

Potential sources of *Triatoma infestans* reinfesting peridomiciles identified by morphological characterization in Los Llanos, La Rioja, Argentina

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The presence of Triatoma infestans in habitats treated with insecticides constitutes a frequent problem in endemic areas. Basing our study on the hypothesis that descendants of a residual population should be more similar to the pre-treatment population than to any other, we compared the indications of two quantitative morphological approaches. This study seeks to find the origin of 247 T. infestans from three populations found in two chicken coops and a goat corral after treatment with insecticides. The results obtained by quantitative morphology suggest that the T. infestans found between three-34 months after the application of insecticides formed mixed populations with insects derived from residual foci and neighbouring habitats. Our analyses also showed the presence of a phenotype which does not resemble neither the pre-treatment phenotype nor the one from neighbouring populations, suggesting the presence of a particular post-treatment phenotype. The heads size showed some variations in males from different populations and remained unchanged in females, which reinforces the hypothesis of an intraspecific competition for food with priority for females. This article presents, for the first time, the combined analysis of geometric morphometry of heads and antennal phenotypes to identify the composition of reinfesting populations.

Key words: *Triatoma infestans* - reinfesting populations - morphometry - antennal phenotype - peridomicile

Triatoma infestans (Hemiptera: Reduviidae) is a haematophagous insect that is a serious health problem in the southern cone of South America because it is the main transmission vector for *Trypanosoma cruzi*, the etiological agent of Chagas disease (Lent & Wigodzinsky 1979, Zeledón 1983). This species is found in rural domestic habitats of impoverished areas of South America, where the vector is common, representing a high risk for the disease transmission. Other habitats frequently infested by *T. infestans* are chicken coops, goat corrals, rabbit hutches and peridomestic storerooms, which host abundant vector populations a few metres away from dwellings.

Peridomestic areas are important Triatomine shelters, creating potential sources of reinfestation and recolonisation in households and habitats that have been treated with insecticides (Cecere et al. 1997, 2006, Gürtler 1999). The importance of each peridomestic habitat in the process of reinfestation is not clear. Because of their high architectural complexity, peridomestic sites often retain residual or reinfesting populations after a thorough insecticide treatment. In these peridomestic sites, the insecticide does not penetrate deeply into the structure and it degrades more quickly than in the interior of the domicile.

Los Llanos, in the province of La Rioja, is located south of the Gran Chaco and is one of the most arid and poor regions in Argentina. Within Los Llanos, goat breeding is one of the most important agricultural activities. Goat corrals are popular refuges for peridomestic *T. infestans* populations because insecticides have low efficacy, leaving residual populations that are able to recover after one or two years (Cecere et al. 1997, Gürtler et al. 2004, Porcasi et al. 2006). The availability of hosts, the peridomestic complexity, the lack of systematic vector control and vigilance, high temperatures in warm seasons and other factors allow for the high peridomestic density of this vector in Los Llanos. The campaigns to control *T. infestans* have not been sustained in this region and because operational responsibility for vector control interventions was transferred from the national government to the provinces in the 1990s, the Chagas programme was interrupted in this endemic area.

After a 15-year interruption of systematic vector control activities by the Chagas Programme, insecticide treatments were resumed in La Rioja province in 2006. Despite these efforts, peridomestic populations frequently reappeared, generating new questions about the risk that they represent for human health. In some cases, there are peridomestic habitats with severe *T. infestans* infestations within a few metres of an intradomestic habitat with low or no infestation. Therefore, each habitat may influence the *T. infestans* source that invades and successfully colonises nearby habitats, particularly those that are intradomicile. It is of utmost importance to understand the origin of the *T. infestans* populations that appear in a habitat after insecticide treatments. In places where there

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is neither resistance to pyrethroid insecticides nor wild populations, reinfesting populations may result from errors in insecticide application that leave residual foci or from insects that spread from untreated foci.

