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Population genetics of *Glossina palpalis palpalis* in sleeping sickness foci of Côte d'Ivoire before and after vector control

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24 **Abstract**

25 *Glossina palpalis palpalis* remains the major vector of sleeping sickness in Côte
26 d'Ivoire. The disease is still active at low endemic levels in Bonon and Sinfra foci in the
27 western-central part of the country. In this study, we investigated the impact of a control
28 campaign on *G. p. palpalis* population structure in Bonon and Sinfra foci in order to adapt
29 control strategies. Genetic variation at microsatellite loci was used to examine the
30 population structure of different *G. p. palpalis* cohorts before and after control campaigns.
31 Isolation by distance was observed in our sampling sites. Before control, effective
32 population size was high (239 individuals) with dispersal at rather short distance (731 m
33 per generation). We found some evidence that some of the flies captured after treatment
34 come from surrounding sites, which increased the genetic variance. One Locus, GPCAG,
35 displayed a 1000% increase of subdivision measure after control while other loci only
36 exhibited a substantial increase in variance of subdivision. Our data suggested a possible
37 trap avoidance behaviour in *G. p. palpalis*. It is important to take into account and better
38 understand the possible reinvasion from neighbouring sites and trap avoidance for the
39 sake of sustainability of control campaigns effects.

40

41 Running title: Tsetse genetics before and after control

42

43 **Introduction**

44 Human African Trypanosomiasis (HAT) is a centuries-old disease that has affected
45 the lifestyle of people in sub-Saharan Africa (Steverding, 2008). It is a parasitic disease
46 due to two subspecies of *Trypanosoma brucei* transmitted by tsetse flies belonging to the
47 *Glossina* genus. There are two forms of HAT: one, known as gambiense HAT, due to *T.*
48 *brucei gambiense*, is endemic in West and Central Africa and causes over 95% of current
49 cases; the other, known as rhodesiense HAT, due to *T. brucei rhodesiense*, is endemic in
50 East and Southern Africa and accounts for the remainder of cases (Büscher et al., 2017).

51 The disease reemerged at the end of the 1990s, but renewed efforts from endemic
52 countries brought the disease under control again (Franco et al., 2018). In this context,
53 sustainable elimination of the gambiense HAT was considered as a feasible target for
54 2030 (Franco et al., 2014).

55 Tsetse control has recently become a key component of the overall activities of HAT
56 control (Solano et al., 2013; Bouyer et al., 2015; Courtin et al., 2015). However, many
57 tsetse control efforts were not sustainable due to either flies surviving the initial
58 interventions, or flies immigrating from untreated regions, or both (Hargrove, 2003; Adam
59 et al., 2014; De Meeûs et al., 2019), except when control itself is sustained (Simo and
60 Rayaisse, 2015; Meyer et al., 2016). This lead to the fact that in 2015, animal
61 trypanosomiasis was still an important issue in West and Central Africa (Simo and
62 Rayaisse, 2015). The knowledge of the genetic structure of a target population can
63 facilitate decision-making (Mccoy, 2008; Solano et al., 2010a; Solano et al.,
64 2010b). Quantifying exchanges of individuals among subpopulations gives information on
65 the isolation status and structure of the studied population. These population parameters
66 are important for medical entomologists as they may have consequences on the
67 epidemiology and control of vector borne diseases (De Meeûs et al., 2007; Solano et al.,

68 2010b; Chevillon et al., 2012; Krafur and Maudlin, 2018; De Meeûs et al., 2019;
69 Manangwa et al., 2019a).

70 In Côte d'Ivoire, the development of cash crops (coffee and cocoa) in western part
71 of the country has led to profound changes in the biotopes that are favorable to tsetse flies
72 (Courtin et al., 2008; Cecchi et al., 2009) so that the country is the second most affected
73 by HAT in West Africa (Simarro et al., 2010). Bonon and Sinfra foci are still active at low
74 endemic level, since 11 cases have been reported in both foci in the last five years
75 according to National Program for HAT Elimination. In order to accelerate elimination, in
76 addition to active and passive HAT cases screening, a tsetse control campaign based on
77 the use of impregnated tiny targets (Esterhuizen et al., 2011; Rayaisse et al., 2011;
78 Rayaisse et al., 2012) has been firstly set in the Bonon focus in February 2016, then in
79 Sinfra in May 2017.

80 In this study, we aimed to assess how vector control can affect the population
81 genetic structure of *Glossina palpalis palpalis* in the Bonon focus. Sinfra subsamples were
82 added to allow widening the studied area and get more precision on the initial structure of
83 tsetse populations before any control in the Marahoué region.

84

85 **Material and Methods**

86 *Study site*

87 The study was carried out in the Bonon and Sinfra HAT foci located in the western-
88 central part of Côte d'Ivoire (Figure 1). These foci belong to the Marahoué region, which is
89 located in a mesophilic forest area, although forest has now almost disappeared, replaced
90 by coffee and cocoa plantations. In these foci and surroundings, many livestock farms
91 raising pigs, goats, sheep and cattle are present (Courtin et al., 2005; N'Djetchi et al.,
92 2017). Due to the cash crops interests (coffee, cocoa, bananas, etc.) and associated
93 human settlements, favoring tsetse-human contact, HAT found ideal geographic conditions

94 for its development, and Bonon and Sinfra became the most active foci in Côte d'Ivoire at
95 the beginning of the 1990s (Dje et al., 2002; Solano et al., 2003; Kaba et al., 2006).
96 Thanks to control efforts, only few cases are now reported each year in these foci (Koffi et
97 al., 2016).

98

99 *Sampling*

100 Sampling extended from May 2015 to March 2017. If we consider a 2 months length
101 for a tsetse generation (Williams, 1990; Krafsur, 2009), our sampling contain different
102 tsetse fly cohorts: cohorts 1, 7, 8, 9, 10 and 11. In Bonon focus, the first entomological
103 survey was carried out in June 2015 (T0, cohort 1), before the control campaign. Tsetse
104 control campaign took place in February 2016 and then 30 sentinel traps were deployed at
105 chosen sites in order to monitor the evolution of tsetse flies densities, among which 25
106 were used for the present study. Evaluations were done every three months allowing us to
107 define T1, T2, T3 and T4. T1 (June 2016, cohort 7) corresponded to the first survey after
108 control, T2 (September 2016, cohort 8) to the second survey, T3 (December 2016, cohort
109 10) to the third survey and T4 (March 2017, cohort 11) to the fourth entomological survey
110 after control. In Sinfra, two entomological surveys were done before tsetse control. The
111 first was done in May 2015 (T0, cohort 1) and the second in November-December 2016
112 (T0_bis, cohort 9). Sinfra subsamples allowed getting more precision on the initial
113 structure of tsetse populations of the Marahoue region before control. Tsetse flies were
114 sampled using Vavoua traps deployed during two consecutive days in tsetse fly favorable
115 biotopes. In total, 17 and 8 traps were used in Bonon and Sinfra respectively (Table 1). For
116 the population genetics analyses, we used lies captured in 14 traps: 6 in Bonon at T0 and
117 8 in Sinfra. In Bonon, we used captured flies from 4 traps at T1, 5 traps at T2, 3 traps at
118 T3, and 3 traps at T4. These 30 subsamples varied in size from 1 to 25 flies (13 on
119 average), but most (21) contained more than 9 flies. Surface of sampling was computed

120 for each site (Table 1) with the longer distance between two traps (D_{max}) taken as the
121 radius of the disc that contained all traps of the site ($S_S = \pi \times D_{max}^2$). Census densities (D_c)
122 were computed as the number of captured flies per site (N_c) divided by S_S (Table 1). The
123 comparison between densities at T0 and the others (T1-T4) was undertaken with a
124 Wilcoxon rank sum test with continuity correction with R-Commander package (Fox, 2005;
125 Fox, 2007) for R (R-Core-Team, 2018).

126 Three legs were removed from each fly and stored in 70% ethanol tubes labelled
127 with a code containing the trap number followed by individual fly number and the sampling
128 date. In total 403 tsetse individuals were analyzed (Table 1 and supplementary Table S1).
129 All flies were identified as *Glossina palpalis palpalis* according to morphological criteria
130 (Pollock, 1982).

131

132 *Genotyping*

133 Tsetse flies were genotyped at 10 microsatellite loci (Supplementary Tables S1 and
134 S2). Locus X55-3 is from (Solano et al., 1997). Loci XpGp13 and pGp24 come from (Luna
135 et al., 2001) and GPCAG from (Baker and Krafur, 2001). Loci, B3, XB104, XB110 and
136 C102 were kindly supplied by A. Robinson, Insect Pest Control Laboratory (formerly
137 Entomology Unit), Food and Agricultural Organization of the United Nations/International
138 Atomic Energy Agency [FAO/IAEA], Agriculture and Biotechnology Laboratories,
139 Seibersdorf, Austria. Finally, loci pGp20 and pGp27 came from a microsatellite bank of *G.*
140 *palpalis gambiensis* (S. Ravel, personal communication). Those the name of which begins
141 with the letter X are X-linked. We thus analyzed two data sets: one without these loci but
142 with all individuals and the other with females only and all loci. More characteristics for
143 describing the loci used can be found in the Supplementary Table S2.

