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Demographic evolution and phylogenetic relationship of three populations of *Bactrocera dorsalis* from three host plants: *Mangifera indica*, *Citrus reticulata* and *Anacardium occidentale* in the Niayes area

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Abstract

Bactrocera dorsalis (Diptera, Tephritidae) is a very polyphagous quarantined species that causes enormous damage to fruit plants. The objective of this study is to investigate the demographic evolution and the phylogenetic relationship of three populations of *Bactrocera dorsalis* from three host plants.

Methodology and Results: Populations of *Bactrocera dorsalis* were defined based on host fruits: BdA (Citrus), BdM (Mango) and BdP (Cashew apple) in the Niayes area in Senegal. Each population consists of 15 individuals. The DNA of each individual was extracted and the COI was amplified by the Standard Zymo method (Kit Zymo research) and then sequenced. The results showed that populations from mango and citrus fruits are expanding demographically from a stable ancestral population. However, cashew apple is in moderate demographic expansion; it has undergone a sweep. Gene flow between different populations and isolation of some individuals forming private haplotypes through phylogenetic trees has also been demonstrated.

Keywords: Demographic evolution, phylogenetic relationship, *Bactrocera dorsalis*, Niayes area, fruit fly, COI

Introduction

Agriculture is a very important industry for most countries. The food sector in developing countries has grown rapidly over the past 30 years [1]. Global trade in these main fresh tropical fruits was estimated at 6.3 million tones in 2015, valued at USD 6.1 billion [2]. In the Economic Community of West African States [3], mango is mostly cultivated in the savannah on an area of 540,000 km² [3]. In Senegal, mango production annually ranges from 125,000 to 130,000 tones. Citrus production increased from 50,000 t to 58,400 tones between 2015 and 2022 [4]. As for cashew plantations (*Anacardium occidentale*), they are a lever for socio-economic development for local communities in Senegal. Thus, tropical fruits contribute significantly to food security through export-generated revenues [2]. These fruits provide basic inputs that complement agricultural production and can help reduce or avoid certain food deficiencies [5]. The sector creates several thousand jobs estimated at 23,600, including 13,050 men and 10,550 women for plantation maintenance, harvesting and post-harvest, and processing activities [6, 7]. With the intensification of international trade in fresh produce and the expansion of geographical trading areas despite increasingly draconian quarantine controls, the risk of pest species being introduced through contaminated fruits is increasing [8]. This sector is now threatened by insect pests, particularly the fruit fly *Bactrocera dorsalis* (Diptera, Tephritidae) [9]. *Bactrocera dorsalis* is a highly polyphagous, quarantined insect that causes enormous damage to fruit plants [10, 11, 12, 39]. *Bactrocera dorsalis* is a species complex [14] that was first reported in Senegal in 2004 [15]. It quickly adapted and was found on a range of 17 fruit species (mango, citrus, mahogany apple, wild plants, etc.), most of which have successive ripening periods of each other [12]. Thus, the damage caused by this fly results from three successive phenomena: the egg-laying sting in the fruit, the nutrition of the larvae inside the fruit and finally the attack by several phytopathogens via the egg-laying point. The fruit thus pricked shows an accelerated maturity and falls frequently [40].

These losses affect all players in the sector ^[17], in terms of both yield and fruit quality ^[39]. The adaptation of introduced or invasive species via international or regional trade is governed by various geographical, climatic and biological parameters, among others. Thus, any living species with an ability to survive in different living conditions sees, in most cases, its evolved genetic information ^[18]. It is obvious that from a practical viewpoint, the approach that one can have to a population must necessarily involve a study of its structures. This is particularly true for populations of phytophagous insects dependent on several plant species such as *Caryedon serratus* studied by Sembène ^[19] and the insect *B. dorsalis* of this study. Despite the different control methods (Chemical, prophylactic, genetic, biological and even integrated) implemented to maintain wild populations of these fruit pests below the economic thresholds of harmability, these flies still constitute the main constraint on the production of quality fruits and vegetables in West Africa in particular and in tropical and subtropical regions in general ^[20]. These findings are in favor: a more in-depth study of economically important insect while respecting the environment and public health, a study to characterize the bio-ecology of this insect.

Table I: Summary table of studied populations and sampling code.

