Trends in Parasitology

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Opinion

Negative Density-dependent Dispersal in Tsetse Flies: A Risk for Control Campaigns?

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Tsetse flies are vectors of parasites that cause diseases responsible for significant economic losses and health issues in sub-Saharan Africa, including sleeping sickness in humans and nagana in domestic animals. Efficient vector-control campaigns require good knowledge of the demographic parameters of the targeted populations. In the last decade, population genetics emerged as a convenient way to measure population densities and dispersal in tsetse flies. Here, by revealing a strong negative density-dependent dispersal in two dimensions, we suggest that control campaigns might unleash dispersal from untreated areas. If confirmed by direct measurement of dispersal before and after control campaigns, area-wide and/or sequential treatments of neighboring sites will be necessary to prevent this issue.

Highlights

A strongly negative density-dependent dispersal was found in tsetse fly populations.

Reducing tsetse fly densities may unleash dispersal and recolonization from neighboring sites.

The long-term benefits of control campaigns may be jeopardized as a result.

Tsetse Fly Control and Population Genetics Tools

Flies of the genus *Glossina* are the vectors of trypanosomes, parasites responsible for diseases that lead to a significant disease burden and economic losses in sub-Saharan Africa. In humans, tsetse flies transmit *trypanosomiasis*, also known as sleeping sickness, which is usually fatal if the patient is not treated [1]. In animals, particularly cattle, it causes nagana, a disease known to cause considerable economic losses in sub-Saharan Africa [2,3]. Vector control has become a recognized key component in the management of these plagues [3–5]. Nevertheless, to optimize control campaigns, good knowledge of the biology of the target populations is needed, in particular their density and dispersal ability [6]. Lack of such knowledge has indeed been implicated in the failure of some elimination campaigns due to the raid reinvasion of treated populations by local flies or by flies from neighboring sites [7]. **Population genetics** (see Glossary) offers useful tools [8] in particular for tsetse flies [3].

Considerable work has been undertaken in recent years to study the population biology of different species of tsetse flies in a range of different countries with varying success in terms of population density and dispersal estimates (e.g., see [4,7,9–12]).

The best way to accurately estimate dispersal of a given population is isolation by distance. Isolation by distance is a common feature of population structure in which the genetic relatedness (or genetic distance) between individuals or subpopulations (Box 1) is a decreasing function of the geographic distance separating them (the shorter the distance between them, the more similar they are). When such genetic distances are known, an isolation by distance model can be built and its significance tested (Box 2). If it is significant, and if **effective population density** D_e can be estimated, then dispersal δ can be extracted from the model (Box 2).

In the past decade, several data sets have provided the opportunity for such inferences for different tsetse fly species in different countries in Africa: *Glossina palpalis gambiensis* along the northern reaches [13] and the southern reaches of the Mouhoun River [14] in Burkina-Faso (in 1D, Box 2); *G. palpalis palpalis* in Cameroon (2D, Box 2) [11,15]; *G. palpalis gambiensis* and

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Box 1. Measuring Genetic Distances

Several genetic distances exist that are used in isolation by distance procedures (see [37] for a more complete overview). The most popular, Wright's F_{ST} [38], is not a real genetic distance but a measure of the effect of subdivision on **inbreeding**. The parametric definition of this parameter is (e.g., [8]):

$$F_{ST} = \frac{Q_S - Q_T}{1 - Q_T} \qquad [I]$$

where Q_S is the probability of identity between two **alleles** from two individuals of the same subpopulation and Q_T is the probability of identity between two alleles from two subpopulations of the total population. Its value varies between 0 (no subdivision) and 1 (all subsamples fixed for one or the other allele present, that is, absolute subdivision). It is mainly influenced by subpopulation sizes and **immigration rate** (or dispersal). This parameter is estimated with Weir and Cockerham's unbiased estimator θ [39]. For isolation by distance situations, it has been shown that the use of $F_{ST\_R} = \theta/(1-\theta)$ is more useful because it is linearly related to geographic distances without losing its relation to other demographic parameters [40]. An equivalent measure between individuals a_r, and its unbiased estimator $\hat{a}$, was also designed by Rousset [41] as:

$$a_r = \frac{Q_w - Q_r}{1 - Q_w} \qquad [II]$$

where Q_w is the probability of identity of two genes within an individual, and Q_r is the probability of identity of genes at (geographical) distance r. Another statistic $\hat{e}$ can be used in case of very important **neighborhood** (see [23] for more details).