144 In the laboratory, legs were dried and then subjected to chelex treatment as
145 previously described (Ravel et al., 2007). The 10 PCR reactions were then carried out in a

146 thermocycler (MJ Research, Cambridge, UK) in 20 μ l final volume, using 10 μ l of the
147 diluted supernatant from the extraction step as template. After PCR amplification at the
148 microsatellite loci, allele bands were routinely resolved on ABI 3500 XL sequencer
149 (Applied Biosystems, USA). This method allows multiplexing and the use of four dyes
150 (blue, red, green and yellow). Allele calling was done using GeneMapper V 4.1 software
151 (Applied Biosystems) and the size standard GS600LIZ short run.

152 In order to confirm subspecies determination, we amplified partial sequences of
153 ITS1 following the protocol described by Dyer et al (Dyer et al., 2008).

154 All data and genotypes are available in the supplementary Table S1. In total, 403
155 flies, including 309 females and 94 males, were genotyped.

156

157 *Data analyses*

158 All data were formatted for Create (Coombs et al., 2008) and transformed in the
159 appropriate format for subsequent analyses.

160 Significance of linkage disequilibrium (LD) between locus pairs was assessed with
161 the G -based test of Fstat 2.9.4 (Goudet, 2003) an updated version of Fstat (Goudet, 1995)
162 with 10000 permutations. The G -based test allows obtaining a global test across
163 subsamples (for each pair of loci) and is more powerful than other combining procedures
164 (De Meeûs et al., 2009). There are as many tests as locus pairs, i.e. $L(L-1)/2$ (15 if the
165 number of loci $L=6$) These tests are not independent. We used the Benjamini and Yekutieli
166 false discovery rate (FDR) procedure (Benjamini and Yekutieli, 2001) that is appropriate in
167 case of non-independent test series. The corrected p -values were computed with R (R-
168 Core-Team, 2018).

169 For a hierarchy with three levels (individuals in subsample in total sample), three F -
170 statistics (Wright, 1965) can be defined: F_{IS} , which measures inbreeding of individuals
171 relative to inbreeding of subsamples; F_{ST} , which measures inbreeding of subsamples

172 relative to total inbreeding; and F_{IT} , which measures inbreeding of individuals relative to
173 total inbreeding. Deviations of genotypic proportions expected under local panmixia are
174 measured by F_{IS} , while F_{ST} measures the effect of subdivision (genetic isolation between
175 subsamples) and F_{IT} reflects the combination of both (e.g. (De Meeûs et al., 2007)). Under
176 the null hypothesis (panmixia and no subdivision), all these statistics are expected to be
177 null. Otherwise F_{IS} and F_{IT} can vary from -1 (heterozygote excess) to +1 (homozygote
178 excess) and F_{ST} from 0 (all subsamples share similar allele frequencies) to +1 (all
179 subsamples fixed for one or the other allele). In any case, the three statistics are linked by
180 the famous relationship: $1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$ (e.g. (De Meeûs, 2018b)).

181 In dioecious species (like tsetse flies), heterozygote excess is expected over all loci
182 in small random-mating subpopulations (e.g. (De Meeûs et al., 2007)). Multilocus positive
183 F_{IS} (homozygote excess) can be produced by systematic mating between related
184 individuals like sib-mating (e.g. (De Meeûs et al., 2007)). It can also come from the
185 admixture, in each subsample, of individuals that belong to genetically divergent entities
186 (subpopulations, subspecies or species) (Wahlund effect) (e.g. (De Meeûs et al., 2007; De
187 Meeûs, 2018a)).

188 Technical problems, like null alleles, stuttering, short allele dominance or allele
189 dropouts unevenly affects some loci, producing a positive F_{IS} with an important variation
190 across loci (De Meeûs, 2018a).

191 A positive value for F_{ST} suggests that the total population is subdivided, for
192 instance, into n subpopulations of effective size N_e and an immigration rate of m . In an
193 Island model of migration (with no spatial structure) (Wright, 1951) at mutation-drift
194 equilibrium, we expect $F_{ST} = 1/[4N_e(m + u) + 1]$, where u is the mutation rate of the locus
195 (e.g. (De Meeûs et al., 2007)). If $u \ll m$, the number of immigrants $N_e m$ can be extracted as
196 $N_e m = (1 - F_{ST})/(4F_{ST})$.

197 Wright's F -statistics were estimated through Weir and Cockerham's unbiased
198 estimators (Weir and Cockerham, 1984). It is worthy of note that θ , F_{ST} estimator, can
199 display negative values. This happens when subsamples share more similar genetic
200 composition than would be expected if the different subsamples were randomly drawn
201 from the same subpopulation, i.e. when alleles are more related between than within
202 subsamples ((Weir, 1996) page 175).

203 For F -statistics, significant departure from 0 was tested by randomizing alleles
204 among individuals within subsample (deviation from local random mating test) or of
205 individuals among subsamples within the total sample (population subdivision test) (10000
206 permutations in each case). The p -value then corresponded to the number of times a
207 statistic measured in randomized samples was as big as or bigger than the observed one
208 (unilateral tests). For F_{IS} , the statistic used was f (Weir and Cockerham's F_{IS} estimator). To
209 test for subdivision, we used the G -based test (Goudet et al., 1996) over all loci, which is
210 the most powerful procedure when combining tests across loci (De Meeûs et al., 2009).

211 To compute 95% confidence intervals (95%CI) of F -statistics, we used the standard
212 error of F_{IS} (StrdErrFIS) and F_{ST} (StrdErrFST) computed by jackknife over populations, and
213 5000 bootstraps over loci as described elsewhere (De Meeûs et al., 2007). Since
214 jackknife's computation of 95%CI assumes a normal distribution of the parameters and
215 because F -statistics do not follow such a distribution, these confidence intervals were only
216 used to graphically visualize parameter variation across subsamples and not for statistical
217 comparisons. Bootstrap's 95%CI does not require that the data follow any distribution and
218 is thus statistically valid.

219 In case of significant homozygote excess and linkage disequilibrium we have tried
220 to discriminate demographic from technical causes with the determination key proposed by
221 De Meeûs (De Meeûs, 2018a). In case of null alleles, both F_{IS} and F_{ST} are augmented,
222 StrdErrFIS is at least twice StrdErrFST (jackknives over loci), a positive correlation is

223 expected between F_{IS} and F_{ST} as is expected a positive correlation between F_{IS} and the
224 number of missing data (putative null homozygotes). If such correlations do not exist and if
225 $StrdErrF_{IS} > StrdErrF_{ST}$, then a Wahlund effect better explains the data. The significance of
226 correlations was tested with a unilateral ($\rho > 0$) Spearman's rank correlation test with R. The
227 presence of null alleles was also looked for with MicroChecker v 2.2.3 (Van Oosterhout et
228 al., 2004) and null allele frequencies estimated with Brookfield's second method
229 (Brookfield, 1996). The adjustment between observed and expected numbers of missing
230 data was tested with a unilateral exact binomial test under R with the alternative
231 hypothesis: "there are not enough missing data as expected if heterozygote deficits were
232 entirely explained by null alleles under panmixia". MicroChecker also checks for stuttering
233 and short allele dominance (SAD). Short allele dominance was also assessed with
234 unilateral ($\rho < 0$) Spearman's rank correlation test between allele size and F_{IT} , which is
235 more powerful than other alternatives (Manangwa et al., 2019b). In case of SAD, a
236 negative correlation is expected (unilateral tests).

237 For local population structure studies, we used all subsamples, while for population
238 subdivision studies we kept only comparisons between subsamples from the same cohort.

239 When null alleles are present, unbiased estimation of subdivision and/or of isolation
240 by distance were obtained with the ENA correction for F_{ST} estimates and the INA
241 correction for Cavalli-Sforza and Edwards' chord distance (Cavalli-Sforza and Edwards,
242 1967) D_{CSE} computed with FreeNA (Chapuis and Estoup, 2007). For this, all missing
243 genotypes were converted into homozygous individuals for allele 999 as recommended
244 (Chapuis and Estoup, 2007).

245 Isolation by distance was undertaken with Rousset's approach (Rousset, 1997)
246 where, in a two dimension framework Rousset's Index $F_{ST_R} = F_{ST} / (1 - F_{ST})$ follows
247 $F_{ST_R} = a + b \times \ln(D_G)$ where a is a constant (intercept), $\ln(D_G)$ is the natural logarithm of the
248 geographic distance between two sites and b is the slope of the regression. Rousset also

249 demonstrated that the product of the effective population density D_e by the average of the
250 squared axial distance between reproducing adults and their parents $\overline{\sigma^2}$: $D_e \times \overline{\sigma^2} = 1/(4\pi b)$;
251 that the neighborhood size is $Nb=1/b$; and that the number of immigrants from neighboring
252 sites at each generation is $N_e m = 1/(2\pi b)$, where N_e is the effective subpopulation size and
253 m is the immigration rate. The parameter σ represents half the average parent offspring
254 axial distance. A proxy for dispersal distances per generation $\bar{\delta}$ can be obtained if N_e and
255 average surface of a subpopulation (S) are known: $D_e = N_e/S$ and $\bar{\delta} \approx 2 \times \sqrt{1/(4\pi b D_e)}$. For
256 S , we took either the surface computed above (S_S). Another possibility is to use the
257 threshold distance (D_T) between two sites for F_{ST} to become positive, hence $D_T = e^{-a/b}$. This
258 new parameter was then considered as the distance between the center of two
259 neighboring subpopulations and hence to their diameter. The corresponding surface was
260 then $S_T = \pi \times (D_T/2)^2$.

