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Capture of culicids in urban areas: evaluation of the resting box method

ABSTRACT

OBJECTIVE: To evaluate the occurrence of adult culicid populations in urban areas and measure the sensitivity of the resting box collection method.

METHODS: Mosquito were collected in 1999 and 2000, in two cities in the State of São Paulo, Brazil: Ocauçu and Uchoa. In each city, 15 blocks were drawn by lots and then one home in each block was drawn. Two resting boxes were installed at each home: one inside and the other outside the house. Monthly collections were carried out at each home, over a 13-month period, using manual aspirators inside and outside the home and inside the boxes. The captured specimens were taken to the laboratory for screening and identification according to species and sex.

RESULTS: Out of the 2,112 culicid specimens collected, 99.7% were of four species: *Culex quinquefasciatus*, *Aedes aegypti*, *Cx. declarator* and *Cx. coronator*. The distribution of these species in Ocauçu was 83.3%, 3.2%, 10.8% and 24%, respectively, and in Uchoa it was 83.8%, 8.4%, 4.4% and 3.0%, respectively. Among the females of the genus C*ulex*, 34.3% were captured in the resting boxes and 59.9% were collected from inside the house. Among the females of *Ae. aegypti*, 17.6% were found in the resting boxes and 82.4% inside the home.

CONCLUSIONS: The great majority of the specimens collected belonged to four species of culicids, and *Cx. quinquefasciatus* was the most common. Proportionally, the females of *Ae. aegypti* were found more inside the home than were those of the genus *Culex*. Resting boxes present potential for use as surveillance devices, but their use needs to be more thoroughly evaluated.

KEYWORDS: Culicidae. Insect vectors. Spatial behavior. Estimation techniques. Evaluation studies. Sensitivity and specificity. *Aedes aegypti*. *Culex*.

INTRODUCTION

Among the culicids living in urban areas, *Aedes aegypti* is considered to be the principal vector for the dengue virus and a potential vector for the yellow fever virus,[18] also it has been targeted in surveillance and control actions throughout Brazilian national territory.

Mosquitoes of the genus *Culex*, especially the species *Cx. quinquefasciatus*, are also present in human urban environments. As well as representing an important nuisance factor for people, their role as a vector for filaria (*Wuchereria bancrofti*) also makes them a target for control. Recently, the involvement of these mosquitoes in the transmission of West Nile virus (WNV) in the Americas[12] has represented a potential risk that this disease might be introduced into Brazil.

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In Brazil, surveillance of *Ae. aegypti* is based on larval index measurements,[13] by determining the presence, frequency of occurrence and abundance of larvae in the environment.[3] However, the entomological indicator that is most associated with the risk of dengue virus transmission is the relationship of females with the human population. Information on the bioecology of the females in urban areas is important, since it helps in selecting control measures for this vector. Furthermore, knowledge of adult population dynamics is believed to allow more accurate epidemiological evaluations. Although these questions have frequently been covered in the worldwide literature, they have rarely been dealt with in Brazil.[2]

The method of catching adult culicids using manual aspirators[16] in home environments is laborious and low-yielding and has the disadvantage of depending on the operator's dexterity.[11,19] The method proposed by Edman et al[10] for entomological surveillance of *Ae. aegypti* was by means of capture in resting boxes, which gets round the operational difficulties of aspiration and also optimizes the fieldwork. This method has been successfully tested in Asia for *Ae. aegypti* and in the United States for *Cx. quinquefasciatus*.[7,10,15]

The objective of the present study was to evaluate the occurrence of adult populations of culicid species according to density, sex and capture location; and to measure the sensitivity of capture in resting boxes for detecting adults of these species. In addition to this, for the genus *Aedes*, the number of females was correlated as an indicator of larval infestation.