In other instances it may be more appropriate to use another genetic distance, for example, Cavalli-Sforza and Edward's chord distance [42]. D_{CSE} is more appropriate for tree topology design [43] and more powerful in some cases of isolation by distance testing [37]:

$$D_{CSE} = \frac{2}{r\pi} \sum_{j=1}^{r} \sqrt{2\left[\sum_{i=1}^{mj} \sqrt{x_{ij} y_{ij}}\right]} \qquad [III]$$

where r is the number of loci, j the **locus** name (from 1 to r), i the allele name (from 1 to mj), mj the number of alleles at locus j, x_{ij} and y_{ij} are the frequencies of allele i at locus j for subpopulations x and y, respectively.

G. tachinoides in Southern Burkina Faso (2D) across river basins [9]; *G. tachinoides* in Ghana (2D) [7]; *G. fuscipes fuscipes* from Uganda [12,16], in Tanzania and Kenya [17] (2D); *G. pallidipes* from Kenya Nguruman escarpment and Ruma [18] and from the Serengeti Park Reserve in Tanzania (2D) [10]. This offered the opportunity to check if any relationship existed between these different inferences of population density and dispersal. The objective of the present paper is to discuss the evidence that tsetse dispersal may be density dependent.

Regression between Dispersal and Effective Population Density

The detailed protocol for using or reanalyzing available data and computing the necessary parameters is given in File S1 in the supplemental information online. Positive density-dependent dispersal occurs when dispersal increases with density, as expected if individuals leave to escape overcrowding. Negative density-dependent dispersal happens when crowded populations can no longer accept any immigrants. To investigate evidence for density-dependent dispersal in the tsetse, we computed effective population densities as the ratio of **effective population size** (N_e) to the average surface area occupied by a population (Box 2). For the sake of homogeneity across studies, N_e was estimated using **linkage disequilibrium** methods [19–22] and the surface area occupied by a subpopulation corresponds to the surface of subsamples, when available, or the disc defined by the minimum distance between the closest subsamples taken as the diameter of a population (Box 2). As for dispersal, we extracted **slope** estimates and 95% **bootstrap** confidence intervals over loci (95% CI) (when available) to compute dispersal (δ) as explained in Box 2. Because the relationship is exponential, we log-transformed both geographic and genetic distances to perform the **regression**. We kept only data with

Glossary

Allee effect: a phenomenon in which the individual fitness (survival and/or reproduction) is positively correlated with population size or density.

Alleles: the different states of a locus or a gene (e.g., the three alleles of the ABO system for blood groups).

Bootstrap: a randomization procedure where an item series (e.g., loci) are resampled with replacement (the same item can be resampled several times) until the number of items present in the raw data is reached. At each resampling, a statistic is measured (e.g., genetic distance, see Box 1). The procedure is repeated a great number of times (e.g., 5000), which produces a distribution of possible values for the statistic. Excluding the 2.5% smallest and the 2.5% biggest values of the bootstrap distribution provides the 95% bootstrap confidence interval of the statistic.

Correlation coefficient: a measure of the covariation between two variables.

Effective population density: the ratio of effective population size to the surface area occupied by a given population.

Effective population size: quantifies the rate at which a population loses its genetic diversity. Indeed, the reciprocal of the effective size (1/Ne) gives the long-term probability that two randomly sampled genes in the population are replicates (or descend) from a single gene in the parental generation. It can be roughly defined as the number of adults in a population that will leave a genetic signature to the next generation. It is generally smaller than the census size N_c, except when coalescence is delayed due to a particular system of mating (negative assortative mating) or in very small dioecious populations. In any case, N_e and N_c must be strongly positively correlated in most, if not all, situations.