Every habitat has a different number and type of host organisms and environmental conditions. These characteristics affect the morphophysiological traits of *T. infestans* in ways that are useful for identifying recolonising insects (Dujardin et al. 1997, López et al. 1999, Catalá & Dujardin 2001, Schachter-Broide et al. 2004, Abrahan et al. 2008, Hernández et al. 2011). The present study identified the origins of *T. infestans* populations found in two chicken coops and a goat corral that were treated with insecticides. We determined whether *T. infestans* from different post-treatment populations originated from residual foci or emigrated from neighbouring sites. Measurements of head and antennae phenotype similarities were used to test the hypothesis that post-treatment adult *T. infestans* originated from residual individuals. In contrast, if the post-treatment adults were more similar to populations external to the habitat, reinfestations produced the post-treatment populations. It was also possible that both sources, i.e., residual population and reinfestation, contributed to the post-treatment population.

MATERIALS AND METHODS

Study area - The study was carried out in the department of Independencia in western Los Llanos. This department is located in the southern end of the Gran Chaco Region (arid Chaco) (Fig. 1). Most of the structures proximal to domiciles are chicken coops and goat corrals, which are frequently infested with *T. infestans*. Specimens in both of these habitats were sampled in Patquia Viejo, La Torre and La Aguadita. Patquia Viejo is 570 m above sea level (masl) and separated by 80 km from La Torre (1.182 masl). La Aguadita is situated at 900 masl and 60 km from Patquia Viejo. This locality had only one house with a chicken coop and a goat corral and this home is 5 km from the nearest population.

The three habitats selected for the study had high levels of infestation before and after treatment with insecticides. The chicken coop in La Torre (chicken coop A) was a 6 m² structure consisting of branches arranged vertically and intersected by a roof also made of plant material (Fig. 2A). The chicken coop located in La Aguadita (chicken coop B) covered approximately 2 m² and it was built with wooden walls, plastic fabric, sticks and a roof made of local plant material and leather (Fig. 2B). The third habitat was a goat corral in Patquia Viejo. This habitat comprised a 35 m² area and was made of a cardboard roof supported by branches from common local shrubs (Fig. 2C).

To select the localities and the habitats where the insect collection was performed, places with a high *T. infestans* infestation after 15 years without insecticide treatments were prioritised. This criterion allowed the largest number of *T. infestans* to be collected for morphometric studies.

All of the habitats selected for study were theoretically high-risk habitats based on the high infestation rate

(high infestation rates were found in these locations both pre- and post-treatment) and habitat characteristics supportive of *T. infestans* populations.

Insects - The insects were collected in October 2006 (pre-treatment), January, April and October 2007 and August 2009 (3, 6, 12 and 34 months after insecticide application, respectively). All insects collected after October 2006 were designated as the post-treatment group. In October 2006, the Chagas Provincial Program sprayed the domiciles within the department of Independencia with a concentrated beta-cypermethrin suspension at a nominal dose of 60 mg/m². The peridomestic structures were not sprayed, but the animals within them were treated with topical formulations of cypermethrin (6% active ingredient) on goats and chickens (5 cm³ and 1 cm³ treatment areas, respectively) and fipronil (1% active ingredient) on dogs.

The habitats (chicken coops A and B and the goat corral) were manually inspected for 30 min by two experienced persons using a dislodging agent (tetramethrin 0.2%) to collect *T. infestans*. On the same pre-treatment date, the neighbouring habitats representing potential sources of *T. infestans* reinfestation (goat corrals, chicken coops, incubating chicken sites and storerooms) were also sampled. In the case of the La Aguadita chicken coop (chicken coop B), the only neighbouring population was a goat corral belonging to the same owner. The

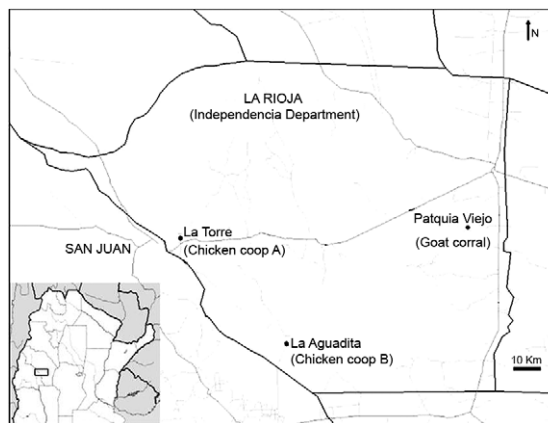


Fig. 1: map of study area showing the locations where the insects were collected (La Torre, La Aguadita and Patquia Viejo) and the habitats selected for study. The inset shows the location of the study area (black box).



