Effects of vector control on the population structure of tsetse (*Glossina fuscipes fuscipes*) in western Kenya

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Highlights

• Tsetse populations can recover even after being suppressed by over 90%.

• Island subpopulations of tsetse demonstrate intra-population fly size variability.

• Tsetse fly populations that recover after control intervention are smaller in size.

• Over time size and wing shape of tsetse changes.

• Tsetse control intervention could influence changes in wing shape.

Abstract

Displacement rates of tsetse affect performance of targets during vector control. Fly size, one of the indicators of population structure usually obtained from wing measurement, is among the determinants of displacement rates. Although recovery of tsetse in previous intervention areas has been widely reported, the population structure of tsetse that recover is rarely evaluated despite being associated with displacements rates. Previously, intervention trials had reduced tsetse densities by over 90% from >3 flies/trap/day to <1fly/trap/day on Big Chamaunga and Manga islands of Lake Victoria in western Kenya. In this study, we assessed the recovery in densities of *Glossina fuscipes fuscipes* on the two islands and evaluated the effects vector control might have on the population structure. A before and after intervention study was undertaken on four islands of Lake Victoria in western Kenya; Small and Big Chamaunga, Manga and Rusinga Islands, two of which tsetse control intervention had previously been undertaken. Three years after intervention average *G. f. fuscipes* catches in biconical traps were estimated on each island. Wing centroid size (CS) (a measurement of fly size) and shape, indicators of the population structure of flies from the four islands were compared using geometric morphometric analyses. CS and shape of available female but not male tsetse wings obtained before the intervention trial on Big and Small Chamaunga islands were compared with those from the same islands after the intervention trial. *G. f. fuscipes* apparent density on the previous intervention islands were >9 flies/trap/day. Irrespective of sex, wing shape did not isolate tsetse based on their islands of origin. The fly size from Big and Small Chamaunga did not differ significantly before intervention trials (P=0.728). However, three years after the intervention flies from Big Chamaunga were significantly smaller than those from Small Chamaunga (P<0.003). Further, there was an increase in the divergence of wing morphology between flies collected from Big Chamaunga and those from Small Chamaunga after tsetse control. In conclusion, even though populations are not

isolated, vector control could influence the population structure of tsetse by exerting size and wing morphology differential selection pressures. Therefore, we recommend further studies to understand the mechanism behind this as it may guide future vector control strategies.

Key words: Displacement rates; apparent tsetse densities; Recovery; Fly size; Centroid size; Wing shape; Geometric morphometrics

1. Introduction

Tsetse flies (*Glossina* species) are important cyclical vectors of protozoan parasites, trypanosomes which cause animal and human African trypanosomiasis (Vreysen et al., 2013). Animal African trypanosomiasis (AAT) is mainly caused by *Trypanosoma brucei brucei, T. congolense* and *T. vivax* with 50 to 70 million cattle at risk (Geerts et al., 2001). Direct and indirect losses due to AAT in the agricultural sector are estimated at about USD 4.75 billion in sub Saharan Africa (Budd, 1999; Vreysen et al., 2013). On the other hand, human African trypanosomiasis (HAT) is caused by *T. brucei rhodesiense* in eastern and southern Africa and *T. brucei gambiense* in central and western Africa (Holmes, 2013). Whereas Rhodesian HAT (rHAT) is acute and usually causes death within weeks, Gambian HAT (gHAT) is chronic and infections can last as long as 29 years (Sudarshi et al., 2014; Welburn et al., 2015). In fact, it has been suggested that the chronic carriers harbouring low levels of *T. brucei gambiense* which is undetectable by conventional diagnostic techniques are the ones who sustain gHAT foci (Checchi et al., 2008). HAT has an impact of 1.59 million disability adjusted life years (DALYs) with about 8.5 and 55.1 million people at risk of rHAT and gHAT (Esterhuizen et al., 2011; WHO, 2015).

Tsetse flies are distinguished into three taxonomic groups based on their habitat, host preference and morphology of the external genitalia (Vreysen et al., 2013). These taxonomic groups include morsitans, palpalis and fusca. Of the three taxonomic groups, palpalis and

morsitans are of economic importance as they transmit most of the cases of AAT and HAT (Omolo et al., 2009; Tirados et al., 2011; Vreysen et al., 2013). In the palpalis group, *Glossina fuscipes* subspecies which include but not limited to *G. f. fuscipes* and *G. f. quanzensis* are responsible for transmission of over 90% of HAT cases while, *G. palpalis* subspecies and *G. tachinoides* mainly transmit AAT in central and western Africa (Omolo et al., 2009; Tirados et al., 2015, 2011). From the morsitans group, *G. morsitans* subspecies and *G. pallidipes* are responsible for transmission of both AAT and HAT in eastern and southern Africa (Vreysen et al., 2013).

Tsetse flies from the palpalis group (subgenus Nemorhina), are associated with riverine habitat and wetlands as well as lowland rain forest (Vreysen et al., 2013). Species within the palpalis group are opportunistic feeders and have shown flexibility by tolerating high degree of disturbance in landscape (Van den Bossche et al., 2010; Vreysen et al., 2013). Among *G. fuscipes* subspecies, *G. f. fuscipes* are the most widely distributed with ranges spanning from northern Democratic Republic of Congo, DRC, and its neighbouring countries extending through to the eastern shore of Lake Victoria (FAO, 1992a; Tirados et al., 2011). However, insect species do not generally inhabit their geographic space in a uniform manner but strategically arrange themselves according to needs such as, reproductive, dispersion, availability of food resources, adaptation to local conditions and survival to treatments which may give rise to structuring in populations occupying same or separate geographical space (Dujardin, 1998; Getahun et al., 2014; Lorenz et al., 2017; Schofield et al., 1999). This structuring could result in subpopulations with phenotypic and genetic variation (Getahun et al., 2014). In medical entomology, it is important to quantify existing exchange of individuals among subpopulations and to give information on the population isolation status and structure as these may have consequences on epidemiology and control of vector borne diseases (Dujardin, 2008). Thus, the use of a fast and low cost tool of morphometrics is

critical in population structure studies. Morphometrics, defined as "an interwoven set of largely statistical procedures for analysing variability in size and shape of organs and organisms" focuses on variation, its description in terms of parameters and relation to extrinsic factors of organs and organisms under study (Cardillo and Reyment, 2010). Previous studies on population structures of tsetse have shown a strong correlation between morphometrics results with methods that are based on genetics and cuticular hydrocarbons (Bouyer et al., 2007b; Camara et al., 2006; Getahun et al., 2014; Kaba et al., 2012).

