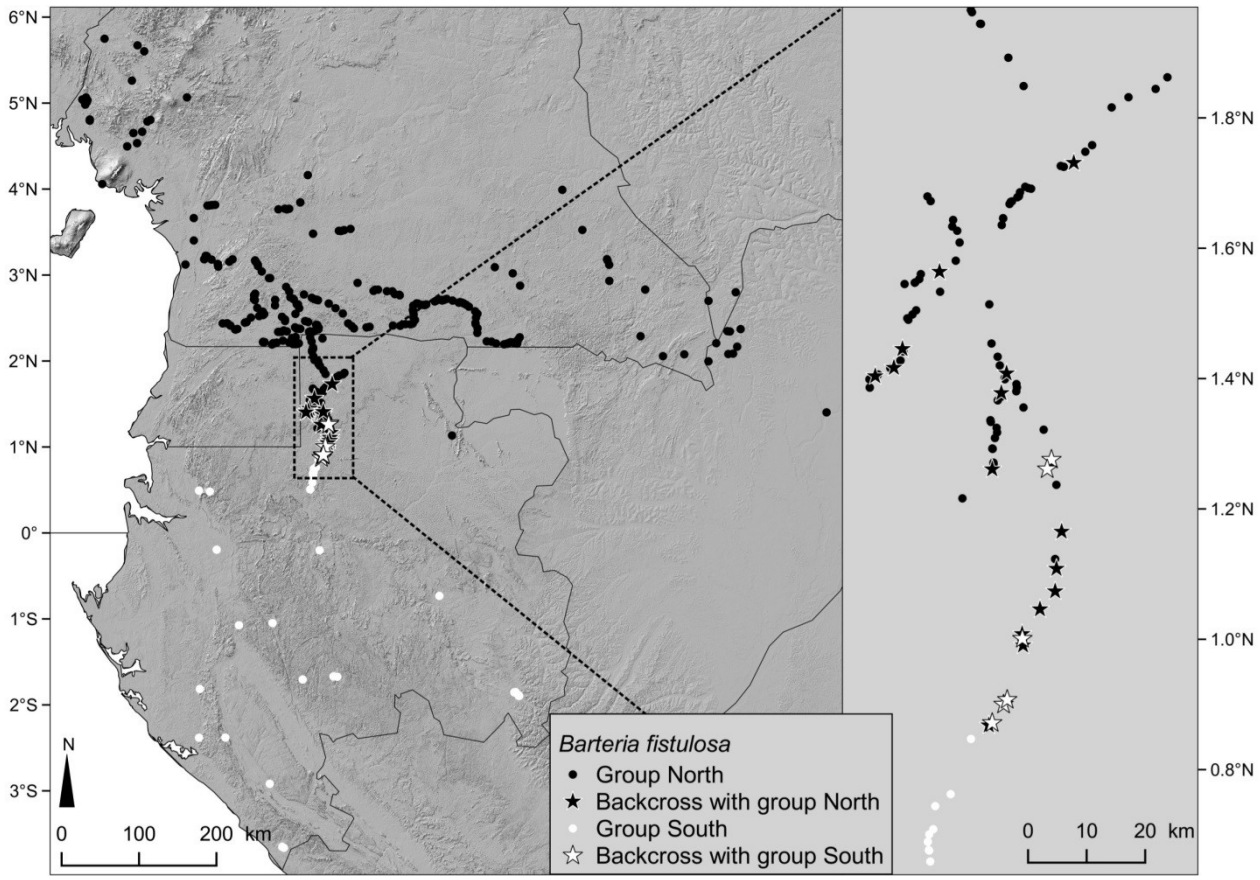

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