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Aggregation Behaviour and Interspecific Responses in Three Species of Triatominae

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The response to intra- and interspecific assembling signals was tested in three species of Chagas' disease vectors. As previously described for Triatoma infestans, *larvae of both species,* T. sordida *and* T. guasayana, *aggregated on papers impregnated with their own excrement. Moreover, bugs belonging to each of the three species also aggregated on papers contaminated with faeces from the other two, with the only exception of the larvae of* T. guasayana, *which did not assemble on faeces of* T. sordida. *In all cases, the response to interspecific excrement was as strong as that to the intraspecific one. The non-specificity of the signal is discussed in the context of the ecological association of the three species and their role as vectors of Chagas' disease.*

Key words: Triatominae - aggregation behaviour - interspecific communication - faeces

Triatoma infestans, *T. sordida* and *T. guasayana* are haematophagous bugs distributed over Bolivia, Brazil and Argentina. All three species are extremely important from a sanitary point of view because they transmit the flagellate *Trypanosoma cruzi* and are thus vectors of Chagas disease in South America. Among them, *T. infestans* is the best adapted to domestic environments. *T. sordida* and *T. guasayana* are closely related species that inhabit mainly peridomestic environments, but can also colonise human habitations, together with *T. infestans* (Lent & Wygodzinsky 1979). *T. sordida* and *T. guasayana* have been frequently mistaken with each other because of their morphological similarity and overlapping distribution. However, the two species can be discriminated by different variables, mainly, by the relative length of the second rostral segment and the anteocular length (Lent & Wygodzinsky 1979, Gorla et al. 1993).

It has been argued that if *T. infestans* could be eliminated from human housing, *T. sordida* and *T. guasayana* would colonise its empty niche. Indeed, such a replacement of *T. infestans* by *T. sordida* has been reported in Brazil (Schofield 1994). Therefore the knowledge of the factors affecting the ecological interaction between species becomes relevant. In addition, given their role as Chagas vectors, this knowledge could aid in the design of control strategies.

Aggregation behaviour, as a response to chemical signals, has been reported in several species of Triatominae: *Rhodnius prolixus*, *T. infestans*, *T. mazzottii*, *T. longipennis*, *T. pallidipennis* and *T. barberi* (Velázquez Antich 1968, Baldwin et al. 1971, Schofield & Moreman 1976, Schofield & Patterson 1977, Ondarza et al. 1986, Cruz-López et al. 1993, Lorenzo Figueiras et al. 1994, Manrique & Lazzari 1995). The specificity of these signals has been studied by Cruz-López et al. (1993), who reported that an "interspecific aggregation pheromone" is present in the faeces of larvae and adults of several species of the subfamily Triatominae (*T. barberi*, *T. mazzottii*, *T. longipennis*, *T. pallidipennis* and *R. prolixus*).

The present study was undertaken to examine the response of *T. sordida*, *T. guasayana* and *T. infestans* to aggregation substances present in their faeces. Experiments were conducted by testing the response of the bugs to both, intra- and interspecific putative aggregation signals present in bugs excrement.

MATERIALS AND METHODS

Larvae of *T. sordida* and *T. guasayana*, reared on chicken, were obtained from the laboratory stock of the "Centro de Reservorios y Vectores, Servicio Nacional de Chagas, Córdoba, Argentina". Larvae of *T. infestans*, reared at 28°C and fed on heparinized bovine blood by means of an artificial feeder (Núñez & Lazzari 1990), were obtained from our laboratory colony.

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For the obtention of the excrement, insects were placed in plastic containers bearing a plastic mesh at their bottom, and fed using the artificial feeder. Faeces were collected on pieces of filter paper, placed below the insect containers, for 3 to 5 hr after feeding. Any contact between the animals and the filter papers was carefully avoided to exclude other potential cues (Lorenzo Figueiras & Lazzari 1996). The collected faeces were used 24 hr later (dry faeces). This time interval guarantees their aggregating activity (Lorenzo Figueiras et al. 1994).

The larval excrement of different species was examined for their ability to induce aggregation of the insects. The procedure has been previously described by Lorenzo Figueiras et al. (1994). Briefly, a circular glass arena (12 cm diameter) was divided in three sectors, and three pieces of filter paper (3x2 cm) folded once, were placed one in each sector, 2 cm away from the wall. One paper was contaminated with dry faeces, while the other two remained clean, as controls. A group of larvae was carefully released in the centre of the arena. After 1 hr the position of the bugs was recorded by counting the number of insects on each sector (i.e., 1/3 of the total area of the arena). Nine to 12 insects were used for each trial. Room temperature was 24-25°C.