Immigration rate: the proportion of individuals in a subpopulation that come from other subpopulations of the total population.

Inbreeding: a concept describing how alleles, individuals or subpopulations can be related. Formally, it corresponds to the probability of randomly drawing two identical alleles that are identical by descent, that is, that come from a common ancestor.

Linkage disequilibrium: a measure of the statistical correlation between alleles at two or more loci. If in equilibrium, then the occurrence of alleles at two loci is simply equal to the product of

Box 2. Measuring and Testing Isolation by Distance and Parameter Inferences

Isolation by distance is measured through a regression of geographic distances (explanatory variable) D_{Geo} between individuals or subsamples and corresponding genetic distances (response variable) D_{Genet}. Inferences follow particular models of regression, depending on whether the population structure occurs in 1D or 2D [23,40,41]. 1D structures correspond to shores, ecotones or river courses, as is the case for *Glossina palpalis gambiensis* along forest galleries in savannas. 2D structures are more common, or at least more often reported. Three dimension models (dense forests or water columns for aquatic organisms) remain poorly explored. In 1D, the model is $D_{\mathrm{Genet}} = a+b\times D_{\mathrm{Geo}}$, where a is the intercept, b the slope of the regression, and D_{Genet} stands for $F_{\mathrm{ST\_R}}$ (for between subpopulations distances), $\hat{a}$ or $\hat{e}$ (between individuals). In 2D, the model is $D_{\mathrm{Genet}} = a+b\times\ln(D_{\mathrm{Geo}})$, where $\ln(D_{\mathrm{Geo}})$ is the natural logarithm of D_{Geo}. The slope b is linked to the effective population density D_e and the average squared axial parent–offspring distance $\overline{\sigma^2}$ with a neighborhood estimated as $Nb = 4D_e \times \overline{\sigma^2} = 1/b$ in 1D and $Nb = 4\pi D_e \times \overline{\sigma^2} = 1/b$ in 2D [40].

The average surface (S) occupied by a subpopulation can be computed as the surface area occupied by the different traps used in a given survey site. If only one trap is available per site, the distance between the two closest sites (D_{min}) can be taken as the raw proxy of the distance between the centers of two neighboring subpopulations and hence as their diameter: $S = \pi\times(D_{\mathrm{min}}/2)^2$. If the average effective population size N_e is computed with appropriate algorithms (e.g., see [20,44–46]), then $D_e = N_e/S$, and a rough proxy of parent–offspring average distance (dispersal) can be computed as [37]:

$$\delta \approx 2 \times \sqrt{\frac{1}{4\pi b D_e}} \qquad \text{[I]}$$

In 2D, immigrants from neighboring subpopulations at each generation can be estimated as $N_e m = 1/2\pi b$ [40].

For isolation by distance between individuals, $\hat{a}$ should be used instead of $\hat{e}$ when $Nb<10000$ in 1D or when $Nb<50$ in 2D [23].

Significance testing cannot be undertaken with a **parametric test** since distance measures are autocorrelated (paired comparisons). The significance of the slope can be tested by a bootstrap over loci based 95% confidence interval (95% CI). If 0 is not included in 95% CI, then the slope is significantly above 0. Otherwise, **a correlation coefficient** (e.g., Pearson) is computed between the two distance matrices and cells of one of those permuted a great number of times (Mantel test [47]). The P value of the test is the proportion of time the randomized correlation was as big as, or bigger than, the observed one. If the 95% CI is not above 0, the Mantel test may be more powerful if the genetic distance used is D_{CSE}, at least for highly variable markers like microsatellite loci [37].