Fig. 2: habitats selected for study. A: chicken coop A; B: chicken coop B; C: goat corral.

distances between the analysed habitats and the neighbouring habitats ranged from 2-900 m.

Antennal phenotype - The antennae from adult insects were extracted and processed as described previously by Hernández et al. (2011). For each specimen, the ventral side of the pedicel and flagellum 2 were drawn and the sensilla were identified and counted as in Catalá and Schofield (1994). Bristles (BR) from the pedicel, thin-walled (TH) trichoids from the pedicel and flagellum 2 and basiconica (BA) from flagellum 2 were quantified. These receptors were selected because they are associated with dispersal (TH trichoids and BA) and are involved in the olfactory uptake of host or partner odours. The BR (mechanoreceptors) provided information about the insect density in the habitat where the specimens developed as nymphs (Catalá 1993, Catalá & Schofield 1994). The number of sensilla per segment (antennal phenotype) enabled comparisons between the *T. infestans* collected pre and post-insecticide treatment for each sex.

The statistical analysis and software used were previously described by Hernández et al. (2011).

Geometric morphometrics of *T. infestans* heads - Each specimen was dissected and preserved in 70% alcohol for further analysis. Each head was excised at the collar and mounted on a pin attached to a metal support. Photographs were taken through a stereomicroscope (10X magnification) with a digital camera (Kodak C613, 6.2 MP). Ten type II landmarks were selected on the ventral surface of the adult heads, according to Hernández et al. (2011). The average size of landmarks on both sides of the head (5 landmarks for each side) was calculated to reduce intra-individual variation and minimise digitising errors.

The shape variables were obtained by a generalised Procrustes analysis that was subsequently projected into a Euclidean space. Uniform and non-uniform components (e.g., partial warps) (Rohlf 1990) were used as the shape variables. Uniform components describe the global variation due to stretching and compression and non-uniform components correspond to the local variation (Zelditch 2004). These two components describe differences in shape as deviations from an average configuration of landmarks.

When sample sizes were smaller than the number of shape variables, a restricted shape representation, i.e., a set of principal components (relative warps) derived from the shape variables, was used. These relative warps were used as input into a discriminant analysis. The significance in the discriminant analysis was evaluated using the values of Wilks and Mahalanobis distances. These values were evaluated using non-parametric tests based on permutations (5.000 runs). The Bonferroni correction was used for multiple comparisons.

The percentage of phenotypic similarity was calculated using the cross-check test of discriminant analysis. In this test, each individual in the post-treatment population is removed and reinserted into the analysis and they are later assigned into a group according to their phenetic similarity (PAD software).

To compare head size among groups, the isometric size estimator [centroid size (CS)] was derived from coordinates data. This constant is defined as the square root of the sum of squared distances between the centre of the configuration of landmarks and each individual landmark (Bookstein 1991).

The size and shape variables of heads were used to compare pre and post-treatment insects and neighbouring populations by sex and for each habitat. The statistical significance of CS differences was assessed with non-parametric tests based on permutations (5.000 runs).

The relationship between CS and shape discrimination among groups (allometry) was estimated by regression on size variation from the discriminant axes derived from shape.

Bioinformatics - For the morphometric analysis, the modules COO, MOG, COV and PAD were used as described by Hernández et al. (2011). These modules were developed by JP Dujardin and are freely available (mpl.ird.fr/morphometrics).

The STATISTICA package (StatSoft Inc. 2005) was used for the univariate comparisons (parametric ANOVA and Kruskal-Wallis).

RESULTS

***T. infestans* captured before and after insecticide treatment in three habitats** - Table I summarises the number of *T. infestans* collected in chicken coop A, chicken coop B and the goat corral on different sampling dates. Due to the low number of specimens on post-treatment sampling dates, post-treatment data were aggregated for each habitat.