METHODS

The municipalities of Ocauçu and Uchoa were chosen for studying. Ocauçu is located at 22º25'S and 49º56'W, at an altitude of 551 m above sea level and a distance of 46 km from the city of Marília, which is the principal municipality in the region of the same name, located in the western central part of the State of São Paulo. In the year 2000, it had 4,164 inhabitants and 1,100 homes in the urban area. The presence of *Ae. aegypti* was first detected in 1993 and, up to February 2007, the municipality had not notified any autochthonous cases of dengue.*

Uchoa is located at 20º57'S and 49º10'W, at an altitude of 485 m above sea level and a distance of 33 km from the city of São José do Rio Preto, which is the principal municipality in the region of the same name, located in the northwest of the State of São Paulo. In the year 2000, it had 9,035 inhabitants and 3,020 homes in the urban area. The presence of *Ae. aegypti* was first detected in the municipality in 1988. Dengue cases occurred in 1995 (rate of 164 cases/100,000 inhabitants) and 2001 (77 cases/100,000 inhabitants).*

In each municipality, 15 blocks in the urban area were drawn by lots. In each block, one home was drawn for installing two resting boxes:[10] one inside the home and the other outside the home. The boxes were made of cardboard of matt black color (both inside and outside), with dimensions of 30 cm in width, 30 cm in depth and 90 cm in height. At the front, there was a rectangular opening of 30 cm in width and 50 cm in height. To increase the attractiveness, an oviposition receptacle was placed inside each box. This consisted of a matt black flask of approximate capacity one liter that was filled with 500 ml of water and also contained a wooden pallet.

Between June 1999 and June 2000, monthly collections were carried out at each of these 15 homes, using manual aspirators inside and outside the home, and inside the boxes. The properties selected had fixed points for monitoring the study variables, and the resting boxes were installed one week before the collections. Two pairs of operators carried out the collections: one pair working inside the home and the other outside the home, during the period between 8:00 a.m. and midday. Each collection took a mean of 30 minutes. The adult mosquitoes were collected using aspirators with rechargeable batteries, in conformity with the model proposed by Nasci.[16]

After capture, the mosquitoes were placed in entomological boxes that were kept in a polystyrene box containing recyclable ice until arrival in the laboratory for screening and identification of the culicids according to species and sex.

Monthly measurements of the Breteau Index (BI; the number of receptacles with larvae per hundred houses)[4] were made in the two municipalities for *Ae. aegypti* and *Ae. albopictus*, using cluster sampling (per block). All the homes in the blocks that were drawn were visited to look for receptacles with mosquito larvae.[1] Each of the samples for measuring the Breteau Index (around 250 homes) was drawn independently of the sample of 15 homes for collecting adults.

For the females and males of the culicids captured, and for each species and genus, the numbers of specimens in relation to place of capture (inside or outside the home) and presence in the resting boxes were registered. The numbers of female specimens per home were calculated. The sensitivity of culicid detection using resting boxes was represented as the percentage of specimens captured in the boxes in relation to the total collected using aspirators,[16] according to genus and/or species, sex, capture location and municipality. The species of

* Information obtained from the Endemic Disease Control Superintendency. Unpublished data.

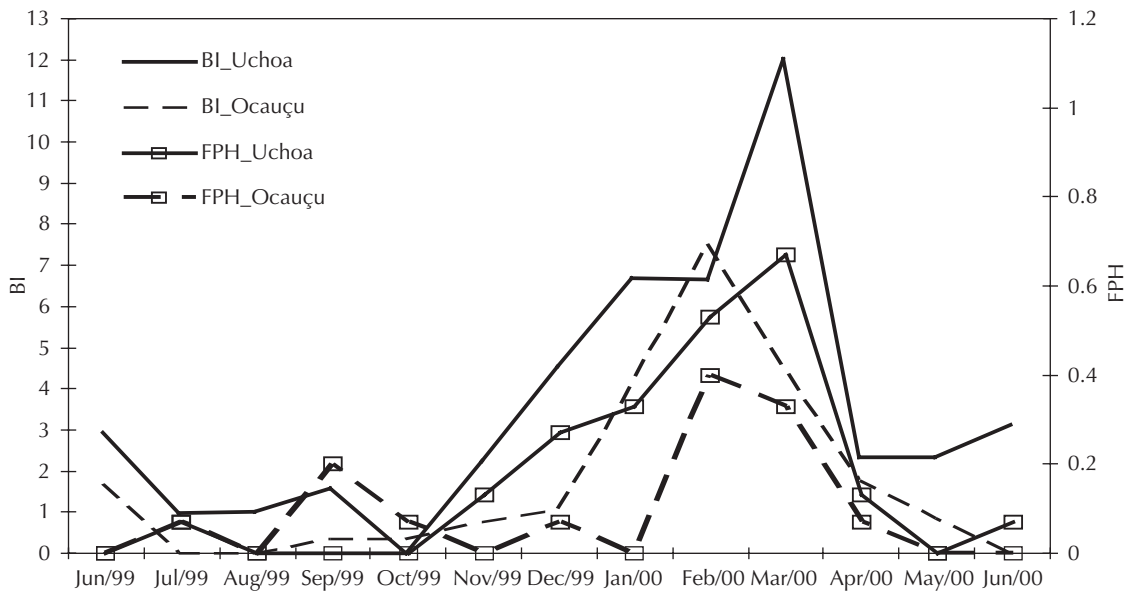
the genus *Culex* were grouped in this analysis and in the evaluation according to capture environment, because all of them were potential vectors for WNV infection.

