



SHORT NOTE

The reallocation of the ant species *Dinoponera lucida* Emery (formicidae: ponerinae) population increasing its local genetic diversity

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Abstract

The aim of the current study is to describe the genetic consequences of the reallocation process in order to preserve an entire *Dinoponera lucida* Emery (1901) population living in a forest fragment supposed to be deforested to give area for a seaport construction site. The sample collection and the mitochondrial data analysis were conducted before and after the reallocation of all found nests to two conservation units in Espírito Santo State, Southeastern Brazil. It was hypothesized that the reallocation of an exogenous population of *D. lucida* would increase the genetic variability - which is naturally very low - of populations living in isolated forest fragments. The data analysis showed that the genetic variability within the receiving populations has considerably increased, above the natural levels, due to the introduction of a new population. The biological viability of this effect was observed during the five years of monitoring program. The importance of the present study relies on information and reports from an unprecedented study on insect populations, and it will provide essential knowledge to future studies.

Introduction

The Atlantic Forest is one of the richest biomes in the world and one of the 25 biodiversity hotspots (Myers et al., 1999), but most of its remaining biome is distributed in small forest fragments (Ribeiro et al., 2009). This habitat fragmentation process may represent a constant threat to endemic species (Brooks et al., 2002), such as the giant ant *Dinoponera lucida* Emery 1901, whose geographic distribution is limited to Bahia and Espírito Santo States, in Northeastern and Southeastern Brazil, respectively (Campiolo & Delabie, 2008). This region is also known as the Central Corridor of the Atlantic Forest.

Female ants belonging to this species have no wings, whereas the male ants are winged (Paiva & Brandão, 1995), although they do not perform an efficient flight (Teixeira, M.C. personal communication, February, 2009). New colonies are

created through the fission of bigger colonies. Thus, nests are established in an aggregated spatial distribution pattern inside the forest fragment (Mariano et al., 2008; Peixoto et al., 2010), and are probably genetically related to each other (Araújo, 1994). Such distribution pattern increasingly limits the species' dispersal ability.

Habitat fragmentation causes the isolation of populations and increases endogamy, which results in low genetic variability inside inbreeding groups and high genetic divergence between isolated groups (Packer & Owen, 2001). In addition, populations with dispersion limitations, such as *D. lucida*, are under high extinction risks due to stochastic events (Burkey, 1989). Although the process that causes stochastic events may naturally occur, the anthropic exploration accelerates its natural course and leads to the extinction of populations haplotypes. Because of the high forest fragmentation, not just entire forest fragments



disappear, but a habitat might become no longer suitable to host highly adapted populations (Fahrig, 1997; Brooks et al., 2002). Endemic species are even more threatened under these circumstances, and this is why the species *D. lucida* requires ultimate population genetic studies.

The ecological theory features fragmented populations as a set of subpopulations form a metapopulation. These metapopulations experience extinction and recolonization events within subpopulations, and they persist in landscapes depending on the dynamic flow of the individuals. From a genetic point of view, small subpopulations are prone to extinction because of endogamy. However, individuals from other large subpopulations can migrate to a small subpopulation, and it could increase the genetic diversity in and avoid the extinction of this small subpopulation. Such effect is known as rescue effect (Begon, 2006); the large populations are called “donor” or “source”, and the small ones, “sink” or “receiver”.

As it was previously mentioned, *D. lucida* populations have special dispersion limitations. In the framework of fragmentation and metapopulation theory, it is important understanding how the migration of individuals from a donor (or source) population affects the genetics of a receiver (or sink) population. Therefore, a rescue and reallocation process was designed using an entire *D. lucida* population. The experiment took place in 2009, when a seaport construction site was set in an Atlantic Forest fragment. We tested the hypothesis that the reallocation of an exogenous *D. lucida* population would increase the genetic variability of populations located in isolated forest fragments.

