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Determination of prey antigen half-life in *Polistes metricus* using a monoclonal antibody-based immunodot assay

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Abstract

In order to derive quantitative estimates of predation rate from serological gut analysis data, one must have an estimate of the interval during which a meal can be detected after feeding. In practice this has turned out to be 'D_{max},' defined as '...the time from finishing a meal until that meal could just no longer be detected in any individuals.' However D_{max} substitutes an absolute limit for what is really a continuous variable with significant variation. We examined this problem in a study of the detectability of *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) fifth instar remains in the guts of *Polistes metricus* Say (Hymenoptera: Vespidae). Wasps were maintained on *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) fifth instars before being fed a single *H. zea* fifth instar. They were killed and frozen at 0, 24, 48 and 96 h intervals, with those held for more than 24 h fed a single *T. ni* fifth instar at 24 h intervals in order to simulate continued feeding. Wasp abdomens were assayed by immunodot, using a monoclonal antibody to *H. zea* arylphorin. There was a logarithmic decay in the proportion of *P. metricus* positive over time, a single *H. zea* fifth instar meal having a detectability half-life of 19.4 h at field temperatures. If prey antigen detectability decays exponentially, then a detectability half-life is a more appropriate unit of detectability than an absolute detectability period.

Introduction

Serological gut analysis is our most powerful tool for determining the impact of arthropod predators on prey populations (Sunderland, 1988; Greenstone, 1989). In order to derive ecologically useful data from serological assay, one must have an estimate of the interval during which a meal can be detected after feeding. In practice this has turned out to be what Sunderland *et al.* (1987) have called the maximum detection period, 'D_{max},' defined as '...the time from finishing a meal until that meal could just no longer be detected in any individuals.' It has been found to range from a little less than a day to more than two weeks in a variety of situations (Dempster, 1960; Greenstone, 1983; Lovei *et al.*, 1985, 1990; Sopp & Sunderland, 1989).

The difficulty with D_{max} is that it substitutes an absolute limit for what is really a continuous var-

iable with significant variation. A further difficulty with all but a few studies (Fichter & Stephen, 1981; Lovei *et al.*, 1987, 1990) is that the predators were starved from the time they ceased feeding until they were killed for gut analysis, whereas had they been in the field they would doubtless have continued to hunt and feed. For at least one major group of arthropod predators, the spiders (Araneae), starvation reduces the metabolic rate (Anderson, 1970), which is bound to reduce the digestive rate as well. Furthermore the digestive rate is profoundly affected by temperature (McIver, 1981; Sopp & Sunderland, 1989; Hagler & Cohen 1990), and while a few attempts have been made to simulate field temperature regimes by holding predators at separate daytime and nighttime temperatures (Greenstone, 1979; Sopp & Sunderland, 1989), most have been run at a single constant temperature.

We have addressed some of these shortcomings in a laboratory study employing fifth instar *Helicoverpa zea* Boddie as prey and the paper wasp *Polistes metricus* Say as predator. This is a realistic model predator-prey system, since *Polistes* spp. are well known polyphagous predators of Lepidoptera larvae, congeners of *P. metricus* are known from field studies to feed on fifth instar *H. zea* (Quaintance & Brues, 1905; Rabb & Lawson, 1957; Whitcomb & Bell, 1964), and some individuals feeding upon *H. zea* larvae and identified as *P. fuscatus* (Fabricius) were probably *P. metricus* (Rabb & Lawson, 1957). The animals were maintained under a simulated field temperature and photoperiod regime and were fed serologically negative alternative prey from the time they ceased feeding on *H. zea* until they were killed.

Materials and methods

Wasp collection and feeding. Adult *P. metricus* were collected in midsummer 1987 and 1988 during the day in St. Louis and Boone Counties, Missouri, and held in a rearing room on an L14:D10 photoperiod. Fresh water, diluted *Apis mellifera* honey, and live *Trichoplusia ni* (Hübner) fifth instars were provided daily. Wasps collected in 1988 were used for detectability period experiments; all other experiments utilized the 1987 wasps (below).

At least 12 h before feedings were to begin, wasps were isolated in individual cages and deprived of food and water. They were offered homogenates of fifth instars with a Pasteur pipette or dissecting needle held directly in front of the mandibles and fed until they refused to feed further. In order to verify that we could distinguish *H. zea* meals from other species so that we could continue to feed wasps serologically negative species after the consumption of a single *H. zea* meal, we fed wasps the following noctuid species, maintained on artificial diets in long-term continuous culture (Lenz & Greenstone, 1988): *H. zea*, *Heliothis virescens* (F.), *H. subflexa* Grote, *Anticarsia gemmatalis* (Hübner), *T. ni*, and *Spodoptera frugiperda* (J. E. Smith). Groups of four wasps fed each species were placed immediately in gelatin capsules and killed by freezing at −80 °C.

