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Local Genetic Co-Structuring of the Ant *Petalomyrmex phylax* and its Host Plant *Leonardoxa a. africana*: No Role for a Sixty Meter River Width in Separating Social Forms

by

G. Léotard*, E. Defossez*, C. Debain, D. McKey, F. Kjellberg[1] & R. Blatrix

ABSTRACT

The ant *Petalomyrmex phylax* is a protective mutualistic ant associated with *Leonardoxa a. africana*, a small tree of coastal rainforests of Cameroon. The association has expanded southwards during the last few centuries. Northern colonies of the ant are secondarily polygynous while in southern populations, colonies are strictly monogynous. This shift in social behavior seems to be associated with selection for dispersal along a colonization front. The Lobé river seems to constitute a geographic barrier for the system. In all populations north of the river, many polygynous colonies are observed, while mostly monogynous ones are observed south of the river. As the river flows towards the north-west, populations located close to the coast, but just south of the river, present a social structure mainly observed in more southern populations. We investigated, using microsatellite markers, whether the river constituted a genetic barrier thus explaining the rupture in social structure. For both plant and ant, the river did not appear to be an obstacle. These observations suggest that the distribution of social structure in the ant is not explained by physical obstacles. This distribution may rather reflect the historical dynamics of colonization. We showed also that the pattern of genetic structuring was the same for both plant and ant at a scale of a few hundred meters.

Keywords: ant-plant mutualism, monogyny, polygyny, dispersal

INTRODUCTION

Mutualistic associations between ants and plants are widespread in the tropics. They may be diffuse or specific (Labeyrie *et al.* 2001). When the

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association is obligate, ant and plant share the same history. If plant and ant dispersal rates are different, the patterns of genetic structuring of their populations could be somewhat distinct. Nevertheless ant and plant genetics should globally reflect the same biogeographic history. Indeed, a fine description of the distribution of host plants is also a fine description of the habitat available to the ant and the phylogeography of the host is a record of the historical distribution of favorable ant habitat. This situation provides unique opportunities to investigate the ecological and historical determinants of variation in ant social structure. Genetics provides a formidable tool to investigate past historical events (*e.g.* McKern *et al.* 2007), and species structuring into populations (*e.g.* Husen *et al.* 2006). In this contribution we use genetics to answer a riddle in the social structure of *Petalomyrmex phylax* Snelling, a mutualistic ant specifically associated with the treelet *Leonardoxa africana* subsp. *africana*. Genetic and paleoclimatic data demonstrate that the association has expanded southward in coastal Cameroon over the last few centuries (Dalecky *et al.* 2007a). In northern populations, colonies become secondarily polygynous, while southern colonies of *P. phylax* are strictly or almost strictly monogynous (Dalecky *et al.* 2005). Sexual females from southern populations present a number of traits associated with enhanced capacity for claustral foundation, such as larger size (Dalecky 2003). This has been interpreted as the result of repeated foundation of new populations on the colonization front by more dispersive forms of alate females, and hence their selection (Dalecky *et al.* 2007a). This is an example of classical selection for dispersal traits due to range expansion driven by climate change (Dynesius
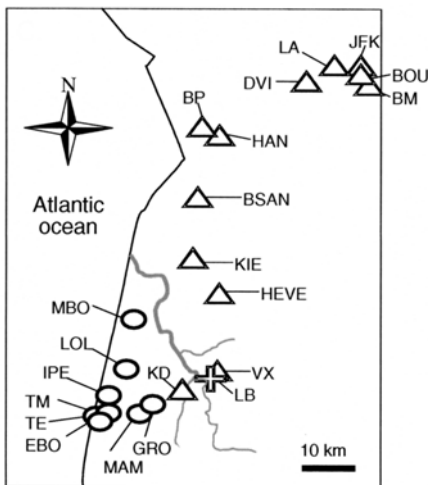

Fig. 1. The ant populations previously analyzed for social system, genetic structuring and cuticular compounds. The populations are located in the southern part of coastal Cameroon. The Lobé river is represented. All or almost all colonies in populations represented by a circle are monogynous. In populations represented by a triangle, colonies become secondarily polygynous. The cross represents a population of uncertain social structure. The Lobé river seems upon first inspection to be an obstacle separating the two types of populations.

