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Changes in *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) Functional Response as a Consequence of Host Density Choice

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Abstract

Most predator and parasitoid functional response studies have been carried out by using experimental designs where insects are confined to an arena and subsequently exposed to different host densities, which are evaluated individually. In the case of a parasitoid that looks for profitable patches, this design forces it to use the single host density patch available, and therefore the possibility of selection by the parasitoid is not considered at all. A selective functional response, in which the host is distributed in discrete patches at different densities, could be a solution to avoid such a limitation. However, the disadvantage of this design is that it does not meet the independence assumption required to perform a parametric statistical analysis. Nevertheless, the use of nonparametric analyses such as GAM and GAMM models allows the performance of this kind of design, making the relationship between the response and the explanatory variable more flexible, looking for general behavioral patterns. The behavior of the fruit fly parasitoid *Diachasmimorpha longicaudata* (Ashmead) in a patched condition was assessed in order to demonstrate that nonparametric analyses are useful tools when studying the selective functional response. Results showed that the functional response changed from a "sigmoid curve" to a "bell-shape curve" when the parasitoid had the chance to choose freely among different host densities. The female parasitoid distributed their ovarian load among the eight host densities. The present study suggests that the bell-shape curve displays a general behavior pattern of the parasitoid population.

Key words: functional response, *Diachasmimorpha longicaudata*, host–parasitoid system, patchy environment, fruit fly

The functional response is described as the number of parasitized or attacked host or prey by a parasitoid or predator according to the host or prey density (Solomon 1949). Oaten and Murdoch (1975) considered the functional response as a cornerstone of any study of systems in which either parasitoid or predator are involved; the number of parasitized hosts or consumed prey will determine the development, survival, and reproduction of their population.

From the Solomon's (1949) definition and from the different types of functional response described by Holling (1959) to date, researchers have sought the mathematical model that better fits the functional response; thus, in order to assign values to biological parameters such as the attack rate, handling time, and searching time, mathematical parameters have been developed (Juliano 2001, Nachman 2006, Okuyama 2012). Most functional response studies

have been carried out using experimental designs, where insects are confined to an arena and exposed to different host densities, one at a time (Takahashi 1968, Collins et al. 1981, Bezemer and Mills 2001).

Nevertheless, this kind of design does not show the effect derived from the parasitoid choice of patches with different host densities. van Lenteren and Bakker (1978) considered that the traditional functional response design forces the parasitoid to return to the same site and to search in it again, increasing the probability that hosts are found even at the lowest densities. This hypothesis is the basis to prove how highly conditioned the functional response design without options is. Therefore, a design involving a selective functional response, in which the parasitoid has the opportunity to choose between different host densities, would be more suitable for

reducing the risk that the parasitoid concentrates its activities on the same host density. In addition, a selective functional response analysis would make it easier to find a general population behavioral pattern in response to different host densities, under the hypothesis that this pattern arises from groups of individuals with different foraging strategies. In natural conditions, parasitoids and predators have the chance to search, select, and make use of different patches with host or prey available, and each patch could yield a different number of hosts or preys (Lessells 1985; Bernstein et al. 1988, 1991). Several authors measured the functional response of parasitoids or predators in patchy environments (May 1978, Luck et al. 1979, Hassell et al. 1985, Nachman 2006). However, the parameters used in these studies to describe the functional response are not reliable when conditions change; moreover, the parametric statistical analysis used for curve fitting assumes data independence, which is difficult to achieve in a patch design.

Taking into account each and every aspect aforementioned, the aim of the present study is to describe the functional responses of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), reared on larvae of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae), in host density choice test, as well as no choice test. The host–parasitoid system *A. fraterculus*–*D. longicaudata* is appropriate for this kind of study because the fly host is naturally distributed and confined to finite patches (e.g., fruit), the number of host (larvae) per fruit usually varies, and the fruits are closely spaced. For this reason, the use of a nonparametric statistical method in the functional response analysis instead of a parametric method is proposed. Both the normality and independence assumptions of data are avoided in nonparametric analyses, whereas they are required in parametric analyses.