For the detectability period study, additional groups of wasps were fed *H. zea* homogenate, placed in a Conviron Model 123 programmable incubator (Controlled Environments, Inc., Pembina, ND) and held at temperatures simulating mean hourly shade temperatures and photoperiod in a Stoneville, Mississippi cotton field during the first ten days of July, 1986 (K. R. Hopper, pers. comm.; Fig. 1). Surviving wasps were killed and frozen at 24 h intervals; those held for more than 24 h were fed *T. ni* homogenate at each interval in order to simulate continued feeding.

Because insect venoms are rich in enzymes (Schmidt *et al.*, 1986), we were concerned that the presence of the wasp venom gland in the homogenate prepared for immunoassay might hasten digestion of *H. zea* antigen in the wasps' guts. In an experiment to test this possibility, the portion of the body containing the gland was isolated and homogenized separately from that portion containing most of the gut. This was achieved by cutting the abdomens with a razor blade behind the third abdominal segment (i.e., the second segment of the gaster), thereby dividing the abdomen into anterior (primarily gut) and posterior (con-

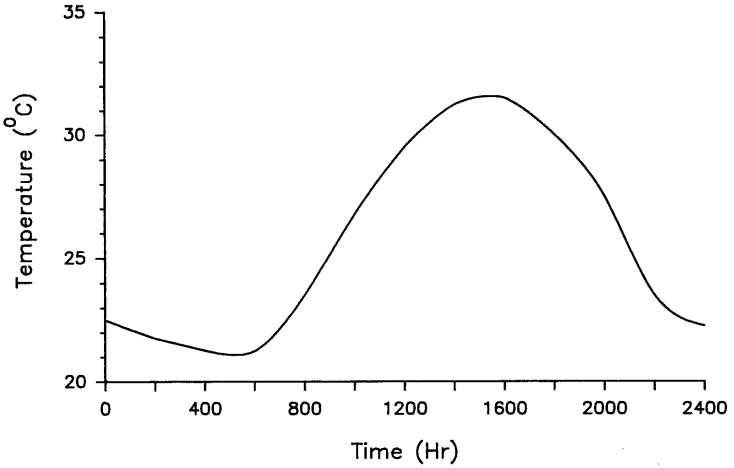

Fig. 1. Temperature trace from the programmable incubator. Lights were off from 2100 through 0500.

taining the venom gland) portions of roughly two-thirds and one-third volume, respectively. The anterior portion was homogenized in 2.0 ml of PBST-BSA (Greenstone & Morgan, 1989) while the posterior portion was homogenized in 1.0 ml of the same buffer. These homogenates were prepared for final assay by diluting them 1:5 in TTBS-BLOTTO (Stuart & Greenstone, 1990).

Homogenates of the anterior portions of the abdomens of a series of wasps that had been positive by immunodot were assayed alone or with the addition of an equal quantity of the posterior abdominal homogenates of the same animals, either immediately or after 4 h incubation at 37 °C with rotation at 100 RPM. As a control for the dilution effect of the addition of the posterior portion, a sample in which the anterior portion was incubated with an equal quantity of buffer (TTBS-BLOTTO) was also run.

Immunoassay procedure. We used the immunodot assay with ZetaProbe membranes (Bio-Rad Laboratories, Richmond, CA), as described by Stuart & Greenstone (1990). Monoclonal antibody (MAB) HZ5-1, specific for the arylphorin of *H. zea* (Lenz & Greenstone, 1988), was used for both capture antibody and conjugate. The MAB was mass produced by tissue culture in serum-free medium (DME/High, JRH Bioscience, Lenexa, KS), concentrated by ultrafiltration, and purified to remove phosphatase activity (Greenstone *et al.*, 1991).

Results and discussion

Species-specificity of the immunodot assay. All wasps fed *H. zea* fifth instars were positive in the immunodot assay; those fed other noctuid fifth instars were all negative (data not shown). This makes *P. metricus* the third predator species, after *Phidippus audax* Hentz (Araneae: Salticidae) and *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), for which the assay is species-specific (Greenstone & Morgan, 1989; Stuart & Greenstone, 1990). Since these predators belong to three arthropod orders in two classes, the assay should be generally useful for studying arthropod predation on fifth instar *H. zea*. This information enabled us to choose any of the non-target species as the serologically negative one for the detectability period studies; we chose *T. ni* because it is the most economical one for us to rear.