261 Significance of isolation by distance was assessed through the 5000 bootstraps
262 confidence intervals from FreeNA and with a Mantel test between D_{CSE} and $\ln(D_G)$ or
263 F_{ST_R} , computed with the INA or ENA correction by FreeNA, as recommended for
264 microsatellite markers (Séré et al., 2017). Because some subsamples are not
265 contemporaneous and because we kept only contemporaneous pairs, the final matrices
266 were not squared and were thus analyzed with the menu "Mantelize it" of Fstat. Since
267 Fstat handles bilateral tests, we converted the resulting p -value into a unilateral one
268 (correlation between geographic and genetic distance is positive) by halving the bilateral p -
269 value in case of positive correlation.

270 Effective population sizes (N_e) were estimated through several methods and
271 softwares. The heterozygote excess method (Balloux, 2004) could not be used due to the
272 excessive presence of null alleles in almost all loci (see below). The Linkage disequilibrium
273 method (Waples and Do, 2010; Peel et al., 2013) and the coancestry method (Nomura,
274 2008) were undertaken with NeEstimator v 2.1 (Do et al., 2014). For LD method, several

275 threshold values are proposed for minimal allele frequencies to be used (above 0, 0.01,
276 0.02 and 0.05). We used the average across usable values obtained with all methods. We
277 also used the inter and intra correlation method (Vitalis and Couvet, 2001b) with the
278 software Estim (Vitalis and Couvet, 2001a) (available at [http://www.t-de-
280 meeus.fr/ProgMeeusGB.html](http://www.t-de-
279 meeus.fr/ProgMeeusGB.html)). We then computed the average, minimal and maximal
281 (MiniMax range) effective population sizes across methods weighted by the number of
282 usable values obtained in each case. Effective population size reflects the demography
283 and other phenomena as reproductive strategy and/or historical perturbations and its
284 estimation also varies across methods (Krafsur and Maudlin, 2018). We nevertheless
285 expected a good correlation between N_e and the census size (N_c) of the corresponding
subpopulations (De Meeûs et al., 2019).

286 The surface occupied by a subpopulation was inferred with S_S and S_T described
287 above.

288 Effective population densities could then be computed as $D_e = N_e / S$.

289 The effect of control campaigns on the population genetic structure of *G. p. palpalis*
290 was assessed first with a principal component analysis (PCA) undertaken with PCA-Gen
291 1.2.1 (Goudet, 1999) for which the metrics of principal axes correspond to Nei's G_{ST} (Nei
292 and Chesser, 1983). Significance of axes was tested according to the broken stick criterion
293 (Frontier, 1976) and 10000 permutations of individuals across subsamples. We also
294 compared F_{ST} (corrected for null alleles) between T0 subsample pairs and pairs between
295 T0 and TX (i.e. sampled after control campaign at times T1, T2, T3 and T4) with a Mann-
296 Whitney-Wilcoxon U test with Rcmdr.

297

298 **Results**

299

300 *Fly densities*

301 In Bonon, fly densities decreased significantly after the control campaign (less than
302 10% of its initial value) as show in Figure 2 (p -value=0.0269) (see also Table 1).

303

304 *Local population genetics with autosomal loci only in the Marahoue region*

305 There was only one pair of loci in significant LD (p -value=0.0406) that did not stay
306 significant with Benjamini and Yekutieli correction (p -value=1). We thus safely concluded
307 that all markers are statistically independent.

308 There was an important heterozygote deficit that varied considerably from one locus
309 to the other (Figure 3). Null alleles only explained partly these results with a StdErrFIS
310 more than four times StdErrFST , a positive though not significant correlation between
311 the number of observed missing genotypes and F_{IS} ($\rho=0.1429$, p -value=0.4014); and a
312 positive though not significant correlation between F_{IS} and F_{ST} ($\rho=0.4058$, p -
313 value=0.2123). No stuttering could be evidenced and a significant short allele dominance
314 was detected for locus pGp27 (Figure 3). This locus thus seems problematic.

315

316 *Local population genetics with females only in the Marahoue region*

317 There were four pairs of loci in significant LD (p -values<0.05) that did not stay
318 significant after Benjamini and Yekutieli correction (p -values>0.9).

319 The variation of F_{IS} was very important across the 10 loci with a global highly
320 significant heterozygote deficit (Figure 4). There was some evidence of the presence of
321 null alleles with a StdErrFIS eight times StrdErrFST , positive correlations between the
322 number of missing data and F_{IS} ($\rho=0.4681$) and between F_{IS} and F_{ST} ($\rho=0.4667$) though
323 marginally not significant (p -value=0.0862 and p -value=0.0891 respectively). Three loci
324 displayed short allele dominance: Loci X55-3, pGp27 and XB110 (Figure 4). This could be
325 expected for the first two, which displayed the highest F_{IS} but with too few missing
326 genotypes. Nevertheless, for XB110, the important number of missing genotypes (the

327 highest observed) was in agreement with null alleles as the explanation for the important
328 F_{IS} observed at this locus ($F_{IS}=0.201$ with 26 missing genotypes and null allele frequency
329 estimated as $p_n=0.2$). Furthermore, it is worth noting that the SAD signature was mainly due
330 to the fact that all longest alleles were rare (average frequency < 0.006) and displayed a
331 very small F_{IT} (average < 0.042). Considering that with a frequency of $p_n=0.2$, the
332 probability for heterozygous individuals between such alleles and a null allele is
333 $2 \times 0.2 \times 0.006 = 0.0024$ and the average of homozygote frequencies for these rare alleles is
334 ~ 0.00005 , with a total sample size of 296, this means a total expected number of falsely
335 interpreted or true homozygous individuals for these long and rare alleles is 0.725. Thus,
336 because null alleles affect preferentially the F_{IS} of the most frequent alleles, and because
337 here these are also the shortest ones, this observation invalidates the SAD test for XB110.
338 This locus can simply be considered as only affected by null alleles. Heterozygote deficits
339 observed at other loci can be explained by null alleles. Subsequent subdivision measures
340 and testing were thus done with corrections implemented by FreeNA.

341 Variations of F_{ST} across loci can be seen in the Figure 5. From this figure, it can be
342 seen that locus GPCAG's F_{ST} appeared completely outside the range of the nine other
343 loci. Here, these subdivision measures are presented only for the sake of examining loci
344 behavior. Indeed, values presented here both include temporal and spatial subdivision.
345 Interestingly, this locus corresponds to a trinucleotide motive. It probably responds to
346 some kind of selection.

347 In order to avoid possibly biased estimates, we removed loci X55-3, pGp27 and
348 GPCAG from subsequent analyses.

349 To keep a number of loci above 5, we thus only studied female subsamples.

350

351 *Population subdivision in the Marahoue region before control of female subsamples with*

352 *the seven loci retained*

353 The regression of isolation by distance is presented in the Figure 6. The F_{ST_R}
354 based tests with 95% confidence interval of the slope or with the Mantel test are significant
355 while the D_{CSE} based Mantel test is not. The 95% confidence interval of the slope is a very
356 good indicator of what occurs and F_{ST_R} based methods should be less powerful than with
357 D_{CSE} (Séré et al., 2017), but it is highly significant here. It is thus safer to accept the
358 alternative hypothesis of isolation by distance in this tsetse population.

359 Using the slope and its 95% confidence interval ($b=0.0015$ in
360 $95\%CI=[0.0011..0.0021]$), we could estimate a neighborhood size $Nb=667$ individuals in
361 $95\%CI=[476..909]$ and an immigration of $N_e m=106$ individuals from neighboring sites (in
362 $95\%CI=[76..145]$).

363 Average effective subpopulation sizes was $N_e=239$ ranging in MiniMax= $[135..325]$.
364 Threshold geographic distance was $D_T=875$ m, leading to a surface $S_T=0.602$ km² while
365 the average surface of sampling in sites was $S_S=1.417$ km². Then effective population
366 density $D_{e_S}=169$ appeared much smaller than $D_{e_T}=397$, which in turn displayed values
367 that appeared closer to the average census density $D_c=534$ individuals per km² (Figure 7).
368 Such inferences allowed computing dispersal as represented in the Figure 8. Dispersal
369 inferred from different effective population densities (Minimal, Average and Maximal) and
370 methods (with threshold distance, sampling surface or census density) varied between 500
371 and 1800 meters per generation. Dispersal obtained with sampling surfaces were
372 significantly higher (1122 m on average in $95\%CI [948..1310]$) than those computed with
373 the threshold surface (731 m in $95\%CI=[618..854]$), the last being very close to values
374 calculated with the census population density (630 m in $95\%CI=[533..636]$).

375

376 *Effects of control in Bonon*

377 The PCA graphic is represented in the Figure 9. Although no axis was significant, it
378 can be seen that subsamples before control campaigns occupy less space than

379 subsamples after control campaigns, which suggests a greater genetic variance after
380 control.

381 Accordingly, genetic subdivision appeared smaller between traps from T0 ($F_{ST}=-$
382 0.005) than between traps at T0 and traps at TX ($F_{ST}=0.0035$) but the difference was not
383 significant (p -value=0.1225).

384 Modelling isolation by distance between contemporaneous subsamples after control
385 was uneasy because of the small number of available points. It nevertheless provided a
386 slope the bootstrap confidence interval of which did not contain 0 ($b=0.0086$ in
387 95%CI=[0.0024..0.0219]. From Figure 7, effective population density significantly
388 increases after treatment according to the distance threshold method (D_{e_T}). On the
389 contrary, effective population density using sampling surface (D_{e_S}) significantly drops to
390 values similar to the census density.