***Adult T. infestans* collected post-treatment in chicken coop A** - Antennal phenotypes: univariate analysis of the antennal phenotypes of pre and post-treatment insects revealed significant differences ($p < 0.01$) in all four male sensilla and one female sensilla. The TH of pedicel and multiporous olfactory of pedicel receptors were significantly increased in post-treatment insects. There was also a significant difference ($p < 0.01$) between pre and post-treatment males and females, as determined by discriminant analysis the four antennae variables. The canonical functions 1 and 2 accounted for 79% and 17% of the total variation, respectively. Head morphometry - size: the average head size of post-treatment insects was significantly smaller than in insects from neighbouring populations ($p < 0.05$), but it did not differ between pre and post-treatment populations. Shape: post-treatment males and females showed significant differences from pre-treatment and neighbouring populations that were independent of size ($p < 0.05$, using the total conformation). Fig. 3B shows the percentage of post-treatment males and females with head-shape phenotypes similar to pre-treatment and neighbouring populations.

***Adult T. infestans* collected post-treatment in chicken coop B** - Antennal phenotypes: univariate analysis of the antennal phenotypes of pre and post-treatment insects showed that there were significant differences ($p < 0.01$) in one sensillum type in males (F2BA). In fe-

males, antennal phenotypes were not significantly different. Whereas there was a significant difference ($p < 0.01$) between pre and post-treatment males, females did not exhibit these differences. The canonical functions 1 and 2 accounted for 72% and 25% of the total variation, respectively. Head morphometry - size: head size of post-treatment insects was not significantly different from pre-treatment insects and neighbouring populations. Shape: when the effect of size was excluded post-treatment males did not show significant difference from pre-treatment and neighbouring populations ($p > 0.05$, using the total conformation). Post-treatment female head shapes were not different from the other groups. Fig. 3C shows the percentage of post-treatment males and females with head-shape phenotypes similar to pre-treatment and neighbouring populations.

Adult T. infestans collected in the goat corral - Antennal phenotypes: univariate analysis of pre and post-treatment insects revealed significant differences ($p < 0.01$) in both pedicel receptors in males (TH and BR). Females did not show differences in their antennal phenotype. Discriminant analysis with all four antennae variables confirmed that there was a significant difference ($p < 0.01$) between pre and post-treatment males. Females did not show significant differences between these groups. The canonical functions 1 and 2 accounted for 72% and 25% of the total variation, respectively. Head morphometry - size: head size of post-treatment insects showed no significantly different from that of pre-treatment insects and neighbouring populations. Shape: after removing the allometric effect, post treatment males did not show significant difference ($p > 0.05$, using the total conformation). The first two canonical functions account for the 79% and 21% of the total variation. Due to the low number of females in one of the groups (6 females in pre-treatment), the post-treatment and neighbouring groups were used in the discriminant analysis and the pre-treatment insects were added afterwards as an external group. The post-treatment insects were different from those in neighbouring populations ($p < 0.05$). Only one factor accounted for 100% of the variation, with the allometric effect contributing 0%. Fig. 3A shows the percentage of post-treatment males and females with head-shape phenotypes similar to pre-treatment insects and neighbouring populations.

DISCUSSION

When *T. infestans* are present in the peridomestic environment a few months after pyrethroid insecticide treatment, it is important to know their origin, the efficacy of the applied treatment and the neighbouring habitats that harbour these triatomines. To identify the origin of *T. infestans* populations, this study analysed the morphology of adult individuals in areas treated with insecticides. Insect head geometric morphometry and antennal phenotype analyses indicated that the *T. infestans* found three-34 months after insecticide treatments were mixed populations of survivors from the treatment area and the surrounding habitats. A similar result was found