& Jansson 2000). In this general pattern, the Lobé river seems to play an important role: the lower part of the river could constitute an obstacle to ant progression (see Fig. 1), (modified after Dalecky *et al.* 2007b). Indeed in terms of genetics and social structure, a population such as MBO located south of the lower part of the Lobé river is much more similar to populations such as GRO and MAM than to populations that are geographically closer but located on the other side of the river. The geographic variation of cuticular compounds tells a similar story (Dalecky *et al.* 2007b). However, data are missing along the riverbanks in the lower part of the Lobé river so that we cannot tell whether the river is really the site of a genetic, morphological and behavioral transition. In this contribution we address the question: does the lower part of the Lobé river effectively constitute an obstacle to ant and plant dispersal, explaining the differences observed in social structure?
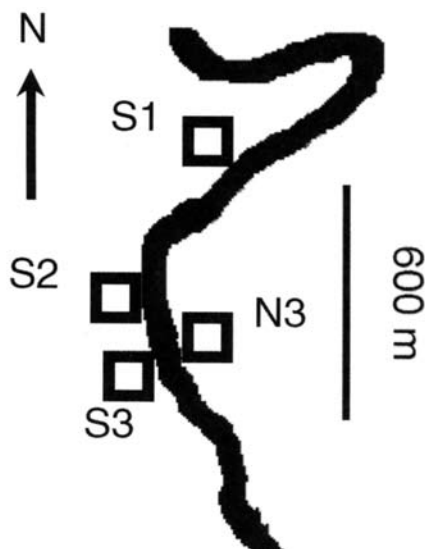
MATERIAL AND METHODS

Fig. 2. The study site. Along this short portion of the river, S1, S2, S3 and N3 were the sole patches of *Leonardoxa a. africana*. At this site the river flows northwards.

Study sites and sampling

L. a. africana is characterized by a distribution in discrete patches. Each plant of *L. a. africana* can be occupied by a single *P. phylax* colony. The study site (Fig. 2) was composed of four patches located on both sides of the Lobé river, three on what we will call the southern bank of the river (S1, S2, S3) and one on what we will call the northern bank of the river (N3). The precise locations were, S1: 2°51'03"N, 9°54'38"E; S2: 2°50'49"N, 9°54'32"E; S3: 2°50'42"N 9°54'34"E; N3: 2°50'44"N, 9°54'35"E. We used southern and northern instead of south-western and north-eastern, because the river separates the southern and the northern part of the range of the mutualism. At the studied site, the river is 60 m wide.

A leaf and one domatia containing ants were sampled from each plant. One hundred and forty plant samples, of which 83 hosted *P. phylax* ants, were collected and analyzed.

Molecular analyses

Nine microsatellite markers isolated from *L. a. africana* were used (Leo24; Leo79; Leo52 ; Leo64 ; Leo68; Leo4; Leo35; Leo73;Leo21 [Debout *et al.* 2005 and G. Leotard, unpublished]) and 12 for *P. phylax* (pet90a; pet16b; pet29; pet3; pet30b; pet32; pet37; pet41; pet44; pet81; pet83; pet90a [Dalecky *et al.* 2007a]). Using these markers we analyzed genetic differentiation between the different sample sites.

For the plants, total DNA was extracted using DNeasy® Plant Kit (Qiagen, Hilden, Germany), following the DNeasy™ Tissue Kit Handbook protocol, with two 50 µL final elutions and subsequent dilution at 1/50. PCR and sizing were carried out following Debout *et al.* (2005), but amplifications were conducted in four steps: Mix1: Leo24 and Leo79; Mix2: Leo52, Leo64, and Leo68; Mix3: Leo4, Leo35, and Leo73; and finally Leo21 alone. For the ants the procedures followed Dalecky *et al.* 2002.

Genetic differentiation among the four patches was analysed by calculating pairwise Fst values (Gaggiotti *et al.* 1999) using the sofware Genetix, version 4.05.2 (Belkhir et al., 1996-2004). Significance of the results was tested using an Analysis of Molecular Variance (AMOVA) implemented in the Genalex software (Peakall & Smouse 2006). The correlation between genetic distance and geographic distance was tested with a Mantel test. We used the Geneland statistical package (Coulon *et al.* 2006) to detect genetic discontinuities among the four patches. The software optimises the number of homogeneous subgroups into which the patches should be partitioned. The patterns detected using Geneland were then tested with an AMOVA.

Morphological analysis

A previous study had shown that the size of queens increased southwards, a trait interpreted as the result of selection for increased dispersal capacity (Dalecky 2003). We tested whether the size of queens differed between the two banks of the Lobé river by measuring head length of five, nine, ten and five queens collected in patches S1, S2, S3 and N3, respectively. Head length was used to estimate body size because in our study species it is the trait most

correlated with five other size measurements (dry weight, head width, full length, thorax width and partial length of anterior wing; Dalecky 2003). Each queen was sampled in a different colony. Because of slightly heterogeneous variances, sizes were compared using a Kruskal-Wallis non-parametric test.