Several methods of managing African trypanosomiasis exist. These include screening and curative treatments in humans and prophylactic and curative treatments with trypanocidal drugs in animals (Vreysen et al., 2013). Other methods include promotion of trypanotolerant cattle and suppression or eradication of the vector, the tsetse fly (Vreysen et al., 2013). However, controlling the vector is considered the most desirable way of managing African trypanosomiasis (Bouyer et al., 2010; Leak, 1998; Vreysen et al., 2013) but, in the absence of area-wide control interventions covering biologically relevant areas and targeting isolated tsetse populations, re-invasion is commonly reported (Bouyer et al., 2007b; Kaba et al., 2012; Schofield and Kabayo, 2008). Some vector control techniques such as the use of targets exploit the host seeking behaviour which to a larger extent depends on the displacement rates of the tsetse fly (Vale et al., 2014). Among the factors that influence displacement rates is fly size, with the displacement potential increasing as fly size increases (Vale et al., 2014). Fly size is one of the indicators of tsetse population structures and can be obtained from wing measurement (Kaba et al., 2012; Solano et al., 1999). Inter-species variation in tsetse fly size has been associated with differences in displacement rates, responses to attractive and repellent odours, availability to tiny or large targets, persistence and landing responses (Torr et al., 2011; Vale, 1974; Vale et al., 2014, 1984). Interestingly, the fly size of tsetse populations that recover in previously controlled/suppressed areas are rarely reported.

Environmental conditions such as temperature in a living organism's habitat have a direct effect on its size (Dujardin, 2008). However, size in insects has shown high heritability values and can be selected for experimentally to produce subpopulations that are genetically distinct for size an indication that it could have trans-generational effects (Anderson, 1973; Dujardin, 2008; Lehmann et al., 2006; Partridge et al., 1994).

Wing shape, another indicator of population structure, is a more stable trait than size and less influenced by environmental changes (Dujardin, 2008; Lorenz et al., 2017). It is strongly determined by genes and is a polygenic trait (Breuker et al., 2006; Dujardin, 2008; Klingenberg and Leamy, 2001; Patterson and Klingenberg, 2007).

In Western Kenya on some islands of Lake Victoria, where *G. f. fuscipes* thrives along the shores of the lake, densities of the flies were reduced drastically by over 90% on the islands of Big Chamaunga (June 2011 to December 2012) and Manga (January 2012 to October 2013) from 3.9 and 28.2 flies/trap/day to <0.1 and <1 fly/trap/day respectively during a tsetse control intervention trial using tiny targets (Tirados et al., 2015). The tiny targets were deployed at densities of 20/km on Big Chamaunga and 10/km on Manga. Therefore, in this study, which was carried out three years later, we assessed the recovery of fly densities on Big Chamaunga and Manga Islands. We also evaluated the impact of vector control on the population structure of *G. f. fuscipes* using wing geometric morphometrics.

2. Materials and Methods

2.1 Study area

G. f. fuscipes were captured from the following Islands on Lake Victoria in western Kenya: Small Chamaunga (surface area of about 0.2 km^2), Big Chamaunga (surface area of about 0.2 km^2), Manga (surface area of about 1 km^2) and Rusinga (surface area of about 43 km^2)

(Figure 1) (Mwangelwa, 1990; Tirados et al., 2015). These Islands were selected as study sites based on the fact that there was both anecdotal and documented evidence on studies previously undertaken on *G. f. fuscipes* (Mohamed-Ahmed and Odulaja, 1997; Mwangelwa, 1990; Omolo et al., 2009; Tirados et al., 2015) . The islands' vegetation consists of a mixture of *Aeschynomene eraphroxylon* (freshwater mangrove), *Dombeya spp.* and *Lantana camara* (Tirados et al., 2015).Whereas Manga and Rusinga Islands are inhabited by humans, Big and Small Chamaunga Islands are not. Tsetse fly populations mainly take their blood meals from *Varanus niloticus* (monitor lizard) and *Hippopotamus amphibius* (common hippopotamus) but can also feed on cattle and humans on the inhabited Islands (Tirados et al., 2015).