Material and Methods

The forest fragments that have donated and received the reallocated population are located in Aracruz County, Espírito Santo State, Southeastern Brazil. The research team visited conservation units in Aracruz County aiming to assess the viability of reallocating the rescued nests to these units, before moving the population in. The criteria for selection were fragments under environmental protection policies, the shortest distance from the original area as possible, and the occurrence of the species. The Barra do Riacho Terminal Waterway (BRTW, $-19^{\circ}50.548'$, $-40^{\circ}03.827'$) was the donor forest fragment supposed to be deforested, and it comprised 11 hectares. The conservation units selected to host the nests were: David Victor Farina Municipal Natural Park (DVFMP, $-19^{\circ}55.850'$, $-40^{\circ}07.774'$, 44 hectares) and Aricanga Waldemar Devens Municipal Natural Park (AWDMNP, $-19^{\circ}48.827'$, $-40^{\circ}19.959'$, 515 hectares). These fragments belong to a highly fragmented landscape, and the two CU fragments are located approximately 25 kilometers from each other (in a straight line) (Fig 1).

All the 19 nest found in the BRTW forest fragment were open, and every individual (eggs, young and adults forms) were removed and reallocated to artificial nests constructed in two Conservation Units (CU), according to the protocol designed by Ferreira et al. (in prep). The reallocated population was monitored throughout the following five years in order to observe its behavioral and ecological reactions.

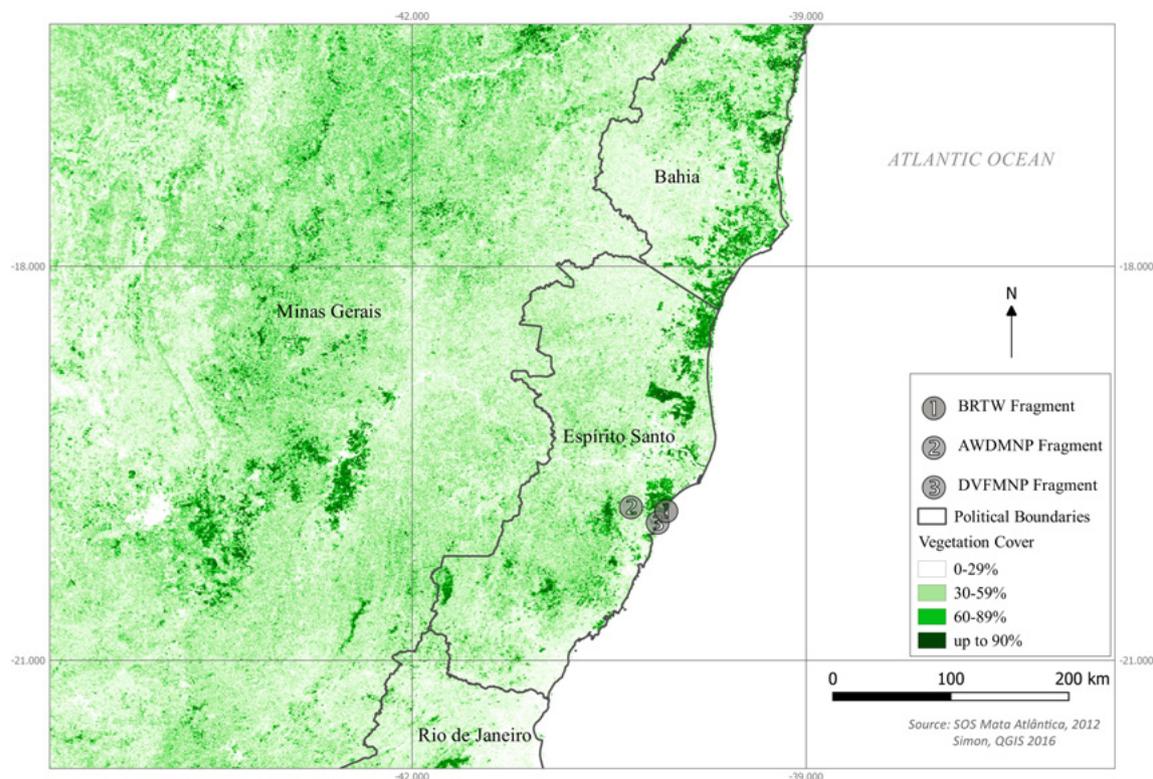


Fig 1. Map of Espírito Santo State indicating the study area. (1) BRTW: Barra do Riacho Terminal Waterway; (2) AWDMNP: Aricanga Waldemar Devens Municipal Natural Park; (3) DVFMP: David Victor Farina Municipal Natural Park.