RESULTS

Queen size was homogeneous among the different patches (Kruskal-Wallis, $P = 0.57$). The global AMOVA showed that most of the genetic variance was observed within patch (96% for the plant, 94% for the ant). Nevertheless, for both plant and ant, differentiation among patches was highly significant ($P < 0.0001$).

Pairwise Fst values indicated that S2 was the patch most distinct from the others (Table 1). Indeed for the plant the two highest Fst values were for comparisons of S2 with S3 and with N3. For the ant the three highest Fst values were obtained for the comparisons of S1, S3 and N3 with S2. We detected no correlation between genetic differentiation and geographic distance (Mantel test, $P = 0.54$ for *L. a. africana* and $P = 0.17$ for *P. phylax*).

Table 1. Genetic differentiation (Fst values) between the four patches. Despite its intermediate position, very close to patch S3, patch S2 was genetically the most differentiated for both plant and ant.

Patch	plant			ant		
	S1	N3	S3	S1	N3	S3
S2	0.05607	0.08423	0.10093	0.06414	0.04459	0.05056
S1		0.05343	0.06796		0.02772	0.03918
N3			0.05317			0.01051

The software Geneland was used to jointly analyze spatial and genetic data. The software detected two groups of patches in both plant and ant. One included S1, S3 and N3, the second one included only S2. The same pattern was found for both plant and ant. An *a posteriori* AMOVA confirmed the significance of the separation between the two groups: partitioning the patches into these two groups accounted for 3% of the variance in both species ($P < 0.0001$ for both species).

DISCUSSION

Our aim was to determine whether the Lobé river constituted a barrier to gene flow in the plant and in the ant. Through careful exploration we managed to locate four patches of *Leonardoxa a. africana* in close proximity to one another, three on one side of the river, one on the other. Genetic differentiation among our four study patches was limited, reflecting gene flow between the two sides of the river. Pairwise Fst values for a series of populations using the same genetic markers are available for *P. phylax* (Dalecky *et al.* 2007a) and for *L. a. africana* (G. Léotard, unpublished data) thus allowing scaling of our data. For both ant and plant, our patches were separated by Fst values that fall within the range typical of those associated with neighboring populations. Nevertheless, our patches showed some genetic differentiation. This again fits the global picture as genetic differentiation at a similar scale has been observed in the plant (G. Léotard, unpublished data) and in *P. phylax* (Dalecky *et al.* 2007a), as well as in other ant species (Clemencet *et al.* 2005).

The similarity in queen size between the two sides of the Lobé river confirms the genetic results. Head size, which is a good predictor of queen size and social systems (strictly monogynous versus secondarily polygynous; Dalecky 2003) was identical on both sides of the river. Hence we can conclude that the river does not constitute a natural obstacle limiting gene flow and thus separating strictly monogynous from facultatively polygynous populations. Further field data will be needed to understand why populations such as MBO resemble more southerly populations.

The host plant, *L. a. africana*, presented higher Fst values than the ant. However, Fst values cannot be compared among species, because variation among species in the polymorphism of the analyzed microsatellite loci affects Fst values. Nevertheless, both species showed the same pattern of genetic structuring. Instead of classical genetic isolation by distance, frequent in social insects and plants (*e.g.* Clemencet *et al.* 2005), population S2 differed from the other populations, for both plant and ant. This was totally unexpected given its very close topographic proximity with population S3.

Joint differentiation into populations of both ant and host plant opens up opportunities for local coevolution. We may have to envision the mutualistic

association between *P. phylax* and *L. a. africana* in terms of a geographic mosaic of coevolution (Thompson 2005).

Further studies will have to focus on fine grained differentiation among populations to establish a local history of colonization and of extant local gene flow. Given the surprising differentiation of ant and plant populations in patch S2, we would have discarded such findings for either one of the partners alone as accidental were it not for the similarities in structuring between ant and plant. Hence combining data on the two partners of the mutualism gives exceptional analytical power.

In conclusion, our data suggest that the distribution of social structure in the ant is not explained by physical obstacles. Previous studies had already suggested that social structure was also not determined by the most obvious potential ecological determinants. Hence this distribution may simply reflect the historical dynamics of colonization.

ACKNOWLEDGMENTS

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