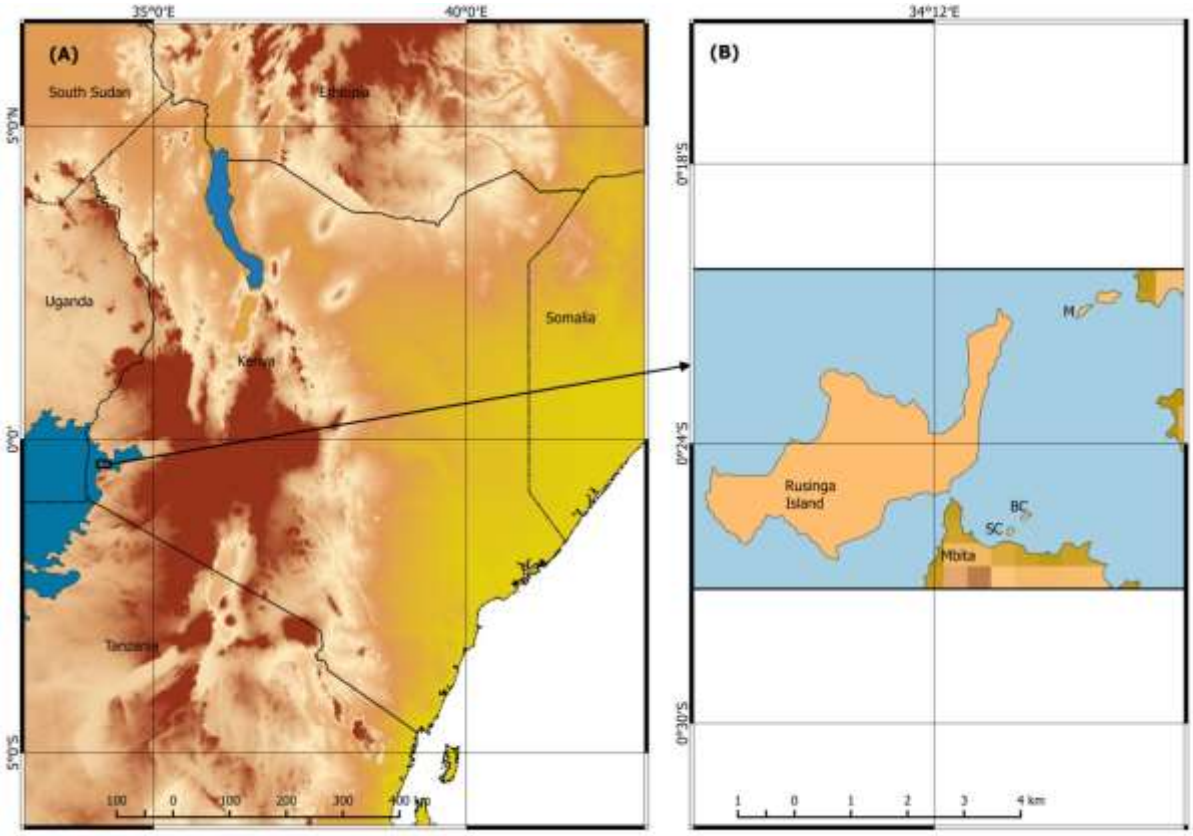

Fig 1: (A) Study area in western Kenya. (B) Islands on Lake Victoria where the study was undertaken: BC is Big Chamaunga; SC is Small Chamaunga; and M is Manga.

2.2 Study design, sample collection and wing preparation

A before and after intervention study design (Goldenhar et al., 2001) was undertaken on Big Chamaunga, Manga , Rusinga and Small Chamaunga islands. Between June 2011 to December 2012, during the tsetse control intervention using tiny targets Small Chamaunga served as the control island (non-intervention) for Big Chamaunga (Tirados et al., 2015). Un-baited biconical traps (Challier and Laveissiere, 1973) were used to catch tsetse for the periods before and after the intervention. For the period before the tsetse control intervention using tiny targets, we used samples collected between April 2010 and May 2011 (Tirados et al., 2015). From those only female wings of *G. f. fuscipes* caught on Big Chamaunga and Small Chamaunga were available. Three years after tsetse control intervention male and female flies were collected for three days during the months of March and April 2016. A total of 35 biconical traps (8 on Small Chamaunga, 9 on Big Chamaunga, 6 on Manga and 12 on Rusinga) were set in *G. f. fuscipes* suitable habitat within a meter from the lake shore at minimum and maximum distances of about 50 and 4000 meters apart respectively on the four islands. Flies collected were sorted and thereafter sexed according to the island they were collected on. All fly wings collected before and after intervention that were intact and had the 8 landmarks of interest (Figure 2) for morphometric measurements were selected. The wings were mounted between microscope glass slides. To avoid asymmetry bias (Rohlf and Slice, 1990) only one side of the pair of wings was mounted.

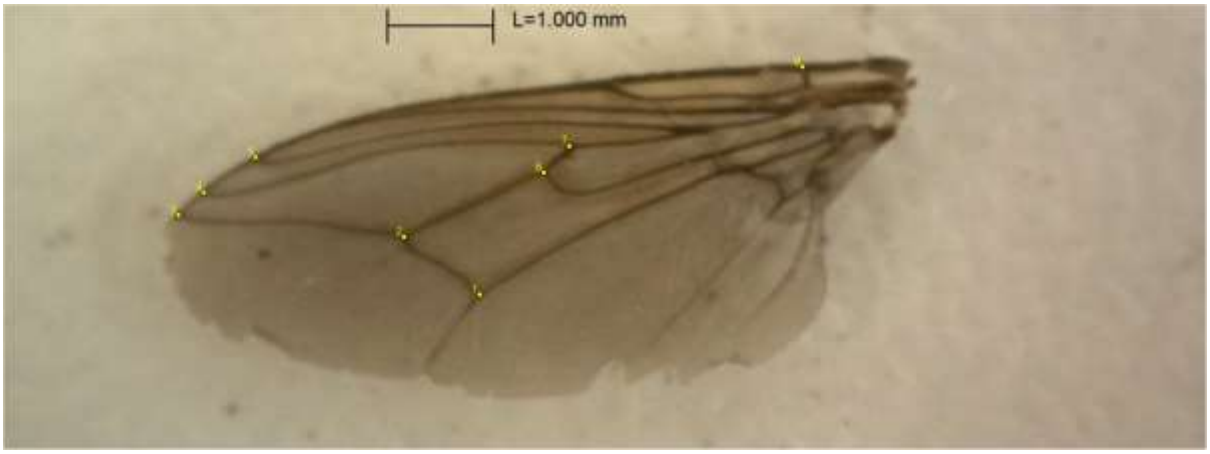

Fig 2: Eight landmarks and order of their collection from male and female right wings.

Table 1: Number of *G. f. fuscipes* wings used from island and time of collection

Island	**Before intervention**		**After intervention**	
	No. Female	**No. Males**	**No. Female**	**No. Males**
Big Chamaunga	89	N/A	162	226
Manga	N/A	N/A	126	151
Rusinga	N/A	N/A	317	276
Small Chamaunga	92	N/A	291	256

N/A represents not available

2.3 Wing morphometric measurements

A total of 1,986 right wings for both male and female flies (Table 1) mounted on glass slides from Big Chamaunga, Manga, Rusinga and Small Chamaunga Islands were photographed using a Dino-Lite digital microscope (AnMo Electronics Corporation, Taiwan) at a magnification of ×34, image size of 1,280 × 1,024 pixels and 96 dots per inch. Scaling of the image in pixels to millimetres was done and thereafter eight land marks defined as

junctions of wing veins were collected using the COO module of Collection of landmarks for identification and characterisation (CLIC) software (Dujardin and Slice, 2007) (Figure 2). To avoid individual bias, all measurements were taken by the same person. The data was then formatted in TET module and the wing shape (partial warps, PW) and size (centroid size, CS) (Dujardin and Slice, 2007) variables were generated in MOG module of CLIC software (Dujardin, 2008).