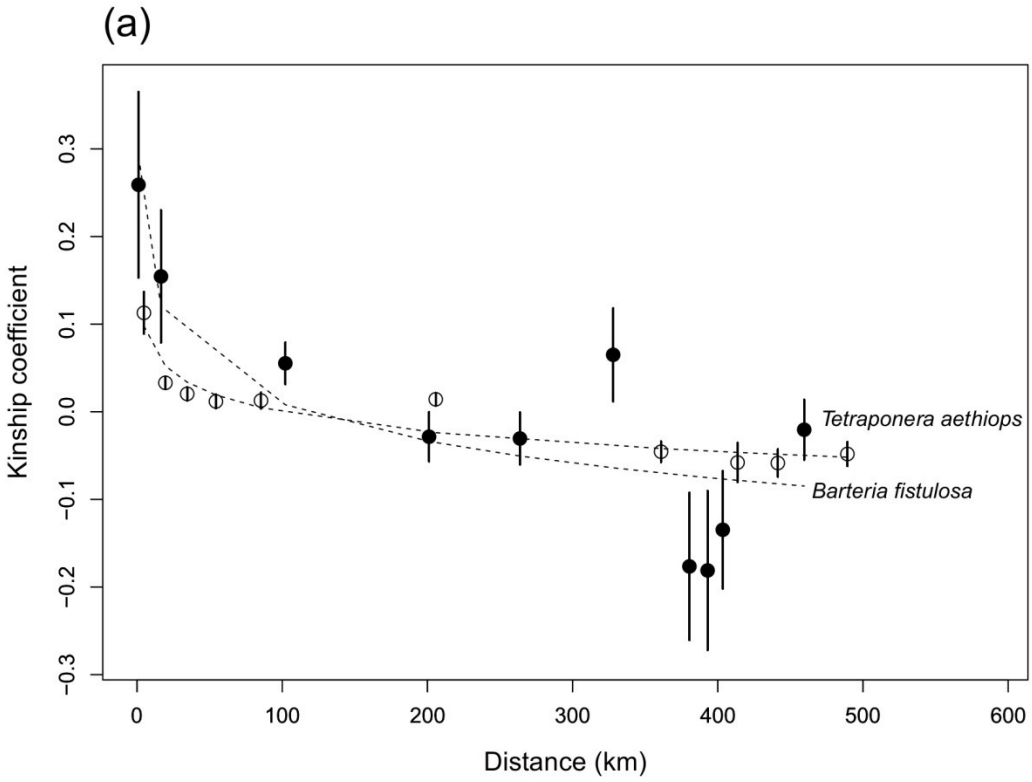

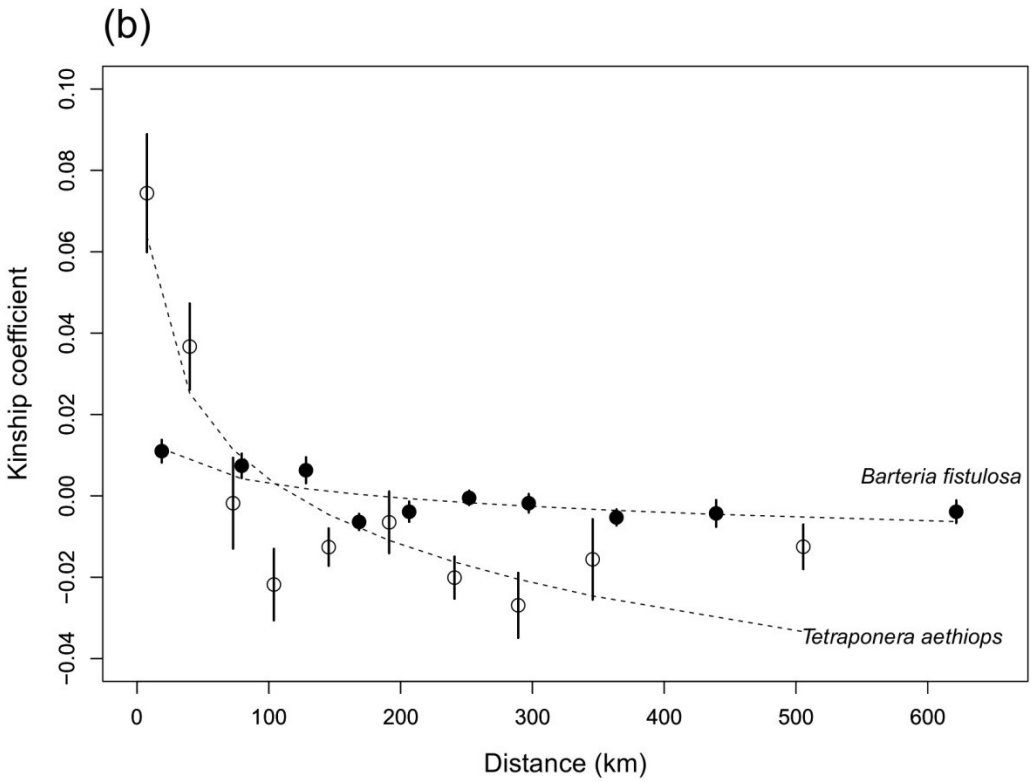

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