391 Regarding dispersal (Figure 8), it significantly drops with the threshold distance
392 method after the control campaign, while dispersal from the sampling surface method and
393 from the census density are not significantly affected after the control campaign, with
394 bootstrap 95%CI containing all the range of densities estimated at T0.

395

396 *Comparison between the seven loci and GPCAG for T0/TX genetic differentiation in Bonon*

397 As can be seen from Figure 10, Locus GPCAG displayed a slightly higher
398 subdivision measure as compared to the other loci for subsamples from T0 but displayed a
399 1000 % increase when measured between T0 and TX, while other loci displayed an
400 unchanged average though with a much higher variance. By examining population
401 subdivision between T1-T4 subsamples revealed absence of genetic differentiation
402 ($F_{ST_FreeNA}=-0.0127$, p -value=0.5843). After pooling all T0 traps together and all T1-4
403 together, we obtained a highly significant (p -value<0.0001) subdivision ($F_{ST_FreeNA}=0.1286$)
404 for GPCAG consistent with what can be seen in Figure 10. A glance at allele frequencies

405 evolution reveals that this is mainly due to a 328% increase of allele 219 (from 0.146 to
406 0.625) at locus GPCAG after treatment.

407

408 **Discussion**

409 In the Marahoue region (Bonon and Sinfra), before control, we noted relatively high
410 population densities and short dispersal distances as compared to other studies (De
411 Meeûs et al., 2019). Traps selected for this study were located in villages' edge with
412 sacred forest (i.e. religiously protected from any human activity with economic purposes)
413 that seems to constitute highly suitable environments for tsetse flies: ideal hygrometric and
414 shade conditions, protection from insecticide pressure existing in cultivated fields and
415 abundant hosts as wandering pigs (Laveissière et al., 1985; Sané et al., 2000b, a). Flies
416 may not need to disperse much because they get all they need there and/or, high densities
417 limit settling possibilities of immigrants. Dispersal is indeed negatively density dependent
418 in tsetse flies (De Meeûs et al., 2019).

419 In Bonon, the increase of genetic variance after control campaigns suggests that
420 flies sampled after controls may come from surrounding sites, at least partly, as was
421 observed 35 years ago for the same species in the same area (Randolph et al., 1984;
422 Rogers and Randolph, 1984; Rogers et al., 1984). The drastic drop of more than 91% in
423 flies' density may have freed up space for surrounding flies. So the area that seemed
424 saturated before control can receive new colonizers. Because of recent treatment and/or
425 immigration, T1-4 subsamples were harvested from subpopulations in strong
426 disequilibrium where effective population density and isolation by distance are hard to
427 measure. The very odd results obtained for D_{e_T} and δ_T after treatment can illustrate this.
428 Alternatively, it may also reflect the inappropriateness of defining the surface occupied by
429 a subpopulation with the threshold distance for genetic subdivision. The relevance of such
430 method was indeed strongly questioned (Rousset, 1997). On the other hand, before

431 control campaigns, these D_{e_T} and δ_T appeared very close to values using census
432 population sizes. When we used the sampling surface, effective population densities
433 appeared significantly smaller than census density before control but dropped to values
434 similar to those computed with census population sizes, while dispersal remained
435 unchanged but with an important increase of the confidence interval. This may reflect a
436 classical result where $N_e < N_c$ (e.g. (Krafsur and Maudlin, 2018)), before treatment, while
437 both N_e and N_c drop to similar very low values after control and only the variance of
438 dispersal is affected but not the average. So, estimating effective population density and
439 dispersal with the sampling surface method may be much more accurate than the
440 threshold distance for genetic subdivision method.

441 Locus GpCAG seems selected by the control technique used in Bonon, by a
442 mechanism that may include either insecticide resistance, behavioral avoidance of
443 trapping device or another unknown mechanism. This would mean that some flies with
444 specific GpCAG profiles (allele 219) were able to escape from control measures: either
445 because this trinucleotide locus may code for something, or because it is part of a selected
446 gene as an intronic sequence, or very close to it. It would be interesting to identify what
447 caused this result. We have been unable to find to what corresponded the sequence
448 where this locus is exactly. Indeed, a "highly similar sequences (megablast)" in GenBank
449 at <https://blast.ncbi.nlm.nih.gov/Blast.cgi> (with the accession number AY033512.1)
450 outputted no result but the sequence deposited by (Baker and Krafsur, 2001). A
451 discontinuous megablast in GenBank, using the Program BLASTN 2.8.1+ (Zhang et al.,
452 2000) did not provide very useful information. It matched at 75% with a nuclear receptor
453 coactivator of *Salvelinus alpinus* (a salmonid fish), with a putative mediator of RNA
454 polymerase II transcription subunit 26 of *Ceratitis capitata* (at 69%) and *Rhagoletis*
455 *zephyria* (at 72%), two Dipteran insects of the sub-order Brachycera like tsetse flies.
456 Deeper investigations would be required to decipher the mechanism involved in the

457 possible resistance associated with GPCAG allele 219. Insecticide resistance has never
458 been reported in tsetse flies to our knowledge and we could find nothing more than
459 recommendations on that issue in an old FAO report (Georghiou et al., 1993) and
460 assumptions that the likelihood of insecticide resistance evolution is negligible in tsetse
461 (Krafsur and Maudlin, 2018). Moreover, the slight superiority of subdivision measured at
462 GPCAG locus even before the control campaign presented here suggests the signature of
463 preceding past treatments. This can come from the different sampling campaigns that
464 have preceded this study between 2000 and 2001 (Courtin et al., 2005; Ravel et al., 2007),
465 using Vavoua traps without insecticide, which resulted in drastic reduction in tsetse
466 densities (Courtin et al., 2005). Alternatively, the massive use of insecticides and
467 pesticides in cocoa and coffee plantation may also explain these results, hypothetically.
468 Resistance evidenced here is possibly behavioral. Insecticide-avoidance behavior is
469 known for different mosquito species (Chareonviriyaphap et al., 1997; Chareonviriyaphap
470 et al., 2013; Tainchum et al., 2013; Porciani et al., 2017). As for tsetse flies, physiological
471 or behavioral resistance is only suspected, if not speculated (Georghiou et al., 1993) but,
472 to our knowledge, was never documented.

473 If resistance is behavioral, the fact that a substantial proportion of resistant tsetse
474 flies harboring the GpCAG-219 allele were captured in the trapping device after treatment
475 suggests: i) that resistance is not absolute but statistical; and ii) that the proportion of
476 resistant tsetse flies must be much higher in the pool of uncaptured flies.

477 More researches are needed to locate this locus in the *Glossina* genomes and
478 check if it is in or near a coding gene, determine its nature, and clarify if this trinucleotide
479 microsatellite itself is responsible for what we have evidenced in the present paper.

480

481 **Conclusion**

482 The work presented here shows that control campaigns has modified tsetse flies
483 population structure. Although it has allowed reducing considerably tsetse fly's densities, it
484 may also have selected for the emergence of flies resisting the treatment by a mechanism
485 that remains to be identified. This result should be taken into account and new strategies
486 developed to prevent reinvasion.

487

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496

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Tables

743 Table 1: Number of *Glossina palpalis palpalis* sampled in each site - N_c , description of
 744 sampling, surface of sampling (S_s) (in km²), observed densities (D_c) and number of
 745 genotyped flies (N_G). Treatment status (T) are indicated and GPS coordinates
 746 (Long, Lat) are given in decimal degrees

Focus	T	Site	Trap	Long	Lat	S_s	N_c	D_c	N_G
Bonon	T0	Biegon	BE4017	-6.0298	6.7966	2.9326	9	85	0
Bonon	T0	Biegon	BE4019	-6.0377	6.7930	2.9326	38	85	0
Bonon	T0	Biegon	BG4018	-6.0344	6.7962	2.9326	203	85	22
Bonon	T0	Blanou	BE4015	-6.0250	6.7933	1.3513	38	33	0
Bonon	T0	Blanou	BG4013	-6.0239	6.7979	1.3513	0	33	0
Bonon	T0	Blanou	BG4014	-6.0231	6.7989	1.3513	5	33	0
Bonon	T0	Blanou	BG4016	-6.0249	6.7975	1.3513	1	33	0
Bonon	T0	Dianou	BE4008	-6.0170	6.7996	2.1569	259	336	15
Bonon	T0	Dianou	BE4012	-6.0124	6.7962	2.1569	63	336	0
Bonon	T0	Dianou	BG4005	-6.0149	6.7989	2.1569	0	336	0
Bonon	T0	Dianou	BG4006	-6.0157	6.7997	2.1569	7	336	0
Bonon	T0	Dianou	BG4007	-6.0170	6.8007	2.1569	79	336	0
Bonon	T0	Dianou	BG4009	-6.0164	6.8018	2.1569	111	336	0
Bonon	T0	Dianou	BG4010	-6.0171	6.8020	2.1569	206	336	14
Bonon	T0	Kangreta	BE4021	-6.0422	6.7989	0.3256	53	1056	16
Bonon	T0	Kangreta	BE4022	-6.0399	6.7978	0.3256	190	1056	21
Bonon	T0	Kangreta	BG4020	-6.0409	6.8006	0.3256	101	1056	15
Bonon	T1	Biegon	BG4018	-6.0344	6.7962	2.9326	22	8	18
Bonon	T1	Dianou	BE4008	-6.0170	6.7996	2.1569	1	1	1
Bonon	T1	Dianou	BG4010	-6.0171	6.8020	2.1569	2	1	2