2D population structure regressions [7,9–11]. For some studies [12,15–18] we needed to re-compute several statistics, as explained in detail in File S1. Only two studies where 1D dispersal inferences were available along the Mouhoun River in Burkina Faso [13,14] were not included. According to Watts *et al*. [23], high mutation rates have a stronger effect on the accuracy of the method in linear habitats than in two-dimensional habitats, and this may affect analyses of **riparian** systems. The fact that the metric in 1D is in m, and in 2D in m^2, also makes comparisons difficult. Additionally, we wanted to check that the relationship was not due to a systematic bias in D_e and δ estimates by using independent estimates of D_e and census densities (see below). N_e can strongly depend on the reproductive system, on fluctuations in population sizes and/or generation overlap. This last point should be minimized if samples taken at intervals of not more than 2 months are used, as we recommend (File S1). We also expect that N_e is strongly correlated with the census size of the population, otherwise all population genetics studies of tsetse flies would need to be called into question. Finally, a census of flies captured during the studies, when available, was also analyzed and, likewise, was seen to be correlated with the real census of the corresponding tsetse populations [24].

The results of the regressions are presented in Figure 1, in 1A with effective population densities (D_e), and in 1B with census population densities (D_c). An exponential, tight and negative relationship can be seen between population density and dispersal. The regression explains most of the

corresponding allele frequencies in the population for the two loci. The main forces influencing linkage disequilibrium are reproductive systems, selection, and genetic drift. It can be used to measure effective population size (small populations generate and maintain higher linkage disequilibria than bigger ones).

Locus: a specific segment of the genome, not necessarily a coding sequence (gene).

Neighborhood: the number of individuals connected through migration in an isolation by distance framework.

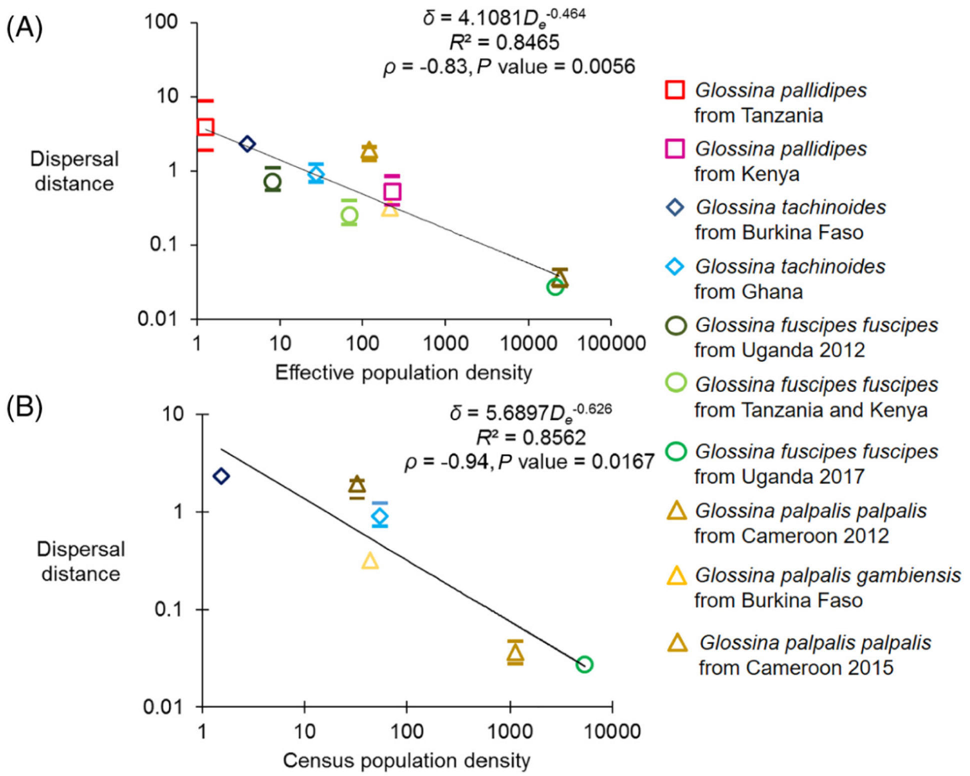
Parametric tests: statistical tests using population parameters (average and variance). If the constraints to apply such tests (e.g., normality of data, homogeneity of variances) are not met, nonparametric tests must be applied instead (e.g., rank tests or Mantel tests).