2.4 Statistical analyses

Daily tsetse catches (n) from biconical traps were normalised using a $\log_{10}(n+1)$ transformation and detransformed apparent densities were reported. A negative binomial regression was performed to measure any associations between fly catches and the status of human habitation on the Islands. A test for association between sex of flies with Islands where they were caught from and human habitation status were performed using Fisher's exact test. Differences between the overall proportion of male and female flies were tested using a Student's t-test. Analysis of variance (ANOVA) and Bonferroni tests were used for multiple comparisons of mean CS of groups (according to the islands) for each sex. A multiple linear regression was used to model CS for females collected on Big and Small Chamaunga with tsetse control status as an explanatory variable while controlling for the island and time of collection of flies (either before or after control). There were a total of 12 PW as shape variables and the principal components of these (relative warps, RW) were used as input for discriminant analysis of the groups of flies from the four islands. A cross validation procedure was undertaken to determine the success of discriminant analysis in assigning specimen to groups whereby each individual after being omitted from the initial calculation of the discriminant factors and introduced as supplementary data. CS variation was regressed against the first two discriminant functions to estimate its contribution to their variation (Bouyer et al., 2007b). The residue allometry was approximated by a multivariate

regression with CS and PW as the independent and dependent variables respectively. For this, statistical significance was estimated by 1,000 permutations (Bouyer et al., 2007b; Good, 2000). Procrustes distance matrix was used to build a neighbour joining tree in order to illustrate divergence of wing shape among the group of flies from the islands. Statistical software used for analyses were R (R Core Team, 2016) ,PAD and COV modules of CLIC (Dujardin and Slice, 2007). *P* values of < 0.05 were considered statistically significant.

3. Results

3.1 Fly densities and sex structure after intervention

A total of 3,367 flies were caught of which 1,599 were males and 1,768 females from 35 trapping sites. The overall apparent fly densities (number of flies/trap/day) on the islands were as follows: Big Chamaunga 9.2 (95%CI:8.4-9.9); Manga 22.7 (95%CI: 22.1-23.3); Rusinga 25.6 (95%CI:25.3-25.8) and Small Chamaunga 24.8 (95%CI: 24.3-25.2).The highest apparent fly density was recorded on Small Chamaunga for both males (12.7; 95%CI: 12.3-13.1) and females (13.6; 95%CI: 13.1-14.1) while Big Chamaunga recorded the lowest for both sexes (Figure 3). Overall the total apparent fly density on Big Chamaunga was significantly different from those of the flies on Small Chamaunga, Manga and Rusinga islands (ANOVA, $df_{104,}$ F=5.94, *P*<0.001). There was no significant difference in fly catches between human un-habited and human inhabited islands (Catch index=1.1; 95%CI: 0.8 -1.5; *P*>0.05). The overall proportion of females (52.5%; 95%CI: 50.8-54.2%) was significantly higher than those of males (47.5%; 95%CI: 45.8-49.2%) with *P*< 0.01. Higher male catches were only observed on Big Chamaunga Island (Table 2). A test of association using Fisher's exact test showed a statistically significant association between sex of the flies and the islands (*P* < 0.05). However, when sex and human habitation status were tested using Fischer's exact test, no significant associations were observed (*P*>0.05).

Table 2: Proportions by sex of *G. f. fuscipes* and its association with island

Island	n	Number of males (%;95%CI)	Number of females (%;95%CI)
Small Chamaunga	882	402 (45.6; 42.3-48.9)	480 (54.4; (51.1-57.7)
Big Chamaunga	615	324 (52.7; 48.7-56.6)	291 (47.3; 43.4-51.3)
Manga	530	261 (49.2; 45.0-53.5)	269 (50.8; 46.5-55.0)
Rusinga	1340	612 (45.7; 43.0-48.3)	728 (54.3; 51.7-57.0)
Total	N= 3367	1599 (47.5; 45.8-49.2)	1768 (52.5; 50.8-54.2)
P-value	0.016		

CI: Confidence interval

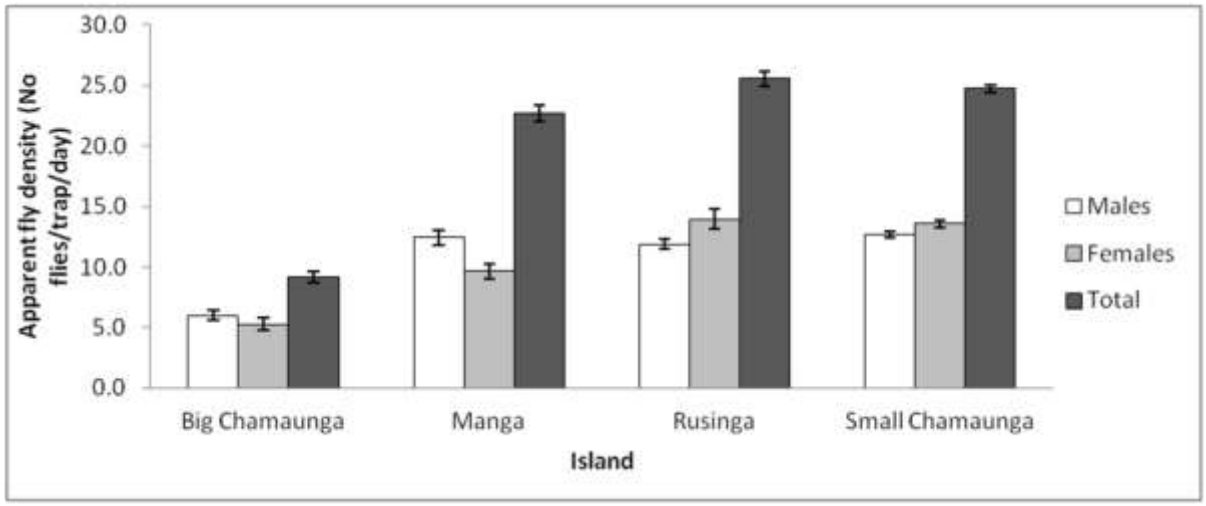

Fig 3: Detransformed apparent *G. f. fuscipes* density on Big Chamaunga, Manga, Rusinga and Small Chamaunga Islands.