The response to intra- and interspecific excrement was tested. The aggregation behaviour of *T. sordida* and *T. guasayana* to their own faeces and on *T. infestans* excrement was recorded. Cross-aggregation of *T. infestans* on faeces of *T. sordida* or *T. guasayana* was also tested. In addition, we analyzed the cross-aggregation of *T. sordida* to faeces of *T. guasayana* and vice-versa. Test insects were 2nd and 3rd instar larvae of *T. sordida*; 2nd and 4th instar larvae of *T. guasayana*, and 4th instar larvae of *T. infestans.* Experimental insects were used 2-3 weeks after ecdysis.

For each experimental series the distribution of the insects in the arena was statistically analyzed by means of a G-test for goodness of fit to a random distribution (i.e., 1/3 for experimental zone and 2/3 for control zones). After testing for homogeneity (G-test for heterogeneity), data corresponding to the different replication of each assay were pooled. The aggregation responses were compared by means of two-factors ANOVA (factor 1: species origin of faeces; factor 2: species of test insects) and Tukey's test performed *a posteriori* for multiple comparisons.

The data about aggregation of *T. infestans* on their own faeces used in this paper for comparison, have been previously published (Lorenzo Figueiras et al. 1994).

RESULTS

Figure depicts the aggregation responses of the three species evoked by their own excrement, as well as by that belonging to the others. As previously shown for *T. infestans* (Lorenzo Figueiras et al. 1994), both, *T. sordida* and *T. guasayana* aggregated around papers impregnated with their own dry excrement. Larvae from both species chose those papers contaminated with their own faeces rather than clean ones. It could be established that *T. sordida* larvae significantly responded to *T. guasayana* excrement. However, the inverse response did not occur, i.e., faeces belonging to *T. sordida* were not able to assemble larvae of *T. guasayana.* When tested for their aggregation response to faeces belonging to *T. infestans,* both species, exhibited a significant tendency to aggregate around the experimental papers. A similar assembling response was observed in *T. infestans* larvae tested with faeces of *T. sordida* or *T. guasayana.* The insects significantly aggregated around contaminated papers (Table).

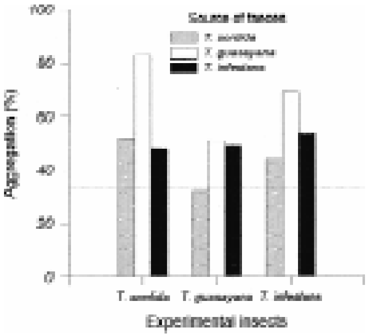
The two-factor ANOVA showed that the responses of the three species differ significantly (F=4.021; df=2, 60; P=0.023). In general, larvae of *T. guasayana* exhibited a weaker aggregation response than *T. sordida* and *T. infestans* (Fig.). No difference between the last two named species was observed. Concerning the origin of faeces, they revealed a significant difference of attractiveness on the insects (F=6.604; df=2, 60; P=0.003). The faeces of *T. guasayana* evoked a stronger response than those collected from *T. sordida* and *T. infestans* (Fig.). The interaction species-faeces was not significant (F=1.224; df=4, 60; P=0.31).

TABLE

Aggregation response of *Triatoma guasayana T. sordida* and *T. infestans* to larvae faeces of these species. G value and its significance were computed against a random distribution (i.e., 1/3 experimental, 2/3 control sectors). The letter N shows the total number of insects for each series

Faeces of	*T. sordida*			*T. guasayana*			*T. infestans*		
Test insects	N	G	P	N	G	P	N	G	P
T. sordida	63	7.8	<0.01	41	42.9	<0.001	64	6.2	<0.025
T. guasayana	57	0.09	N. S.	45	6.0	<0.025	84	11.4	<0.025
T. infestans	89	4.2	<0.05	59	32.0	<0.001		*a*	

a: see Lorenzo Figueiras et al. (1994).



The aggregation response of triatomine larvae to intra- and interspecific larval faeces. The horizontal line indicates the value waited from a random distribution.

DISCUSSION

The results presented here extend our knowledge about the aggregation behaviour of Triatominae bugs. An aggregating substance is present in the dry faeces of *T. infestans, T. sordida* and *T. guasayana*, which acts in an intra-, as well as interspecific fashion. We found that faeces of *T. infestans* elicit assembling in larvae of *T. sordida* and *T. guasayana*. In a similar way, the excrement of the last two named species induces aggregation in larvae of *T. infestans*. On the other hand, the faeces of *T. guasayana* elicit assembling behaviour in larvae of *T. sordida,* but the inverse is not true: faeces of *T. sordida* do not promote the aggregation of *T. guasayana* larvae.

Cruz-López et al. (1993) obtained comparable results in their analysis of the aggregation of *T. mazzottii*, *T. longipennis*, *T. pallidipennis*, *T. barberi* and *R. prolixus.* The named authors postulated the existence of an "interspecific aggregation pheromone" that would be present in the faeces of larvae and adults of these species. They found that *T. longipennis* larvae showed a stronger response than the other triatomine larvae tested. However, there were no significant differences in faecal attractiveness to larvae.