The South American fruit fly *A. fraterculus*, native of the Neotropical region, is one of the main pests of commercial fruit and vegetable crops in Latin America (Aluja et al. 2003). In Argentina, *A. fraterculus* and *Ceratitis capitata* (Wiedemann) are responsible for 20% of the losses caused by direct damage in commercial fruit (Guillén and Sánchez 2007). *Diachasmimorpha longicaudata*, a larval–prepupal, koinobiont, solitary endoparasitoid native of Southeast Asia, is one of the most widely used parasitoid species in fruit fly biological control programs (Wharton 1989, Ovruski et al. 2000). This braconid species was introduced throughout much of the American continent, from southern United States to Argentina (Ovruski et al. 2000). This exotic parasitoid is being studied in detail as a potential fruit fly biological control agent in fruit-growing Argentinean regions (Viscarret et al. 2006, Ovruski et al. 2012, Suarez et al. 2012). It has proved a good capacity for successful development on both *C. capitata* and *A. fraterculus* larvae (Ovruski et al. 2012), and also a good host-searching ability at different host densities on a wide variety of fruit species at canopy and ground levels (García-Medel et al. 2007). Thus, the present study is part of ongoing biological research in Argentina, focused on mass production and augmentative releases of *D. longicaudata*.

Materials and Methods

Insect Rearing

Parasitoids and fruit fly larvae were reared at the Biological Control Division of the Planta Piloto de Procesos Industriales Microbiológicos y Biotecnología (PROIMI) located in San Miguel de Tucumán, Argentina. In the PROIMI's laboratory, the *D. longicaudata* colony, which takes 108 generations in rearing, was held in Plexiglas cages (30 by 30 by 30 cm) that were covered on both lateral walls by an organdy screen. Parasitoids were provided with water and honey every other day. The parasitoid colony was reared on laboratory-reared third-instar larvae of *A. fraterculus* at $25 \pm 1\,°C$, $75 \pm 5\%$ relative humidity, and a photoperiod of 12:12 (L:D) h. Host larvae were exposed to parasitoids every other day for a 1.5-h period in artificial rearing units with a diameter of 12 cm consisting of a double ring frame with two meshes of *voile* that allows access to the hosts on both sides. After exposure, larvae were placed in plastic containers (8 cm diameter, 5 cm deep) with a 1-cm vermiculite layer as pupation substrate and kept inside them until adults emerged. Rearing procedures of an *A. fraterculus* colony were carried out as described by Vera et al. (2007).

Experimental Procedure

Two experiments were carried out to examine the effects of host density choice on the functional response of *D. longicaudata*. In the first one the functional response was assessed by exposing separately parasitoid females at different host densities (no-choice test). In the second experiment, the parasitoid females were simultaneously exposed to eight patches with different host densities (choice test). In both experiments, parasitoid males and females (1:1 proportion) were kept in plastic cages for 5 d after emergence. After that period, 7-d-old mated *D. longicaudata* females were used in the experiments. One day before the experiment, female wasps were exposed to hosts in order to homogenize the physiological and behavioral status of the females, as recommended by Samson-Boshuizen et al. (1973) and van Lenteren and Bakker (1978). Once the parasitoid females were released into each test cage, they were allowed to forage for 4 h to avoid any restrictions on the host search time. This exposure time was based on preliminary observations that indicated a residence time up to 3 h in a particular patch for some *D. longicaudata* females (Sergio Marcelo Ovruski, unpublished data). In addition, Montoya et al. (2000) had already indicated that *D. longicaudata* females could remain over the parasitism unit for 2.30 h. Laboratory-reared third instars of *A. fraterculus* (11 d old) were used as hosts. The experiments were performed under similar laboratory conditions as described above for insects rearing process.