3.2 Wing morphometrics before and after intervention

Male tsetse wings from Big and Small Chamaunga for the period before tsetse control intervention were not available for comparison with those collected during the period after intervention because only female wings for that period were preserved. Before tsetse control

intervention, there was no significant difference (P>0.003 after Bonferroni correction) between mean CS of female tsetse flies collected from Big and Small Chamaunga (Figure 4; Table 3). However, 3 years after tsetse fly control intervention on Big Chamaunga, the mean CS for female flies significantly differed between Big and Small Chamaunga with P<0.003 after Bonferroni correction. CS data for female flies caught on Big and Small Chamaunga Island before and after tsetse control were combined and subjected to a multiple linear regression. The results showed that tsetse control intervention significantly lowered CS of females by an average of 0.07mm (95% CI: 0.02 – 0.12mm; P<0.01) while accounting for island and time of collection (either before or after intervention) of the flies. As an explanatory variable, time of collection whether before or after intervention significantly affected CS with female flies collected after intervention having CS which were smaller by 0.06mm (95%CI: 0.02 –0.09mm; P<0.001). Island of collection (Big or Small Chamaunga) did not have any significant effect on CS (P>0.05).

With regard to the four islands the CS of female and male flies caught after tsetse control intervention (in March to April 2016)significantly varied according to the island (ANOVA, $df_{905,}$ F=18.96, P<0.001 and $df_{892,}$ F=23.7, P<0.001 respectively). Males were smaller than females (Figure 4). The mean CS for males from Big Chamaunga and Manga were significantly smaller than those from Small Chamaunga and Rusinga (P<0.008 after Bonferroni correction (Table 3) while that for females from Big Chamaunga were significantly smaller than those from Manga, Rusinga and Small Chamaunga (P<0.003after Bonferroni correction ;Table 3). The CS of female flies collected on Rusinga island and those collected from Big and Small Chamaunga before control were not significantly different (P>0.003 after Bonferroni correction).

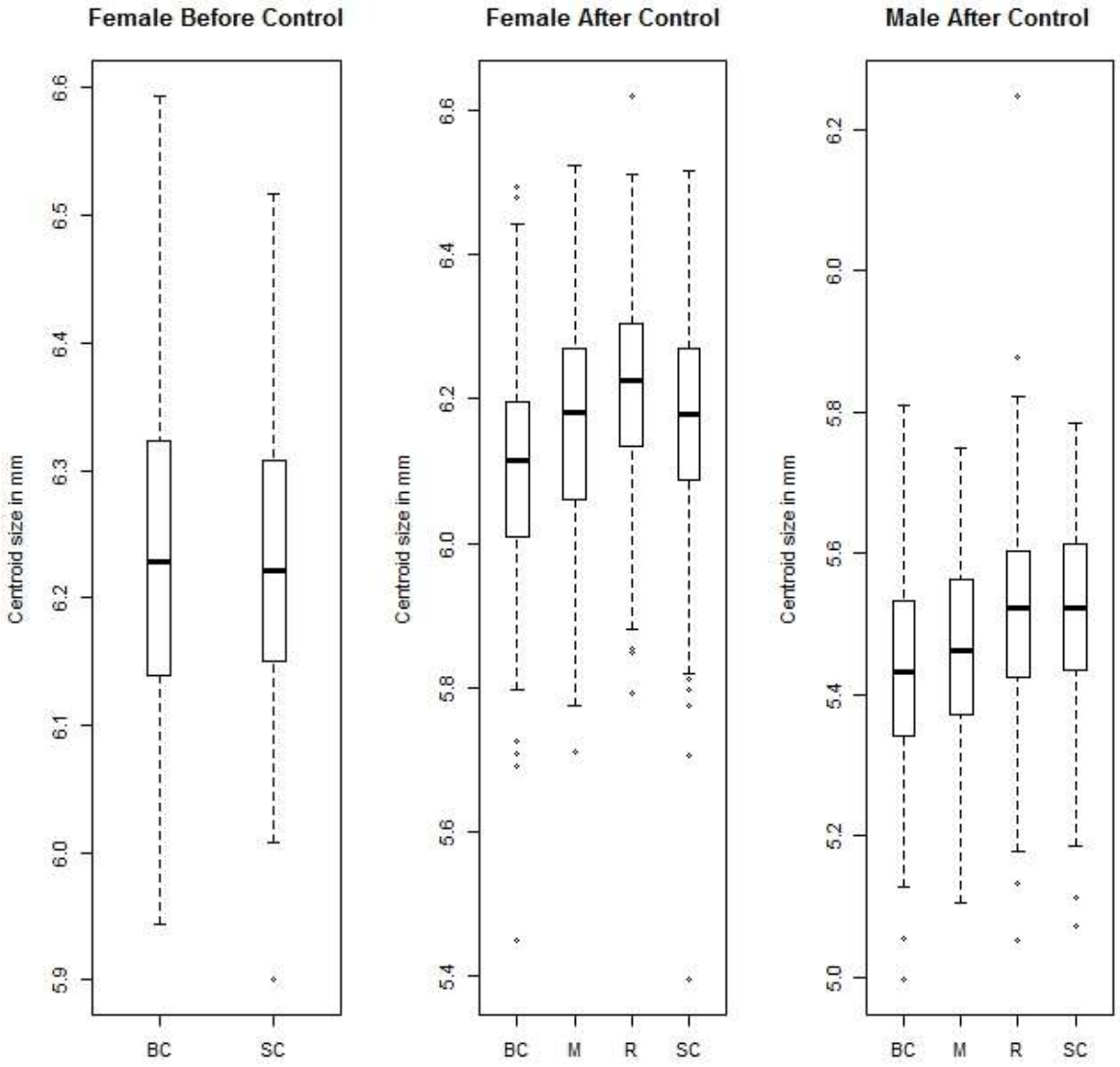

Fig 4: Wing centroid size distribution of *G. f. fuscipes* by location. BC, SC, M and R, stand for Big Chamaunga, Small Chamaunga, Manga, and Rusinga islands respectively. The boxes indicate the 25th and 75th percentiles; the solid line in the box shows the median while the capped bars are the 10th and 90th percentiles; data points outside these limits are shown as circles.