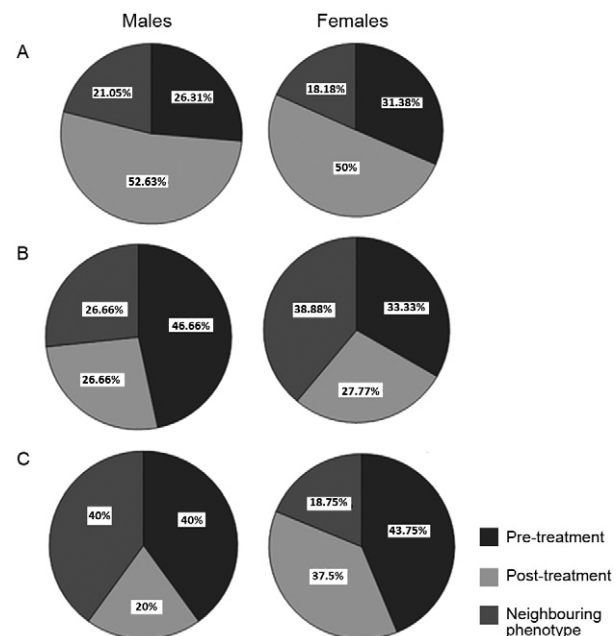


Fig. 3: phenotypic composition of the population of *Triatoma infestans* after treatment, test based on cross-check, using shape variables of the head. The percentage of pre-treatment phenotype indicates the amount of residual population in this habitat, the percentage of neighbouring populations phenotype indicates the amount of reinfestant phenotype and the percentage of population post treatment indicates the proportion of the population produced by ancient and residual population. A: goat corral; B: chicken coop A; C: chicken coop B.

TABLE I
Adults of *Triatoma infestans* collected by active search method man-hour

Habitat	Sex	Pre-treatment	Post-treatment (3 months)	Post-treatment (6 months)	Post-treatment (12 months)
Chicken coop A	M	23	6	16	43
	F	38	3	7	18
Chicken coop B	M	15	5	14	7
	F	12	1	10	8
Goat corral	M	16	13	8	0
	F	5	16	5	2

F: female; M: male.

when the geometric morphometry of *Rhodnius prolixus* wings in post-treatment populations was analysed by Feliciangeli et al. (2007); these populations consisted of survivors of the applied insecticide and reinvasions from the sylvatic habitat. Our analysis also discovered phenotypes that differed from both the pre-treatment phenotype and the post-treatment neighbouring populations. This result suggests that a new phenotype originated shortly after the treatment from uncharacterised populations, previous reinfestation populations, residual insects or a combination of all three. Alternatively, the post-treatment phenotype may have originated from genetic derivation or selection due to the insecticide. Reinfestation from wild foci is not likely because sylvatic *T. infestans* have not been detected in light traps or active searches in the study area (G Marti, unpublished observations). However, the classification of insects in the three years post-treatment may have erroneously assigned the sources of reinfestation in the analysed habitats.

The origin of post-treatment *T. infestans* populations and the dynamics of their spread in the Argentine and Bolivian Chaco have been analysed using genetic markers (Pérez de Rosas et al. 2007, Marcet et al. 2008, Quisberth et al. 2011).

These studies suggest that there are genetically similar populations after the vector control and support the residual source hypothesis, as suggested by Dujardin et al. (1997) and Pizarro et al. (2008). However, Marcet et al. (2008) suggested that populations with sporadic control actions were more homogeneous, with the dispersal of *T. infestans* between and within the communities studied.

Phenetic and genetic markers have been widely used to study the population structures and reinfestation dynamics of Chagas disease vectors (Dujardin et al. 1997, Schachter-Broide et al. 2004, Pérez de Rosas et al. 2007, 2008, Abraham et al. 2008, Marcet et al. 2008, Villacís et al. 2010, Hernández et al. 2011, Quisberth et al. 2011, Gaspé et al. 2012). The correlations between these markers have been verified in triatomines (Dujardin et al. 1999, Villegas et al. 2002, Hernández et al. 2008, Martínez-Hernández et al. 2010, Ceballos et al. 2011, Villacís 2011) and other medically important insects (Dujardin et al. 2004, Bouyer et al. 2007). In some cases, the phenotypic differences observed in triatomines (Moreno et al. 2006) have been subsequently corroborated with genetic markers (Campos et al. 2012). Moreover, Villacís (2011) showed greater microgeographic differentiation of *Rhodnius ecuadoriensis* populations when antennal phenotypes were used instead of microsatellite markers. This author suggested repetition of the genetic analyzes with additional primers. Although a pattern of reinfestation was expected in peridomestic habitats, the results show that there was heterogeneity. Each habitat behaved in its own particular manner after the application of insecticide. These differences can result from the variety of construction materials, type and quantity of hosts, level of infestation before insecticide treatment in the habitat and in the neighbouring habitats and *T. infestans* preferences. The two chicken coops contained fewer residual *T. infestans* than the goat corral. The construction materials and design of goat corrals favoured the survival of

residual *T. infestans*, as they offered more refuge than the chicken coops. Alternatively, because the insecticide was only applied to adult goats, their offspring (free of insecticidal treatment) may have promoted the growth of residual *T. infestans* populations in different seasons. Another hypothesis is that that more residual insects emigrated to nearby habitats.