Populations Sample	Code	Number of individuals sequenced
Citrus <i>Bactrocera dorsalis</i>	BdA	15
Mango <i>Bactrocera dorsalis</i>	BdM	15
Cashew apple <i>Bactrocera dorsalis</i>	BdP	15

Mass breeding

Batches of fruit infested with mangoes, mandarins and cashew apples are incubated in tents or in buckets of five or twenty liters. After eight to ten days of incubation, the eggs pass from the L1, L2 and L3 stage larvae to the pupa stage in the sand. Currently, pupae extraction is done every two days. The imagoes born within twenty-four hours and not fed are collected and put in 7 ml tubes containing alcohol 96 °C for genetical studies.

Genetic Study

A mitochondrial gene, Cytochrome oxidase I (COI), was chosen for this genetic study.

DNA extraction

DNA was extracted using the Standard Zymo method (Zymo research kit). The abdomen of each insect and the wings were removed to prevent any bacteria residing there from contaminating the DNA. The rest of the insect (head, thorax and legs) was crushed and collected in tubes for DNA extraction and amplification. Two primers; Forward (5'-CCAGGTAAAATTTAAATATAAACTTC-3') and Reverse (5'-GGATCACCTGATATAGCATTCCC-3') were used for the classical PCR method. The mix solution consisted of 18.3 µl of MiliQ water, 2.5 µl of 10X buffer, 0.5 µl of dNTP, 0.25 µl of each primer (COI F and COI R), 0.2 µl of DNA polymerase or Taq, 1 µl of MgCl₂ (Mg²⁺ ions). Thus, the reaction volume to be distributed in each well is equal to 23 µl where 2 µl of DNA is added, which will make a total of 25 µl per well. The plate is then covered with adhesive paper and tightly closed. Amplification is performed by repeating cycles, each of which multiplies the amount of target DNA by 2. Each cycle consists of a succession of 3 phases: Denaturation, hybridization and elongation. First there is the initial denaturation at 94 °C for

Materials and Methods

Sampling

The biological materials used for this work are infested fruits and young adults of *B. dorsalis* from these fruits. The samples of *B. dorsalis* which are the subject of this study were collected in three localities of the Niayes area which are Sangalkam (14° 47'58"N and 17° 14'17"W), Sebikotane (14° 44' 17"N and 17° 00' 15"W) and Notto (14° 57' 56.8"N and 17° 00'25"W). It was done during the period of July-November 2018 and that of July-November 2019. Several orchards were sampled without taking into account the area to have heterogeneous individuals and avoid families. The targeting of these localities was done according to the high production areas, insect infestation and taking into account the geographical distance between the localities. Samples of *B. dorsalis* were taken from mangoes, tangerines and cashew apples in orchards to be incubated in the laboratory at room temperature. The choice of fruits was made according to the successive ripening season for the three species, their infestation rate and the economic and nutritional interest. The end of the cashew apple season spans the mango season and the end of the mango season corresponds to the beginning of the citrus season.

three minutes, followed by 35 cycles of denaturation at 94 °C for one minute; then the hybridization of the primers is carried out at 48 °C for 1 minute; finally the elongation of the complementary 5'3' strands from the primers by the DNA polymerase which begins with an elongation at 72 °C for 1 minute then ends with a final elongation at 72 °C for 10 minutes. Migration was performed on a 2% agarose gel in the presence of 10 µl safe view. The samples, 7 µl of DNA extract added to 2 µl of blue charge, were deposited in wells and migrated at 100 Volts for 35 minutes.

Genetic analysis

The demographic evolution of *B. dorsalis* populations in Senegal has been described using different parameters of demogenetic testing. Tajima D ^[21, 22] tests the hypothesis that all mutations are selectively neutral ^[23]. It is based on the differences between polymorphic sites (Sp) and the average number of nucleotide differences (K). The Fs de Fu ^[24] is based on the probability of having an expected number of haplotypes greater than or equal to the number observed in a sample drawn from a population of constant size. These two parameters were generated by the software Harlequin v. 3.5.2.2 ^[25] and the values found can be interpreted as follows: if the values found are significantly equal to zero, there is demographic stability and a lack of selection so the population is of constant size; if the values found are significantly negative, this explains a (recent) demographic expansion with an excess of rare variants resulting in negative or purifying selection (disappearance of deleterious mutations) or selective scanning (positive selection: appearance and propagation of an advantageous mutant in the population); if the values found are significantly positive, this suggests a bottleneck that corresponds to a drastic reduction in the population following an ecological or environmental event. The Ramos-Onsins and Rozas R2