To investigate possible significant differences between the proportions obtained, Fisher's exact test, the proportions test or the Chi-square test was used. The significance level taken was 5%. When proportions were not compared using statistical tests, they were presented with their 95% confidence intervals (95% CI). The relationship between the number of females of *Ae. aegypti* per home and the Breteau Index was evaluated using Spearman's correlation coefficient.

The Stata and Epi Info 2002 software were used for the statistical data analysis.

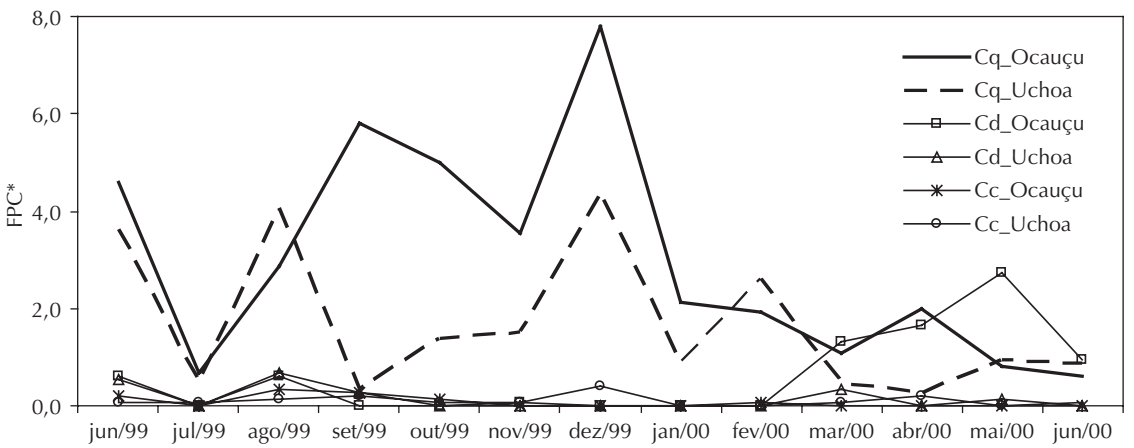
RESULTS

Between June 1999 and June 2000, 1,386 adult mosquito specimens were collected in Ocauçu: 1,155 (83.3%) of *Cx. quinquefasciatus*, 150 (10.8%) of *Cx. declarator*,



* Monthly collections from approximately 250 homes
** Collected from 15 homes per municipality

Figure 1. Breteau Index* (BI) per municipality and mean monthly numbers of females of *Aedes aegypti* per home** (FPH) inside and outside the home and in resting boxes. Municipalities of Ocauçu and Uchoa, Southeastern Brazil, 1999 to 2000.



* Collected from 15 homes per municipality using Nasci aspirators

Figure 2. Mean monthly numbers of females per home* (FPH) of *Culex quinquefasciatus* (Cq), *Culex declarator* (Cd) and *Culex coronator* (Cc), inside and outside the home and in resting boxes. Municipalities of Ocauçu and Uchoa, Southeastern Brazil, 1999 to 2000.

Table 1. Distribution* of adults of *Aedes aegypti*, according to collection environment and sex. Municipalities of Ocauçu and Uchoa, Southeastern Brazil, 1999 to 2000.