Back to 2009, at the time the procedure was put in place, one sample was collected from each nest for genetic analysis, as well as samples from each of the 18 AWDMP and 17 DVFMNP nests of the native populations in the CU, in order to gather genetic data of the *D. lucida* populations before the reallocation. The research team collected new samples from all monitored nests in the receiving fragments in 2011. The DNA extraction was conducted according to the protocol by Waldschmidt et al. (1997) in the Insect Molecular Biology Laboratory of Federal University of Viçosa. Sequences of the *Cox1-Cox2* mitochondrial gene and intergenic *tRNA_{leu}* regions were obtained using primers developed by Resende et al. (2010), which were later aligned in the Mega 5.0 software (Tamura et al., 2011).

The haplotype network analysis was conducted in the Network 4.6 software, through the median-joining method (Bandelt et al., 1999). The Analysis of Molecular Variance (AMOVA) was performed using the Arlequin 3.5 (Excoffier & Lischer, 2010) applied to the data collected before and after the reallocation process. The “Among populations” hierarchical level corresponded to the BRTW, AWDMP and DVFMNP populations in the AMOVA conducted before the reallocation. The same hierarchical level consisted of AWDMP and DVFMNP populations in the AMOVA conducted after the reallocation process, since the BRTW population was reallocated to those both receivers. The AMOVA was applied to three hierarchical levels in order to take the potential subpopulations into account, because previous reviews refer to aggregate distribution patterns within a single forest fragment.

Results

Four haplotypes were identified in the data set: one in each park (named H1 in AWDMP; H4 in DVFMNP) and two in the reallocating population (H2 and H3, both from BRTW) (Fig 2). As it was expected, the AMOVA conducted before the reallocation process has shown high genetic variance between populations from different forest fragments (83%) when the three populations, namely: BRTW, AWDMP and DVFMNP, were studied as a single one (Table 1).

Table 1. Analysis of Molecular Variance (AMOVA) applied to three hierarchical levels on data set before reallocation. **VS:** Variation Source; **DF:** degrees of freedom; **SS:** sum of squares; **E (MS):** Estimate medium squares - variance components.

VS	DF	SS	E (MS)	% Variation
Among populations	2	17.500	0.42226	83.00
Within population	5	1.724	0.04212	8.28
Within the potential sub-population	53	2.350	0.04434	8.72
Total	60	21.574	0.50873	100.00

10.000 permutations, $P < 0.000001$

$F_{ST} = 0.91284$

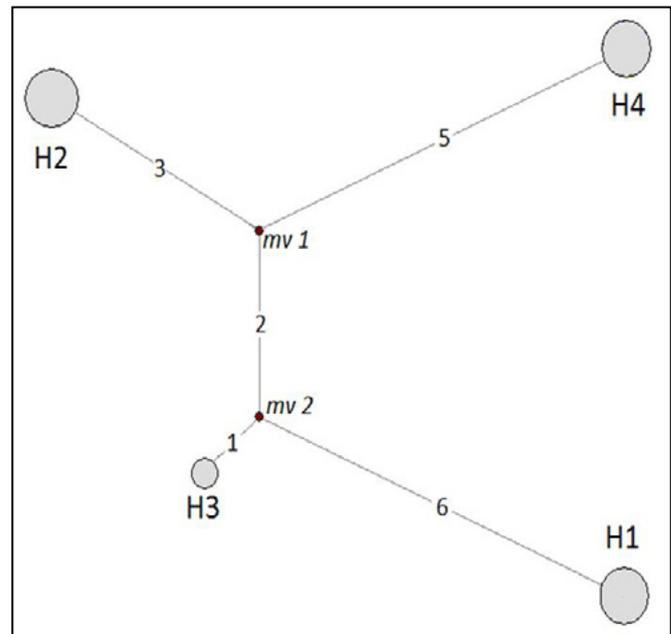


Fig 2. The haplotype network of the *Cox1-tRNA_{leu}-Cox2* region from 54 *Dinoponera lucida* individuals. The numbers represent mutational steps. H1, H2, H3 and H4 represent the haplotypes. *mv1* and *mv2* (median vector) represent the extant or the non-sampled haplotypes.

The second AMOVA has shown that the genetic variance between AWDMP and DVFMNP has decreased to 55% after the reallocation, whereas the diversity within populations and subpopulations has increased.

It is worth mentioning that after the five-year monitoring, 82% of the reallocated nests has survived in the receiver fragments (Ferreira et al., in prep).