Table 3: Wing size comparison between groups of male and female *G. f. fuscipes* from Small Chamaunga, Big Chamaunga, Manga and Rusinga Islands

Group 1	Group 2	Absolute difference in mean CS between groups (P-value)	
		Female	Male
BC	M	0.06 (**0.000**)	0.03(0.02)
BC	R	0.12 (**0.000**)	0.08 (**0.000**)
BC	SC	0.07 **(0.000)**	0.08(**0.000**)
BC	BCB	0.13 **(0.000)**	N/A
BC	SCB	0.13 (**0.000**)	N/A
M	R	0.06 **(0.000)**	0.05 **(0.001)**
M	SC	0.01 (0.728)	0.05 **(0.000)**
M	BCB	0.07 **(0.000)**	N/A
M	SCB	0.06 **(0.002)**	N/A
R	SC	0.05 **(0.000)**	0.00 (0.849)
R	BCB	0.02 (0.305)	N/A
R	SCB	0.01 **(**0.538)	N/A
SC	BCB	0.07 **(0.000)**	N/A
SC	SCB	0.06 **(0.000)**	N/A
BCB	SCB	0.01 (0.728)	N/A

CS denotes centroid size. All *P*-values <0.003 and <0.008 for females and males respectively are significant (in bold) after Bonferroni correction. N/A= Not applicable, BC=Big Chamaunga, M= Manga, R=Rusinga, SC=Small Chamaunga, BCB= Big Chamaunga before control, SCB= Small Chamaunga before control.

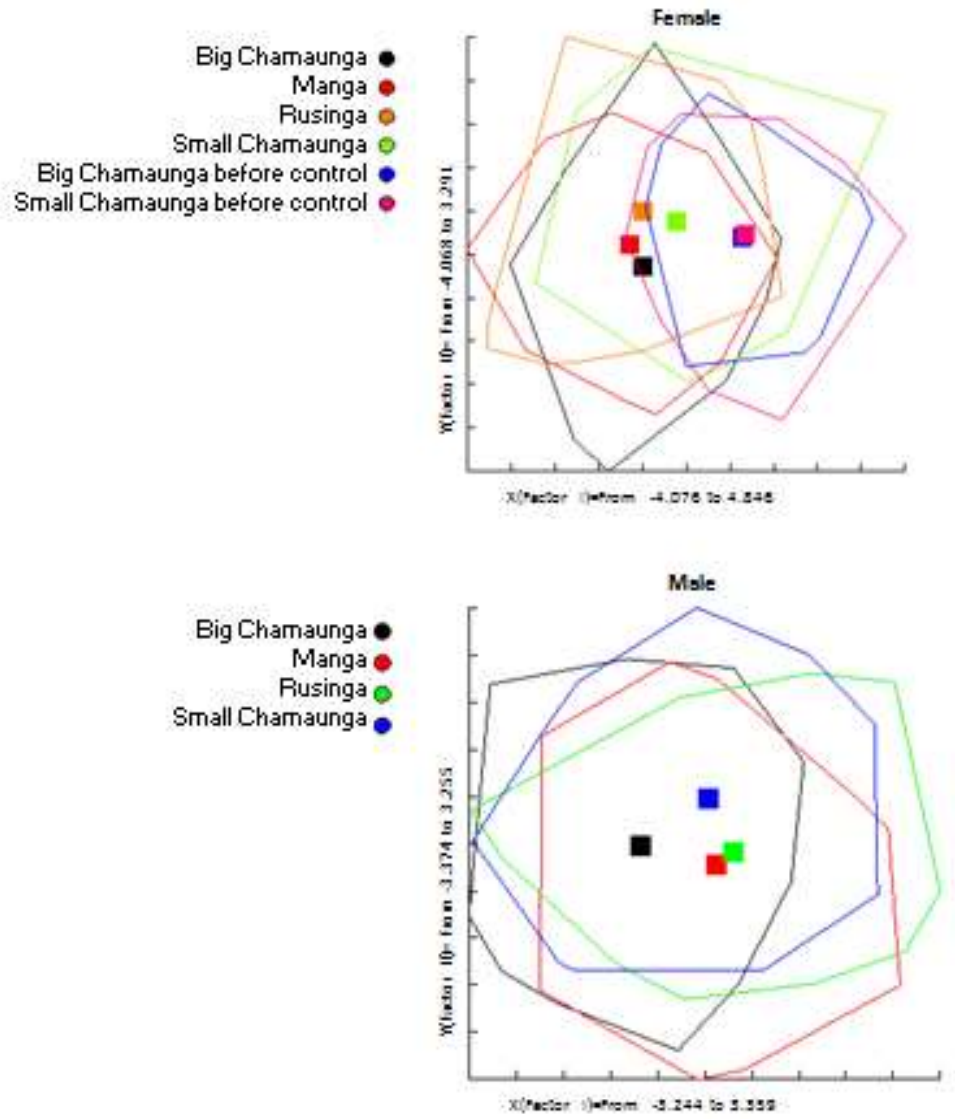

Fig 5: Plots of *G. f. fuscipes* wing shape for females and males in the morphospace. The X-axis is first discriminant factor and the Y-axis is the second discriminant factor. Both discriminant factors account for 93% and 94% of variation for females and males respectively.

Discrimination among groups (according to island) in the morphospace defined by the first two discriminant functions derived from the shape variables were projected without evidence of separation irrespective of sex and whether the flies where collected before or after tsetse control intervention (Figure 5). CS for female flies contributed 1.3% and 6.3% to the variation of the first and second discriminant factors while for males it contributed 6.2% and 0.1% to the first and second discriminant factors. The residue allometry which was estimated

by a multivariate regression of PW on CS was significant for both females and males (P<0.001). Discriminant analysis based on Mahalanobis distance resulted in assignment of *G. f. fuscipes* ranging from 28% to 42% for females and 32% to 52% for males (Table 4).

Table 4: Individual reclassification rates based on the wing shape

Island	**Before intervention**		**After intervention**	
	Female %	**Males %**	**Female %**	**Males %**
Big Chamaunga	35	N/A	38	52
Manga	N/A	N/A	38	33
Rusinga	N/A	N/A	35	32
Small Chamaunga	42	N/A	28	50

N/A represents not available

Neighbour joining tree derived from Procrustes distances analysis produced two clusters representing female populations collected before and after tsetse control intervention on Big Chamaunga (Figure 6). The wing morphology of female populations collected from Big and Small Chamaunga Islands before the tsetse control intervention were less divergent than after tsetse suppression on Big Chamaunga (Figure 6). Actually the female population from Manga, another island where tsetse control intervention was undertaken were grouped with those from Big Chamaunga (Figure 6). The male population collected on Big Chamaunga was the most distant from those collected on Small Chamaunga, Rusinga and Manga Islands (Figure 6).