Bonon	T1	Kangreta	BE4021	-6.0422	6.7989	0.3256	1	126	0
Bonon	T1	Kangreta	BE4022	-6.0399	6.7978	0.3256	3	126	3
Bonon	T1	Kangreta	BG4020	-6.0409	6.8006	0.3256	37	126	7
Bonon	T2	Biegon	BG4018	-6.0344	6.7962	2.9326	20	7	18
Bonon	T2	Dianou	BE4008	-6.0170	6.7996	2.1569	7	6	5
Bonon	T2	Dianou	BG4010	-6.0171	6.8020	2.1569	6	6	6
Bonon	T2	Kangreta	BE4021	-6.0422	6.7989	0.3256	14	86	11
Bonon	T2	Kangreta	BE4022	-6.0399	6.7978	0.3256	1	86	0
Bonon	T2	Kangreta	BG4020	-6.0409	6.8006	0.3256	13	86	11
Bonon	T3	Biegon	BG4018	-6.0344	6.7962	2.9326	0	0	0
Bonon	T3	Dianou	BE4008	-6.0170	6.7996	2.1569	10	21	6
Bonon	T3	Dianou	BG4010	-6.0171	6.8020	2.1569	36	21	17
Bonon	T3	Kangreta	BE4021	-6.0422	6.7989	0.3256	39	141	24
Bonon	T3	Kangreta	BE4022	-6.0399	6.7978	0.3256	1	141	1
Bonon	T3	Kangreta	BG4020	-6.0409	6.8006	0.3256	6	141	6
Bonon	T4	Biegon	BG4018	-6.0344	6.7962	2.9326	8	3	7
Bonon	T4	Dianou	BE4008	-6.0170	6.7996	2.1569	17	9	15
Bonon	T4	Dianou	BG4010	-6.0171	6.8020	2.1569	2	9	2
Bonon	T4	Kangreta	BE4021	-6.0422	6.7989	0.3256	0	3	0
Bonon	T4	Kangreta	BE4022	-6.0399	6.7978	0.3256	0	3	0
Bonon	T4	Kangreta	BG4020	-6.0409	6.8006	0.3256	1	3	0
Sinfra	T0	Benhuafla	SE2002	-5.8812	6.5960	0.2813	154	874	22
Sinfra	T0	Benhuafla	SE2004	-5.8796	6.5938	0.2813	92	874	19
Sinfra	T0	Bintifla	SE1012	-5.8189	6.5685	1.1297	55	283	19
Sinfra	T0	Bintifla	SE1013	-5.8178	6.5710	1.1297	207	283	25
Sinfra	T0	Bintifla	S1023	-5.8232	6.5712	1.1297	20	283	16

Sinfra	T0	Bintifla	S1031	-5.8178	6.5710	1.1297	38	283	14
Sinfra	T0	Ville	S5030	-5.9232	6.6272	0.0351	27	2279	13
Sinfra	T0	Ville	S5031	-5.9233	6.6263	0.0351	53	2279	12

747

Figure legends

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749

750 Figure 1: Location map of study area with selected traps.

751

752 Figure 2: Evolution of tsetse flies apparent density per km² (D_c) from T0 (before
753 control campaign) in Bonon and Sinfra to T4 in Bonon. In Bonon, the
754 difference between densities at T0 and TX (X=1-4) was significant (p -
755 value=0.0269) (Wilcoxon rank sum test with continuity correction).

756

757 Figure 3: F_{IS} observed in subsamples of *Glossina palpalis palpalis* from Ivory Coast
758 for autosomal loci only with jackknife's over subsamples 95% confidence
759 intervals for each locus and 95% Bootstrap over loci confidence interval for the
760 average. Results of panmictic tests, short allele dominance tests, stuttering
761 detection and possible null allele frequencies are also given.

762

763 Figure 4: F_{IS} observed in subsamples of females only of *Glossina palpalis palpalis*
764 from Ivory Coast with jackknife's over subsamples 95% confidence intervals
765 for each locus and 95% Bootstrap over loci confidence interval for the
766 average. Results of panmictic tests, short allele dominance tests, stuttering
767 detection and possible null allele frequencies are also given.

768

769 Figure 5: Variation of F_{ST} across loci for females *Glossina palpalis palpalis* from Ivory
770 Coast.

771

772 Figure 6: Isolation by distance for contemporaneous subsamples of *Glossina palpalis*
773 *palpalis* from Bonon and Sinfra in Ivory Coast before treatment (T0). The
774 average regression line is represented as a straight line and the 95%
775 confidence intervals (95%CI), obtained after 5000 bootstraps over loci, are in
776 dotted lines. Abscisses are the natural logarithms of geographic distances in
777 km. Ordinates are Rousset's F_{ST_R} corrected for null alleles by FreeNA.
778 Average slope was 0.0015 in 95%CI= [0.0011...0.0021]. Mantel test p -values
779 were 0.4158 and 0.00795 for D_{CSE} and F_{ST_R} respectively.

780

781 Figure 7: Population densities of *Glossina palpalis palpalis* with three methods:
782 effective population density using the threshold distance for genetic
783 subdivision (D_{e_T}), effective subpopulation size using the average sampling
784 surface of a site (D_{e_S}) and census density (D_c) (thin straight line). Effective
785 population densities are represented with their average minimal and maximal
786 values. Estimates are given for subsamples before the control campaign (T0)
787 and after control (T1-4). Ordinates were scaled in log.

788

789 Figure 8: Dispersal (δ) of *Glossina palpalis palpalis* in Bonon and Sinfra (Ivory Coast)
790 for different effective population densities (minimal, averaged and maximal)
791 and different methods: using the threshold distance for genetic subdivision for
792 defining the surface occupied by a subpopulation (grey), using the average
793 sampling surface of the different sites (empty symbols) and using the census
794 population density. The 95% confidence intervals computed from the isolation
795 by distance slope after 5000 bootstraps over loci are represented as small
796 dashes. Estimates are given for subsamples before control campaigns (T0)

797 and after (T1-4) and were all made from Rousset's isolation by distance model
798 between contemporaneous subsample pairs only.

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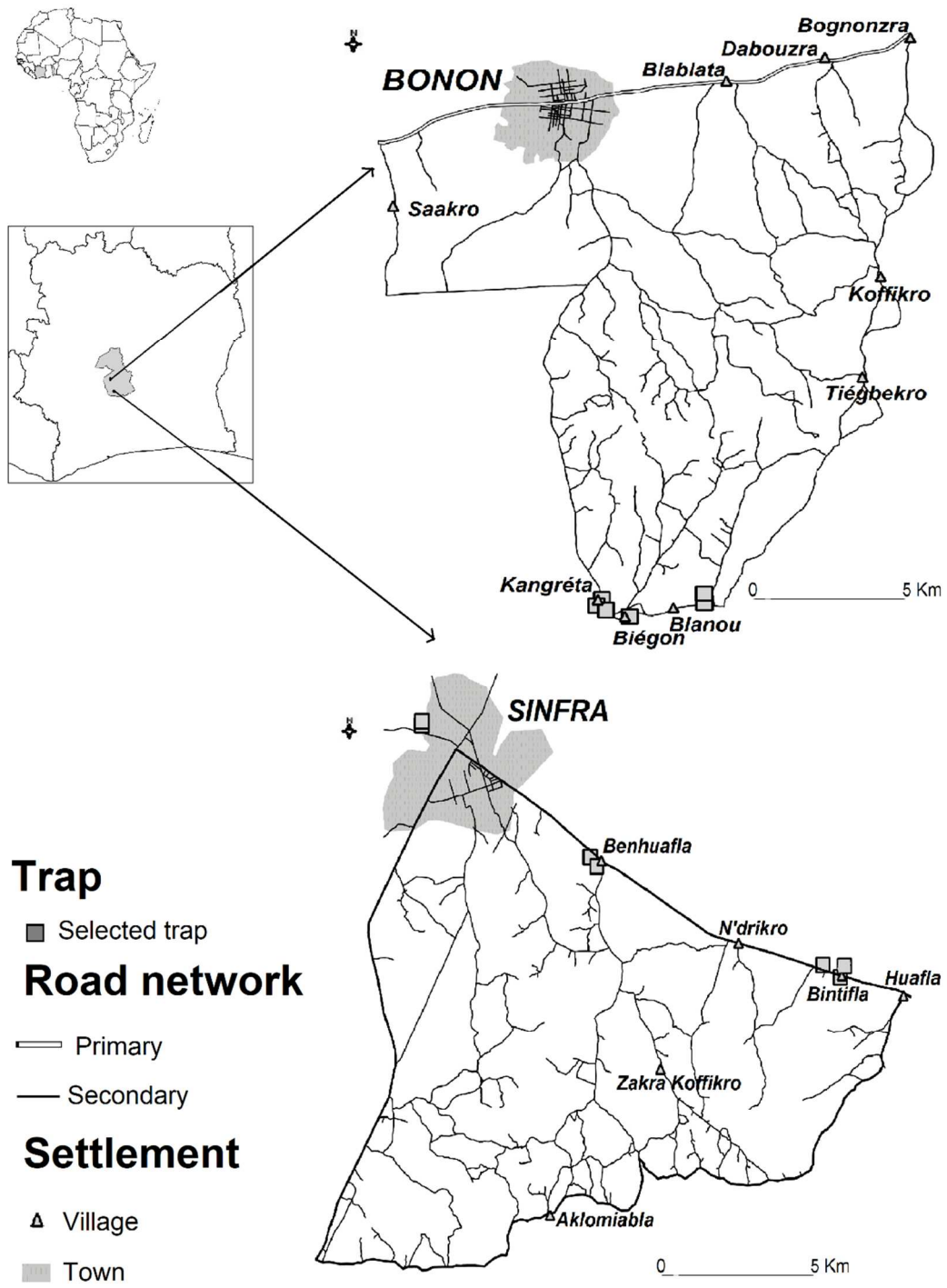
800 Figure 9: PCA Analysis result on the two first principal axes for the different
801 subsamples before control campaigns in Bonon and Sinfra (T0, in blue) and
802 after control campaigns (T1, T2, T3, and T4, other colors). Axis percent inertia
803 values are 11.68 and 8.08 for axis 1 and 2 respectively.