When we compared similar habitats (chicken coops), we observed that the post-treatment females were phenotypically similar to those of the neighbouring populations, suggesting recent reinfestations (chicken coop B) and that the phenotypical differences between these populations were significant, indicating isolation or previous reinfestations that had already produced different phenotypes (chicken coop A).

Lastly, head geometric morphometry and the antennal phenotype analyses were used to determine the origins of *T. infestans* populations reinfesting insecticide-treated habitats. Although both techniques showed the morphological changes that occurred within three years after treatment, different selective pressures forces act on these organs, as observed for heads and wings in *Rhodnius pallescens* (Caro-Riño et al. 2009) and in *T. infestans* (Hernández et al. 2011). The allometry-independent shape analysis of heads detected phenotype variations due to genetic components, whereas the antennal phenotypes showed both genetic variations and environmental adaptations. Thus, antennal phenotypes showed evidence of faster population changes. As shown in Table II, the antennae changed even when the head conformation manifested little or no differences between populations. The converse was not observed, i.e., any change in the heads also appeared in the antennae. Both techniques showed a common result: post-treatment populations arose mainly from residual populations with some input from *T. infestans* in neighbouring peridomestic habitats.

The CS of the heads, which mainly reflects environmental variations, showed some variation in males from different populations, but was unchanged in females. This result supports the hypothesis that intraspecies competition for food compels females to feed themselves independently from the host or the availability of blood in the habitat.

During the last sampling at 34 months after the insecticide applications, the domiciles studied contained only one adult male *T. infestans* (Hernández 2012). *T. infestans* may prefer peridomestic habitats, where the movement of individuals preferentially occurs. There may also be little vector exchange via active dispersal between peri and intradomestic habitats, as shown by Ceballos et al. (2005), Vazquez-Prokopec et al. (2006) and Abraham et al. (2011).

In this study, we found evidence for more extensive movement of males between peridomestic habitats. According to Hernández (2012), the chances of dispersal are not equal in both sexes, as the males appear to have less spatial structure regarding their habitats and enhanced probabilities of dispersal due to flight. However, previous work (Hernández et al. 2011) in a similar study area showed a greater population structure of males between habitats. The dispersal pattern may change in

TABLE II
Results of univariate and multivariate analysis based in antennal phenotypes and geometric morphometry of head in chicken coop A and B and the goat corral to compare populations pre, post-treatment and neighbours

Habitat	Sex	Antennal phenotype			Head morphometry			Conclusion
		Univariate	Multivariate	Size	Shape	Size	Shape	
Chicken coop A	M	Pre#post (4/4)	Pre#post	Pre#post	Pre#post	Pre#post	Pre#post	Post and pre populations are dissimilar.
	F	Pre#post (1/4)	Pre#post	Pre=post	Pre#post	Pre#post	Pre#post	Post and pre populations are dissimilar.
Chicken coop B	M	Pre#post (1/4)	Pre#post	Pre=post	Pre#post	Pre#post	Pre#post	Post is dissimilar to pre and neighbouring populations.
	F	Pre=post	Pre=post	Pre=post=neighbouring	Pre=post=neighbouring	Pre=post=neighbouring	Pre=post=neighbouring	Populations pre, post and neighbours are similar.
Goat corral	M	Pre#post (2/4)	Pre=post	Pre=post=neighbouring	Pre=post=neighbouring	Pre=post=neighbouring	Pre=post=neighbouring	Populations pre, post and neighbours are similar.
	F	Pre=post	Pre#post	Pre=post=neighbouring	Pre=post=neighbouring	Pre=post=neighbouring	Pre=post=neighbouring	Pre and post populations would be dissimilar. The two techniques show that changes appear in a different order in the organs.

F: female; M: male; #: statistically different populations; =: statistically similar population, $p < 0.05$.

time and space, as *T. infestans* populations behave differently even in nearby geographic areas and in similar habitats. This dispersal pattern can be influenced by the particular environment, history of spraying, nutritional status of the population at the time of sampling, degree of infestation and season, among other factors.

This novel analysis combined head geometric morphometry and antennal phenotypes to identify reinfesting populations, thus reinforcing the utility of these inexpensive techniques to evaluate reinfestation sources.

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