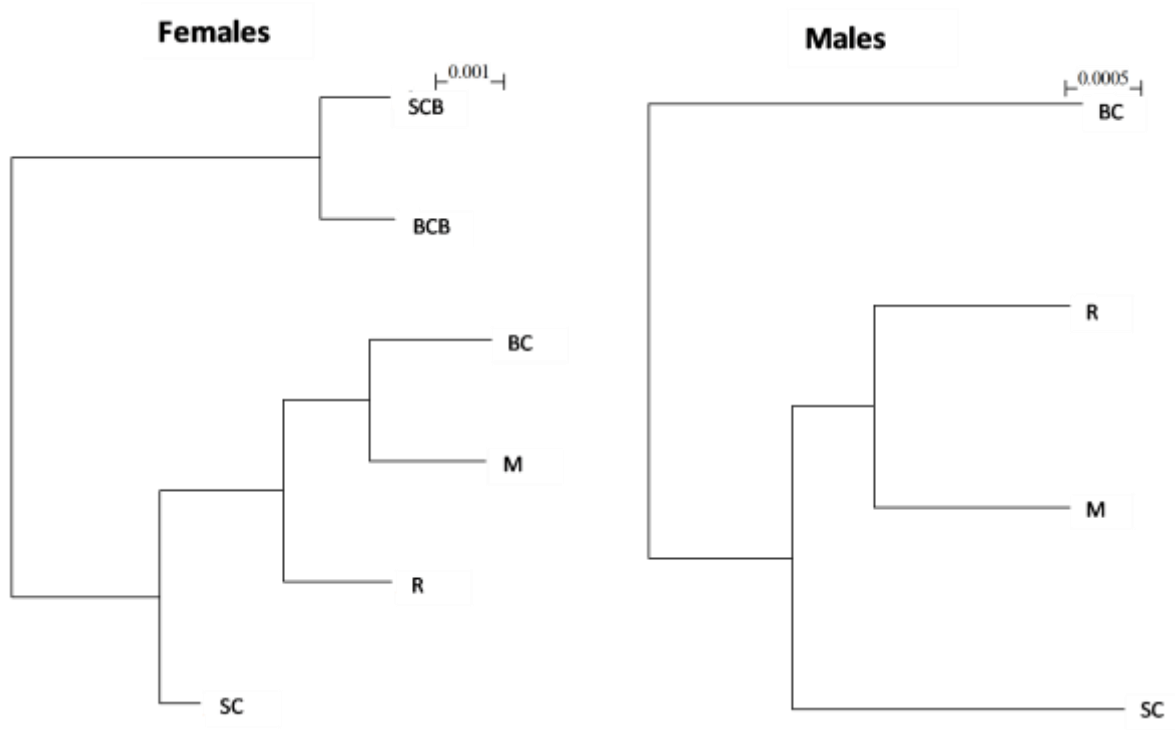

Fig 6: Phenetic trees of female and male *G. f. fuscipes* derived from Procrustes distances. BC=Big Chamaunga, M= Manga, R=Rusinga, SC=Small Chamaunga, BCB= Big Chamaunga before intervention, SCB= Small Chamaunga before intervention.

4. Discussion

This study clearly demonstrated the absence of separation of *G. f. fuscipes* groups on the four islands as evidenced from the morphospace of the first two discriminant factors which was supported by the observed reclassification rates. It also demonstrated the divergence of wing shape, an indication of population structuring of *G. f. fuscipes* on the islands, as seen from the phenetic tree derived from Procrustes distances. CS derived from wing measurement can be used as an estimate for adult insect body size (Dujardin, 2008; Lorenz et al., 2017). In tsetse, fly size is among the factors associated with displacement rates, with larger flies having a higher displacement potential than smaller flies (Vale et al., 2014). Displacement rates affect performance of targets (Vale et al., 2014). In the recent past, preliminary trials to determine the number of tiny targets required to reduce the population of *G. f. fuscipes* by more than

90% were undertaken on Big Chamaunga (2011 to 2012) and Manga (2012 to 2013) (Tirados et al., 2015). During these trials, tiny targets deployed at 20/km and 10/km reduced the apparent fly densities from 3.9 and 28.2 flies/trap/day to less than 0.1 and 1 flies/trap/day on Big Chamaunga and Manga respectively (Tirados et al., 2015). When mean fly size of the available females obtained prior to tsetse control intervention were compared, there was no significant difference in size between those from Small and Big Chamaunga. Three years after tsetse control intervention on Big Chamaunga, the flies that recovered had a significantly smaller mean size than those from Small Chamaunga. This suggests that vector control could have had an influence on the observed smaller size of tsetse that recovered after control intervention. Furthermore, the observed lack of significant difference in size of females from Rusinga Island with those from Big and Small Chamaunga before the suppression of flies seems to supports this. It is possible that with a higher density of tiny targets more of the larger tsetse whose mobility and displacement potential is higher (Vale et al., 2014, 1984) and had a higher chance of encountering the killing devices were eliminated more than the smaller flies. Killing the larger flies could have exerted an increased selection pressure for smaller flies paving way for a new generation of smaller flies once tsetse control intervention was ceased. This could have led to the observed smaller size in tsetse that recovered and were caught on Big Chamaunga compared to Small Chamaunga and the other island. Apart from density dependant factors, the lower mobility and displacement rate of smaller flies (Vale et al., 2014, 1984) and their reduced chance of encountering targets (thereby increasing their chance of survival) could be among the factors that explain why the use of targets alone as a tsetse control method has rare reports of successful elimination of tsetse populations (Meyer et al., 2016; Vreysen et al., 2013). Probably the use of targets only could achieve more successes in elimination by incorporating strategies in the tsetse control approach that also aim at killing the smaller flies that do not encounter targets. However,