804

805 Figure 10: Average subdivision measures corrected for null alleles with FreeNA
806 (F_{ST_FreeNA}) between T0 subsample pairs (T0-T0) (before treatment
807 campaigns) (black circles) with the seven loci kept for all analyses and 95%
808 confidence intervals (dashes), for GPCAG alone (crosses) and between T0
809 and TX subsample pairs (T0-TX) (X=1, 2, 3 or 4).

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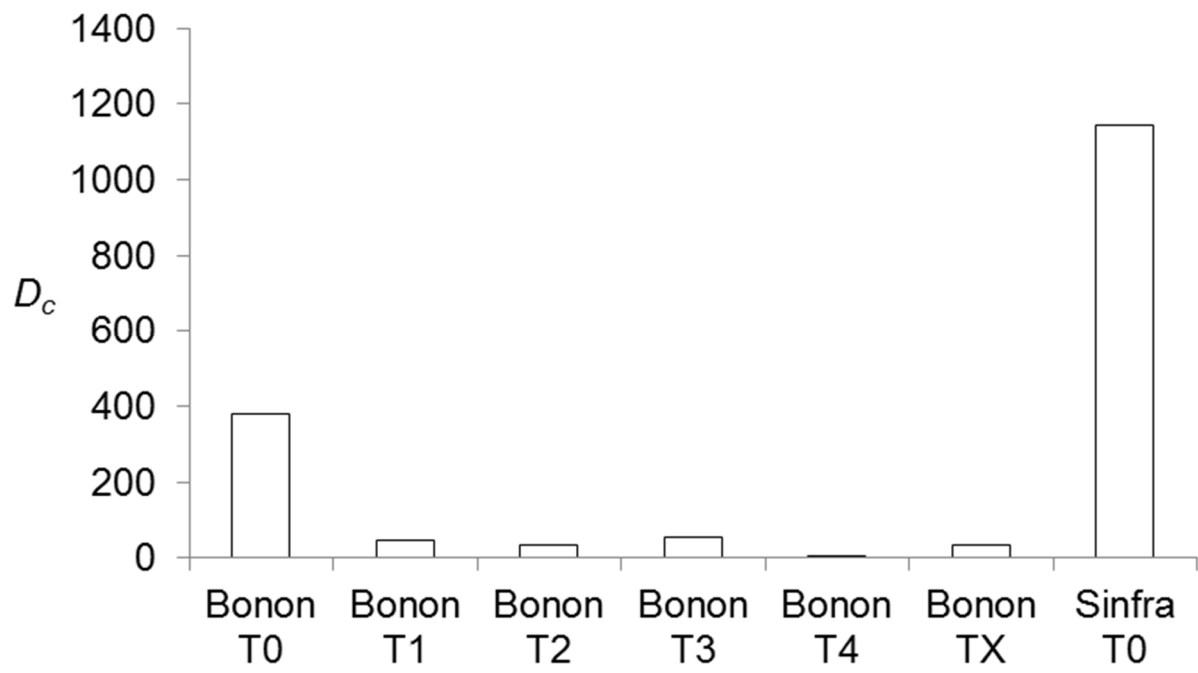
Figure 1



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Figure 2

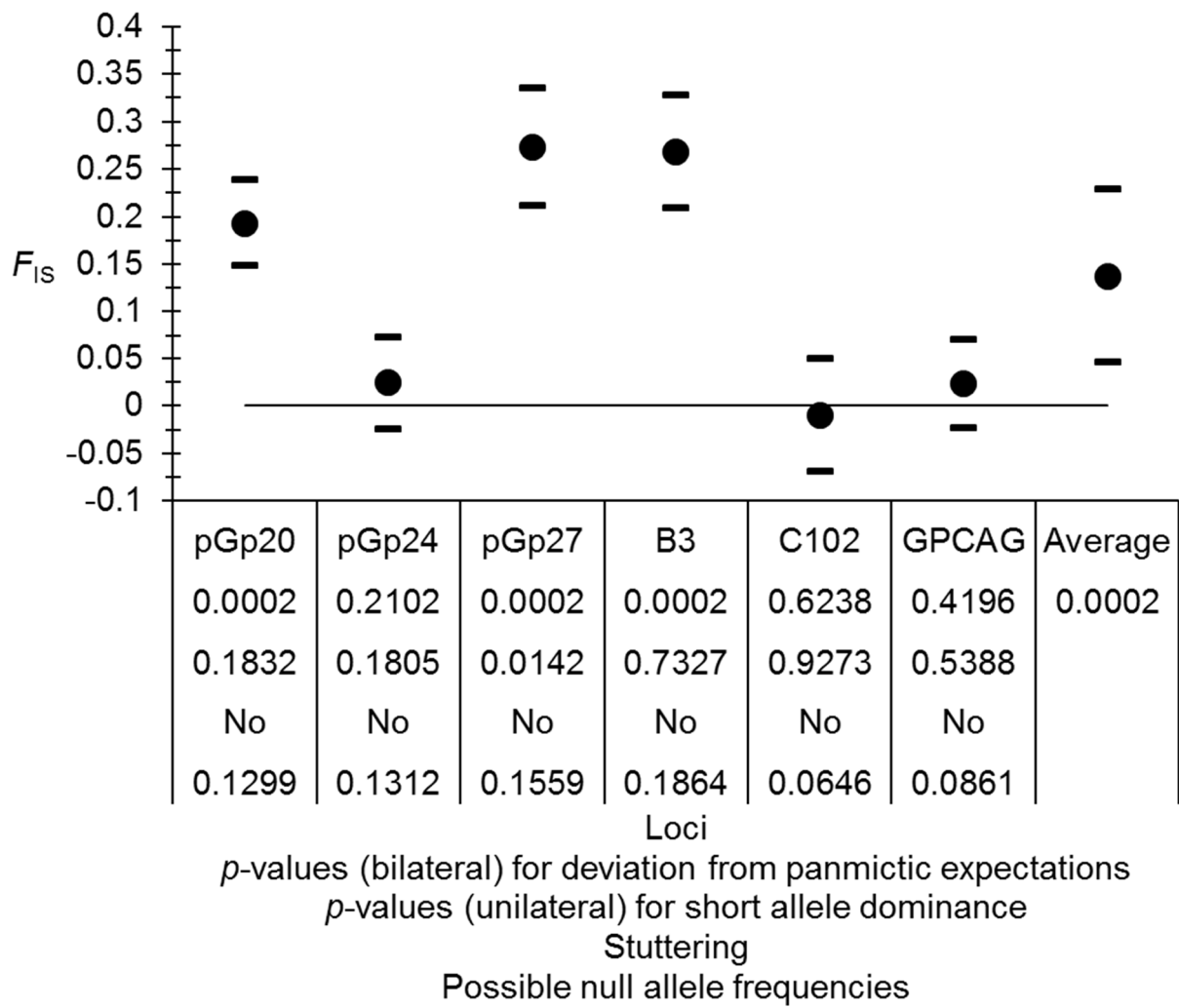
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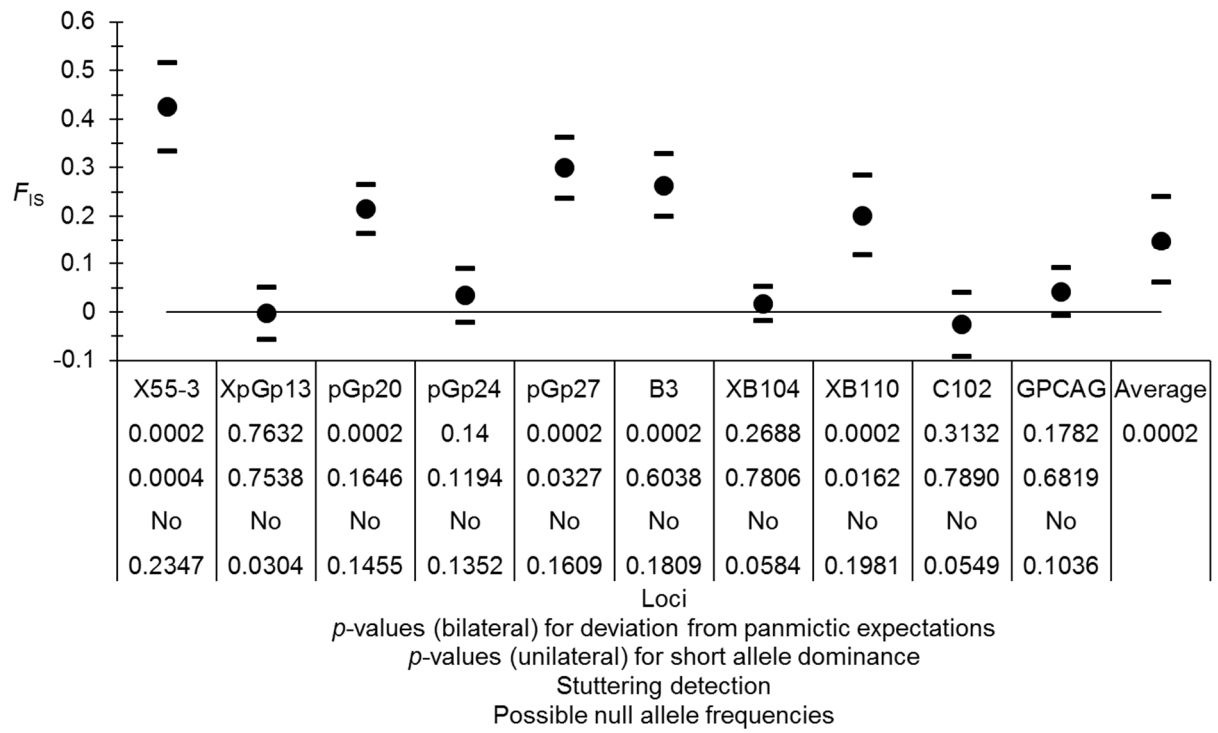
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Figure 3