Functional Response Without Patch Choice

Eight different host densities (1, 2, 3, 5, 15, 30, 60, and 120 *A. fraterculus* larvae densities) were individually exposed to a parasitoid female in transparent plastic cages (10 by 8 by 15 cm) for a 4-h period. Host larvae were previously placed inside an oviposition unit (an organdy screen-covered petri dish, 6 cm diameter, 0.8 cm depth) without an artificial diet and then exposed to the parasitoid. Each female parasitoid and host larvae were used only once. After host exposure, host larvae were placed in plastic containers (8 cm diameter, 5 cm depth) with a 1-cm vermiculite layer as pupation substrate until adult emergence. The number of parasitoids and flies emerged from pupae was recorded. Nonemerged pupae were dissected two weeks after the last parasitoid emergence in order to determine the presence of adult and preimaginal stages of *D. longicaudata* that died before emergence; in this case the host was recorded as parasitized. Densities 1 and 2 were replicated 71 times, density 3 was replicated 70 times, density 5 was replicated 68 times, and densities 15, 30, 60, and 120 were replicated 41 times. In order to determine if there was a range of host density dependence, low host density units were further replicated (Juliano 2001), considering that the dependent variable is a proportion and the relative variability of observations should increase at low density.

Functional Response With Patch Choice

The same eight host densities tested in the first experiment were also used in this experiment. Host larvae from each evaluated density were placed in oviposition units as described above. A single female parasitoid was exposed to all host densities at the same time in a cubic transparent plastic cage (30 by 30 by 30 cm). Both lateral sides of the experimental cage were covered with an organdy mesh to facilitate internal aeration. The eight different patches with hosts were located in a circle. Each patch was distanced 14 cm from each other. A female parasitoid was released in the center of the circle at the start of the experiment. The different densities were randomly settled in each replica in order to allow a random search of the parasitoid. The host exposure time to the female parasitoids was 4 h for each replica. The experiment was replicated 51 times. After exposure, the host larvae processing, the insect adult recovery, and the puparia dissection were similar to that described above for experiment 1. Behavioural observations were carried out to determine the frequency of female parasitoid visits (females that landed on the oviposition unit surface) to each host density patch. The parasitoid female was observed once every 15 min during the experimental time (4 h) and each observation lasted 5 min. The frequency data were recorded in a binomial way considering 1 as "success" and 0 as "failure" for each visit observation.

Statistical Analysis

For both experiments the statistical analyses were done in R 3.1.3 (R Core Team 2014), by using additive modeling in order to avoid *a priori* assumptions about the shapes of the functional response curves. This nonparametric approach enables full flexibility in the relationship between the response and the explanatory variable (Hastie and Tibshirani 1990, Nilsen et al. 2009). The no-choice test was analyzed with generalized additive models (GAMs) with cubic smoothing splines from the "gam" function of "mgcv" package (Wood 2006, 2012) with Poisson error distribution and log link function. This procedure was done to examine the relationship between density and the number of parasitized host larvae. Binomial error structure and logit link function were used to model the relationship between density and the proportion of parasitized host larvae. In both cases, quasi-GAMs were fitted because data present a significant amount of overdispersion.

In the choice test, generalized additive mixed-effect models (GAMMs) were used to incorporate the random effects and to take into account the lack of independence among wasp choices (Breslow and Clayton 1993, Krawchuk and Taylor 2003, Zuur et al. 2009). The parasitoid identity (effect of each female parasitoid behavior) was introduced as a random factor in the model (Zuur et al. 2009). For this analysis, the "gamm4" function from the "gamm4" package was used to run separate models in order to determine the best random structure, one of them with random intercept and the other one with random intercept and slope. The best fitting model was chosen based on their Akaike's information criterion (AIC; Burnham 2004). Poisson error distribution and log link function were used to examine the relationship between density and the number of host larvae attacked. Binomial error structure and logit link function were used to model the relationship between density and the proportion of parasitized host larvae. Due to the statistical limitation to compare the two different models (GAM and GAMM), the superposition of their confidence intervals (estimated as 1.96 times of the standard errors) was examined. Binomial data obtained from the frequency of parasitoid female visits were analyzed using a GAMM with binomial errors and logit link function. A Spearman correlation was performed in order to measure the interaction between the three highest host density patches with the aim to determine the effect of the individual behavior of parasitoid females predicted by the GAMM analysis.