Municipality / sex		Collection environment								
		Inside the home			Outside the home			Both		
		Box	Home	Total	Box	Home	Total	Box	Home	Total
Ocauçu										
Females	N	3	12	15	0	3	3	3	15	18
	%	20.0	80.0	100.0	0.0	100.0	100.0	16.7	83.3	100.0
Male	N	0	16	16	1	9	10	1	25	26
	%	0.0	100.0	100.0	10.0	90.0	100.0	3.8	96.2	100.0
Uchoa										
Females	N	6	21	27	0	6	6	6	27	33
	%	22.2	77.8	100.0	0.0	100.0	100.0	18.2	81.8	100.0
Male	N	1	22	23	1	4	5	2	26	28
	%	4.3	95.7	100.0	20.0	80.0	100.0	7.1	92.9	100.0
Both										
Male	N	9	33	42	0	9	9	9	42	51
	%	21.4	78.6	100.0	0.0	100.0	100.0	17.6	82.4	100.0
Male	N	1	38	39	2	13	15	3	51	54
	%	2.6	97.4	100.0	13.3	86.7	100.0	5.6	94.4	100.0

* Collected monthly from 15 homes per municipality using Nasci aspirators.

44 (3.2%) of *Ae. aegypti*, 33 (2.4%) of *Cx. coronator*, two (0.1%) of *Ochlerotatus scapularis*, one (0.1%) of *Anopheles brasiliensis* and one (0.1%) of *Coquillettidia* sp.. In Uchoa, over the same period, 726 specimens were collected: 608 (83.8%) of *Cx. quinquefasciatus*, 61 (8.4%) of *Ae. aegypti*, 32 (4.4%) of *Cx. declarator*, 22 (3.0%) of *Cx. coronator*, two (0.3%) of *Ochlerotatus scapularis* and one (0.1%) of *Anopheles* sp..

With regard to the four species of greatest frequency (99.7% of the total), the distribution in Ocauçu presented a statistically significant difference with the distribution found in Uchoa (χ^2=49.75; p=0.0000). The species that contributed towards this difference were *Cx. declarator*, with numbers in Ocauçu that were greater than expected (χ^2=24.83; p=0.0000), and *Ae. aegypti*, with numbers in Uchoa that were greater than expected (χ^2=26.54; p=0.0000). The other two species did not present significantly different distributions.

The proportions of the females of *Ae. aegypti* that were captured between the months of November 1999 and April 2000 were 72.2% (95% CI: 46.5;90.3) and 93.9% (95% CI: 79.8;99.3), respectively in Ocauçu and Uchoa. The proportions of the males captured over this same period were 76.9% (95% CI: 56.4;91.0) and 96.4% (95% CI: 81.7;99.9), respectively in Ocauçu and Uchoa. For the three *Culex* species captured in Ocauçu, greater densities were observed in specific months: females and males of *Cx. quinquefasciatus* were more frequent between August and December 1999, respectively 64.4% (95% CI: 60.4;68.3) and 75.2% (95% CI: 71.5;78.7); females and males of *Cx. declarator* were more frequent between March and May 2000, respectively 72.3% (95% CI: 63.3;80.1) and 80.8% (95% CI: 62.5;92.5); and females and males of *Cx. coronator* were more frequent between August and October 1999, respectively 73.3% (95% CI: 44.9;92.2) and 88.9% (95% CI: 65.3;98.6). In Uchoa, no groupings of specimens at certain times of the year were identified for the genus *Culex*.

It can be seen in Figure 1 that the largest value for the Breteau Index and the largest mean number of females per home in Ocauçu were, respectively, 7.5 and 0.4 and they occurred in February 2000. The maximum Breteau Index and number of females per home in Uchoa were, respectively, 12.0 and 0.7 and they occurred in March 2000. The Spearman correlation coefficient between these two variables, paired by month, was not significantly different from zero for Ocauçu (p=0.208). For Uchoa, the coefficient obtained was 0.77, which was significantly different from zero (p=0.002). Contrary to the adult findings (capture of *Ae. aegypti* alone), the Breteau index measurements revealed the presence of *Ae. albopictus*. This species was detected in Ocauçu in February, March and April 2000 and in Uchoa in January, February and March 2000.

Figure 2 presents the mean numbers of females per home for the three species of *Culex*, by month. In Ocauçu, the highest numbers were 7.8 females per

Table 2. Distribution* of adults of the genus *Culex*, according to collection environment and sex. Municipalities of Ocauçu and Uchoa, State of São Paulo, 1999 to 2000.