[26] is based on the difference between the number of singletons (mutations at one site) and the average number of nucleotide differences. When the R^2 value is low and significantly positive, there is a demographic expansion. On the other hand, if the R^2 value is significantly negative, it suggests that the population is constant. It is calculated using the DNASP software v. 5.10.01 [27]. The analysis of «Mismatch distribution» [28, 29] is the qualitative graphical representation of the distribution of genetic distances between individuals of a population taken in pairs. Two models have been defined to give the demographic evolution of a study population from the Mismatch distribution graph. A unimodal distribution reflects demographic stability (population of constant size) while a multimodal distribution is the signal of a population expanding [29; 30]. These graphs are generated by DNASP v. 5.10.01 [27]. They were constructed under the assumption of a constant population. The haplotype network was built using the software Network v. 5.0 [31] which allows a visualization of the link between the different haplotypes, the number of mutation steps and the number of median vectors if it exists. Thus, groups can be made in the form of haplogroups. Haplogroups or lineages are those that share mutations that are specific to them. In other words, it is a monophyletic cluster of haplotypes [32]. The probabilistic approach was chosen to trace the phylogenetic tree using the Bayesian inference method. Phylogenetic reconstruction methods are based on hypotheses of DNA or amino acid substitution processes [33]. The best HKY model was found with the j Model Test software version 2.1.10 [34]. Bayesian inference calculates subsequent probabilities from probabilities defined a priori. This probabilistic method uses the concept of likelihood. The tree was rooted with a

sequence of *Bactrocera tryoni* (Diptera: Tephritidae). It is built with the software MrBayes v. 3.2.6 [35]. The distribution of the posterior probabilities in the tree reconstruction is estimated by MCMC using four chains simultaneously (Three of which were "Heated" gradually and one cold). Ten million (10,000,000) generations will be performed for each of the chains by sampling the various parameters every 1000 generations. The degree of convergence of the chains can be verified by examining the evolution of the likelihood function during the "cold" chain run to determine the ignition period. Generations completed during this period are eliminated from subsequent analyses and estimates. Conservatively, the first 2,500,000 generations are eliminated (25%) and inferences are then realized on the 7,500,000 next generations. The tree generated by MrBayes was visualized using the Fig Tree software v.1.4.4 [36].

Results

Demographic change

Demogenetic testing

Demogenetic tests show a negative Tajima D for all populations. It is not significant for populations from citrus and mango but significant for the population from cashew apple (-1.744). The SSD demographic index is non-significant for the first two populations and significant for the cashew apple population (0.277). The Rag demographic index is significant only for the first population (0.604). F_s de Fu is not significant for all populations but positive for citrus and mango populations and negative for the cashew apple population. The R^2 for all populations is positive (Table I).

Table I: indices of demographic change

Pop	D de Tajima	Fs de Fu	R ²	SSD	Rag
BdA	-1.092 NS	2.649 NS	0.2023	0.171 NS	0.604 *
BdM	-1.249 NS	0.596 NS	0.1323	0.032 NS	0.1 NS
BdP	-1.744 *	-0.877 NS	0.1312	0.277 **	0.179 NS
Average	-1.362 NS	0.789 NS	0.0667	0.160 NS	0.294 NS
s.d	0.278 NS	1.446 NS		0.1 NS	0.222 NS

NS: non-significant (Pv 0.05); *: significant (Pv 0.05); ** moderately significant (Pv 0.001)

Mismatch distributions per pair of individuals

Mismatch distributions graphs are constructed under the assumption of a constant population. Analysis of these shows multimodal curves for the overall population of *B.*

dorsalis (Figure 1a) and for populations derived from citrus fruits (Figure 1b), mango and cashew apple (Figure 2c and d).