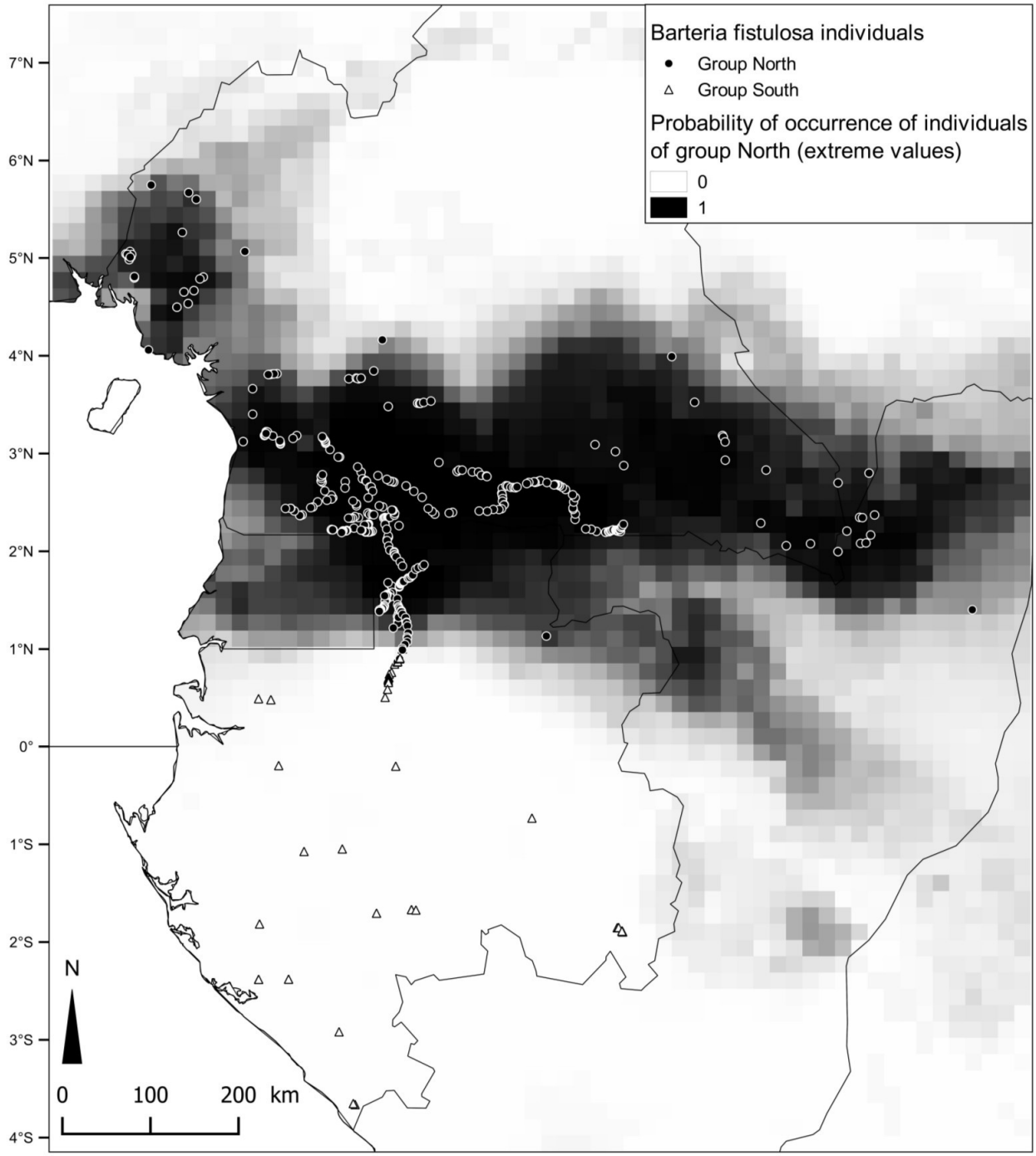
653 Figure 5



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