Effects of the venom gland on prey detectability. Inclusion in the wasp homogenate of the posterior portion of the abdomen containing the venom gland did not affect the ability of the immunodot to detect an *H. zea* meal even after 4 h incubation at 37 °C with rotation (Table 1). In a few cases

Table 1. Results of immunodot assay on the effect of the *P. metricus* venom gland on *H. zea* arylphorin detectability in the gut

A. Animals killed immediately after feeding

Specimen		A′	B′	C′	D′	E′	F′	G′	H′	I′	J′
Abdominal portion(s) assayed and incubation time at 37°C.											
Anterior alone	0 h	+	+	+	+	+	+	+	+	+	+
	4 h	+	+	+	−	+	−	+	+	+	+
Anterior plus posterior	0 h	+	+	+	+	+	−	+	+	+	+
	4 h	+	+	+	+	+	−	+	+	+	+
Anterior plus buffer	0 h	+	+	+	−	+	−	+	+	+	+
	4 h	+	+	+	−	+	−	+	+	+	+

B. Animals killed 24 h after feeding (* = not run due to shortage of homogenate)

Specimen		A″	B″	C″	D″	E″
Abdominal portion(s) assayed and incubation time at 37°C.						
Anterior	0 h	−	+	+	*	*
	4 h	−	−	+	+	+
Anterior plus posterior	0 h	−	+	+	*	*
	4 h	−	+	+	+	+
Anterior plus buffer	0 h	−	−	+	*	*
	4 h	−	−	+	+	+

incubation of the anterior portion alone hastened digestion of the antigen (animals D′, B′′, and F′). This might have been due to proteases from pollen ingested by the wasps prior to capture (Grogan & Hunt, 1979; Hunt *et al.*, 1991). The only case in which addition of the venom-containing posterior portion led to a loss of detectability (animal F′) can be explained by the dilution effect of adding the posterior portion. On the basis of these data we recommend that entire *Polistes* abdomens be homogenized for assay.

Since prey antigens detected in stomach analysis are most often proteins, the generalizability of this recommendation boils down to a question of whether a given predator's venom is rich in proteases. Polistine wasps are important medically, and most biochemical studies of their venom have focused on pharmacologically active fractions. However the venoms of three *Polistes* species, unfortunately not including *P. metricus*, have been assayed for protease activity (Said, 1960, cited in Edery *et al.*, 1978; Schmidt *et al.*, 1986); only one of these, *P. infuscatus*, appears to have such activity.

The venoms of some aculeate wasps probably function in both subduing prey and deterring predators. Predator deterrence is very important for the social wasps, with their diurnal conspicuous foraging and dense aggregations of helpless offspring, which may explain their emphasis on pain-causing and tissue-damaging venom components (Schmidt, 1990). The extent to which these functions are enhanced by the inclusion of proteases, or the possibility that proteases serve other functions, will depend upon the evolutionary histories of individual taxa. The polistine wasps do not use venom to subdue their prey, but the enzyme complement of venoms of extant species may in part be a vestige from ancestors who did so (Carpenter, 1982; Cowan, 1991).

Detectability period. Immunodot results for thirty-nine *P. metricus* held for times ranging from zero to 96 h post-feeding are presented in Fig. 2. These

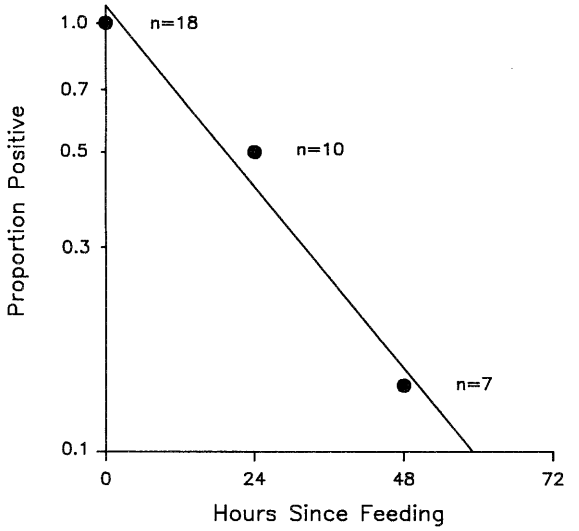

Fig. 2. Proportion of wasps positive 0, 24, and 48 hours after feeding on *H. zea*; none of four wasps held for 96 hours were positive. The line is a least-squares regression with $R^2 = 0.973$.