Municipality / sex		Collection environment								
		Inside the home			Outside the home			Both		
		Box	Home	Total	Box	Home	Total	Box	Home	Total
Ocauçu										
Females	N	143	283	426	116	174	290	259	457	716
	%	33.6	66.4	100.0	40.0	60.0	100.0	36.2	63.8	100.0
Male	N	49	164	213	179	230	409	228	394	622
	%	23.0	77.0	100.0	43.8	56.2	100.0	36.7	62.3	100.0
Uchoa										
Females	N	39	189	228	77	71	148	116	160	376
	%	17.1	82.9	100.0	52.0	48.0	100.0	30.9	69.1	100.0
Male	N	13	78	91	62	133	195	75	211	286
	%	14.3	85.7	100.0	31.8	68.2	100.0	26.2	73.8	100.0
Both										
Male	N	182	472	654	193	245	438	375	617	1,092
	%	27.8	72.2	100.0	44.1	55.9	100.0	34.3	65.7	100.0
Male	N	62	242	304	241	363	604	303	605	908
	%	20.4	79.6	100.0	39.9	60.1	100.0	33.4	66.6	100.0

* Collected monthly from 15 homes per municipality using Nasci aspirators.

home in December 1999 for *Cx. quinquefasciatus,* 2.7 in May 2000 for *Cx. declarator* and 0.3 in August 1999 for *Cx. coronator.* In Uchoa, the peaks occurred in December 1999 for *Cx. quinquefasciatus* (4.4), August 1999 for *Cx. declarator* (0.7) and December 1999 for *Cx. coronator* (0.4).

Table 1 presents the distribution of males and females of *Ae. aegypti* according to capture environment. No females of *Ae. aegypti* were captured in the boxes placed outside the home. The sensitivities of the resting boxes in relation to accommodating females inside the home were, respectively for Ocauçu and Uchoa, 20.0% and 22.2% (p=1.000). For the two municipalities together, the sensitivities were 21.4% (95% CI: 10.3;36.8) for inside the home and 17.6% (95% CI: 8.4;30.1) for the total collected. Among the females of *Ae. aegypti* captured in Ocauçu and Uchoa, respectively, 15 (83.3%) and 27 (81.8%) were found inside the home (p=1.000).

Among the 13 Breteau Index measurements in Ocauçu, 64 receptacles were found to contain larvae of *Ae. aegypti*, of which two (3.1%) were inside the home and 62 (96.9%) were outside the home. In Uchoa, among the 114 receptacles found to contain larvae of this vector, 14 (12.3%) were inside the home and 100 (87.7%) were outside the home. All the receptacles found to contain larvae of *Ae. albopictus* (21 in Ocauçu and six in Uchoa) were outside the home.

Table 2 presents the distribution of males and females of *Culex* according to the capture environment. The sensitivities of the resting boxes for collecting females of *Culex* inside the home were, respectively, 33.6% for Ocauçu and 17.1% for Uchoa (p=0.040). For the two municipalities together, the sensitivities were 27.8% (95% CI: 24.4;31.4) for inside the home and 34.3% (95% CI: 31.5;37.2) for both environments.

DISCUSSION

The coincidence of predominant species of culicids and of their distributions in the urban areas of two municipalities in different regions of the State of São Paulo indicate the possibility of establishing similar mechanisms for the surveillance and control of these vectors.

The great majority of the females and males of *Ae. aegypti* were captured between the months of November and April, which is in accordance with the climatic pattern of the areas studied and with the seasonal behavior of this species. The two municipalities studied are in regions where the climate is characterized by the existence of two defined seasons: a hot and rainy season between November and April and a dry and cooler season between May and October.* In relation to *Culex*, there were only concentrations at certain times of the year in Ocauçu, but without any apparent relationship with the climatic seasons.

* Information from the Agricultural Department of the State of São Paulo. Unpublished data.

The findings from the present study relating to the distribution of females of *Ae. aegypti* inside and outside the home confirm the results from Barata et al[2] in a study conducted in São José do Rio Preto. In this medium-sized city in the northwest of the State of São Paulo, the proportion of females inside the home was 87.3%. In the present study, which was carried out in two small-sized municipalities on different regions of this State, no differences were found between the proportions of females inside the home. It can be deduced that remaining in the indoor environment is a characteristic of the females' behavior, since such locations provide their needs for food and shelter.