despite previous control on Manga Island the size of female flies caught were not significantly different with those from Small Chamaunga Island. The probable explanation could be that a target density of 10/km on Manga did not exert a strong pressure for selection of smaller female flies compared to a density of 20/km on Big Chamaunga. Even though insect size is a reversible character and can vary due to but not limited to environmental factors, population density and diet , it has often shown high heritability estimates (Dujardin, 2008; Lehmann et al., 2006). Some studies have shown that insect size can be experimentally selected for to produce subpopulations that are genetically distinct (Anderson, 1973; Partridge et al., 1994). This can take place through a process referred to as "genetic assimilation" whereby a phenotypic trait initially expressed as a response to some environmental factor is taken over by the genotype through selection such that it is found even when the environmental factor is absent (Waddington, 1953). Through the concept of "genetic assimilation" it is asserted that phenotypic plasticity could acquire evolutionary significance (Dujardin, 2008). Thus (Dujardin, 2008) cautions against excluding the possible trans-generational effects of size. Wing shape shows strong genetic determinism and is a good indicator of population structure of insects (Bouyer et al., 2007b; Dujardin and Slice, 2007; Lorenz et al., 2017). The observed increase in divergence of wing shape (Figure 6) between female *G. f. fuscipes* population collected on Big Chamaunga and those from Small Chamaunga before and after tsetse control intervention on the former supports our assertion that vector control could lead to population structuring. Further, the phenetic tree was differentiated into two clusters clearly separating female populations collected before and after the tsetse control intervention, could be an indication of the influence of environmental elements on the population structure over time. The phenetic tree for male *G. f. fuscipes* clearly separated the population collected on Big Chamaunga from the other three islands. However, due to the unavailability of male samples for the period before tsetse suppression

intervention, it is not possible to ascertain whether vector control and environmental elements could have the same effect as it has on wing shape of the female population.

When determining the effect of tsetse suppression on size using females from Big and Small Chamaunga, our results could have been biased by the effect of environmental elements over time (before and after control) on the size. However, this confound was addressed during statistical analysis by accounting for it in the multiple linear regression. The multiple linear regression showed an increased size in the absence of tsetse control using tiny targets. Further, with only female wing samples available for the period before control using tiny targets, it is possible that our results for males could be different from those of females. Nevertheless the observed size for both female and male collected on Big Chamaunga after control were significantly smaller compared to Small Chamaunga and Rusinga islands where no tsetse suppression intervention was undertaken. This could be an indication that the factors that influence size in both female and male tsetse could be the same. However, further investigations are needed to ascertain this.

In other dipteran vectors, size has been associated with fecundity, longevity and blood volume intake, all factors that affect epidemiology of vector borne diseases (Ameneshewa and Service, 1996; Maciel de Freitas et al., 2007; Mwangangi et al., 2004; Schneider et al., 2007). With the observed intra-population variation of tsetse fly size in our study, further studies are needed to investigate how fly size could influence the epidemiology of African trypanosomiasis.

Our results indicate that, the tsetse populations on Big Chamaunga and Manga islands have recovered from the previously reported apparent densities of less than 0.1 and 1 fly/trap/ day after their suppression during trials using tiny targets to control *G. f. fuscipes* (2011 - 2012 and 2012 - 2013 respectively) (Tirados et al., 2015) to 9.2 and 22.7 flies/trap/day

respectively. Recovery could be due to suppressed population growing back to pre-suppression levels or re-invasion from neighbouring areas (Meyer et al., 2016). The significantly smaller size of both females and males collected three years after tsetse control intervention on Big Chamaunga suggests that recovery in this case could have been mainly due to the suppressed population growing back. However, on Manga the lack of significant difference in the size of female tsetse collected after intervention with those from Small Chamaunga does not rule out re-invasion occurring from neighbouring islands. The apparent fly density on Big Chamaunga was significantly lower compared to the other islands. This could be explained by the higher level of suppression of densities to <0.1 flies/ trap day on Big Chamaunga compared to <1 fly/trap/day on Manga (Tirados et al., 2015). Even so, the rate of recovery on Big Chamaunga was much higher (approximately 91.2 times) compared to that of Manga (approximately 22.7 times). Further as observed elsewhere (Meyer et al., 2016), the recovery in density of *G. f. fuscipes* even after their suppression by over 90% also brings us to the realisation that as long as flies are not completely eliminated we should be wary of the constant threat of fly populations recovering either due to re-invasion from neighbouring and/or growing back in density to pre-suppression levels in previously control areas. Additionally, the apparent fly densities on the other islands were 2.4 to 2.8 fold higher than that of Big Chamaunga. It is possible that density of vegetation and presence of specific plants such as *Lantana camara* which have been shown to attract tsetse (Syed and Guerin, 2004) and or availability of food sources could be responsible for the observed difference in apparent fly density. However, further research is recommended to ascertain this.

The lack of significant association between *G. f. fuscipes* catches and sex with human habitation status seems to support the observation by Van den Bossche et al (2010) that palpalis group tsetse species are able to tolerate high degree of disturbance in their ecological niche (Van den Bossche et al., 2010). The adaptive capacity of palpalis group tsetse species

has been attributed to their capability to utilise microclimatic niches and ability to feed on hosts they encounter first (Bouyer et al., 2007a; Van den Bossche et al., 2010; Vreysen et al., 2013). Although both sexes of tsetse emerge from pupae approximately in equal numbers, females live longer than males in their habitat (FAO, 1992b). As a result, field population of flies comprise of more females than males. This could be the probable explanation for the observed significantly higher proportion of female flies than males in our study.

5. Conclusion

The study showed that no separation of populations of *G. f. fuscipes* from Big Chamaunga, Small Chamaunga, Manga and Rusinga Islands was evident based on wing shape; vector control could induce the diminishing of fly size and divergence of wing morphology in tsetse that recover. Therefore an investigation to understand how this happens is recommended as it may guide future tsetse control strategies. Additionally we recommend further studies on the effect of fly size on the vectorial capacity of tsetse as it could give more insights into the epidemiology of African trypanosomiasis in previous intervention areas where recovery of populations has occurred. Furthermore, given the recovery of tsetse population densities on islands where their densities were previously suppressed (Big Chamaunga and Manga) we recommend sustained area wide tsetse control interventions and those that target isolated populations to prevent population recovery. We further emphasise on the need to undertake population structure studies as part of baseline for both trials and full scale vector control interventions as they may be a reference to assist in determining whether population recovery in previous intervention areas are due to re-invasion from neighbouring areas or the population growing back from suppressed to pre-suppression levels.

Consent for publication

Not applicable

Availability of data and materials

All datasets used and/or analysed during the current study are available from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Authors' contribution

NJM, RKS, BT, JI, AAY and CP conceived and designed the study. NJM collected samples and analysed the data. NJM, RKS, BT, JI, AAY and CP wrote the manuscript. All authors read and approved the final version of the manuscript.

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