data describe an exponential rate of decay in the proportion of animals with detectable prey with time since feeding. There are only two other comparably reported data sets. Fichter & Stephen (1981) found a linear rate of decay in the proportion of positives for *Podisus maculiventris* allowed to feed on plant material after having consumed one prey individual. Ohiagu and Boreham (1978) studied rates of decay, in two different assays, in the proportion of positives of *Coccinella septempunctata* L. which had been starved after having been fed a single prey individual. In a latex assay they found a linear rate of decay, but in a ring test there appeared to be two phases of linear decay, with the first 24 h being more rapid than the final 36 h; this could represent the discontinuous operation of two processes, such as egestion and digestion. The linear decay rates seen in these two studies may be due to the authors having used anti*sera*, which although in one case highly purified (Fichter & Stephen, 1979), were still polyclonal mixtures, so that the decay curves reflect digestion of many antigenic determinants, whereas the MAB used in our work documents digestion of a single determinant on one protein (Lenz & Greenstone, 1988). The rate of decay with time since feeding of prey antigen *mass* has also been found to be exponential (Lovei *et al.*, 1985, 1990; Sopp & Sunderland, 1989), but that could not have caused the exponential decrease in detectability which we report here. The latter is more likely explained by individual differences in prey mass consumption, digestive rate, or some combination of the two.

Positivity and negativity in predator gut assays. The starting point for a quantitative predation estimate based on serological gut analysis is the determination of whether or not each predator in the sample is positive for prey antigen. Some assays are by their nature qualitative, so that there is no apparent arbitrariness in this determination (Ohiagu & Boreham, 1978; Stuart & Greenstone, 1990). But even when the raw test result is quantitative, as in the case, for example, of an ELISA absorbance, it will be compared to a background or heterologous negative reading, or to a distribution of such readings, and designated negative or positive according to an arbitrary rule (Crook & Sunderland, 1984; Sunderland *et al.*, 1987; DuDevoir & Reeves, 1990) or a statistical decision (Fichter & Stephen, 1981; Schoof *et al.*, 1986; Greenstone & Morgan, 1989; Sopp & Sunderland, 1989; Fenlon & Sopp, 1991).

Since the criterion of detectability for the present research is the visual detection of a spot on the membrane, the implicit claim that there is no 'gray area' between positives and negatives bears some scrutiny. Homogenates from twenty-six predators fed *H. zea* or *H. armigera* (Hübner) (positives) and twenty-four fed *H. virescens* or *T. ni* were subjected to both ELISA and immunodot (Stuart & Greenstone, 1990, Table 1 and Figs. 3 & 4 of that paper, respectively). When one compares the immunodot results for the negative homogenate with the highest ELISA absorbance (0.696), C6, with those from the positive homogenate with the lowest absorbance (0.826), D6, one sees that on both the nitrocellulose and nylon membranes D6 produced a faint dot while C6 produced no discernible dye development. Ongoing research utilizing different membranes and other substrates indicates that background can be entirely eliminated, enhancing the distinction be-

tween positive and negative results (Greenstone & M. K. Stuart, unpublished data).

Significance of the results for quantitative estimation of predation rate. In order for serological gut analysis to provide ecologically useful data, assay results must be converted to per-capita estimates of numbers of prey eaten per unit time. The numbers eaten may be derived from a statistical model of feeding (Nakamura & Nakamura, 1977; Greenstone, 1979), or from an estimate of the biomass of prey detected and the shape of the function describing digestion of prey biomass (Sopp *et al;*, in press). The appropriate time unit for antigen detectability will depend upon the manner in which detectability is lost over time. If the decay in detectability is exponential, then a 'detectability half-life,' analogous to the half-life of a radionuclide (Wang *et al.*, 1975) and defined as the time after which only half of the meals eaten can be detected, is more appropriate than an absolute 'detectability period' (Sunderland *et al.*, 1987; Sopp & Sunderland, 1989; Hagler & Cohen, 1990; Lovei *et al.*, 1990). This half-life may be computed by calculating a least-squares linear regression for data of the form:

$$Y = \text{Log}_{10} \text{ Proportion of Guts Positive} = mx + b,$$

where m is the decay rate, x is time since feeding, and b is the Y-intercept. Solving for the value of x when $Y = -0.3010$, which is the $\log_{10}$ of 0.5, gives the half-life. The data in Fig. 2 yield a half-life of 19.4 h for a single *H. zea* meal in the gut of *P. metricus*.

Because a number of wasps died before they could be assayed, these results must be considered preliminary. Additional research with larger numbers of individuals of more robust species will tell us whether the decay in detectability tends to be linear or exponential or of some other form, and whether its shape is influenced by the assay system, predator or prey.

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