Population genetics: the study of the distribution of genetic variation in space and time and its evolution with random genetic drift, selection, mutation, migration etc.

Regression: a mathematical model explaining the relationship between a response variable and one (or several) explanatory variable(s).

Riparian: a riparian system describes the interface between land and a river or stream.

Slope: the parameter of a regression that describes how many units ordinates go up or down for each unit increase in the abscissa.



Trends in Parasitology

Figure 1. Strong Negative Density-dependent Dispersal in Tsetse Fly Populations. The graph shows the relationship between effective population densities D_e (individuals per km^2) and dispersal (δ, in km) (A) or between census population densities (densities of captured flies) (B) across different two-dimensional isolation by distance studies of different tsetse fly species in different African countries. The straight line corresponds to the power regression indicated in the graph with its determination coefficient R^2 and corresponding Spearman's coefficient (ρ) and associated P value. Dashes indicate bootstrap over loci 95% confidence intervals. The same symbols indicate the same species. Scales were log-transformed for both axes.

variance ($R^2 \approx 0.85$ for D_e; $R^2 \approx 0.86$ for D_c). Spearman's rank correlation testing with Rcmdr package [25,26] for R [27] gave significant P values (0.0056 and 0.0167 for D_e and D_c respectively).

Possible Causes for Negative Density-dependent Dispersal

Negative density dependence appears to be much less frequent than positive density dependence (the intuitively expected sign of correlation), in particular in insects [28,29]. Matthysen [30] said "The occurrence of negative density dependent dispersal is in agreement with the 'social fence' and related hypotheses which have been proposed in particular for small mammal populations, where movements are increasingly inhibited by aggression at high densities". Negative density dependence can result from **Allee effects** or because other factors, such as predation, interact with density to negatively influence dispersal [28]. In the case of insects, if there is a trade-off between wing development and reproductive capacity, dispersal may also be promoted by harsh local conditions, which can be correlated with low levels of population density, especially when dispersal is costly [31].

There are nevertheless several reported cases of negative density-dependent dispersal. Experimental studies on insects have shown that juvenile hormone titer, which is influenced by the diet at earlier stages, can influence wing size and hence dispersal capacities [31]. Negative density dependence has also been reported in the northern pine processionary moth *Thaumetopoea pinivora* [29].

Dispersal Is Strongly Density-dependent in Tsetse Flies

Negative density-dependent dispersal in tsetse populations has been known for some time (Box 3) but its intensity has not been measured to date. The exponential negative density dependence of dispersal observed in the present study raises several questions. The phenomenon implies that competition for space is very harsh and probably occurs mainly during feeding on the host (Box 3). When densities are high, all the sites are crowded and both hosts and local tsetse are accustomed to each other (Box 3). Naïve immigrants from remote sites are characterized by low feeding success, thus increasing their mortality. When population densities are low, densities may vary in both space and time, which renders immigration much easier and allows long-range immigration. In an empty spot with no other tsetse competitors and naïve hosts, even exhausted immigrants from remote sites can safely settle. This pattern could also reflect favorable host densities. When densities of favorable hosts are low, tsetse flies need to keep moving to find a suitable blood meal.

Since control campaigns aim at considerably reducing tsetse densities, they may unleash dispersal and relatively rapid reinvasion of treated zones from neighboring or even remote sites, as may have occurred in Ghana [7] and certainly occurred in Ivory Coast [32]. Reinvasion of flies in areas depleted as a result of vector control has been well documented for quite some time and emphasizes the role played by fly movement in the development of control strategies [33]. Some evidence suggests strong density-dependence associated with this phenomenon: the bigger the decrease in the population, the higher the potential for reinvasion in a continuous tsetse belt [33]. These authors also report that Hargrove's models use density-dependent mortality, but not dispersal [34]. This limitation has already been reported and actually concerns all existing models [35]. Density-dependent dispersal was only recently incorporated in a tsetse population dynamics model, based on a sigmoidal density-dependent dispersal rate adapted for individuals competing for access to resources [36]. Such immigrations are dangerous for three reasons: (i) because they can severely jeopardize the sustained success of control campaigns; (ii) these new immigrants can bring with them pathogenic agents or more virulent strains that were not present before the campaign and were unable to invade the zone because of competition for