The continual presence of females of *Ae. aegypti* inside the home contrasts with their behavior in selecting environments for oviposition, since most of the receptacles containing larvae of *Ae. aegypti* were found outside the home. A study[8] that evaluated which locations were best for installing oviposition traps showed that, even with equal availability inside and outside the home, 83.5% of all the eggs were deposited outside the home. These data have important repercussions on activities for controlling *Ae. aegypti*. If the main objective is to act on the adult forms, the environment inside the home must be given priority, but if it is to control the larval forms, the environment outside the home must be given priority. The results from the present study have emphasized the importance of this type of investigation, which in the opinion of Donalísio & Glasser[9] is an instrument that is capable of answering specific questions regarding control programs.

The finding of around 60% of the females of genus *Culex* inside the home in both municipalities confirms its adaptation to the human environment and that humans are its preferred food source. These characteristics, together with the greater or lesser degree of ornithophilic activity among the three species found,[6] opens up the possibility for a scenario of WNV transmission to become established, should its introduction occur.[14]

Although collecting adults using manual aspirators supplies information regarding the numbers of female culicids per home, Focks[11] pointed out the limitation that the relationship between the total number of females present and the number collected is unknown. Another point to be raised concerns the capacity of the captures outside the home to represent the quantity of mosquitoes that exist in the environment. In the present study, the capture of females of the genus *Culex* outside the home in much greater proportions than achieved for females of *Ae. aegypti* shows the usefulness of the aspirator for collecting mosquitoes in this environment.

According to Focks,[11] the quantity of resources involved also constitutes a limitation on the technique. However, when this technique is used in scientific investigations, it furnishes information on the behavior of vectors that may direct the control actions and thereby compensate for the costs. In the case of *Ae. aegypti*, for example, Rodrigues-Figueroa et al[20] considered that the number of females per person or per area was a good indicator of the risk that dengue virus transmission might occur.

Although lower than the figure of 1.2 females per home reported by Barata et al,[2] the numbers of females of *Ae. aegypti* per home found in the present study were greater than what was considered to be the threshold for dengue occurrence in Singapore (0.2 females per home),[5] and therefore compatible with virus transmission. One point to be highlighted is the temporal concordance between the greater densities of females of *Ae. aegypti* per home and the Breteau Index values. In Uchoa, these two measurements presented a significant correlation. Since the peak for the adult indicator is concordant in time with the peak for the larval index, identification of this occurrence in time may help in carrying out entomological surveillance activities and in adopting control measures.

The densities of females of *Cx. quinquefasciatus* in the months of greater infestation reached more significant numbers than those found for *Ae. aegypti* (respectively, 7.8 and 4.4 females per home in Ocauçu and Uchoa). In addition to representing a nuisance for the human population, these densities indicate that this species could act as an important vector for disease transmission and especially for WNV infection.[17]

With regard to sensitivity, in a study carried out in Thailand, 30% to 60% of the adults of *Ae. aegypti* that were captured inside the home were aspirated from two to four resting boxes. These figures were greater than what was found in the present study (21.4% of the females), in which only one box was used in each environment.[10] These differences may be related to the quantity of boxes used, the infestation levels and also the times of day when collections were performed. Whereas in Thailand there was a mean of 14.2 females per home (from two collections per day), in Ocauçu and Uchoa the numbers were lower, from collections that were only performed in the mornings.

For the genus *Culex*, the resting boxes were shown to be a more sensitive detection method than was found for *Ae. aegypti*. The biological behavior of *Culex* (nocturnal hematophagic activity and diurnal resting) favors finding specimens in resting places during the day.[7] On the other hand, the hematophagic activity of *Ae. aegypti* is diurnal, with less likelihood of finding specimens in resting places during collections performed during this period.

New evaluations should be carried out in order to propose or reject the use of resting boxes as an entomological surveillance method, both for *Ae. aegypti* and for the

genus *Culex*. It needs to be taken into account that the sensitivity of the boxes was measured in relation to the number of adult specimens that the operators were able to collect and not in relation to the total number of adults present. Even for the genus *Culex*, which presented higher sensitivity, resting boxes should be compared with other methods for estimating the density of adults, such as traps of CDC type.

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