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Figure 4



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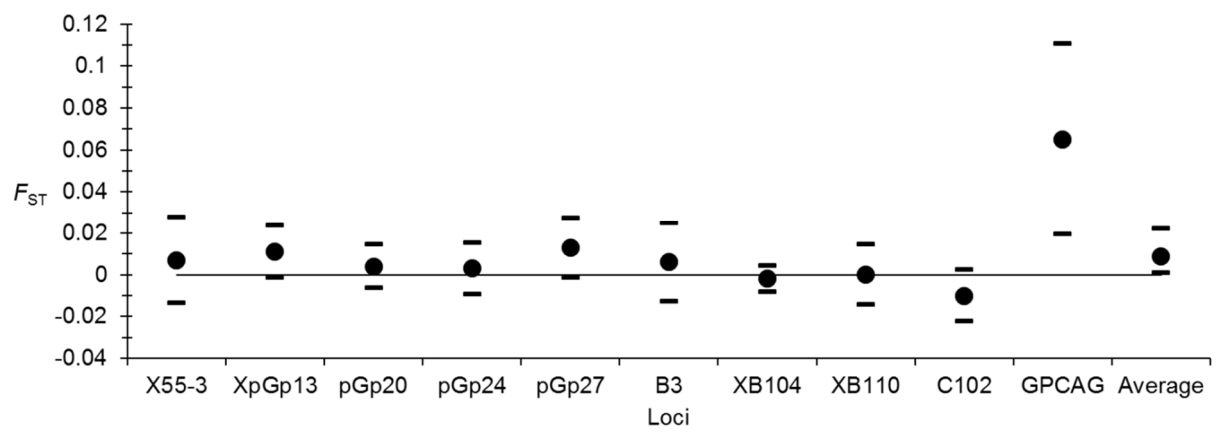
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Figure 5



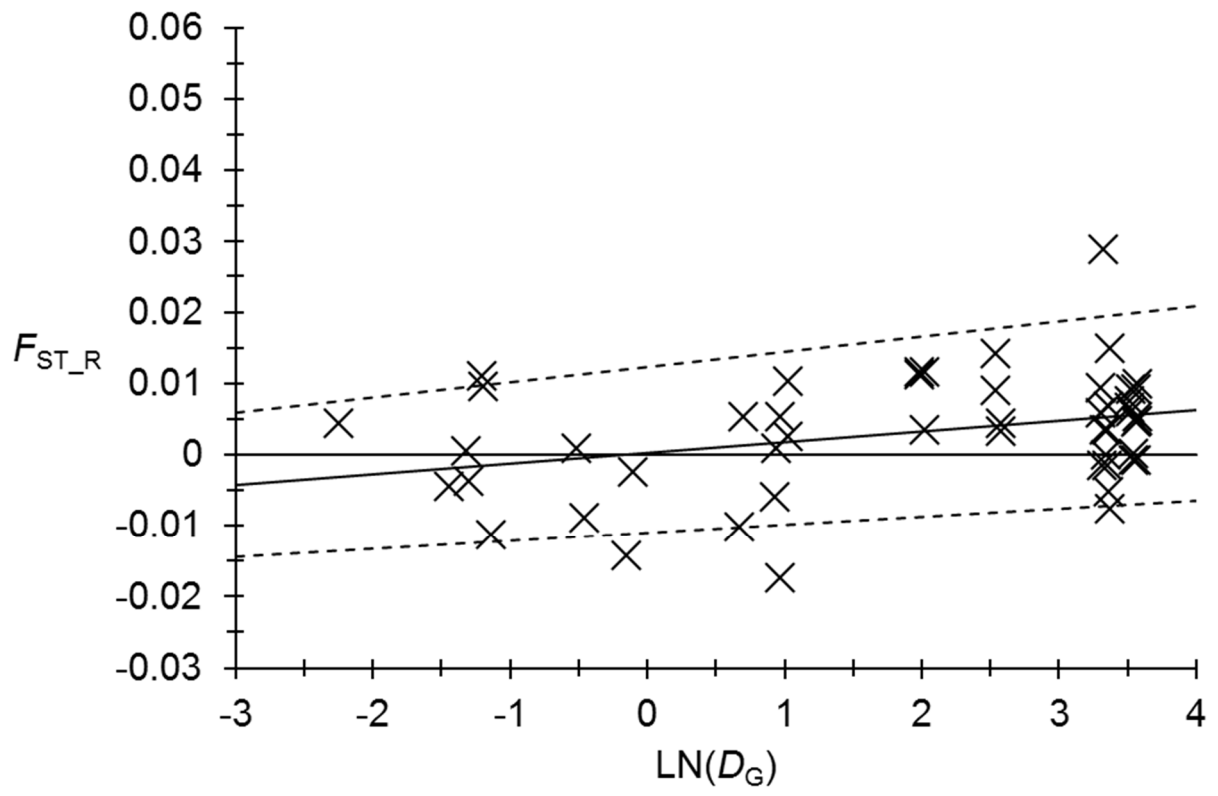
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Figure 6



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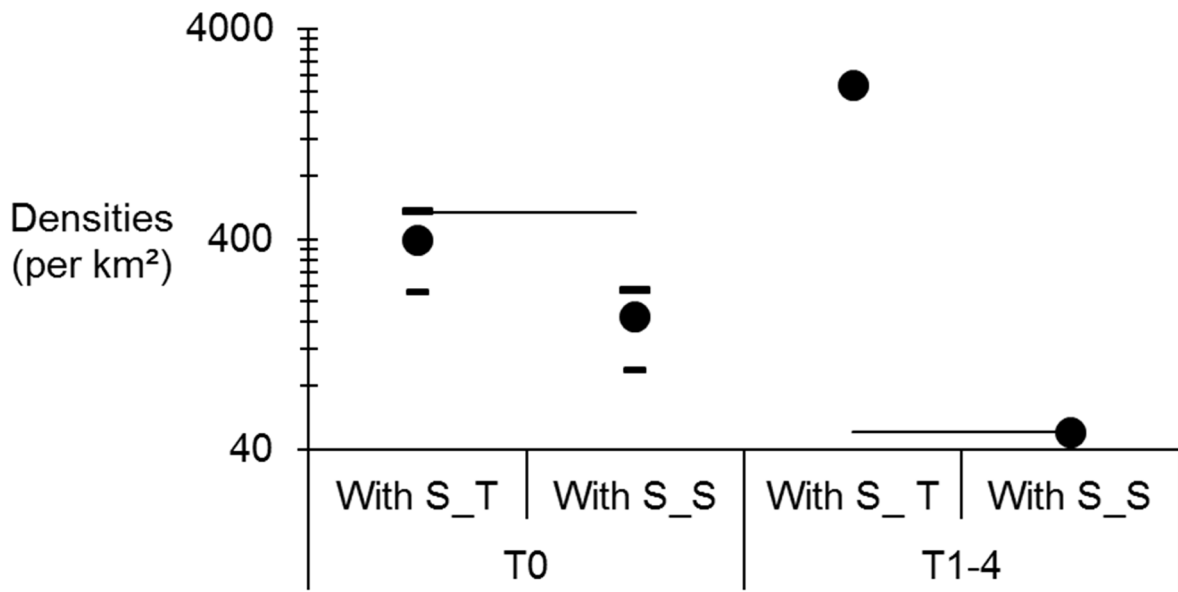
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Figure 7