Results

The GAM analysis generated a sigmoid curve (Fig. 1a). This is supported by the growing proportion of parasitized hosts as density in patches is increased, until it reached a density of 25 hosts (Fig. 1b), from which the curve is asymptotic. The proportion of parasitized hosts increased from 0.026 ± 0.003 (mean $\pm$ SE) for density of 1 host to 0.248 ± 0.009 for density of 30 hosts in the density-dependent zone of the curve (Fig. 1b). The highest number of parasitized hosts occurred at a density of 120 host larvae, averaging 19.5 ± 1.5 parasitized hosts (Fig. 1a). Nevertheless, the highest parasitized host proportion was reached at a density of 30 hosts, which was 1.5 times higher than the parasitized host proportion at a density of 120 host larvae (Fig. 1b). The number of parasitized hosts was significantly related to the host larvae density in the patch
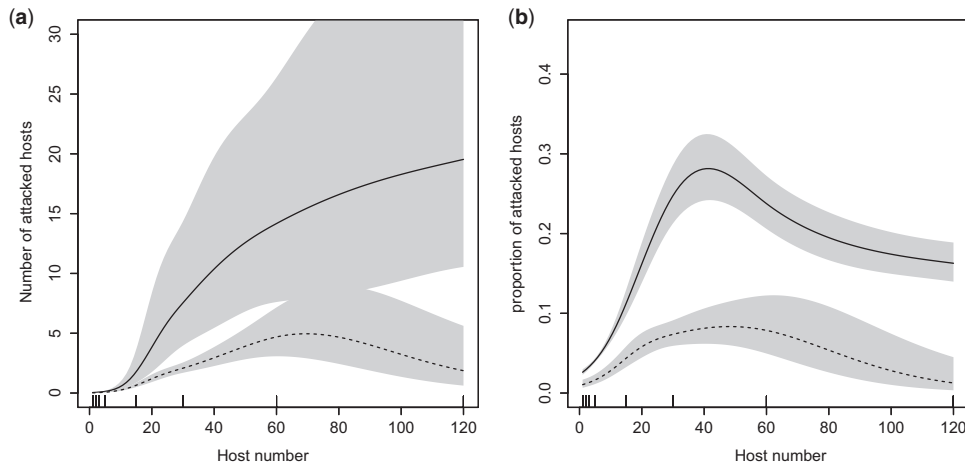
Fig. 1. Effect of host number on the number (**a**) and proportion (**b**) of parasitized host larvae, estimated from a generalized additive model (no choice assay, continuous line) and a generalized additive mixed model (choice assay, dashed line), respectively. Shaded areas correspond to ± 1.96 Bayesian standard errors; values obtained according to Wood (2006).

($F = 55.22$, df $= 3.884$, $P < 0.0001$). Similarly, the proportion of parasitized host larvae was closely associated to the host larvae density ($F = 9.772$, df $= 3.221$, $P < 0.0001$).