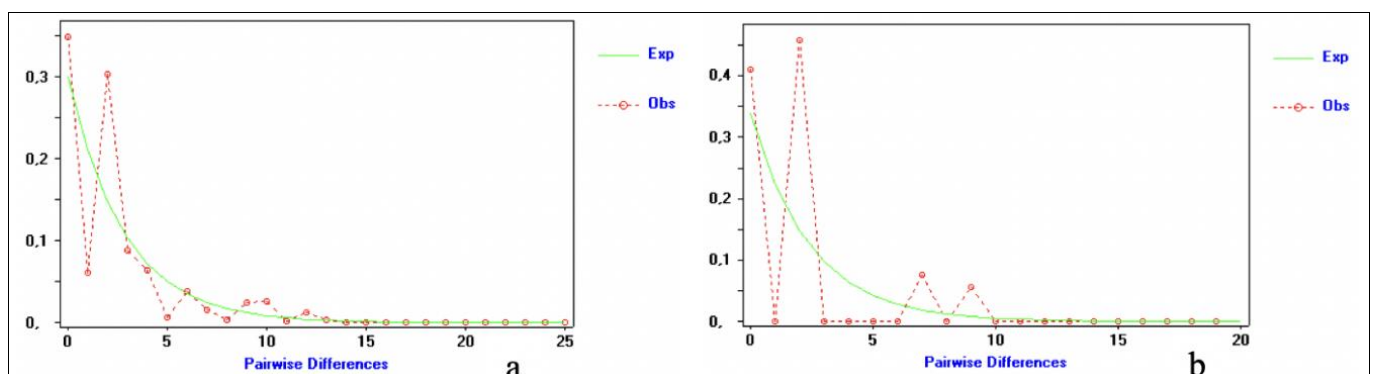


Fig 1: Mismatch pairwise distributions of individuals from the total population (a), and those from citrus fruit (b).

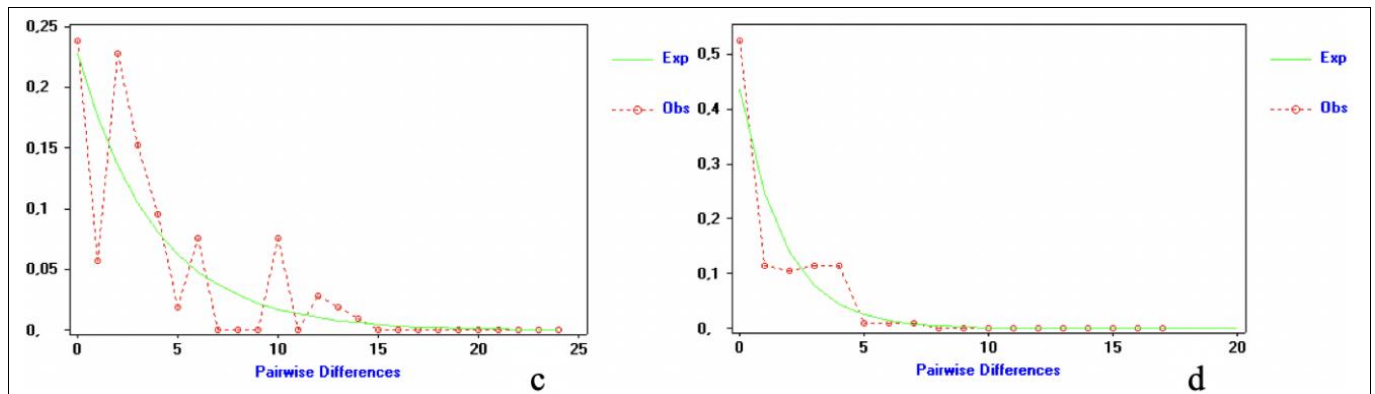


Fig 2: Mismatch pairwise distributions of individuals from mango (c) and cashew (d). Haplotype network and phylogenetic relationships

Phylogenetic relationships

Haplotype network

The majority haplotype is haplotype 1 (BdA1), which has undergone one mutational step to give haplotype 9 (BdP9) and two mutational steps to give haplotype 2 (BdA3). The latter underwent four mutational steps to give haplotype 4 (BdM1), one mutational step to give haplotype 7 (BdM13)

and two mutational steps to give haplotype 5 (BdM2). The latter underwent five mutational steps to give haplotype 3 (BdA9). Haplotype 1 (BdA1) is linked to haplotype 8 (BdP2) by a median vector and has undergone four mutational steps to give haplotype 8. Haplotype 8 underwent six mutational steps to give haplotype 6 (BdM3) (Figure 3).