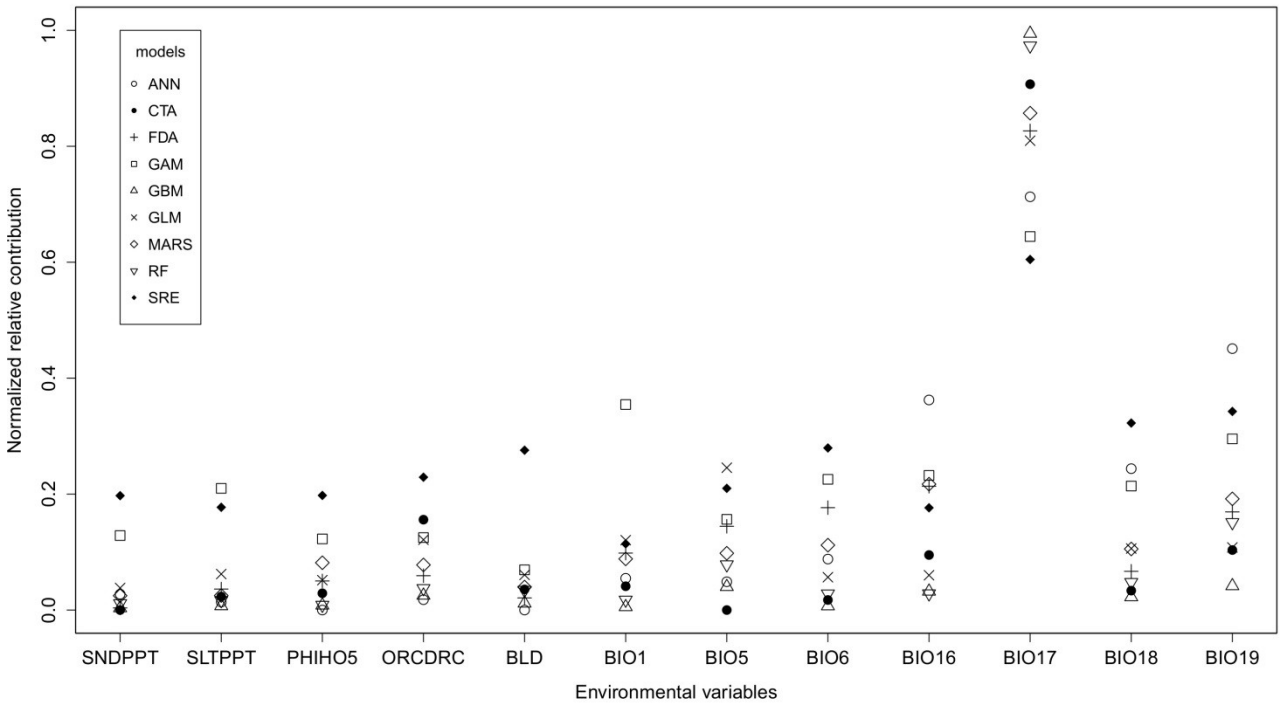
656 Figure 6



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