Discussion

The main aim of the present study was to describe the genetic consequences of the reallocation process involving an entire *D. lucida* population. Instead of collecting genetic samples from all populations and simulate the genetic diversity in order to predict the reallocation impacts, it was made the choice for effectively performing the reallocation procedure and monitoring its effects for the following five years. It was demanding to put this intervention in place in order to save BRTW haplotypes from extinction.

The haplotype network analysis showed that the haplotypes H2 and H3, both from the BRTW fragment, were more similar to each other than the H1 and H4, from AWDMP and DVFMNP, respectively. Such finding may be justified by the geographic distance, because H2 and H3 come from the same area, and it suggests the existence of recent divergence. *D. lucida* populations living in a certain forest fragment often have one or few haplotypes (Resende et al., 2010). There is high genetic diversity from population to population, as well as low variability inside a single population.

Since it is common finding one or few haplotypes in a single population, it is possible inferring that the smaller

the area, the lower the genetic diversity of *D. lucida*. The genetic diversity within the populations and subpopulations of both receivers in the CU has increased after the donor population was reallocated, whereas the variation between AWDMP and DVFMNP populations decreased, due to the homogenization of nests from the BRTW population (Table 2). Thus, the haplotypic diversity in the receiver populations in the present study is higher than natural. It means that the herein studied hypothesis was accepted, because of the high survival rate presented by the reallocated population. The reallocation effectively increased the genetic diversity of subsequent generations. It is a satisfactory result, since the literature shows that the rate of successful reallocation, repatriation and translocation processes is low (Dodd & Seigel, 1991).

Table 2. Analysis of Molecular Variance (AMOVA) applied to three hierarchical levels on data set after reallocation. **VS:** Variation Source; **DF:** degrees of freedom; **SS:** sum of squares; **E(MS):** Estimate medium squares - variance components.

VS	DF	SS	E(MS)	%Variation
Among populations	2	13.162	0.25457	55.10
Within population	5	6.062	0.16306	35.30
Within the potential sub-population	53	2.350	0.04434	9.60
Total	60	21.574	0.46196	100.00
10.000 permutations, P<0.000001				
$F_{ST} = 0.90402$				

It is worth noticing that, although the aggregate distribution within a single forest fragment has been observed in the field (Peixoto et al., 2010), the genetic relationship predicted by Araújo et al. (1994) has not been confirmed by the mitochondrial sequence analysis. The variation within a potential subpopulation and between subpopulation found in the whole fragment is not clearly consistent with this idea (Table 1). It means that nests from the same subpopulations are related to each other, as well as to other nests in the population, alike. It suggests that the aggregation pattern may be due to suitable environmental conditions inside the forest, and that such pattern may be dynamic if these conditions change. However, the predicted genetic relationship between close nests may be assessed through nuclear DNA analysis, which takes into account the disperser role played by the male individuals. If the low diversity between close nests is confirmed, it may be explained by their limited dispersal ability.

Reintroduction programs are a frequent alternative to the recolonization of a sink area when the metapopulation dynamics fails. These interventions often fail due to the founder effect, if the introduced group is highly inbred (Armstrong & Seddon, 2008). Such problem was avoided in the current study, because the introduction was performed in an area hosting its own population and haplotypes, and it increased the total genetic variation. This increased genetic

variation may enable enhancing the population's survival, thus simulating a rescue effect. This result may be representative for the entire *D. lucida* species. In addition, the previously conducted search for suitable and similar environments may have prevented or minimized adaptation issues.

Genetic studies about reallocation and translocation process are rare, mainly studies of this nature involving groups of invertebrates (Sherley et al., 2010). Yet, these studies are essential to assess the persistence of the introduced population (Armstrong & Seddon, 2008). The reallocation program appears to have succeeded after five years monitoring the studied populations. Yet, the herein adopted reallocation procedures shall not be replicated if the basic information provided in the current study are not followed. This is the first time a reallocation program involving an invertebrate population is conducted based on a genetic data analysis associated with a middle time monitoring. It is strongly recommended that future studies do not ignore the importance of the herein presented information.

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CO-AUTHORS CONTRIBUTION

Tânia Maria FERNANDES-SALOMÃO and Marcos da Cunha TEIXEIRA have substantially contributed to every step of this study, since its conception, the acquisition, analysis, and interpretation of data. In addition, they have contributed to the text drafting, revising and its final approval, as well as they have agreed to be accountable for all aspects of the work, ensuring its integrity.