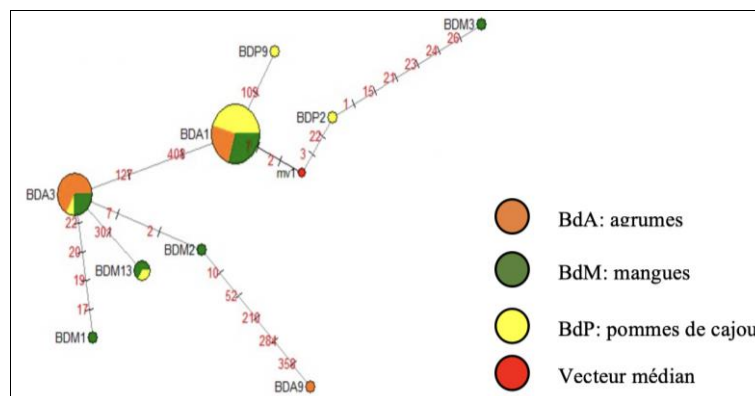
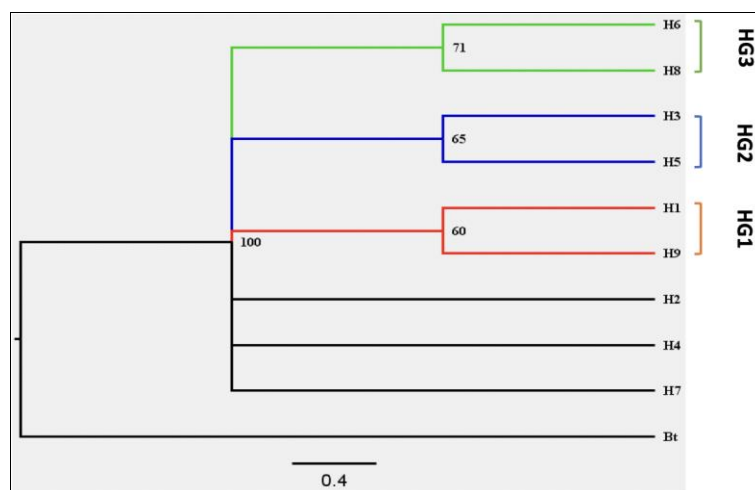


Fig 3: Haplotype network of *B. dorsalis* populations

Phylogenetic relationships

The Bayesian tree shows a typology with three haplogroups (H1 and H9, H5 and H3 and H8 and H6) and three unresolved haplotypes (H7, H4 and H2) (Figure 4). Haplotype 1, formed by BdA1 and BdP9, groups together

a majority of individuals from mango and cashew apple. Haplotype 2, formed by BdA9 and BdM2, is made up mainly of individuals from mango and citrus fruit. Haplotype 3 is made up only of BdP2 and BdM3 individuals.



H1: BdA1; H2: BdA3; H3: BdA9; H4: BdM1; H5: BdM2; H6: BdM3; H7: BdM13; H8: BdP2; H9: BdP9

Fig 4: haplotype tree using the Bayesian method

Discussions

Demographic change

Haplotypic diversity is high for all populations and for the total population of *B. dorsalis* except that of the cashew apple which is low while nucleotide diversity is low for all these populations. This is the signal of rapid population growth from a small, efficient ancestral population for sufficient time for a mutation recovery of haplotypic (Hd) diversity. But this time is too short for the accumulation of strong sequence differences in all populations except cashew apple. The latter has the signal of a sweep. These results are validated by the Ramos R2 all positive that demonstrate population expansion. These results, combined with the analysis of «Mismatch distribution» curves validated by demographic indices (SSD and Rag) as well as genetic diversity indices (Hd and π) and demogenetic tests (D of Tajima, FS of Fu and R2) revealed a population expanding demographically but moderately for the cashew apple population. Indeed, with the Tajima D and the sum of deviation squares (SSD) which are significantly negative, there is excess of rare variants that could be due to a decrease in the population by anthropological effect followed by a rapid demographic reconstruction. The cashew apples are collected by the local population for consumption or to be processed. In the event of an infestation, there will be a decrease in the population of *B. dorsalis* since these future individuals will be subtracted from the population size. The population expansion may be due to the colonization of other territories through the movement of cashew apples by consumers. Cashew apples also ripen in the mid-rainy season. Excess water rots some fruits that become unfit for the development of larvae. Thus, we can witness the drastic reduction of the birth rate of these insects from this fruit and then a demographic increase towards the end of the rainy season; when the amount of water will be optimal for the development of these insects. However, in the overall *B. dorsalis* population and in the mango and citrus populations, analysis of the "Mismatch distribution" curves (multimodal) revealed that these populations are in demographic equilibrium with a constant size. The non-significant demographic indices (SSD and Rag) for these populations, with the exception of the Rag for the citrus population, show that there is no difference between the observed and simulated values, so these are populations undergoing demographic expansion. The genetic diversity indices (Hd and π) and the demogenetic tests (Tajima's D, Fu's FS and R2) show the same result. So, these populations are expanding demographically from populations of constant size. Indeed, according to Ndiaye and Sembène [37], the demographic expansion of a population generally leaves traces in the distribution of haplotypes and could be due to an excess of haplotypes or an excess of singleton type mutations. This would be at the origin of the highest number of haplotypes observed in these *B. dorsalis* populations (mango, citrus and total population) and the high number of singleton mutations (cashew apple). According to Sarr [33], this demographic expansion would be the signal of rapid population growth from an efficient small ancestral population for a sufficient time for a recovery of haplotypic diversity by mutation. But this time is too short for the accumulation of strong sequence differences. Similarly, the existence of a national and local market of mangoes and citrus fruits would be at the origin of this demographic expansion from a small effective size of