Box 3. Tsetse Fly Atypical Reproduction and Density Dependence

Female tsetse flies do not lay eggs but larviposit a single mature larva (3rd instar, L3) in humid soil one at a time. The larva develops feeding from the uterine glands of the mother (adenotrophic viviparity). After larviposition, the larva quickly burrows into the soil surface for pupation. It was shown in *Glossina morsitans* that a larviposition pheromone is deposited to attract other females to the same site, leading to a strong aggregation of pupae [48]. The adult emerges 20–80 days later. Thus, in nature, each female produces no more than three to five offspring during its total life. Lifespan is around 3 months for females, 2 months for males. As a result, the intrinsic rate of tsetse population growth is theoretically low. Both females and males feed on vertebrate blood and are therefore both vectors. Learning capacities of tsetse flies may increase their hunting efficiency with age and encourage those returning to their first host [49].

Evidence for negative density dependence in tsetse flies has been reported in several studies. Interactions between flies and the irritation of the host animal are responsible for a decreased proportion of fed *G. morsitans morsitans* as the numbers of tsetse flies arriving increase, and frequently disturbed spots might encourage tsetse flies to leave without feeding, simultaneously increasing dispersal and associated mortality and decreasing local density [32,50]. Host irritation and tsetse learning are important parameters driving the survival and dispersal of tsetse flies.

In dense tsetse populations, the populations are self-sustained and are considerably reduced after insecticidal spray, but are recolonized from neighboring sites, which takes several months to complete [51]. Alternatively, low-density sites are naturally sustained by immigrants from neighboring sites, and insecticidal treatments do not have as much impact on the total population [32,51,52].

space; and (iii) these immigrants may replace the local population and adapt to local pathogens differently and exhibit new vectorial capacities compared to the former population. What is more, negative density-dependent dispersal may partially explain some observed failures. As underlined by Rogers and Randolph [32]: 'Control programmes must recognize that the efficacy of population suppression may well be reduced at the low levels of density that need to be maintained in order to reduce the rates of disease transmission, because at these low levels, tsetse may show entirely unexpected demographic vigor, due to the absence of normal density dependent constraints'. If suitable host density is the only force driving the observed pattern, then control campaigns would not be expected to have any impact on dispersal.

Concluding Remarks

More data on isolation by distance slopes and effective population density estimates are needed to confirm the exact relationship found so far. One-dimensional isolation (along river courses) is particularly rare up to now. Most species we studied belong to the *palpalis* subgenus, and one species (*G. pallidipes*) to the *morsitans* subgenus. The trends highlighted here probably apply to all tsetse flies, but more studies are needed to generalize the trends we found to the whole genus. Studies should also focus on areas where tsetse control has been implemented. Considering that our results strongly support negative density-dependent dispersal, it will be crucial to evaluate the strength of the phenomenon using direct methods such as mark-release-recapture in the field before and after control, for instance, combined with population genetics analyses. Alternatively, the prediction that neighboring flies will recolonize the treated area after the campaign ends can easily be assessed with sampling and genotyping before and after a control campaign in the treated area and its surroundings. If confirmed, and if one wants to avoid reinvasions and their associated issues (see Outstanding Questions), it will be even more important to find a way to isolate zones after treatment [4] and to sequentially treat neighboring areas harboring tsetse flies. Finally, several published results could not be included in the present review because of data unavailability. Data availability is now mandatory in several journals but we think this should be generalized.

Outstanding Questions

What kind of field study will allow direct measurement of the effect of reduced population density on dispersal distances?

If dispersal is indeed strongly density-dependent, how can we prevent reinvasion of treated zones?

Could isolation of the treated zone with a dense network of traps prevent efficient reinvasion?

How fast must surrounding sites be treated in turn?

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Supplemental Information

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