When the female parasitoid had the option of choosing between different host densities, the GAMM analysis generated a bell-shape curve (Fig. 1a), with a positive density-dependent zone at the lowest host densities and with a negative density dependent at the highest host densities. The positive density-dependent zone occurred between 1 and 60 host densities in which the increasing proportion of parasitized host ranged from 0.010 ± 0.005 to 0.078 ± 0.013 (Fig. 1b). The model predicted a maximum number of parasitized hosts of 4.7 ± 0.7 at a density of 60 hosts (Fig. 1a). From this value, the number of parasitized host decreased toward 1.9 ± 0.8 at a density of 120 host (Fig. 1a), and with a parasitized host proportion of 0.013 ± 0.006 (Fig. 1b). Taking into account the eight host densities, the mean total number of parasitized host larvae by each female parasitoid was 24.9 ± 1.7. The number of parasitized hosts was significantly related to the host larvae density in the patch ($\chi^2 = 559.6$, df $= 4.141$, $P < 0.0001$). Similarly, the proportion of
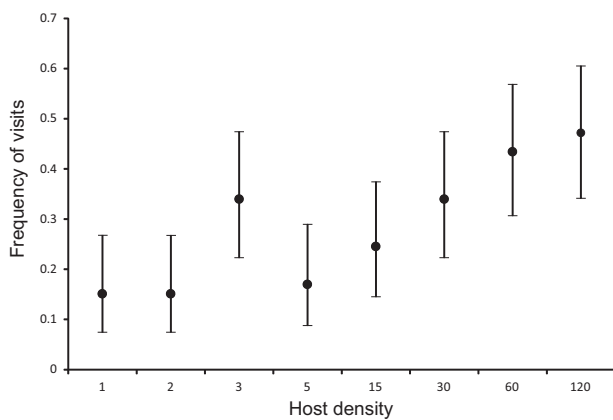
parasitized host larvae was closely associated to the host larvae density ($\chi^2 = 180.3$, df $= 3.702$, $P < 0.0001$).

The frequency of visits of the parasitoid to the patches depended on host density; patches with a higher number of host larvae were most frequently visited than patches with low host densities. Frequency of visits increased significantly 3.1 times from patches with density of 1 to 120 host larvae (Fig. 2). The only exception to the general increase pattern from lower to higher host densities was the patch with 3 hosts, which attained the same frequency of visits as the 30 host density patch. The fixed effect from GAMM showed a significant density effect on the probability of visits ($\beta_1 = 0.011 \pm 0.002$, $z = 4.367$, $P < 0.0001$). Thus, the odds ratios showed that the probability of a patch to be visited by a female parasitoid increased by 1.1% per unit of density increase.

The model showed both random intercept and slope, which would indicate a differentiated behavior among parasitoid females to choose between different host densities. Thus, the 51 female used in the study can be grouped in five differentiated groups. The 5.9, 15.7, 17.6, 27.5, and 33.3% of the parasitoid females parasitized *A. fraterculus* larvae at densities of 2, 15, 30, 60, and 120 hosts, respectively. Each parasitoid female group laid the majority of eggs in one such host density and distributed the remainder between the other seven host densities (Fig. 3). The Spearman correlation analysis showed a significant negative correlation among the three highest densities; thus, when a female of a given group associated with a particular host density (30, 60, and 120) used such a patch, the probability of using the other two highest host densities significantly decreased (see Table 1).

Discussion

Functional response models like those described by Holling (1959) and Ivlev (1961) (see Jeschke et al. 2002 for a review) assume that both parasitoid and host populations are homogeneously distributed in space, and predict that parasitism rates are only a function of the host density. This approach does not take into account that the performance of the parasitoid may depend on the host distribution and the parasitoid responds to such distribution (Nachman 2006). This

Fig. 2. Observed frequency of visits by female *D. longicaudata* to host patches with different number of *A. fraterculus* larvae. Scattering bars correspond to the 95% confidence interval.
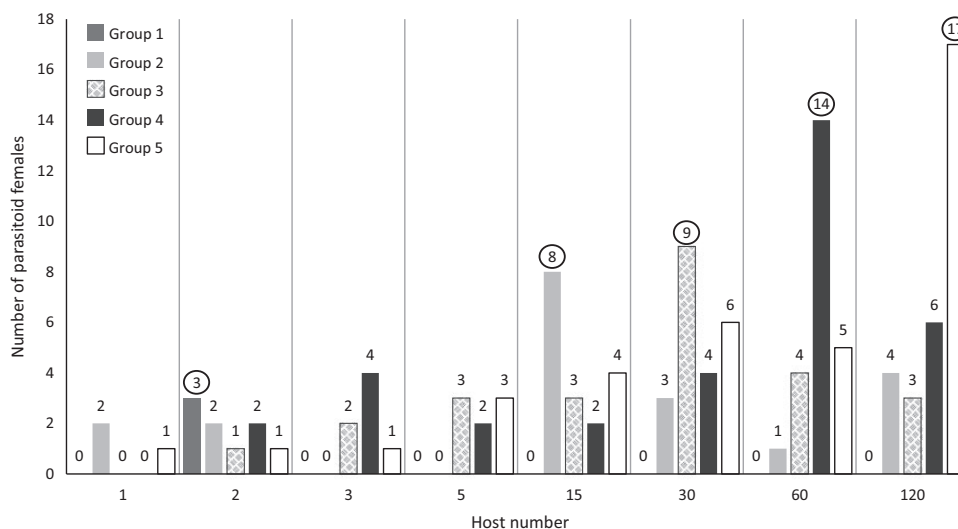