insects of local mangoes and citrus fruits. Indeed, rotten mangoes mixed with fly larvae are thrown either in the garbage or in the open air. The result is the birth of flies in these localities that will then colonize the existing host plants creating new haplotypes.

The demographic evolution of *B. dorsalis* populations in the Niayes area is worrying. Referring to the work of Sarr [33], the observed demographic expansions of *B. dorsalis* can lead to two fears: that of a massive destruction of crops and that of the emergence of strains resistant to control methods, in particular that of chemicals.

Haplotype tree and phylogenetic relationships

The haplotype network shows that haplotype 1 is made up mainly of individuals from which the other haplotypes originate. Haplotype 1, which is in the majority, is also made up of individuals from the three populations. This could be explained by gene flow facilitated by the fruit and vegetable trade. The absence of reproductive isolation favors the meeting of haplotypes and reproduction between individuals from different populations if there is significant gene flow. The roughly radial arrangement of the other haplotypes from haplotypes 1 and 2 shows a total population undergoing moderate demographic expansion. These results are in line with those of Sarr [33] who states that the presence of the majority haplotype in all populations is due to gene flow between them. The most resolving tree is obtained using the likelihood method. The presence of haplogroups in which the majority of individuals from mango and cashew apple are found shows the existence of gene flow between these populations (haplogroup 1). The same is true of the haplogroups, where the majority of individuals from citrus and mango are found. This gene flow may be due to the fact that some *B. dorsalis* insects from mango directly colonize citrus in orchards where the cashew tree is missing. This may explain the phylogenetic relationship between these two populations. On the other hand, it may be explained by the fact that some *B. dorsalis* individuals derived from mango directly colonize cashew apple fruits present in or near the orchard. These results can also be explained by the action of anthropogenic effects and commercial exchanges leading to genetic mixing and genetic characterization of *B. dorsalis* from host plants. In fact, these fruits are transported from one market to another or from one locality to another, encouraging the relocation of certain individuals that will later come into contact with local species. This encourages gene flow between the different individuals during reproduction. The existence of unresolved private haplotypes could be due to the fact that these individuals are almost isolated from the other members of their population. In some orchards, one of the host plants used is present with other host plants that are not considered in this sampling. This would mean that individuals could be mutated to colonize existing plants in the same orchard, such as *Capparis tomentosa* 'yegul ngon' and *Momordica charantia* 'mbeurbeuf' (the names in quotation marks are the local names for these plants). The first, a toxic plant, and the latter, a cucurbit with medicinal uses, are found in the hedgerows bordering most orchards, or in the vicinity of orchards, or even inside them. As a result, some fruit flies lay their eggs there. These insects have been able to detoxify the *Capparis tomentosa* plant in order to develop, which may lead to the existence of private haplotypes owed to mutations that have been beneficial to the insect. The larvae

of these insects could then enter diapause at the end of fruiting of these plants until the next cycle of ripening of the fruits, more particularly mango, to emerge from this diapause and infest them. These results are in line with those of Sembène and al. [38] who found that the scarcity of a fruit to which a species is attached leads the species to reproduce on other plants that are favorable to its development and that are available.

Conclusion

The demographic evolution of the mango and citrus populations shows a demographic expansion from a stable ancestral population. However, the cashew apple population is undergoing moderate demographic expansion. This has the signal of a sweep. Phylogenetic trees have also demonstrated gene flow between the different populations and the isolation of some individuals that form private haplotypes. The distribution of haplotypes shows that part of the mango population of *B. dorsalis* is close to the citrus population, while the second part of the population is closer to the cashew population.

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