The biological action of the assembling signal of triatomines deserves to be discussed in the frame of the present classification of chemosignals. Compounds eliciting inter- or intraspecific responses are known as *semiochemicals.* The term *pheromone* refers to substances that are secreted by an organism to the outside and cause specific reactions in receptor organisms of the *same species*. On the other hand, *allelochemicals* are substances produced, acquired, or released as the result of the activities of an organism, which are capable of evoking a behavioural reaction in a receptor of a *different species* (Dicke & Sabelis 1988). Considering that excrement act as a chemical mark for guiding triatomines towards protected sites (Lorenzo & Lazzari 1996), this landmark (and the refuges) could be exploited for other species, that would benefit from the interaction. Among allelochemicals, such substances that evoke an adaptive response in receptor of other species, have been named *kairomones* (Dicke & Sabelis 1988). Our results show that assembling factors in the faeces of Triatominae act as a *pheromone* for the same species but would also act as a *kairomone* for individuals of other species. The same kind of interaction has been described between different species of the Order Dermaptera (Sauphanor & Sureau 1993), where the cohabitation of shelter-traps by species belonging to the genus *Forficula* has been observed in the field. Other examples are known for such a functional duality of aggregation substances in cockroaches (Appel 1994) and bark beetles (Vité & Renwick 1971).

T. infestans is the most widespread and best adapted domestic species of triatomine bugs. *T. sordida* and *T. guasayana* are frequently infected by *T. cruzi*, and are thus potential vectors of Chagas disease, because they also colonise human habitations. The invasion of human houses is facilitated by the frequently peridomestic condition of the last two species (Lent & Wygodzinsky 1979). In recent years, the invasion by *T. sordida* of houses where other species had been eradicated by successful control campaigns has been reported in Brazil (Schofield 1994). In this context, the chemical signal acting in an interspecific fashion becomes relevant, because the bugs of any of the three species could find and exploit the refuges used by conspecifics, as well as by members of the other species. In addition, *T. sordida* and *T. infestans* has been recently found associated in different localities in Bolivia (Noireau et al. 1995).

The interspecific attractiveness of the faeces of the three species studied here leaves open the question on the origin of the substances involved. The fact that the aggregation "pheromone" acts also interspecifically (i.e., as a kairomone) suggests us that the same, or very similar chemicals, are present in the faeces of the species studied here. It could also indicate that these compounds have a common ancestor. The insects would identify a common compound of the signal as specialists, or could have a generalised response to a family of compounds. For example, the interspecific aggregation produced by the traces of *Blatella germanica* is not strictly a response to a pheromone, since it could be due to the attraction of cockroaches to fatty acids (Ishii 1970).

Regarding the chemical nature of the aggregation substance, although Cruz-Lópes and Morgan (1995) did not succeed in obtaining the active fraction by chromatographic fractionating, they showed that the active compounds could be extracted with polar solvents. This result agrees with data obtained in our laboratory (Lorenzo Figueiras, unpublished observations). Recently, Taneja (1996) found that triatomine faeces release ammonia, and that this compound activates specific sensory receptors in the antennae and evokes a positive anemotactic response in both, *R. prolixus* and *T. infestans*. These results point out future research directions to the characterisation of an interspecific aggregation factor, which becomes of fundamental value for controlling and monitoring bug populations in the field.

In the precedent paragraphs, we referred to the proximate causes of this behaviour in triatomines (e.g., sharing of the same or similar assembling signals by different species, exploitation of a widespread metabolic by-product, etc.). Finally, we can speculate on the ultimate evolutionary causes of intraspecific responses, since this aspect opens several relevant questions. One can ask, for example, what would be the evolutionary benefit for an individual to give a signal for other individuals (belonging or not to the same species) that might represent potential competitors for a blood meal? Triatomine bugs spend light day hours hidden in shelters, where they assemble. As mentioned, excrement functions as a chemical landmark that serves to find the accesses of refuges under use (Lorenzo & Lazzari 1996). So, in the case of individuals of the same species, the question could be reformulated as what would be the evolutionary benefit of living in close aggregation. An obvious benefit is easy finding of symbionts, which are essential normal development in triatomines (Lake & Friend 1968, Eichler et al. 1996), and, in the case of adults, of sexual mates.

The benefit of the interspecific response appears as not so obvious. Again, the advantage for the receiver could be related to the exploitation of protected places, that are in addition contaminated with the necessary symbionts. The need of symbionts could also be argued as a benefit for the emitter. But, in this case, it should be also taken into account that evolutionary constrains (e.g., the lack of certain metabolic pathways) or the balance between costs and benefits of producing a more discrete specific chemical signal could favour the use of a natural metabolic by-product (i.e., ammonia), that is shared by different species.

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