Fig. 3. Number of *D. longicaudata* females parasitizing host larvae in the different patches of host number in the choice design. Each color column represents a different parasitoid female group mainly parasitizing a particular host number. Five female groups can be differentiated. In all, 5.9, 15.7, 17.6, 27.5, and 33.3% of the parasitoid females from the groups 1, 2, 3, 4, and 5 mainly parasitized *A. fraterculus* larvae associated with 2, 15, 30, 60, and 120 host number, respectively. Circles around numbers on the top of the columns indicate the highest number of parasitoid females by group that parasitized a particular host number.

Table 1. Spearman correlation matrix among the three highest host number (30, 60, and 120)

Host densities	30	60	120
30	1.000		
60	−0.150	1.000	
120	−0.152	−0248	1.000

would limit the general applicability of this kind of models when host populations tend to occur in aggregated patterns (Turchin and Kareiva 1989).

Results of the no-choice experiment showed evidence of a female parasitoid response highly conditioned by the design. The higher number of parasitized host as density increase (Fig. 1a) may be due to the fact that the parasitoid has been forced to stay in the same host density patch for a long period (van Lenteren 1976), and probably, the number of host found is by far much higher than that expected in a natural situation. In another case, the parasitoid would certainly leave the patch if it did not find any host after spending some time searching (van Lenteren and Bakker 1978). In contrast, the present study showed that the *D. longicaudata* female response changed when it had the possibility to choose among several patches with different host densities. Thus, the number of parasitized hosts at high densities was lower than that recorded in the no-choice test. The difference between the two functional response curves recorded in this study is another evidence of the host density choice effect on the parasitoid behavior. The optimal foraging theory suggests that parasitoids can maximize their oviposition rate by aggregating in high-density host patches (Charnov 1976, Cook and Hubbard 1977, Pyke 1984). This behavior was clearly developed by *D. longicaudata* females in the no-choice design, but the trend was not followed by parasitoid females in the host densities choice test. Interestingly, the highest numbers of parasitized host ranged between ∼19 and ∼24 host larvae in both experiments. However, in the no-choice test these values were reached only at the patch with the highest host density, but in the choice test the same values were distributed among the eight different patches. This event showed that the female parasitoid distributed their ovarian load in more than one single patch.

The increased frequency of the female parasitoid visits at high host densities recorded in the choice design (Fig. 2) follows the behavior prediction proposed by the foraging theory, which contrasts with the bell-shape functional response curve (Fig. 1b). The bell-shape curve suggests that, more often than not, the female parasitoid uses patches with an intermediate number of hosts, which could be the result of the patch selection effect. In a no-choice design, this result is hidden, as it forces the parasitoid to return to the same and single available patch. Differences between frequency of visits and functional response curve would be a proof of the choice effect that emerges from the individual behavior of a *D. longicaudata* female. Several factors and mechanisms could affect this behavior. Walde and Murdoch (1988) described models that could explain differences with the expected results from the optimal foraging theory. For example, the existence of a partial refuge could be the reason why foraging does not meet the expected pattern by the optimal searching (Hassell et al. 1985, Price 1988). Lessells (1985) expressed that eggs or time limitation, along with a random patch entry, will lead to a "domed" density relationship. Results reported here are consistent with Walde and Murdoch (1988), who concluded that

parasitism distribution (parasitism observed) is more important than individual parasitoid distribution (frequency of visits).