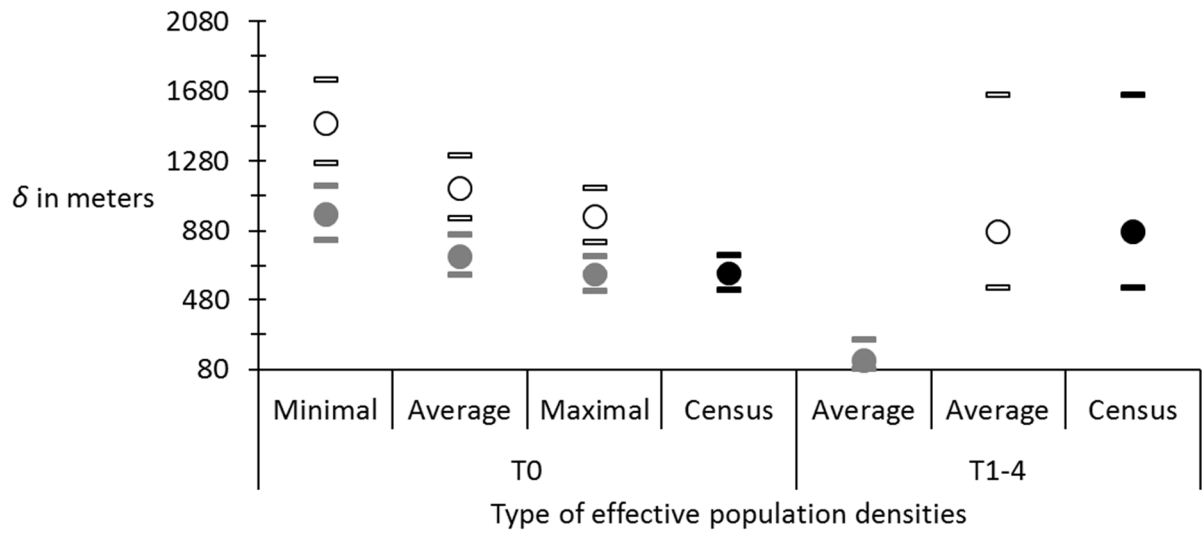


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Figure 8



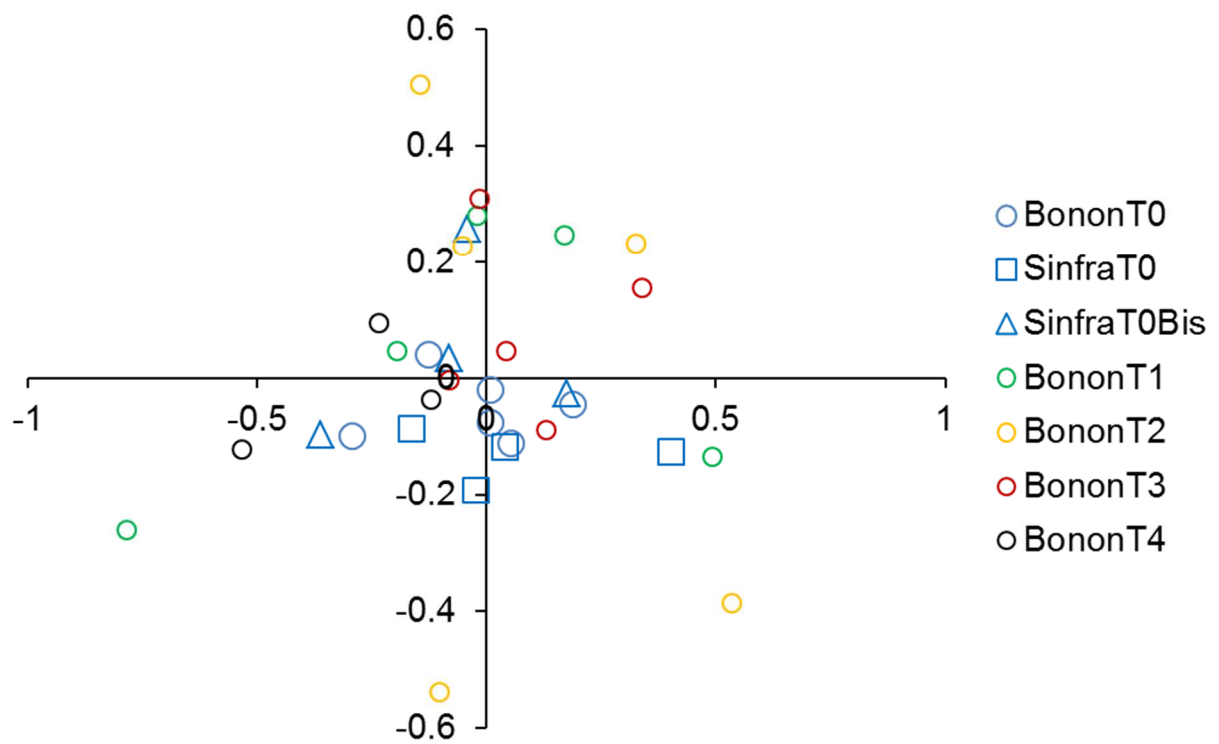
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Figure 9



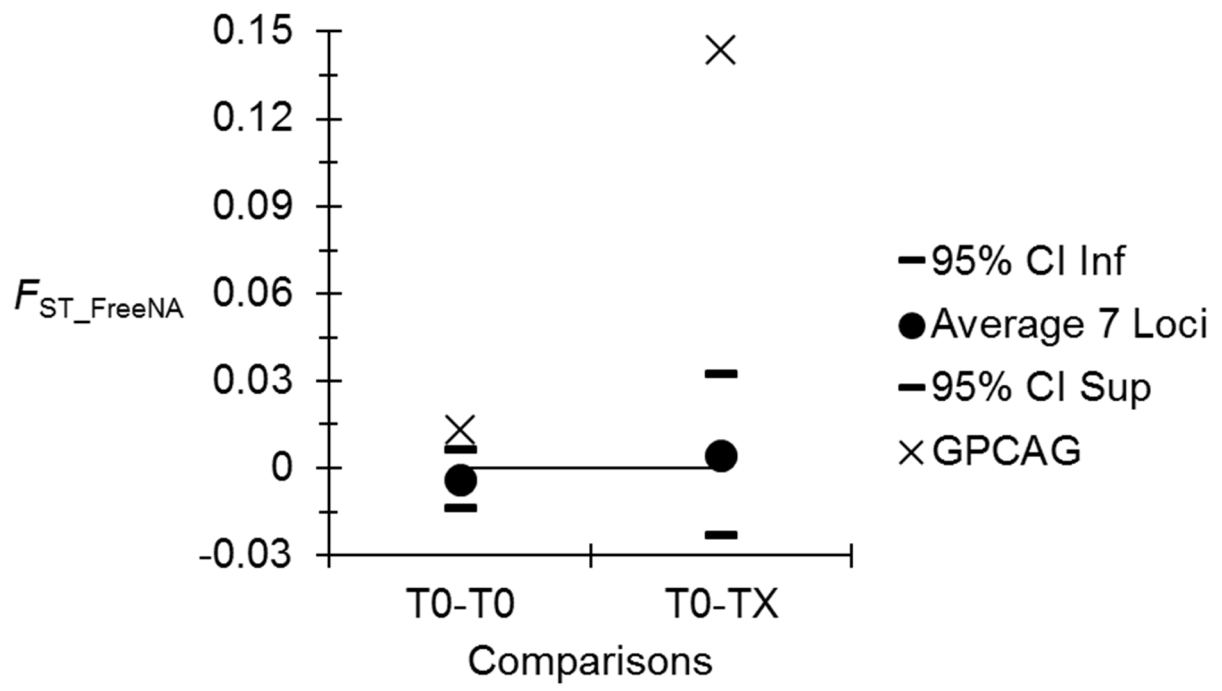
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Figure 10

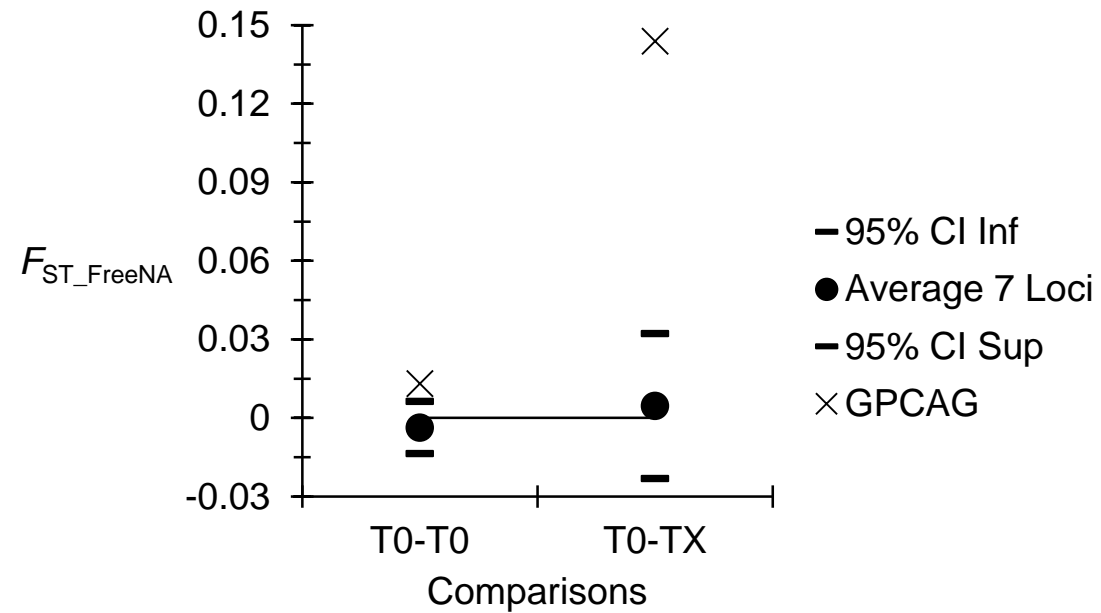


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Population genetics subdivision measure (F_{ST_FreeNA}) only varies in variance (95% CI Inf and Sup) before and after the control campaign (using tiny targets) for the 7 regular loci, but not their average.

For GPCAG, subdivision becomes much higher after control due to a 328% allele frequency increase of one allele at that locus