Considering that the highest host density (120 hosts) used in this study received more visits than lower ones, it would be expected that the female parasitoids spend more time ovipositing there. Several reasons support this idea: 1) the possibility of refuge for the host is minimum, 2) there are enough hosts to empty the egg load without having to move to another patch, and 3) self-superparasitism is a common behavior of *D. longicaudata* that could suppress host immune defenses and consequently reduce egg encapsulation (Kapranas et al. 2012). However, a high self-superparasitism may decrease parasitoid emergence (Montoya et al. 2000, González et al. 2007); thus, the possibility of super-parasitism at high host densities is minimized. Even though the parasitoid had the possibility to choose among patches, the highest host density patch (120 hosts) was not the main target attacked. This no preference of the highest host density leads to the ensuing question: should the parasitoid optimize the number of individuals or the success of its progeny? If the answer is "the number," the parasitoid should parasitize the highest density patch more intensely, but, if the answer is "optimize the success" the parasitoid should: 1) distribute its egg load between more than one patch to ensure that at least the progeny of one patch survives, 2) avoid any possible host density-dependent mortality at higher densities (Ives and Settle 1996), 3) avoid any possible high super-parasitism in lower densities (Montoya et al. 2000), and 4) minimize the possibility of failure in the attempt to search the host at lower densities (Luck et al. 1979). Taking into account the four points discussed above, the patches with intermediate host densities could be making the obvious handicap of the lowest and the highest density patches less evident. In fact, it minimizes the refuge possibility that exists at lowest host densities, and minimizes the probability of dying due to host density-dependent mortality that occurs in patches with highest host density. Bernstein et al. (1991) sustained that the attacks concentrated at intermediate host densities correspond to a patchy environment with small migration costs, in which parasitoids should be able to track spatial changes in the distribution of their resources. Results of the present study are consistent with models proposed by Bernstein et al. (1991), and give a more realistic response of the parasitism produced by the female parasitoids considering the possibility to choose between different host densities.

Results of the GAMM analysis (Fig. 3) showed that the selection preference among patches with different host densities differed among female parasitoids. Thus, the five well-differentiated female groups may be a population strategy of *D. longicaudata*, expressed only in a patched environment and masked with a no-choice design. Therefore, the use of the Information Theoretical Models (Stephens et al. 2007), such as GAM and GAMM analyses, puts aside the exacting idea that a parasitoid population would have an average behavior, disaggregating the underlying individual behavior of the population; furthermore, they also allow experimental designs without data independence as is the case of the functional response with patch choice.

Functional response analyses involving a selective design under field-cage conditions need to be addressed in future studies. It should be expected that *D. longicaudata* females display a similar behavioral pattern in the field as in the present study, conducted under laboratory conditions, since the host larvae are distributed in discrete patches (fruits in the tree) closely spaced and with different host densities. Based on the latter, a series of experiments with artificially infested fruit (unparasitized larvae), hanging from branches or placed

at ground level inside a field cage, will be conducted under field conditions.

The importance of the functional response analysis in the predation and parasitism processes is not questionable (Berryman 1999, Bernstein 2000, Hassell 2000); however, how relevant it is to the success of biological control programs (Fernández-arhex and Corley 2003) remains unclear. Results of the no-choice design showed that *D. longicaudata* attained the highest attack rate at 1:30 (parasitoid/host) proportion; this is good information to establish mass rearing conditions in an augmentative biological control program, where it is desirable to optimize the production of both parasitoid and host, and to provide information for quality control of parasitoid (Montoya et al. 2000, Carey 2001). In addition, data resulting from patch choice could be taken into account to optimize the number of parasitoids for augmentative releasing in the field.

Acknowledgments

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